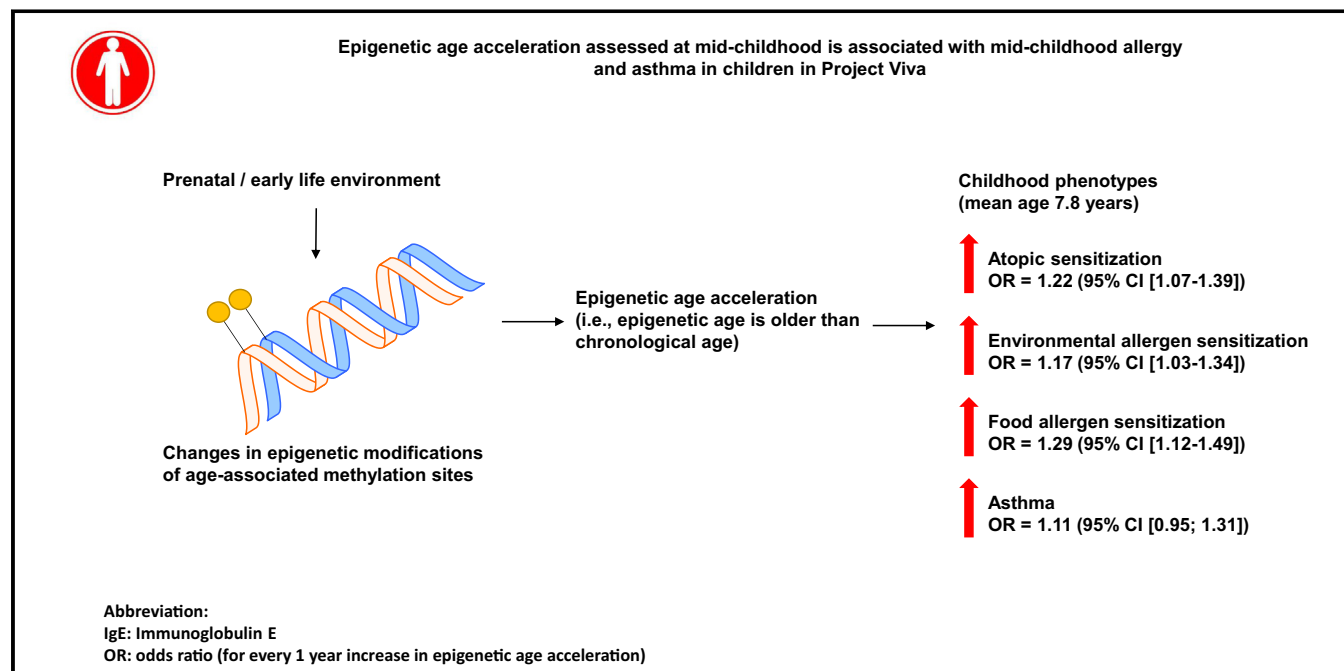


Epigenetic age acceleration is associated with allergy and asthma in children in Project Viva



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GRAPHICAL ABSTRACT



Background: Epigenetic clocks have been suggested to capture one feature of the complexity between aging and the epigenome. However, little is known about the epigenetic clock in childhood allergy and asthma.

Objective: We sought to examine associations of DNA methylation age (DNAmAge) and epigenetic age acceleration with childhood allergy and asthma.

Methods: We calculated DNAmAge and age acceleration at birth, early childhood, and midchildhood based on the IlluminaHumanMethylation450BeadChip in Project Viva. We evaluated epigenetic clock associations with allergy and asthma using covariate-adjusted linear and logistic regressions. We attempted to replicate our findings in the Genetics of Asthma in Costa Rica Study.

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Results: At midchildhood (mean age, 7.8 years) in Project Viva, DNAmAge and age acceleration were cross-sectionally associated with greater total serum IgE levels and greater odds of atopic sensitization. Every 1-year increase in intrinsic epigenetic age acceleration was associated with a 1.22 (95% CI, 1.07-1.39), 1.17 (95% CI, 1.03-1.34), and 1.29 (95% CI, 1.12-1.49) greater odds of atopic sensitization and environmental and food allergen sensitization. DNAmAge and extrinsic epigenetic age acceleration were also cross-sectionally associated with current asthma at midchildhood. DNAmAge and age acceleration at birth and early childhood were not associated with midchildhood allergy or asthma. The midchildhood association between age acceleration and atopic sensitization were replicated in an independent data set.

Conclusions: Because the epigenetic clock might reflect immune and developmental components of biological aging, our study suggests pathways through which molecular epigenetic mechanisms of immunity, development, and maturation can interact along the age axis and associate with childhood allergy and asthma by midchildhood. (*J Allergy Clin Immunol* 2019;143:2263-70.)

Key words: Epigenetic clock, allergy, asthma, DNA methylation

DNA methylation serves as a molecular marker for biological aging and its manifestations.¹ An increasing body of evidence suggests that DNA methylation–derived epigenetic clock metrics (which include DNA methylation age [DNAmAge] and epigenetic age acceleration) cross-sectionally associate with adult diseases, including physical and cognitive decline² and Parkinson disease,³ and are predictive of future onset of lung cancer.⁴ These epigenetic clock metrics have also predicted cancer, cardiovascular, and all-cause mortality independent of chronological age.⁵⁻⁸

More recently, research on the epigenetic clock has been expanded into the early-life spectrum.⁹⁻¹² Studies have demonstrated that birth weight,⁹ birth by means of cesarean section,⁹ and other pregnancy-related exposures (maternal smoking, body mass index, and selenium and cholesterol levels)⁹ associate with greater DNAmAge in children (age range, 7-19 years). Epigenetic age acceleration residuals (residuals from a linear regression of DNAmAge on chronological age) are also prospectively associated with faster increase in weight during childhood and adolescence¹⁰ and are predictive of earlier pubertal development in young girls.¹² However, epigenetic age and epigenetic age acceleration residuals have not been studied in association with allergy and asthma in children.

Furthermore, 2 other epigenetic clock calculations, intrinsic epigenetic age acceleration (IEAA) and extrinsic epigenetic age acceleration (EEAA), have also not been linked to childhood allergic diseases. IEAA measures epigenetic aging independent of major blood immune cell counts, whereas EEAA weights blood cell types that are known to change with age, including naive cytotoxic T cells (CD45RA⁺CCR7⁺), exhausted cytotoxic T cells (CD28⁻CD45RA⁻), and plasma B cells, and measures epigenetic aging in immune-related domains.^{8,13,14}

Development of allergic diseases is likely age dependent during childhood, beginning in some children with food allergy and atopic dermatitis (infancy to early childhood), followed by allergic asthma and rhinitis (early to midchildhood).¹⁵⁻¹⁷ However, chronological age alone does not fully explain disease

Abbreviations used

DNAmAge: DNA methylation age
DNAm-GA: Epigenetic clock for gestational age
EEAA: Extrinsic epigenetic age acceleration
ICC: Intraclass correlation coefficient
IEAA: Intrinsic epigenetic age acceleration

variability. Epigenetic mechanisms can mark allergic disease susceptibility and play a role in the onset, progression, and manifestations of allergy and asthma.¹⁸⁻²² Studying epigenetic signatures at birth or in early childhood has also been postulated as a molecular approach to distinguish transient wheezing from early asthma symptoms and help define age-associated disease endotypes.²³ Because the epigenetic clock likely captures a higher-order property of the methylome²⁴ and reflects the immunologic and developmental components of biological aging, DNAmAge might serve as a molecular marker of allergy and asthma in children.

In this study we sought to evaluate the associations of DNAmAge and epigenetic age acceleration (estimated at birth, early childhood, and midchildhood) with midchildhood allergic phenotypes (including total serum IgE levels, atopic sensitization, environmental allergen sensitization, food allergen sensitization, and asthma) in Project Viva, a longitudinal prebirth cohort in Boston, Massachusetts. We considered both EEAA and IEAA to best capture the age-associated metrics reflective and independent of age-related changes in cell counts, respectively.^{3,8,13} We hypothesized that older DNAmAge and age acceleration (including IEAA and EEAA) are associated with greater risks of midchildhood allergy and asthma. We further postulated that maternal lifestyle factors during pregnancy can influence the epigenetic clock trajectory toward allergen sensitization in midchildhood.

METHODS

Study population

Our study included mother-child pairs from Project Viva, a prospective longitudinal prebirth cohort in Boston, Massachusetts. A detailed description of the protocol has been published elsewhere.²⁵ In brief, Project Viva enrolled 2670 pregnant women at their initial obstetrics visit at Atrius Harvard Vanguard Medical Associates in Boston, Massachusetts, between 1999 and 2002 (2128 women had live births). We included women with a single gestation and a gestational age of less than 22 weeks at enrollment who were able to answer questions in English and intended to stay in the study region before delivery. We performed in-person visits during pregnancy, after delivery, during infancy, and at early childhood and midchildhood.

