Sex-specific associations of asthma acquisition with changes in DNA methylation during adolescence

Running title: Asthma acquisition and DNA methylation changes

Rutu Patel, MPH, MBBS[1]*, Farnaz Solatikia, MPH, MS[1,2]*, Hongmei Zhang, PhD, MS[1]**, Alemayehu Wolde, MS[2], Latha Kadalayil PhD[3], Wilfried Karmaus, MD, Dr.med., MPH[1], Susan Ewart, DVM, PhD, DACVIM[4], Ryan Arathimos, PhD[5,6,7,8], Caroline Relton, PhD[5,6,7], Susan Ring, PhD, BSc[5,6,7], A. John Henderson[6], MD, S. Hasan Arshad, MBBS, DM, FRCP [9,10,11], John W Holloway, PhD[3,11]

[1]Division of Epidemiology, Biostatistics and Environmental Health, School of Public Health, University of Memphis, Memphis, TN, USA.

[2]Department of Mathematical Sciences, University of Memphis, Memphis, TN, USA.

[3]Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK

[4]College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.

[5]MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK.

[6]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK.

[7]National Institute for Health Research Bristol Biomedical Research Centre, University of Bristol and University Hospitals Bristol NHS Foundation Trust, Bristol, UK.

[8]Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK

[9]Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.

[10]David Hide Asthma and Allergy Research Centre, Isle of Wight, UK.

[11]NIHR Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, UK

*: equal contribution, **: corresponding author

Corresponding author: Dr. Hongmei Zhang

Phone: 901.678.4707

Email: hzhang6@memphis.edu

Fax:901.678.1715

Office: 224 Robison Hall, 3825 DeSoto Avenue, Memphis, TN 38152-0001

Abstract (246 words)

Background- Underlying biological mechanisms involved in sex differences in asthma status changes from pre- to post-adolescence are unclear. DNA methylation (DNAm) has been shown to be associated with the risk of asthma.

Objective- We hypothesized that asthma acquisition from pre- to post-adolescence was associated with changes in DNAm during this period at asthma-associated cytosine-phosphate-guanine (CpG) sites and such an association was sex-specific.

Methods- Subjects from the Isle of Wight birth cohort (IOWBC) with DNAm in blood at ages 10 and 18 years (n=124 females, 151 males) were studied. Using a training-testing approach, epigenome-wide CpGs associated with asthma were identified. Logistic regression was used to examine sex-specific associations of DNAm changes with asthma acquisition between ages 10 and 18 at asthma-associated CpGs. The ALSPAC birth cohort was used for independent replication. To assess functional relevance of identified CpGs, association of DNAm with gene expression in blood was assessed.

Results- We identified 535 CpGs potentially associated with asthma. Significant interaction effects of DNAm changes and sex on asthma acquisition in adolescence were found at 13 of the 535 CpGs in IOWBC (p-values$<1.0\times10^{-3}$). In the replication cohort, consistent interaction effects were observed at 10 of the 13 CpGs. At 7 of these 10 CpGs, opposite DNAm changes across adolescence were observed between sexes in both cohorts.

Conclusion- Gender-reversal in asthma acquisition is associated with opposite changes in DNAm (males vs females) from pre- to post-adolescence at asthma-associated CpGs. These CpGs are potential biomarkers of sex-specific asthma acquisition in adolescence.

Keywords: asthma acquisition, ALSPAC, DNA methylation, IOWBC, sex-specificity

Total word count: 3476 words (Introduction-Discussion)

Introduction (427 words)

Asthma is a common chronic condition that affects approximately 339 million people worldwide [1], causing substantial morbidity, reduced quality of life and substantial health-care costs [2]. Asthma predominantly originates in early childhood [3] with an estimated 1.1 million children affected in the UK [4].

There is a male predominance of asthma in early childhood. During adolescence more boys remit asthma than girls, while more girls acquire asthma than boys, which results in gender-reversal of asthma prevalence from pre- to post-adolescence [5-14] with asthma becoming more prevalent and severer among females after puberty [9, 15, 16]. However, the underlying biological mechanisms involved in these sex differences in the natural history of asthma across childhood and adolescence remain unclear.

Although the pathogenesis of asthma reflects a combination of inherited susceptibility and environmental exposures, the etiology and biological mechanisms are poorly understood. The increase in prevalence of asthma in recent decades suggests an important role for environmental exposures in the development of asthma in genetically high-risk individuals, and a number of studies have highlighted the potential for a role of epigenetic programming in response to early life environmental exposures in asthma susceptibility [17-21]. One of the most widely studied epigenetic mechanisms is DNA methylation (DNAm) [22, 23]. Changes in the level of DNAm at specific cytosine-phosphate-guanine (CpG) sites in DNA from both blood and lung tissue has been found to be associated with asthma and related phenotypes in prospective longitudinal [24, 25] and cross-sectional [26-32] studies.

While these studies have established a clear association between DNAm patterns and asthma, they rely on asthma status determined at a single time point. Yet, as previously discussed, asthma phenotype within an individual can be dynamic, new incidence and clinical remission occurring across the life course with gender reversal in asthma prevalence observed in adolescence. In a candidate gene approach, we have previously investigated temporal changes of DNAm at CpG sites in genes encoding proteins in the Th2 pathway and the transition of asthma over adolescence [4]. This study showed that the level of DNAm and the association between specific CpGs (and their interaction with DNA sequence variation) and asthma changes across adolescence. We therefore hypothesized that DNAm changes at specific sites across adolescence might explain the biological basis of sex differences in the natural history of asthma across adolescence and identify biomarkers of asthma acquisition in adolescence that would potentially be beneficial for prediction and prevention. To test this, we have used genome-wide DNAm data to assess sex-specific association of DNAm changes from pre- to post-adolescence with asthma acquisition at asthma-associated CpG sites.

Methods (1202 words)

Study Population

The Isle of Wight birth cohort (IOWBC) consists of children born between January 1, 1989 and February 28, 1990 on the Isle of Wight (IoW), United Kingdom [33]. The IOWBC was established to investigate the natural history of allergic diseases among children residing on a semi-rural island near the UK mainland. Of the 1,536 pregnancies in this period, 1,456 parents consented for further follow-up with survey and clinical data collected at 1, 2, 4, 10, and 18 years.

Asthma acquisition

Detailed questionnaires that included the questions from the International Study of Asthma and Allergy in Childhood (ISAAC) were administered to parents/participants at 10 and 18 years. Asthma was defined as “ever had asthma” and “wheezing or whistling in the chest in the last 12 months” or “current treatment for asthma.” This study focuses on new incidence (i.e., acquisition) of asthma between 10 and 18 years defined as no asthma at 10 years but having asthma at 18 years. Subjects that had no asthma at both ages were used as a reference group.

Of the 1,053 subjects who did not have asthma at 10 years, 275 subjects had DNAm measurements available from peripheral blood samples at both 10 and 18 years and were included in further analyses.

Covariates

Information regarding sex, birth weight, maternal and paternal disease status of asthma was assessed based on questionnaire data and hospital records collected at birth. Socio-economic status was defined based on household income, number of rooms and maternal education. Atopic status was assessed at 10 and 18 years using skin prick test (SPT) for 11 common allergens (house dust mite, cat dander, dog dander, grass pollen mix, tree pollen mix, *Alternaria alternata*, *Cladosporium herbarium*, cow's milk, hen's egg, peanut, and cod), and change of atopic status from 10 to 18 years was recorded. Height and weight were measured at 10 and 18 years, and in cases that a participant did not visit the study center, information was obtained by telephone interviews. Body mass index (BMI) was calculated based on height and weight, and relative changes in height and BMI were calculated for each subject, for instance, relative change in height of a subject is calculated as the difference in height from pre- to post-adolescence divided by their pre-adolescent height.

DNA methylation (DNAm)

DNAm was measured in peripheral blood with samples collected at 10 and 18 years using either the Infinium HumanMethylation450 BeadChips or MethylationEPIC BeadChips (Illumina, Inc., San Diego, CA). Pre-processing of DNAm was carried out using the CPACOR pipeline [34]. Details of DNAm data generation, quality control, and preprocessing, as well as principal components (PC) analyses detecting latent variables for batch and technical variations are in the Supplemental Material S1. After preprocessing, a total of 439,635 CpGs in common between the two platforms were included in the analyses.

Since blood is a mixture of functionally and developmentally distinct cell populations [35], adjusting cell type compositions was needed in analyses to reduce confounding from cell heterogeneity in DNAm measured from blood samples [36]. We estimated cell type proportions using the method proposed by Jaffe and Irizarry [37], adapted from Houseman et al.[38], using the Bioconductor *minfi* package [39]. The estimated cell type proportions of CD4+ T cells, natural killer cells, neutrophil, B cells, monocytes, and eosinophil cells were included in the analyses as confounding factors.

Gene Expression

Gene expression levels from peripheral blood samples collected at 26 years from IOWBC was determined using paired-end (2*75 bp) RNA sequencing. All samples were sequenced twice using the identical protocol and for each sample the output from both runs were combined. Normalized read count were calculated and their log transformed values were used for data analysis. Details on RNA sequencing, transcript reading, mapping and assembly, and normalization are in the Supplemental Material S2.