We collected information on maternal age, smoking habits (never smoker, former smoker, or smoked during pregnancy), education status, marital status, and history of allergy (asthma, eczema, or hay fever) at enrollment. The child's date of birth and sex were extracted from hospital records. We calculated gestational age by subtracting the date of the last menstrual period from the date of delivery; in situations in which gestational age based on the second-trimester ultrasound was greater than 10 days different from that based on the last menstrual period, we estimated gestational age based on ultrasound results.²⁶ Mothers reported their children's race/ethnicity at the early childhood visit. We recorded the child's age at the time of blood draw during the early childhood and midchildhood visits. We defined women who never smoked or smoked less than 100 cigarettes in their lifetime as *never smokers*, women who smoked more than 100 cigarettes in their lifetime but quit smoking 3 months or more before learning they were pregnant as *former smokers*, and women who smoked more than 100 cigarettes and continued to smoke

during the 3 months before learning they were pregnant or reported smoking on the first- or second-trimester questionnaire as *smokers during pregnancy*.²⁷

Of the 2128 mother-child pairs enrolled, 485 samples had cord blood DNA methylation measurements, 120 children had peripheral blood DNA methylation measurements at early childhood, and 460 children had peripheral blood DNA methylation measurements at midchildhood. Of the 460 children with midchildhood methylation measurements, we excluded 52 participants, as follows: 51 mother-child pairs with missing information on covariates or allergy measurements at midchildhood and 1 participant who had a DNAmAge at midchildhood that was greater than 20 years. Thus 408 mother-child pairs were included in the midchildhood analysis.

DNAmAge and age acceleration calculation

Trained personnel collected blood samples at birth (umbilical cord blood), early childhood, and midchildhood (peripheral blood). We extracted DNA from cord blood and the child's peripheral blood using Puregene Kits (Qiagen, Valencia, Calif) and performed bisulfite conversion with the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, Calif). Samples were randomized by chips and plates, and we generated DNA methylation data using the Infinium HumanMethylation450 BeadChip (Illumina, San Diego, Calif). We removed samples that were technical replicates, samples with low quality, and samples with genotype or sex mismatches. We removed low-quality probes (detection $P > .05$), probes on sex chromosomes, 65 single nucleotide polymorphisms probes, probes within 10 bp of a known single nucleotide polymorphism with a minor allele frequency of 1% or greater, non-CpG probes, and nonspecific and cross-reactive probes. We applied the normal exponential out-of-band ("noob") method for background correction and dye bias adjustment²⁸ and performed sample normalization by using the Beta Mixture Quantile Dilation,²⁹ as described previously.³⁰ We controlled for technical variability by using Combat, a nonparametric empiric Bayes method.³¹ We estimated blood cell proportions in peripheral blood from an adult reference panel (Houseman)³² and calculated blood cell proportions in cord blood by using the Bakulski algorithm, which includes nucleated red blood cell counts.^{33,34}

We estimated DNAmAge at birth, early childhood, and midchildhood by using the Horvath method.¹ This method predicted chronological age by using 8000 samples from 82 Illumina DNA methylation array data sets across a broad spectrum of age and tissue samples and selected 353 methylation sites predictive of chronological age by using elastic-net regularized regression.¹ This method assumes a logarithmic dependence of epigenetic age on chronological age until adulthood (age 20 years) and a linear relationship after age 20 years.¹ Based on Horvath's coefficient estimates and intercept values, we estimated DNAmAge at birth, early childhood, and midchildhood separately. Furthermore, we reported 2 methylation age acceleration metrics by using the Horvath and Hannum methods, which we calculated as follows: (1) the residual resulting from a multivariate regression of Horvath DNAmAge on chronological age and blood cell counts (naive CD8⁺ T cells, exhausted CD8⁺ T cells, plasmablasts, CD4⁺ T cells, natural killer cells, monocytes, and granulocytes; IEAA)⁸ and (2) the weighted average of Hannum's estimate with 3 immune cell types: naive (CD45RA⁺CCR7⁺) cytotoxic T cells, exhausted (CD28⁻CD45RA⁺) cytotoxic T cells, and plasmablasts (EEAA)^{8,35} at birth, early childhood, and midchildhood. Specifically, for EEAA, (1) we first calculated DNAmAge from Hannum's estimation (the Hannum epigenetic clock estimation included 656 human samples, aged 19-101 years, and predicts chronological age based on whole-blood methylomic information from Illumina's 450K BeadChip array using elastic-net regression³⁶); (2) we up-weighted the contributions of age-associated blood cell counts (naive cytotoxic T cells, exhaust cytotoxic T cells, and plasmablast) by using the Klemmer Doubal approach; and (3) we calculated the residual variation from the univariate model, regressing the age estimation on chronological age.³⁷ We computed the intraclass correlation coefficients (ICCs) of IEAA and EEAA at birth, early childhood, and midchildhood. We also used the Knight et al³⁸ epigenetic clock for gestational age (DNAm-GA) based on 148 methylation sites selected by using the elastic net in 6 training data sets and 9 testing data sets (a total of 1434 neonates from 15 independent birth cohorts) for cord blood.

Allergy phenotypes

Our study includes several allergic phenotypes in midchildhood, including total serum IgE (on a continuous scale), atopic sensitization, environmental allergen and food allergen sensitization, and asthma. We measured total serum IgE concentrations in peripheral blood by using ImmunoCAP (Phadia, Uppsala, Sweden), a well-established *in vitro* sandwich immunoassay, to quantitatively measure circulating IgE in serum samples. *Atopic sensitization* was defined as any serum specific IgE level of 0.35 IU/mL or greater to common indoor allergens (dust mite, cat dander, dog dander, and cockroach), mold (*Aspergillus* species and *Alternaria tenuis*), outdoor allergens (rye grass and ragweed) or food allergens (egg white, milk, wheat, peanut, and soybean), or total serum IgE concentration of greater than or equal to 100 IU/mL. *Environmental allergen sensitization* was defined as any serum specific IgE level of 0.35 IU/mL or greater to common indoor allergens (dust mite, cat dander, dog dander, and cockroach), mold (*Aspergillus* species and *Alternaria tenuis*), or grass pollen (ryegrass and ragweed),³⁹ and *food allergen sensitization* was defined as any serum specific IgE level of 0.35 IU/mL or greater to one of the food allergens listed (egg white, milk, wheat, peanut, and soybean).⁴⁰ By using a questionnaire, we defined *current asthma in midchildhood* as ever having a doctor's diagnosis of asthma since birth (reported on the midchildhood questionnaire) plus any use of asthma medications or wheezing in the past 12 months.

Statistical analysis

We evaluated the association of DNAmAge and age acceleration (IEAA and EEAA) at birth, early childhood (age range, 2.9-5.3 years old), and midchildhood (age range, 6.7-10.2 years old) with midchildhood total serum IgE levels by using generalized linear regression, assuming a Gaussian distribution with identity link function. We natural log-transformed the total serum IgE level to reduce the skewness of the residuals and meet model assumptions. For the dichotomized outcomes of atopic sensitization, environmental allergen sensitization, food allergen sensitization, and asthma, we performed the analyses using generalized linear regression, assuming a binomial distribution with logit link function and report effect estimates as odds ratios. We adjusted for the following covariates selected *a priori*: maternal covariates (age at enrollment [continuous], smoking status [never, former, or during pregnancy], college graduate [yes/no], and history of atopy [yes/no]), child covariates (sex [female/male], race/ethnicity [white/black/others], and gestational age at birth [continuous]), and estimated cell proportions computed from peripheral blood to capture cellular heterogeneity (percentages of monocytes, CD4 T cells, CD8 T cells, B cells, and granulocytes). For cord blood analysis, we adjusted for cell proportions based on the Bakulski method, which includes nucleated red blood cell counts.³³ As a sensitivity analysis, we further adjusted for the child's age at blood draw when examining the association between DNAmAge and allergic outcomes and tested for effect modification by child's sex, race/ethnicity, and maternal smoking status. For cord blood analysis, we also compared analysis results from the Horvath method with the Knight method for DNAm-GA.

We computed Pearson correlations between chronological age and DNAmAge (the Horvath method) at early childhood and midchildhood. However, because chronological age was set to zero in the Horvath estimation for cord blood, a direct comparison between chronological age and DNAmAge at birth was not possible. We computed Pearson correlations between gestational age and the Knight DNAm-GA value, an epigenetic clock metric specific for gestation. We also calculated the mean and SD (variability) of chronological age and DNAmAge at early childhood and midchildhood.