Statistical analysis

To examine whether the subsample (n=275) included in the study reasonably represented the complete cohort (n=1,053), one sample proportion tests and multinomial tests for categorical variables and one sample t-tests for continuous variables were applied.

Screening analysis to identify asthma-associated CpGs

An R package, *ttScreening*, was implemented to screen for CpGs whose methylation (in M values) was associated with asthma cross-sectionally [40]. Subjects with DNAm and asthma data at one or both ages were included in the screening. This approach was cross-sectional and focused on asthma status rather than asthma transition, to avoid data double dipping, i.e., avoid using the same or a very similar model in screening as well as in final data analyses with the same data. CpGs that survived the screening were treated as asthma-associated CpGs and included in subsequent analyses.

Assessment of DNAm change across adolescence for asthma-associated CpGs

CpGs that passed screening were treated as potentially asthma-associated CpGs. At these sites, M values of DNAm at each CpG were regressed on 15 PCs obtained from control probes (Supplemental Material S1) and the 6 cell type proportions [41] to obtain batch and cell-type adjusted DNAm (residuals). This regression analysis was conducted at 10 and 18 years, respectively. At each of the asthma-associated CpGs, the difference in residuals between 10 and 18 years was then calculated for each subject to represent DNAm change from 10 to 18 years.

Logistic regressions via R function *glm* with a logit link were applied to evaluate the association of asthma acquisition (with asthma-free as the reference) with DNAm changes (independent variable) adjusted for covariates and confounders potentially associated with asthma: maternal and paternal history of asthma, sex, birth weight, socio-economic status, change of atopic status from 10 to 18 years, and relative changes in height and BMI from 10 to 18 years. Additionally, since multiple studies have demonstrated gender reversal on asthma prevalence from pre- to post-adolescence, interaction effects of CpGs and sex on asthma acquisition were assessed. Multiple testing was adjusted by controlling a false discovery rate (FDR) of 0.05.

Replication cohort - the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort

CpGs shown to be associated with asthma acquisition in IOWBC were further assessed in an independent cohort, the Avon Longitudinal Study of Parents and Children (ALSPAC) [42].

DNAm data at 7 and 15 or 17 years and asthma acquisition from 7 to 15 years were included in the replication analyses. Details of these data along with information on covariates are presented in the Supplemental Material S3. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (http://www.bristol.ac.uk/alspac/researchers/our-data/). A p-value<0.05 was deemed as being statistically significant.

Association between DNAm and gene expression

For CpGs with DNAm changes showing consistent associations with asthma acquisition between the two cohorts, we evaluated their biological relevance. Genes annotated to the identified CpGs were extracted from the Illumina's manifest file or SNIPPER (https://csg.sph.umich.edu/ boehnke/snipper/) version 1.2.We tested the association between DNAm at these CpGs and gene expression in blood at 26 years using linear regressions. Gene expression (n=136) was the dependent variable, and DNAm and sex were the independent variables. DNAm at 10 and 18 years were analyzed separately. In addition, to assess sex-specificity of DNAm and expression association, an interaction term of DNAm×sex was also included in the model. Interaction effects were treated as being statistically significant with p-value<0.05.

Results (836 words)

In IOWBC, the analytical subsample was representative of the complete cohort with respect to asthma transition status, demographic variables, and other covariates (p-values >0.05, Table 1). A sex difference in asthma acquisition was observed in the complete cohort; 10.8% of females acquired asthma from 10 to 18 years, as compared to only 7.1% of males (p-value = 0.03).

To identify candidate CpGs potentially associated with asthma at 10 and 18 years for each sex, we applied *ttScreening* to 442,475 CpGs, stratified by sex. In total, 265 (220 for males, 45 for females) CpGs and 290 (40 for males, 250 for females) CpGs passed screening at 10 and 18 years, respectively. CpGs that passed screening at either age of males or females (535 CpGs in total; Supplemental Table S1) were treated as asthma-associated CpGs and included in subsequent analyses.

For each CpG site that passed screening, whether asthma acquisition was associated with changes in DNAm from 10 to 18 years and whether such an association was sex-specific were tested using logistic regressions. Sex and DNAm changes, and their interaction, along with adjusting factors were included in the model. After controlling FDR at 0.05, statistically significant interaction effects were observed at 13 CpG sites (Table 2, left panel of Figure 1). All the coefficients for the interaction effects between sex and DNAm changes were positive. Combined with the estimates of main effect, a potential gender reversal with respect to the effects of DNAm changes on asthma acquisition was identified. For instance, at CpG site cg03269757, a larger increase in DNAm from 10 to 18 years was associated with an increased risk of acquisition in females (log-OR=3.04), but a decreased risk of acquisition in males (log-OR=-1.34). Such opposite associations between males and females were observed at nine of the 13 CpGs. At the remaining four CpG sites, cg11814087, cg12587133, cg18278943, and cg22484084, the association of DNAm changes with asthma acquisition were much stronger in females with larger effect size (increased risk of acquiring asthma). For example, at cg11814087, the log-OR was 6.72 for females, much higher than the log-OR=0.58 for males (interaction effect p-value 8.85×10^{-4} with 95% CI: 2.29, 10.01).

We further tested these 13 CpGs in the ALSPAC cohort. At 10 of the 13 CpG sites, consistent interaction effects with respect to the direction of effects were observed compared to those found in IOWBC, although only one of the 10 CpGs (cg20891917) showed a statistically significant effect (Table 2 and Figure 1). In addition, in the ALSPAC cohort, for eight of these 10 CpGs, the interaction effects were all much stronger than the main effects (Figure 1), the same pattern observed in IOWBC.

To explore underlying mechanisms of the observed interaction effects, for each of the 13 CpGs, we calculated average DNAm changes between 10 and 18 years in IOWBC, and between 7 years and 15 or 17 years in the ALSPAC cohort, for males and females, separately (Figures 2a and 2b). In both cohorts, average DNAm changes in non-asthmatic subjects were all around zero at the 13 CpGs. However, for subjects in IOWBC who acquired asthma between 10 and 18 years, across all the 13 CpGs, the changes in DNAm were opposite between males and females with males showing decrease in DNAm from pre- to post-adolescence (negative differences in males but positive differences in females, Figure 2a). In the ALSPAC cohort, for the first 10 CpGs in Figure 2b, DNAm at these CpGs showed consistent directions of interaction effects with those in IOWBC (Table 2). The pattern of opposite changes in DNAm from age 7 to 15 or 17 years in ALSPAC between males and females were also observed at 7 of these 10 CpGs (Figure 2b).

To assess the biological relevance of the 10 CpGs showing consistent sex-specificity between the two cohorts, we evaluated the association of DNAm at these CpGs with expression of their mapped genes and whether such associations were sex-specific. The 10 CpGs were mapped to 10 genes (Table 2). We did not have expression data for gene *PTPRV.* Statistically significant interaction effects were observed at five of the nine CpG sites (Table 3) with four genes identified based on age 10 DNAm and one based on age 18 DNAm. Combined with the estimates of main effect of DNAm, a potential gender reversal with respect to the association of DNAm with gene expression levels was found at all these five CpG sites (based on opposite signs of the estimated main and interaction regression coefficients). For instance, at CpG site cg11295724, an increase in DNAm at 10 years was associated with increased gene expression levels of *SIRPD* in males, but decreased expression in females. Similar opposite patterns were observed for the CpGs on *CCDC146*, *SLMAP*, and *ZNF385A*. For cg03269757 on *ATL2*, although for both sexes, the regression coefficients were negative (-0.98 for males and -0.06 for females), the effect size for males was more than 16 times as that for females (which was close to zero), representing a potential gender reversal effect as well.

Discussion (1011 words)

We examined the sex-specificity on the association of changes in DNAm with asthma acquisition from pre-adolescence to late- (ALSPAC) or post-adolescence (IOWBC) in two birth cohorts. In IOWBC, 13 CpGs mapping to 13 genes were identified that showed statistically significant interaction effects with sex, of which 10 (77%) CpGs showed consistent directions of interaction effects in ALSPAC with one CpG (cg20891917) being statistically significant. In most of these CpGs, the effects of changes in DNAm on asthma acquisition during adolescence were opposite between males and females, showing gender reversal of DNAm effects. Accompanied by the opposite direction of changes in DNAm between males and females at most of the identified CpGs in both cohorts, this suggests that DNAm may represent a mechanism underlying the well-established gender reversal in asthma prevalence across adolescence [15, 16]. In addition, assessment of the biological relevance for 9 of the 10 CpGs indicated a potential of epigenetic regulatory functionality on gene activities. DNAm at 5 of the 9 CpGs showed sex-specific associations with gene expression, supporting the gender reversal phenomenon found in the association assessment between DNAm changes and asthma acquisition during adolescence. The strength of this study is the availability of DNAm data and asthma status at two important time points, pre- and post-adolescence, enabling the possibility to examine changes in DNAm and its association with asthma acquisition during adolescence. To our knowledge, this is the first study to examine the epigenetics of asthma acquisition during adolescence regarding sex-differences.