We evaluate the association between chronological age at early childhood and midchildhood with midchildhood allergy and asthma and adjusted for the following covariates selected *a priori*: maternal covariates (age at enrollment [continuous], smoking status [never, former, or during pregnancy], college graduate [yes/no], and history of atopy [yes/no]) and child covariates (sex [female/male], race/ethnicity [white/black/others], and gestational age at birth [continuous]). For the continuous outcome (total serum IgE), we used covariate-adjusted linear regression; for

dichotomized outcomes (atopic sensitization, environmental allergen sensitization and food allergen sensitization, and asthma), we used covariate-adjusted logistic regressions.

Maternal socioeconomic factors and midchildhood DNAmAge

As an exploratory analysis, we conducted 1-way ANOVA to examine whether DNAmAge differed by maternal sociodemographic factors, including maternal smoking status, marital status during pregnancy, and maternal education level.

All statistical analyses were performed in R software (version 3.5.0; <https://www.r-project.org/>), except for intraclass correlation calculations, which were performed in SAS software (version 9.4; SAS Institute, Cary, NC).

Replication

We replicated our findings from Project Viva in a subset of subjects from the Genetics of Asthma in Costa Rica Study.⁴¹ In brief, from February 2001 to December 2006, screening questionnaires were sent to 13,125 parents whose children were 6 to 14 years old and enrolled in 113 schools in Costa Rica. Children were eligible if they had physician-diagnosed asthma and at least 2 episodes of respiratory symptoms or asthma attacks in the prior year and a high probability of having 6 or more great-grandparents born in the Central Valley of Costa Rica; 592 subjects subsequently underwent genome-wide genotyping as described, and the methylation set is a subset of these subjects.^{41,42} Our nested study represented 159 subjects with genome-wide DNA methylation measured by using the Illumina 450K platform that passed quality control. We applied “noob” for background correction and dye bias adjustment²⁸ and performed sample normalization using Beta Mixture Quantile Dilation.²⁹ Furthermore, we estimated blood cell proportions in peripheral blood by using the Houseman method.³² Analogous to epigenetic clock estimations in Project Viva, we calculated DNAmAge, IEAA, and EEAA at midchildhood (mean, 9.1; range, 6.0–13.0) by using Horvath’s epigenetic clock calculator.¹ We measured total serum IgE levels with the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, Mich). Atopic sensitization was based on skin test results (positive skin test result to environmental allergens [*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, *Alternaria tenuis*, mixed grasses, mixed trees, *Prunus americana*, dogs, *Blattella germanica*, dust mite, and cockroach] or total serum IgE level ≥ 100 IU/mL) or serum test results (positive serum IgE test result to environmental allergens [*D pteronyssinus*, cockroach, and *Ascaris* species] and total serum IgE level ≥ 100 IU/mL). Given that the cohort was ascertained based on parental report of a physician’s diagnosis of asthma, for the analysis of the association between DNAmAge and age acceleration with asthma, we used a strict definition of asthma based on physician, diagnosis, wheeze in the last year, and airway responsiveness to methacholine (measured as a 20% decrease in FEV₁ after the administration of ≤ 16.8 mg/mL methacholine) or bronchodilator responsiveness. We examined the association of DNAmAge and age acceleration (IEAA and EEAA) at midchildhood with midchildhood total serum IgE level by using generalized linear regression, assuming a Gaussian distribution with an identity link function. We natural log-transformed total serum IgE levels to reduce skewness of the residuals and meet model assumptions. For binary outcomes (asthma and atopic sensitization), we performed the analyses by using generalized linear regression, assuming a binomial distribution with a logit link function and report effect estimates as odds ratios. The study was approved by the Partners Human Research Committee at Brigham and Women’s Hospital (Boston, Mass; protocol no. 2000-P-001130/55) and the Hospital Nacional de Niños (San José, Costa Rica).

All statistical analyses were performed in R software (version 3.5.0; <https://www.r-project.org/>) and SAS software (version 9.4).

RESULTS

Study population

Four hundred eight children had complete information on midchildhood DNA methylation measurements and

TABLE I. Characteristics of children and their mothers in Project Viva

Participants’ characteristics (n = 408)	
Maternal	
Age (y), mean (SD)	32.2 (5.6)
Smoking status, no. (%)	
Smoking during pregnancy	41 (10)
Former smoker	83 (20)
Never smoker	284 (70)
Education, no. (%)	
College or graduate	270 (66)
Not college or graduate	138 (34)
Atopy, no. (%)	
Yes	162 (40)
No	246 (60)
Child (at midchildhood)	
Gestational age (wk), mean (SD)	39.6 (1.6)
Age at blood drawn (y), mean (SD)	7.8 (0.7)
Sex, no. (%)	
Female	201 (49)
Male	207 (51)
Race, no. (%)	
White	255 (63)
Black	77 (19)
Other	76 (18)
Total serum IgE (kU/L), mean (SD)	152.2 (287.0)
Atopic sensitization, no. (%)	
Yes	224 (55)
No	184 (45)
Environmental allergen positive, no. (%)	
Yes	175 (43)
No	233 (57)
Food allergen positive, no. (%)	
Yes	108 (26)
No	300 (74)
Current asthma, no. (%) [*]	
Yes	62 (15)
No	300 (74)

^{*}Number missing = 46.

midchildhood atopic phenotype measurements (Table I). Ten percent of mothers smoked during pregnancy, and 20% were former smokers. Forty percent of mothers reported a history of atopy (including asthma, eczema, and hay fever). Among children, mean gestational age at delivery was 39.6 ± 1.6 weeks, 49% were female, and 63% were white. Mean $\pm$ SD age at blood draw at midchildhood was 7.8 ± 0.7 years. Total serum IgE level at midchildhood showed a right-skewed distribution, with a mean of 152.2 ± 287.0 kU/L. Fifty-five percent of children were classified as atopic, 43% were sensitized to environmental allergens, and 26% were sensitized to food allergens. Fifteen percent of children had asthma at midchildhood.

Association between DNAmAge and age acceleration at birth, early childhood, and midchildhood with midchildhood allergy and asthma

DNAmAge at midchildhood was cross-sectionally associated with allergy and asthma in midchildhood (Fig 1 and Table II and see Figs E1 and E2 in this article’s Online Repository at www.jacionline.org). DNAmAge was a mean of 8.8 years (SD,

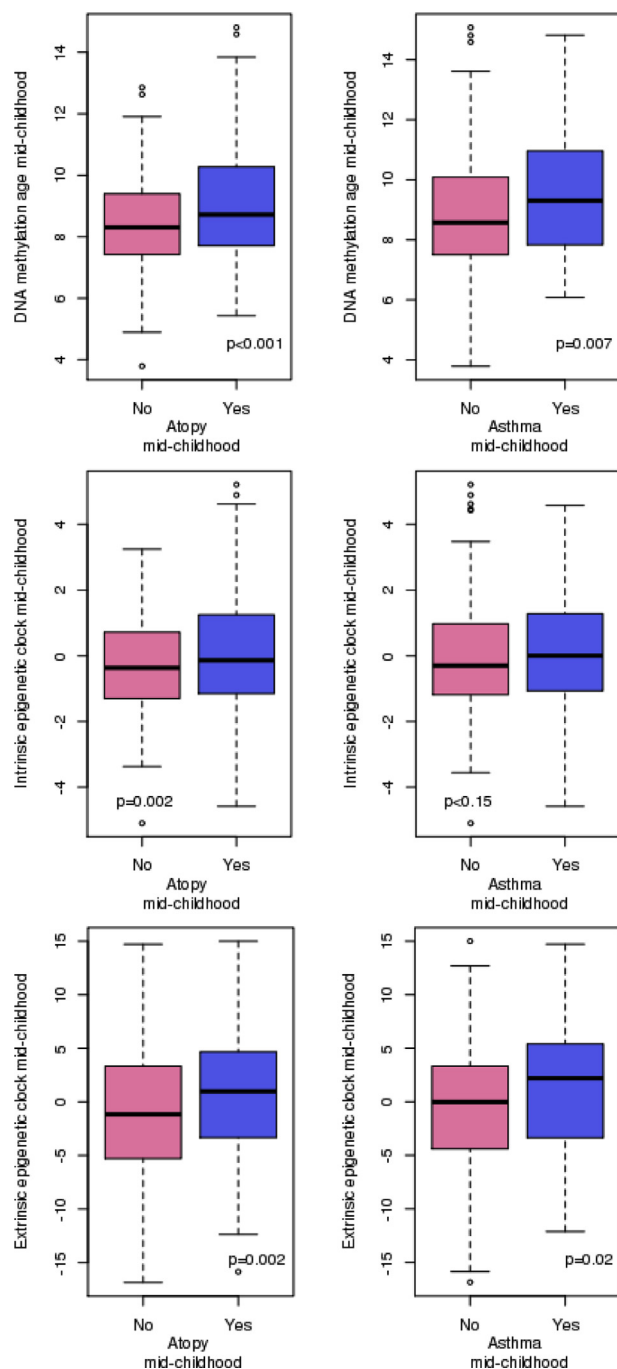


FIG 1. Box and whisker plots of DNAmAge and age acceleration at midchildhood by atopic status.