For each of the genes annotated to the identified CpGs, we performed a literature search for their possible roles that they played in the risk of asthma. Among the CpGs showing consistent direction of interaction effects, DNAm of gene *ZNF385A* and expression of *IFRD1* have been reported as biomarkers of asthma [43]. Our finding on the association of DNAm with expression of *ZNF385A* further strengthens its relationship with asthma. Lund et al. demonstrated that *NDFIP2*, an IL-4 regulated gene, promoted IFN-γ production by the polarized human Th1 lymphocytes [44]. One of our earlier studies in females showed that genes in the Th2 pathway were likely to contribute to an increased risk of asthma and be associated with the risk of asthma transition [4]. Although in the present study we did not identify genes in the Th2 pathway, Th1 and Th2 cells work tightly and interact with other immune cells by regulating their functions with specific cytokine production, associated with the pathogenesis of asthma. As for gene *ZFR*, single nucleotide polymorphisms in *ZFR* have been shown to be associated with asthma or bronchial hyperresponsiveness [45]. However, none of these studies have mentioned sex-specificity in these associations due to the focus of the study and the methods applied in the study. For instance, the study of Zhang et al. [4] only included females; while in the study of Kurz et al. [45], the focus was to identify single nucleotide polymorphisms associated with asthma or bronchial hyperresponsiveness and the effects of sex were not considered. Furthermore, findings in the literature all focused on the risk of asthma instead of the risk of asthma acquisition, which might also explain the limited findings in the literature supporting the identified genes.

At most of the CpGs identified in IOWBC, consistent direction of interaction effects was found in ALSPAC. However, statistical significance was not observed at those CpG sites except for cg20891917. In the ALSPAC cohort, many subjects' DNAm was assessed at 15 years and asthma status change was from 7 years to 15 years. At the age of 15 years, it was likely that children were still in the period of pubertal transition, and thus sex-specificity might not be strong enough to be detected. In addition, we noticed that among the 10 CpG sites showing consistent sex-specificity between the two cohorts, associations in DNAm with expression of genes happened more often with DNAm at age 10 years. We do not have a specific biological explanation for this observation but postulate that this might have been due to larger variations in DNAm data at age 18 compared to DNAm at age 10, and thus we did not have enough power to detect the associations.

In this study, the candidate CpGs were identified based on their associations with asthma status at 10 and 18 years separately. With this approach, we were able to focus on asthma related CpG sites, which was the starting point of the study. On the other hand, we might have missed CpGs that were not related to asthma at ages 10 nor 18 years but were related to asthma acquisition from 10 to 18 years. However, screening candidate CpGs based on asthma acquisition had the risk of double-dipping the data. That is, the screening and final association analyses would share a similar analytical model applied to the same data, which in general is not encouraged. In addition, screening of CpGs and association analyses were applied to each individual CpG sites. CpG sites might be correlated and jointly impact asthma acquisition. Using our approach, correlated CpGs might have presented an issue that we were unable to address. Approaches analogous to linkage disequilibrium and haplotype identification in genetic studies deserve further investigations both methodologically and experimentally. We also would like to point out that the present study was based on a concurrent analysis (i.e. both DNAm changes and asthma acquisition were in the same period). The focus of the study was on associations rather than causality and this analytical approach does not allow predictions or inferring causality.

Nevertheless, the consistency in the results between the two cohorts indicated that the identified CpGs are likely to play a role in the underlying mechanisms of sex-specific asthma acquisition. Furthermore, the sex-specific associations of DNAm at most of these CpGs with expressions of their mapped genes demonstrated their potential of biological relevance and supported our observed sex-specificity related to asthma acquisition. Although future studies are warranted to further examine the credibility of the identified CpGs, these CpGs have the potential to serve as candidate markers in subsequent mechanistic studies on gender reversal of asthma acquisition.

Acknowledgements

This study was supported by the National Institutes of Health research fund R01AI121226 (MPI: H Zhang and JW Holloway). Part of the methylation data generation was supported by R01AI091905 (PI: W Karmaus). The Isle of Wight Birth Cohort assessments have been supported by the National Institutes of Health USA (Grant no. R01HL082925, H. Arshad), Asthma UK (Grant no. 364. S.H. Arshad) and the David Hide Asthma and Allergy Research Trust.

The UK Medical Research Council and Wellcome (Grant ref: 217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). Generation of methylation array data was specifically funded by NIH R01AI121226, R01AI091905, BBSRC BBI025751/1 and BB/I025263/1, MRC MC_UU_12013/1, MC_UU_12013/2, MC_UU_12013/8.