1.8 years) at midchildhood (see [Table E1](#) in this article's Online Repository at www.jacionline.org). A 1-year increase in DNAmAge was associated with a 1.21 (95% CI, 1.07-1.38; $P = .003$), 1.20 (95% CI, 1.06-1.37; $P = .004$), 1.21 (95% CI, 1.06-1.39; $P = .006$), and 1.16 (95% CI, 1.00-1.34; $P = .044$) greater odds of atopic sensitization, environmental and food allergen sensitization, and asthma, respectively ([Table II](#)). Additionally, adjusting for chronological age did not alter the results (see [Table E2](#) in this article's Online Repository at www.jacionline.org). In addition to DNAmAge, epigenetic age

accelerations (IEAA and EEAA) in midchildhood were also cross-sectionally associated with the allergic phenotypes at midchildhood ([Table II](#)). For example, for every 1-year increase in IEAA, we observed a 1.22 (95% CI, 1.07-1.39; $P = .004$), 1.17 (95% CI, 1.03-1.34; $P = .018$), and 1.29 (95% CI, 1.12-1.49; $P < .0001$) greater odds of atopic sensitization, environmental allergen and food allergen, respectively ([Table II](#)). We observed relatively high correlations between DNAmAge and IEAA in midchildhood (see [Table E1](#)). The EEAA at midchildhood, which is thought to capture immune development and response, was additionally associated with asthma at midchildhood (odds ratio, 1.07; 95% CI, 1.02-1.12; $P = .008$). We did not observe effect modification by sex, race/ethnicity, or maternal smoking status with a P value threshold of .05.

We did not observe statistically significant associations of DNAmAge or age acceleration (IEAA and EEAA) at birth or in early childhood with allergic phenotypes in midchildhood (see [Table E3](#) and [E4](#) in this article's Online Repository at www.jacionline.org). DNAm-GA, which is specific for epigenetic age at birth, was also not associated allergic phenotypes in midchildhood (see [Table E3](#)).

To examine the between- and within-person variability of epigenetic age acceleration, we computed ICCs for IEAA and EEAA. For IEAA, the overall ICC across all 3 time points was 0.08. We observed modest ICCs between birth and early childhood (ICC, 0.13), relatively high ICCs of IEAA between early childhood and midchildhood (ICC, 0.44), and low ICCs between birth and midchildhood (ICC, 0.03). For EEAA, the overall ICC across the 3 time points was 0.18. ICCs between birth and early childhood, early childhood and midchildhood, and birth and midchildhood were 0.21, 0.28, and 0.16, respectively.

Chronological age versus DNAmAge and association between chronological age at early childhood and midchildhood with midchildhood allergy and asthma

DNAm-GA (the Knight method) was positively correlated with actual gestational age ($\rho = 0.58$, $P < .0001$; see [Fig E3](#) in this article's Online Repository at www.jacionline.org). DNAmAge (the Horvath method) in early childhood and midchildhood was also positively correlated with chronological age in early childhood and midchildhood ($\rho = 0.55$, $P < .0001$ and $\rho = 0.47$, $P < .0001$, respectively; see [Figs E4](#) and [E5](#) in this article's Online Repository at www.jacionline.org). We observed greater mean levels and wider ranges (SDs) of DNAmAge at early childhood (mean $\pm$ SD, 3.8 ± 0.9 years) and midchildhood (mean $\pm$ SD, 9.0 ± 1.9 years) compared with chronological age at early childhood (mean $\pm$ SD, 3.4 ± 0.5 years) and midchildhood (mean $\pm$ SD, 7.9 ± 0.8 years). A quantile-quantile plot of the association between chronological age and the 353 methylation sites contributing to DNAmAge showed no epigenomic inflation ($\lambda = 0.864$; see [Fig E6](#) in this article's Online Repository at www.jacionline.org).

After adjusting for covariates, chronological age at early childhood visits was not prospectively associated with allergy or asthma in midchildhood (see [Table E5](#) in this article's Online Repository at www.jacionline.org). A cross-sectional analysis of chronological age and allergic phenotypes, both measured at midchildhood, also yielded null results (see [Table E5](#)).

TABLE II. Cross-sectional associations between DNAmAge, IEAA, and EEAA with total serum IgE level, atopic sensitization, environmental allergen sensitization, food allergen sensitization, and asthma measured at midchildhood

Midchildhood outcomes	Epigenetic clock metrics at midchildhood					
	DNAmAge		IEAA (intrinsic)		EEAA (extrinsic)	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Total serum IgE (log-scale)	0.11 (0.02 to 0.20)	.017	0.13 (0.03 to 0.22)	.010	0.024 (−0.002 to 0.05)	.076
Atopic sensitization	1.21 (1.07 to 1.38)	.003	1.22 (1.07 to 1.39)	.004	1.06 (1.02 to 1.10)	.002
Environmental allergen sensitization	1.20 (1.06 to 1.37)	.004	1.17 (1.03 to 1.34)	.018	1.07 (1.03 to 1.11)	<.001
Food allergen sensitization	1.21 (1.06 to 1.39)	.006	1.29 (1.12 to 1.49)	<.001	1.04 (1.00 to 1.08)	.055
Current asthma	1.16 (1.00 to 1.34)	.044	1.11 (0.95 to 1.31)	.193	1.07 (1.02 to 1.12)	.008

For DNAmAge, the model was adjusted for maternal covariates (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and atopic history [asthma, eczema, or hay fever]), child covariates (sex [female or male], race/ethnicity [white, black, or others], and gestational age [continuous]), and cell type proxies estimated for peripheral blood (percentages of monocytes, granulocytes, CD8 T cells, CD4 T cells, and B cells) covariates. For IEAA and EEAA, models were adjusted for maternal (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and atopic history [asthma, eczema, or hay fever]) and child (sex [female or male], race/ethnicity [white, black, or others], and gestational age [continuous]) covariates.

Maternal socioeconomic factors and midchildhood DNAmAge

Maternal educational level and marital status during pregnancy were significantly associated with DNAmAge ($P = .03$ and $P < .0001$, respectively). Children whose mothers had lower educational levels (less than college) or were not married had greater DNAmAge in midchildhood (see Fig E7 in this article's Online Repository at www.jacionline.org). Active maternal smoking during pregnancy was not associated with greater DNAmAge in midchildhood (see Fig E7).

Replication

We replicated our midchildhood findings from Project Viva in an independent cohort, the Genetics of Asthma in Costa Rica cohort ($n = 159$). Participants' characteristics are shown in Table E6 in this article's Online Repository at www.jacionline.org. Age distribution in the Costa Rica study (mean $\pm$ SD, 9.1 ± 1.8 years) denoted that the children were slightly older than the children in Project Viva (mean $\pm$ SD, 7.8 ± 0.7 years). Increases in DNAmAge and age acceleration at midchildhood were cross-sectionally associated with greater odds of atopic sensitization, as measured by using skin tests ($P < .01$ for both IEAA and EEAA; see Table E7 in this article's Online Repository at www.jacionline.org). Increases in IEAA were also positively associated with greater odds of asthma (see Table E7). We observed same signs of direction and comparable effect estimates in Project Viva and the Costa Rica study for the relationship between EEAA, IEAA, and total serum IgE levels and atopic sensitization based on the results of serum specific IgE tests, although these associations did not reach a statistical significance threshold (see Table E7).

DISCUSSION

Our study demonstrates that (1) at midchildhood, DNAmAge in peripheral blood was cross-sectionally associated with greater total serum IgE levels, as well as greater odds of atopic sensitization, environmental and food allergen sensitization, and asthma; (2) midchildhood epigenetic age acceleration metrics (including EEAA and IEAA) were associated with atopic

phenotypes; (3) EEAA, which weights blood cell types and measures epigenetic aging in immune-related domains, was additionally associated with asthma; and (4) maternal socio-demographic factors (lower education level and being unmarried) were associated with older DNAmAge at midchildhood.

Previous epigenome-wide association studies have linked blood, nasal, and bronchial mucosal tissue methylomes with childhood allergy and asthma.^{18–20,22,43–46} However, most of these studies were conducted at a single time point and might not capture postnatal age-related biological processes, which often coincide with early immune development. Indeed, there is increasing evidence showing age-related intraindividual changes in DNA methylation profiles over time, often capturing epigenetic pathways regulating immunity.^{47,48} Hence our study of examining DNAmAge and childhood allergy adds an important piece of evidence to the literature and furthers our understanding of the role of epigenetic regulation in immune system development and allergy.