The authors are thankful to the nurses and staff at the David Hide Asthma & Allergy Research Centre, Isle of Wight, UK, for their help in recruitment and sample collections, and are thankful to all the cohort participants. Our special thanks also go to the High-Performance Computing facility provided by the University of Memphis.

For ALSPAC, DNA extraction and generation of Illumina array data was carried out in the Bristol Bioresource Laboratories at the University of Bristol, UK. We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Data availability statement

The datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declarations

Ethics approval and consent to participate

The IoW birth cohort study was approved by Isle of Wight, Portsmouth and Hampshire Local Research Ethics Committee (now known as the National Research Ethics Service, NRES Committee South Central – Hampshire A) (06/Q1701/34) and the IRB at the University of Memphis (FWA00006815). Written informed consent was obtained from parents during in-person visits. For participants assessed by phone interview, consent was documented on the consent form with the name of the person giving consent, and the name and signature of the person taking the form were recorded.

For ALSPAC, ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees and consent for collection of biological samples was provided in accordance with the Human Tissue Act (2004). For age seven years, United Bristol Healthcare Trust: E4168 (ALSPAC Hands on Assessments at Age Seven), Southmead Health Services: 67/98 (Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) - Hands on Assessments at Age Seven) and Frenchay Healthcare Trust:

98/52 (Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC). Hands on Assessments at Age Seven). For age 15 years, Central & South Bristol Research Ethics Committee (UBHT): 06/Q2006/53 Avon Longitudinal Study of Parents and Children (ALSPAC), Hands on Assessments: Teen Focus 3 (Focus 15+), and for age 17 years, North Somerset & South Bristol Research Ethics Committee: 08/H0106/9 Avon Longitudinal Study of Parents and Children (ALSPAC), Hands on Assessments: Teen Focus 4 (Focus 17+). Full details of ethical approvals (local committees and approval numbers) are available at http://www.bristol.ac.uk/media-library/sites/alspac/documents/governance/Research%20Ethics%20Committee%20approval%20references.pdf

Statement of author contribution

HZ designed the study, FS, AW, RP and RA analyzed the data, LK interpreted bioinformatics findings, WK, HA, CR, JAH, and JWH supervised the study, SE, JH, and SR provided DNA methylation data, RP and HZ drafted the manuscript, and all authors reviewed the manuscript.

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Table 1: Asthma acquisition and non-asthma participants included in the present study compared to the participants in the complete cohort.

Categorical Variables: N (%)		**Females**			**Males**		
		Subsample n=124	**Complete cohort** n=544	*p*-value	**Subsample** n=151	**Complete cohort** n=509	*p*-value
Asthma Transition	Acquisition	12 (9.7%)	59 (10.8%)	0.78	14 (9.27%)	36 (7.1%)	0.37
	Non-asthma	112 (90.3%)	485 (89.1%)		137 (90.7%)	473 (92.9%)	
Maternal asthma	Yes	15 (12.1%)	50 (9.3%)	0.35	23 (15.4%)	53 (10.4%)	0.06
	No	109 (87.9%)	490 (90.7%)		126 (84.5%)	453 (89.5%)	
Paternal asthma	Yes	14 (11.5%)	45 (8.4%)	0.27	15 (10.1%)	50 (9.9%)	1
	No	107 (88.4%)	492 (91.6%)		133 (89.8%)	454 (90%)	
Socio-economic status	Mid-High	42 (33.8%)	193 (36.4%)	0.67	55 (36.4%)	197 (40.2%)	0.60
	Low-mid	35 (28.2%)	153 (28.8%)		51 (33.7%)	149 (30.4%)	
	Low-low	47 (37.9%)	184 (34.7%)		45 (29.8%)	143 (29.2%)	
Change of atopic status from 10 to 18 year	Yes-No	4 (3.6%)	7 (2.37%)	0.53	3 (2.5%)	3 (1.4%)	0.43
	No-Yes	20 (18%)	52 (17.6%)		32 (26.8%)	62 (28%)	
	No-No	87 (78.3%)	236 (80%)		84 (70.5%)	156 (70.5%)	
Continuous Variables: Mean (SD)							
Birth weight		3.32 (0.51)	3.33 (0.50)	0.88	3.45 (0.56)	3.50 (0.52)	0.51
Relative change in height from 10 to 18 years		0.18 (0.04)	0.19 (0.04)	0.27	0.28 (0.03)	0.28 (0.04)	0.39
Relative change in BMI from 10 to 18 years		0.29 (0.15)	0.29 (0.16)	0.64	0.29 (0.15)	0.29 (0.15)	0.88

Table 2: Association of DNAm change with asthma acquisition from pre- to post-adolescence at 13 CpG sites that are sex-specific. Significant interactions between DNAm and sex on asthma acquisition from 10 to 18 years identified in the IOWBC[a] were further tested in the ALSPAC cohort[b]. Males are in the reference group.