The epigenetic clock has been studied extensively in age-related biological processes (weight change during childhood and adolescence),¹⁰ pubertal development in childhood,¹² aging-associated diseases (cognitive decline,² Parkinson disease,³ and lung cancer⁴), and cancer, cardiovascular, and all-cause mortality.^{5–8} Although research on psychosocial outcomes has been performed in childhood cohorts,⁴⁹ little research has been done on chronic diseases of childhood. Here, for the first time, we demonstrated that at midchildhood, DNAmAge and age acceleration were associated with allergic phenotypes. Age-dependent allergic progression in children is well documented in the clinical literature¹⁵ but might be difficult to observe in birth cohorts because participants in birth cohorts usually come to research visits based on their developmental stages within a narrow age window. Therefore in our study we did not observe associations between chronological age and childhood allergy at the 3 research visit windows (at birth, early childhood, and midchildhood) in Project VIVA. On the other hand, DNAmAge integrates additional sources of variabilities from environmental and lifestyle factors and has wider variations at birth, early childhood, and midchildhood.

An ingenuity pathway analysis from the original epigenetic clock manuscript by Horvath¹ suggested the 353 methylation sites used to compute the epigenetic clock showed significant

enrichment for immune cell trafficking, hematologic system development and function, organismal development, embryonic development, and tissue development. In alignment with pathway findings, our study showed DNAmAge and age acceleration were associated with midchildhood allergy. IEAA is suggested to capture cell/intrinsic aging independent of shifts in cell proportion. A shift of T_H1/T_H2 immunodominance toward T_H2 responses has been postulated as one primary mechanism of allergy. Furthermore, the EEAA, which weights blood cell types that are known to change with age,¹³ was additionally associated with asthma. Because of its weighting of age-related blood cell types, EEAA is considered to capture immune-related biological processes and would be a highly relevant biomarker for immune-related phenotypes, such as allergy and asthma. On the other hand, previous literature has shown that anti-inflammatory treatment (eg, oral or inhaled corticosteroids) does not work for every child with allergy or asthma,⁵⁰ suggesting that other biological processes in addition to immune/inflammatory pathways might contribute to the development and progression of allergy and asthma. IEAA might help to capture the nonimmune part of the story.

Our study demonstrated that maternal sociodemographic factors (ie, lower education level and unmarried during pregnancy) were correlated with greater DNAmAge and age acceleration in children and should be considered in future studies of epigenetic age acceleration and chronic diseases in children. A previous study found differential epigenome-wide DNA methylation patterns in children raised since birth in institutional care compared with children raised by their biological parents, with most of the significant methylation sites involved in control of immune responses and cell signaling.⁵¹ Maternal depression, anxiety, and maternal antidepressant use also have been associated with changes in DNA methylation in sites related to oxidative stress, mRNA translocation from the nucleus, and cell division.^{52,53} Among 292 African American youths transitioning into adulthood (ie, spanning ages 17–22 years), lower socioeconomic status (measured as household income of less than the federal poverty line, receipt of Temporary Assistance for Needy Families, caregiver report of income as insufficient to meet all needs, and primary caregiver without high school education or current employment) was associated with greater epigenetic age acceleration.⁴⁹ The effect of maternal factors on asthma and atopy might be partially accounted for by epigenetic effects.

Our study has several strengths. First, we computed a range of epigenetic clock estimators, with a focus on the intrinsic epigenetic clock from Horvath's estimation,^{1,3} the EEAA from Hannum's estimation,³ and DNAmAge for gestational age using the method of Knight et al.³⁸ Our results were consistent across the different epigenetic age estimators.

Second, we examined a comprehensive range of atopic and asthma phenotypes at midchildhood. We showed repeatedly that the epigenetic clock metrics were associated with T_H2 cell–driven phenotypes. This is particularly evident for EEAA, which measures epigenetic aging in immune-related domains.

Finally, our midchildhood associations between atopy and age acceleration in Project Viva, which were replicated in an independent children cohort, the Genetics of Asthma in Costa Rica cohort, support the robustness of our findings.

Our study also has several limitations. First, at midchildhood, peripheral blood DNA methylation and allergic outcomes are measured at the same time, and it is hard to disentangle the

temporal relationships between the 2. However, although not statistically significant (possible because of the small sample size), DNAmAge and age acceleration at early childhood were also associated with greater odds of allergy and asthma in midchildhood, suggesting that the observed associations were unlikely to be caused by reverse causation. As for future direction, better powered longitudinal studies are needed to fully address the issue of reverse causation.

Second, current asthma at midchildhood was defined through a parent-reported questionnaire, and measurement errors are inevitable. Better phenotyping of asthma is warranted in future studies to reduce measurement errors.

Third, we did not find chronological age associated with childhood allergy at the 3 clinic visits (at birth, early childhood, and midchildhood), possibly related to the narrow chronological age windows we studied. However, this did not affect our ability to identify epigenetic age associations, likely because of the wider range of DNAmAge.

Fourth, our null findings at early childhood could also be due to a lack of power, and finally and importantly, our replication cohort was ascertained based on physician-diagnosed asthma, potentially limiting the generalizability of our findings.

In conclusion, our study showed that DNAmAge and epigenetic age acceleration in midchildhood were cross-sectionally associated with greater levels of total serum IgE, as well as greater odds of atopic sensitization, environmental and food allergen sensitizations, and asthma in children. Because the epigenetic clock is thought to reflect the immuno-developmental and developmental components of biological aging, our study sheds light on the potential molecular mechanisms through which immunity, development, and maturation interact along the age axis and associate with childhood allergy or asthma. Taken together, accelerated epigenetic aging in allergic and asthmatic children might reflect perturbations in epigenetic maintenance, and this raises speculation as to whether antiaging therapies might eventually find a role in mitigating the allergic march.

Key messages

- In this study we showed that epigenetic age acceleration at midchildhood is cross-sectionally associated with greater levels of total serum IgE, as well as greater odds of asthma and atopy in Project Viva.
- Accelerated epigenetic aging in allergic and asthmatic children might reflect perturbations in epigenetic maintenance, and this raises speculation as to whether antiaging therapies might eventually find a role in mitigating the allergic march.

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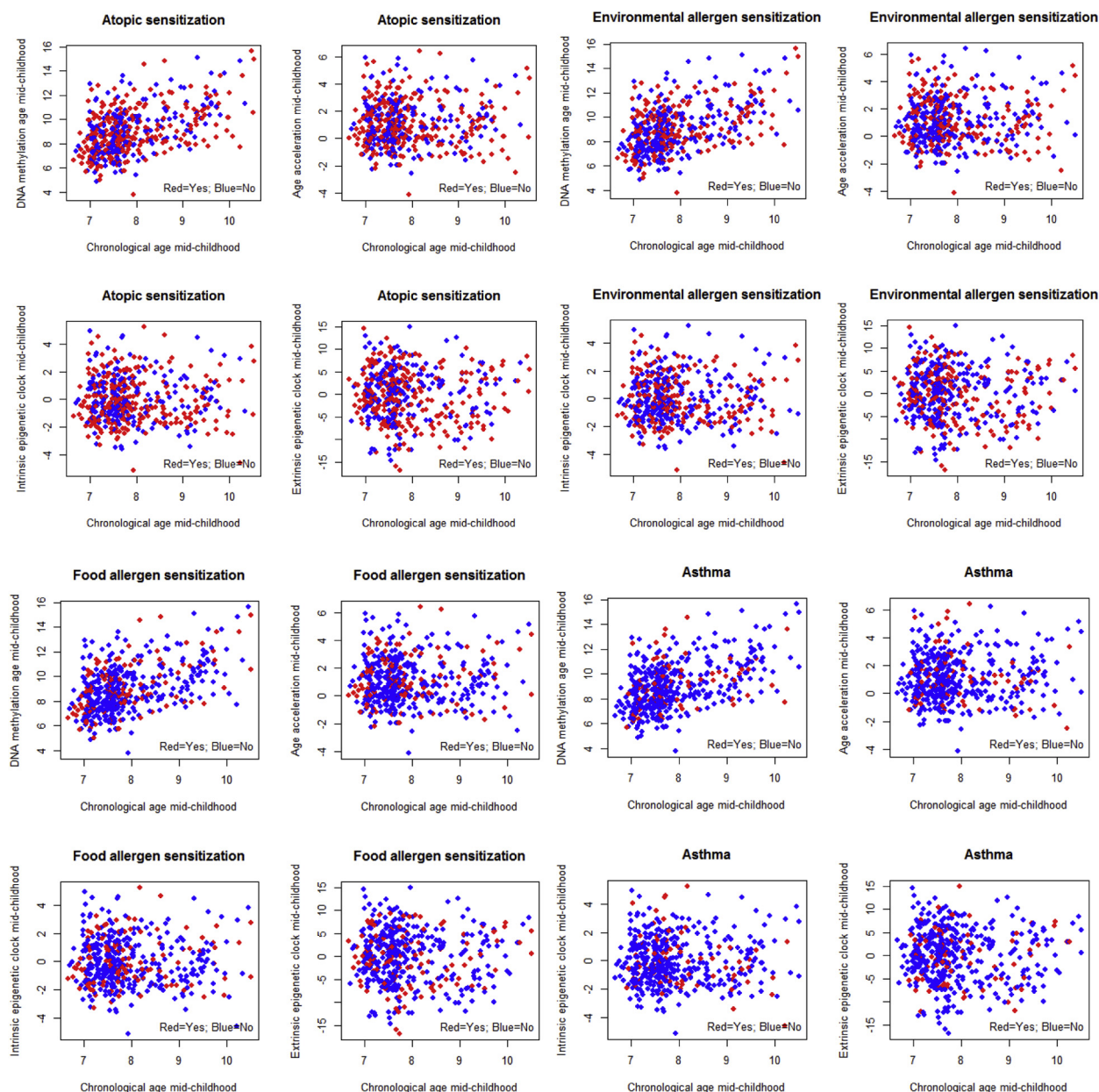


FIG E1. Scatter plot of chronological age versus DNAmAge and age acceleration at midchildhood by atopic sensitization, environmental allergen sensitization, food allergen sensitization, and asthma status (red = yes, blue = no).

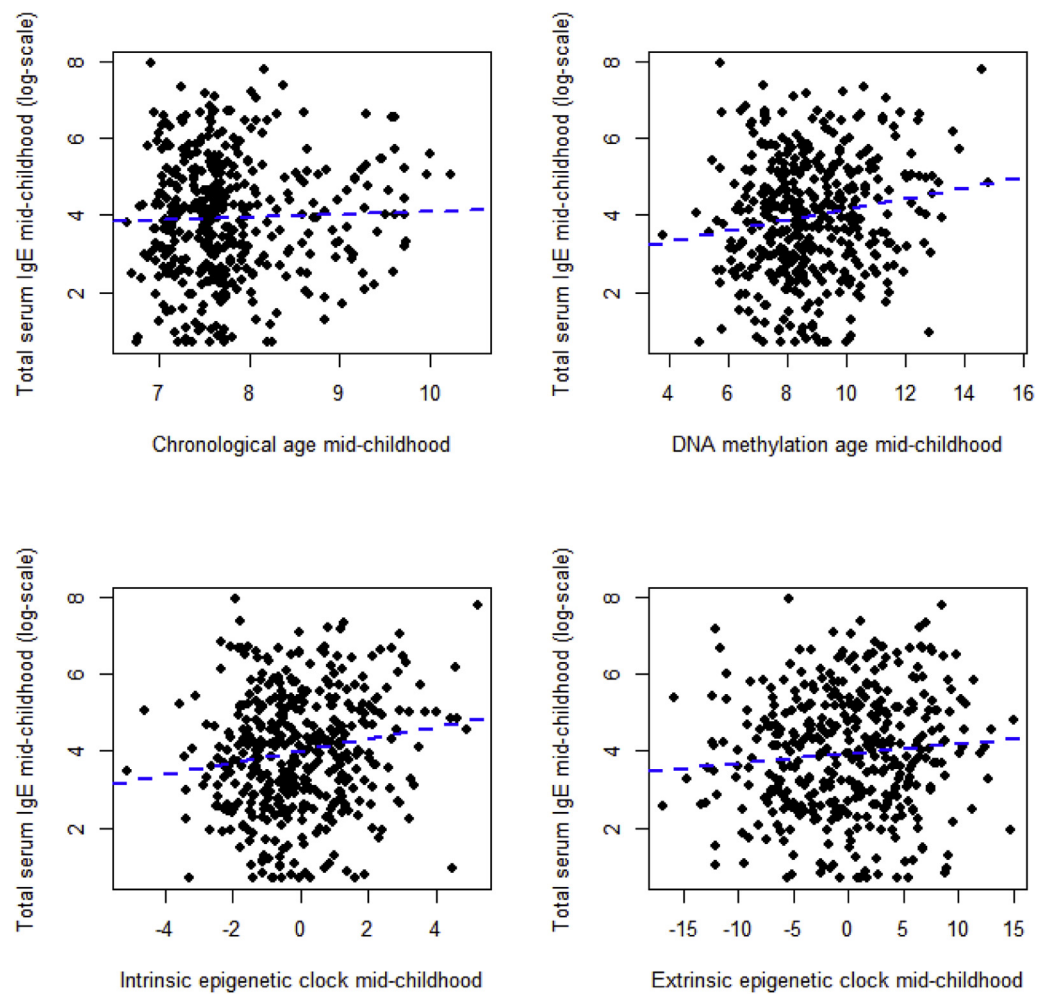


FIG E2. Scatter plots of DNAmAge versus total serum IgE (log-scale) at midchildhood (Horvath estimate).

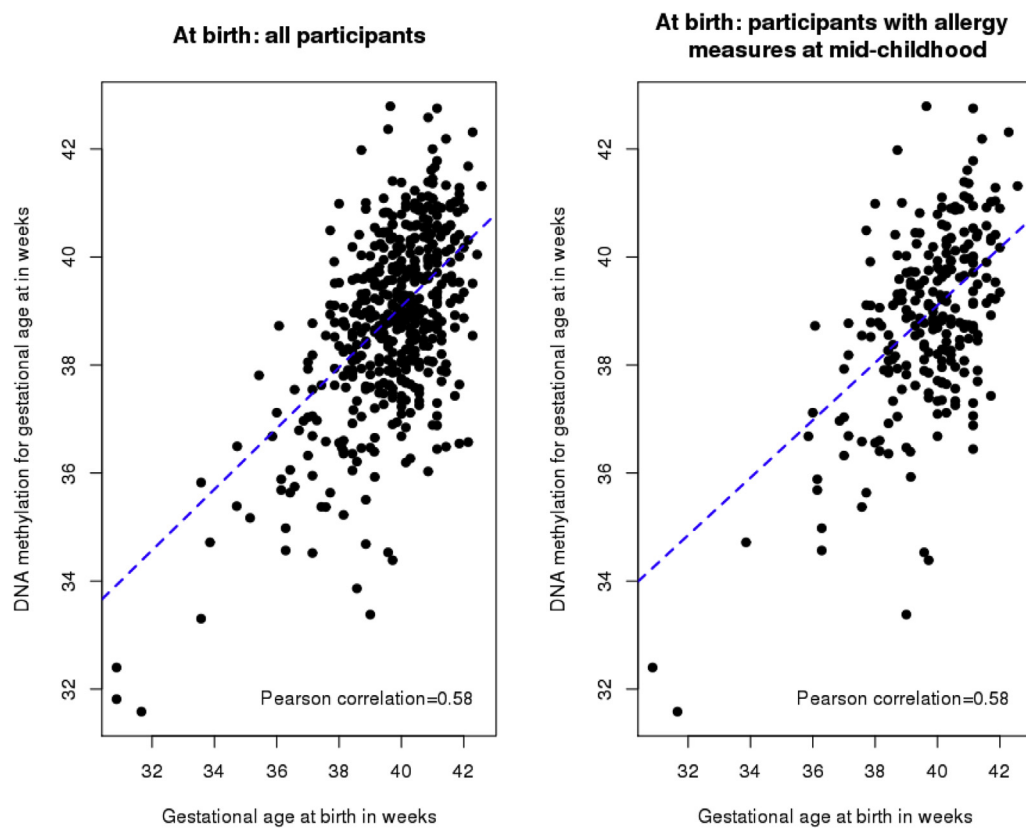


FIG E3. Scatter plots of gestational week at delivery versus DNA methylation for gestational age (in weeks [Knight estimate]).