	IOWBC									ALSPAC cohort		
CpG site	**Est.^{c}**	**Int.^{c}**	**95% C.I.^{c}**	**P_{Raw}^{d}**	**P_{FDR}^{d}**	**Gene**	**CpG islands**	**Gene Location**	**Chr.^{e}**	**Est.^{c}**	**Int.^{c}**	**P_{Raw}^{d}**
cg03269757	-1.34	**4.38**	1.93, 6.83	1.88×10^{-4}	0.02	*ATL2*	N_Shore	Body	2	0.03	**0.59**	0.19
cg11295724	-0.33	**7.82**	4.12, 11.52	5.35×10^{-6}	0.003	*SIRPD*	-	TSS200	20	0.06	**0.76**	0.22
cg12587133	0.34	**4.85**	2.37, 7.32	1.03×10^{-5}	0.003	*AGA*	-		4	0.01	**0.24**	0.5
cg15154628	-0.67	**3.23**	1.14, 5.31	1.15×10^{-3}	0.05	*CCDC146*	-	Body	7	0.24	**0.07**	0.89
cg16301989	-0.48	**4.42**	1.89, 6.93	1.86×10^{-4}	0.02	*FUNDC2P2*^{f}	-		2	0.05	**0.47**	0.26
cg18278943	0.70	**4.55**	1.87, 7.24	4.36×10^{-4}	0.03	*SLMAP*	N_Shelf		3	-0.25	**0.82**	0.18
cg20885063	-0.52	**4.45**	1.86, 7.05	3.78×10^{-4}	0.03	*ATPAF2*	N_Shelf	Body	17	0.007	**0.4**	0.36
cg20891917	-0.43	**5.5**	2.03, 8.96	9.86×10^{-4}	0.04	*IFRD1*	-	5'UTR	7	-0.13	**1.26**	**0.01**
cg21163444	-2.22	**11.13**	4.31, 17.95	8.53×10^{-4}	0.04	*ZNF385A*	S_Shore	Body	12	0.27	**0.14**	0.86
cg22484084	0.29	**7.18**	2.69, 11.67	1.0×10^{-3}	0.04	*PTPRV*	-	TSS1500	1	0.13	**0.84**	0.39
cg06492287	-1.91	10.49	4.73, 16.25	7.78×10^{-5}	0.01	*SNTG2*	-	Body	2	0.002	-0.04	0.96
cg11770323	-0.7	9.18	4.11, 14.25	7.92×10^{-5}	0.01	*NDFIP2*	-	Body	13	-0.75	-0.55	0.29
cg11814087	0.58	6.14	2.29, 10.01	8.85×10^{-4}	0.04	*ZFR*	-	Body	5	0.11	-0.68	0.05

[a] For the analyses in IoW, logistic regression models were adjusted for birth weight, sex, maternal and paternal disease status of asthma, socio-economic status (SES), change of atopic status, BMI, height from 10 to 18 years.

[b] Analyses of ALSPAC used similar covariates: maternal disease status of asthma, sex, birth weight, SES, atopy status at 7 years, and changes of height, and BMI from 7 to 15/17 years.

[c] Est. – Estimated main effect; Int. – interaction coefficient of the CpGs with the sex of a child. Interaction effects consistent between the two cohorts are with bold fonts; C.I. – confidence interval

[d] P_{Raw} – raw p-value; P_{FDR} – adjusted p-value for multiple testing by controlling a false discovery rate (FDR) of 0.05. All the p-values are for interaction effects.

[e] Chr. – Chromosome.

[f] Genes closest to the CpG site annotated using the UCSC genome browser.

Table 3: Association of DNAm at 5 CpGs with their mapped genes' expression levels that are sex-specific. Only results on CpGs showing statistically significant interaction effects of DNAm×sex on gene expression were shown. Males are in the reference group. The p-values are for interaction effects.