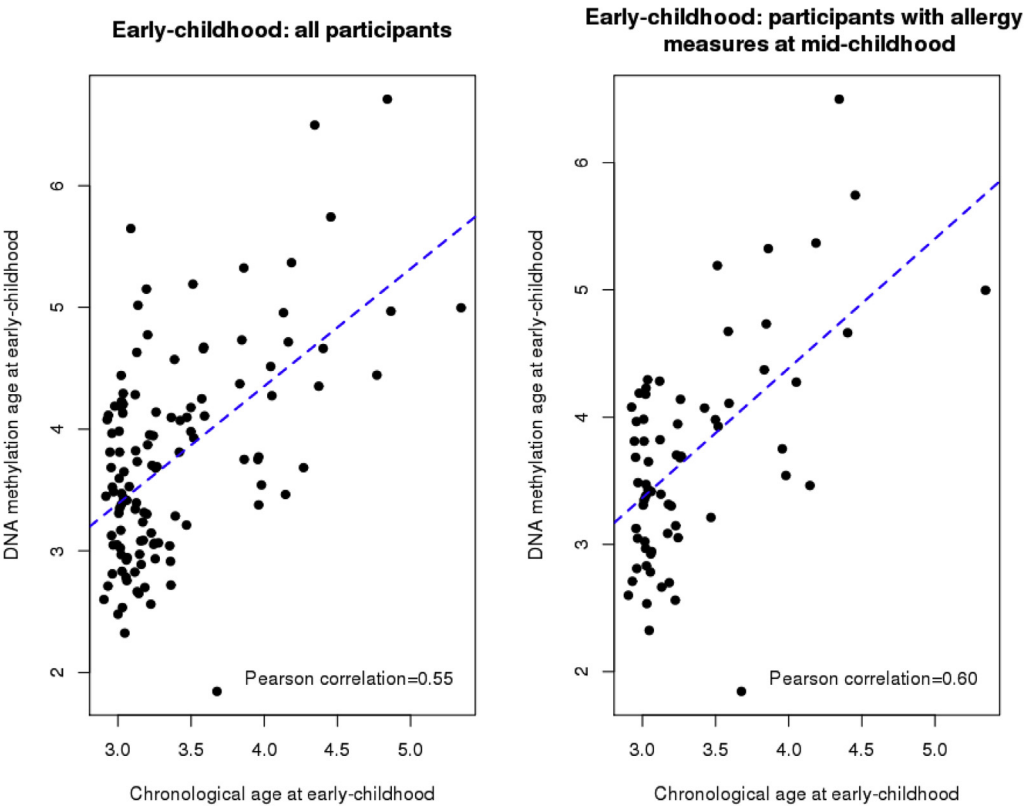


FIG E4. Scatter plots of chronological age versus DNAmAge at the early childhood visit (Horvath estimate).

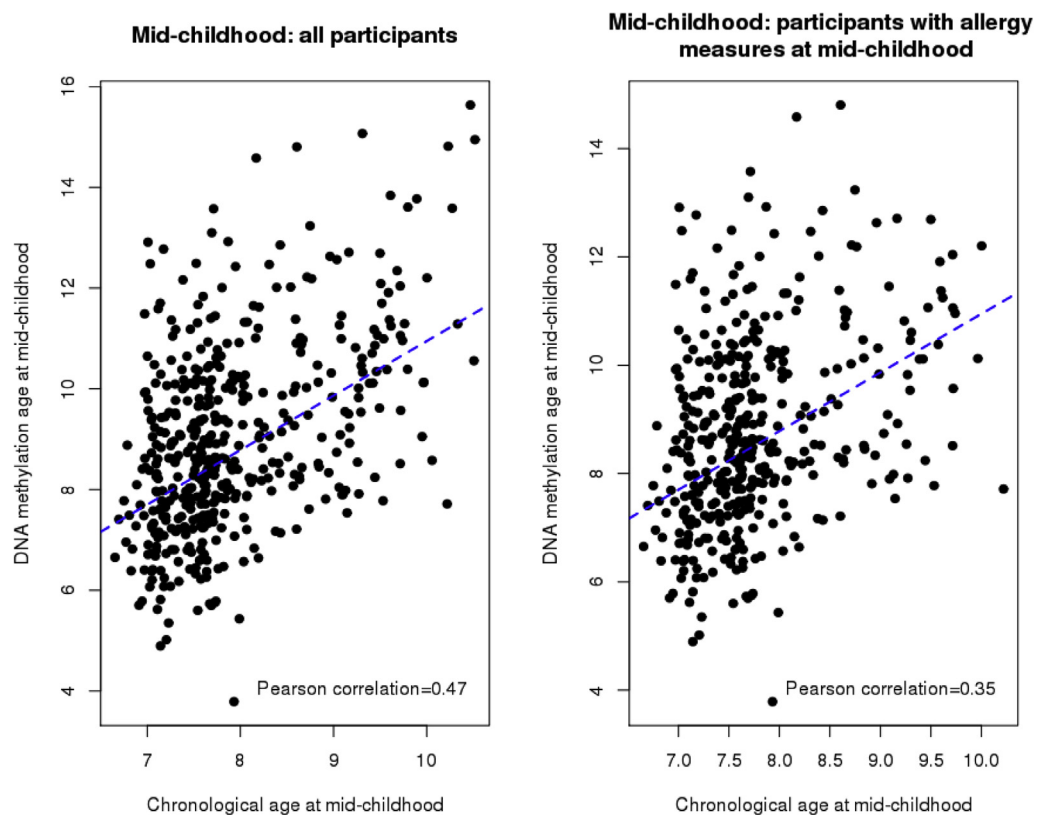


FIG E5. Scatter plots of chronological age versus DNAmAge at the midchildhood visit (Horvath estimate).

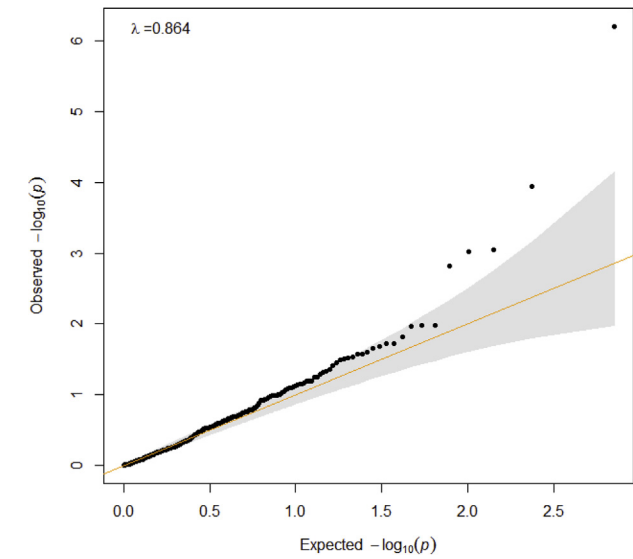


FIG E6. Quantile-quantile plot of association between chronological age and the 353 methylation sites contributing to DNAmAge.

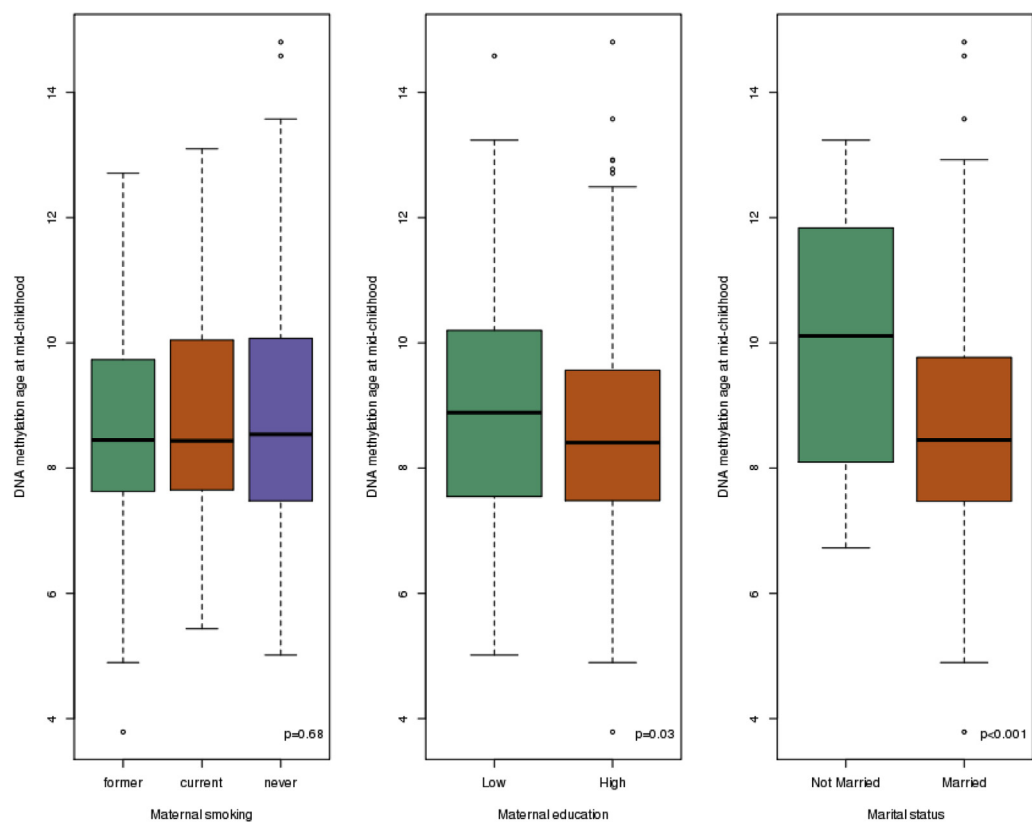


FIG E7. DNAmAge at midchildhood by maternal smoking status and sociodemographic characteristics (maternal education level and maternal marital status) measured during pregnancy.

TABLE E1. Summary statistics and correlation matrix between DNAmAge, IEAA, and EEAA measured at midchildhood (n = 408)

Midchildhood	Summary statistics		Spearman correlations		
	Mean (SD)	Median (IQR)	DNAmAge	IEAA (intrinsic)	EEAA (extrinsic)
DNAmAge	8.8 (1.8)	8.5 (2.5)	1	0.85	0.24
IEAA (intrinsic)	−0.06 (1.6)	−0.3 (2.2)	—	1	0.13
EEAA (extrinsic)	−0.10 (5.8)	0.2 (8.2)	—	—	1

TABLE E2. Cross-sectional associations of DNAmAge in midchildhood with total serum IgE levels, atopic sensitization, environmental allergen sensitization, food allergen sensitization, and asthma at midchildhood, additionally adjusting for chronological age at midchildhood

Midchildhood outcomes	Midchildhood DNAmAge (additionally adjusted for chronological age at midchildhood)	
	Estimate (95% CI)	P value
Total serum IgE (log-scale)	0.11 (0.02-0.21)	.019
	Odds ratio (95% CI)	P value
Atopic sensitization	1.21 (1.06-1.39)	.005
Environmental allergen sensitization	1.17 (1.02-1.34)	.022
Food allergen sensitization	1.28 (1.11-1.49)	.001
Current asthma	1.17 (0.99-1.38)	.065

The model was adjusted for maternal covariates (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and history of atopy [asthma, eczema, or hay fever]), child covariates (sex [female or male], race/ethnicity [white, black, or others], gestational age [continuous], and age at blood draw at midchildhood [continuous]), and cell type proxies estimated for peripheral blood (percentages of monocytes, granulocytes, CD8 T cells, CD4 T cells, and B cells).

TABLE E3. Associations of DNAmAge, methylation age for gestational age, and age acceleration at birth with total serum IgE level, atopic sensitization, environmental allergen sensitization, and food allergen sensitization in midchildhood

	Epigenetic clock metrics at birth							
	DNAmAge		DNAm-GA		IEAA (intrinsic)		EEAA (extrinsic)	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Total serum IgE (log-scale)	−0.52 (−1.59 to 0.54)	.335	0.03 (−0.123 to 0.19)	.692	−0.10 (−1.16 to 0.97)	.856	−0.02 (−0.07 to 0.02)	.295
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Atopic sensitization	0.37 (0.08 to 1.55)	.177	1.13 (0.91 to 1.39)	.271	0.43 (0.10 to 1.76)	.243	0.96 (0.91 to 1.02)	.167
Environmental allergen sensitization	0.40 (0.09 to 1.71)	.223	1.20 (0.97 to 1.49)	.102	0.49 (0.11 to 2.02)	.325	0.97 (0.91 to 1.02)	.252
Food allergen sensitization	0.87 (0.15 to 4.72)	.869	1.01 (0.79 to 1.29)	.946	1.12 (0.21 to 5.81)	.890	0.95 (0.89 to 1.01)	.108
Current asthma	0.55 (0.09 to 2.96)	.494	0.88 (0.70 to 1.10)	.257	0.59 (0.11 to 3.00)	.530	0.96 (0.90 to 1.02)	.212

For DNAmAge and DNAm-GA, the model was adjusted for maternal covariates (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and atopy history [asthma, eczema, or hay fever]), child covariates (sex [female or male], race/ethnicity [white, black, or others], and gestational age [continuous]), and cell type proxies estimated for cord blood (percentages of monocytes, granulocytes, CD8 T cells, CD4 T cells, B cells, and nucleated red blood cells). For IEAA and EEAA, models were adjusted for maternal (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and atopy history [asthma, eczema, or hay fever]) and child (sex [female or male], race/ethnicity [white, black, or others], and gestational age [continuous]) covariates.

TABLE E4. Associations of DNAmAge and age acceleration at early childhood with total serum IgE levels, atopic sensitization, environmental allergen sensitization, and food allergen sensitization in midchildhood

Midchildhood outcomes	Epigenetic clock metrics at early childhood					
	DNAmAge		IEAA (intrinsic)		EEAA (extrinsic)	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Total serum IgE (log-scale)	0.16 (−0.38 to 0.70)	.573	0.23 (−0.38 to 0.84)	.461	0.003 (−0.08 to 0.08)	.949
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Atopic sensitization	1.89 (0.83 to 4.77)	.145	1.71 (0.71 to 4.42)	.241	0.99 (0.88 to 1.11)	.836
Environmental allergen sensitization	1.48 (0.66 to 3.62)	.362	1.87 (0.77 to 4.98)	.182	0.97 (0.86 to 1.09)	.625
Food allergen sensitization	3.40 (0.85 to 18.36)	.108	2.17 (0.62 to 10.10)	.267	0.97 (0.83 to 1.13)	.706
Current asthma	0.64 (0.25 to 1.52)	.332	0.80 (0.32 to 1.96)	.617	1.00 (0.87 to 1.14)	.970

For DNAmAge, models were adjusted for maternal covariates (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and atopy history [asthma, eczema, or hay fever]), child covariates (sex [female or male], race/ethnicity [white, black, or others], and gestational age [continuous]), and cell type proxies estimated for peripheral blood (percentages of monocytes, granulocytes, CD8 T cells, CD4 T cells, and B cells). For IEAA and EEAA, models were adjusted for maternal (age at enrollment [continuous], smoking status [never, former, or during pregnancy], education [college/graduate level or not college/graduate level], and atopy history [asthma, eczema, or hay fever]) and child (sex [female or male], race/ethnicity [white, black, or others], and gestational age [continuous]) covariates.

TABLE E5. Associations of chronological age at early childhood and midchildhood with total serum IgE levels, atopic sensitization, environmental allergen sensitization, and food allergen sensitization in midchildhood

Midchildhood outcomes	Chronological age at early childhood		Chronological age at mid childhood	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Total serum IgE (log-scale)	0.41 (−0.42 to 1.25)	.334	0.03 (−0.20 to 0.26)	.267
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Atopic sensitization	3.47 (0.94 to 17.7)	.088	1.12 (0.82 to 1.55)	.463
Environmental allergen sensitization	0.93 (0.24 to 3.23)	.914	1.32 (0.97 to 1.81)	.078
Food allergen sensitization	0.82 (0.14 to 3.69)	.809	0.81 (0.57 to 1.13)	.222
Current asthma	0.15 (0.009 to 1.06)	.113	1.11 (0.80 to 1.52)	.524

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

TABLE E6. Participants' characteristics in the Genetics of Asthma in Costa Rica cohort

Participants' characteristics (n = 159)	
Maternal	
Current smoking status, no. (%)	
Yes	7 (4)
No	152 (96)
Asthma history, no. (%) [*]	
Yes	45 (28)
No	113 (71)
Child	
Age (y), mean (SD)	9.1 (1.8)
Sex, no. (%)	
Female	64 (41)
Male	95 (59)
Total serum IgE (kU/L), mean (SD)	840.7 (1016.6)
Atopic sensitization (skin), no. (%)	
Yes	144 (91)
No	15 (9)
Atopic sensitization (blood), no. (%) [*]	
Yes	136 (86)
No	23 (14)
Asthma, no. (%) [†]	
Yes	98 (62)
No	44 (28)

*Number missing = 1.

†Number missing = 17.

TABLE E7. Replication: Associations of DNAmAge and age acceleration at midchildhood with total serum IgE level and atopic sensitization in midchildhood in the Genetics of Asthma in Costa Rica cohort

Midchildhood outcomes	Costa Rica cohort					
	DNAmAge		IEAA (intrinsic)		EEAA (extrinsic)	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Total serum IgE (log-scale)	0.01 (−0.03 to 0.05)	.512	0.01 (−0.03 to 0.06)	.492	0.03 (−0.02 to 0.08)	.214
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Atopic sensitization (skin)	1.18 (1.04 to 1.38)	.019	1.19 (1.05 to 1.37)	.010	1.18 (1.05 to 1.35)	.009
Atopic sensitization (IgE)	1.07 (0.98 to 1.17)	.165	1.07 (0.98 to 1.18)	.139	1.09 (0.99 to 1.21)	.074
Asthma	1.07 (1.00 to 1.15)	.066	1.09 (1.01 to 1.18)	.027	1.08 (1.00 to 1.17)	.064

For DNAmAge, models were adjusted for maternal covariates (current smoking status [yes/no] and asthma history [yes/no]), child covariates (sex [female or male]), and plate and cell type proxies estimated for peripheral blood (percentages of monocytes, granulocytes, CD8 T cells, CD4 T cells, and B cells). For IEAA and EEAA, models were adjusted for maternal covariates (current smoking status [yes/no] and asthma history [yes/no]), child covariates (sex [female or male]), and plate.