Gene	**CpG**	DNAm effect	Sex×DNAm interaction	p-value (Sex×DNAm)	DNAm effect	Sex×DNAm interaction	p-value (Sex×DNAm)
			Age 10 years			Age 18 years	
SIRPD	cg11295724	0.89	-1.39	**0.022**	0.24	-0.42	0.45
CCDC146	cg15154628	-0.40	0.72	**0.041**	0.44	-0.43	0.39
SLMAP	cg18278943	-1.43	1.90	**0.006**	-0.28	0.58	0.45
ZNF385A	cg21163444	-0.2	0.72	**0.037**	0.009	-0.24	0.56
ATL2	cg03269757	-0.66	0.80	0.09	-0.98	0.92	**0.040**

Figure 1: Effects of changes in methylation on asthma acquisition in adolescence for 13 CpGs in IOWBC (left) and ALSPAC (right) cohorts, stratified by sex-specific (interaction effects with male as the reference group, black bars) and sex nonspecific effects (main effects, gray bars). X-axis is for the regression coefficients (main effects and interaction effects). For interaction effects (black bars) in the IOWBC, 95% confidence intervals are in Table 2.

Figure 2: Scatter plots showing average DNAm changes from pre-adolescence to post-/late-adolescence, stratified by sex. (a) Average DNAm changes between 10 and 18 years in IOWBC. (b) Average DNAm changes between 7 and 15 or 17 years in the ALSPAC cohort. In both panels, left figure is for males, and right for females.

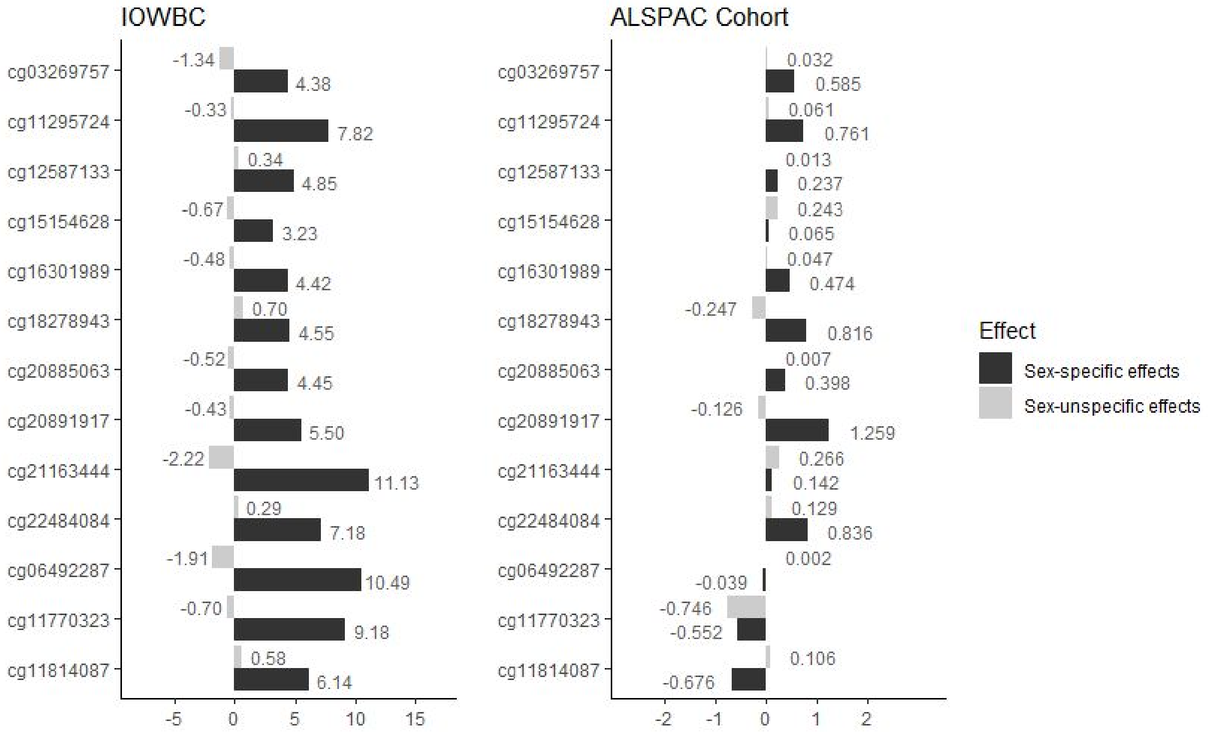

Figure 1: Effects of changes in methylation on asthma acquisition in adolescence for 13 CpGs in IOWBC (left) and ALSPAC (right) cohorts, stratified by sex-specific (interaction effects with male as the reference group, black bars) and sex nonspecific effects (main effects, gray bars). X-axis is for the regression coefficients (main effects and interaction effects). For interaction effects (black bars) in the IOWBC, 95% confidence intervals are in Table 2.

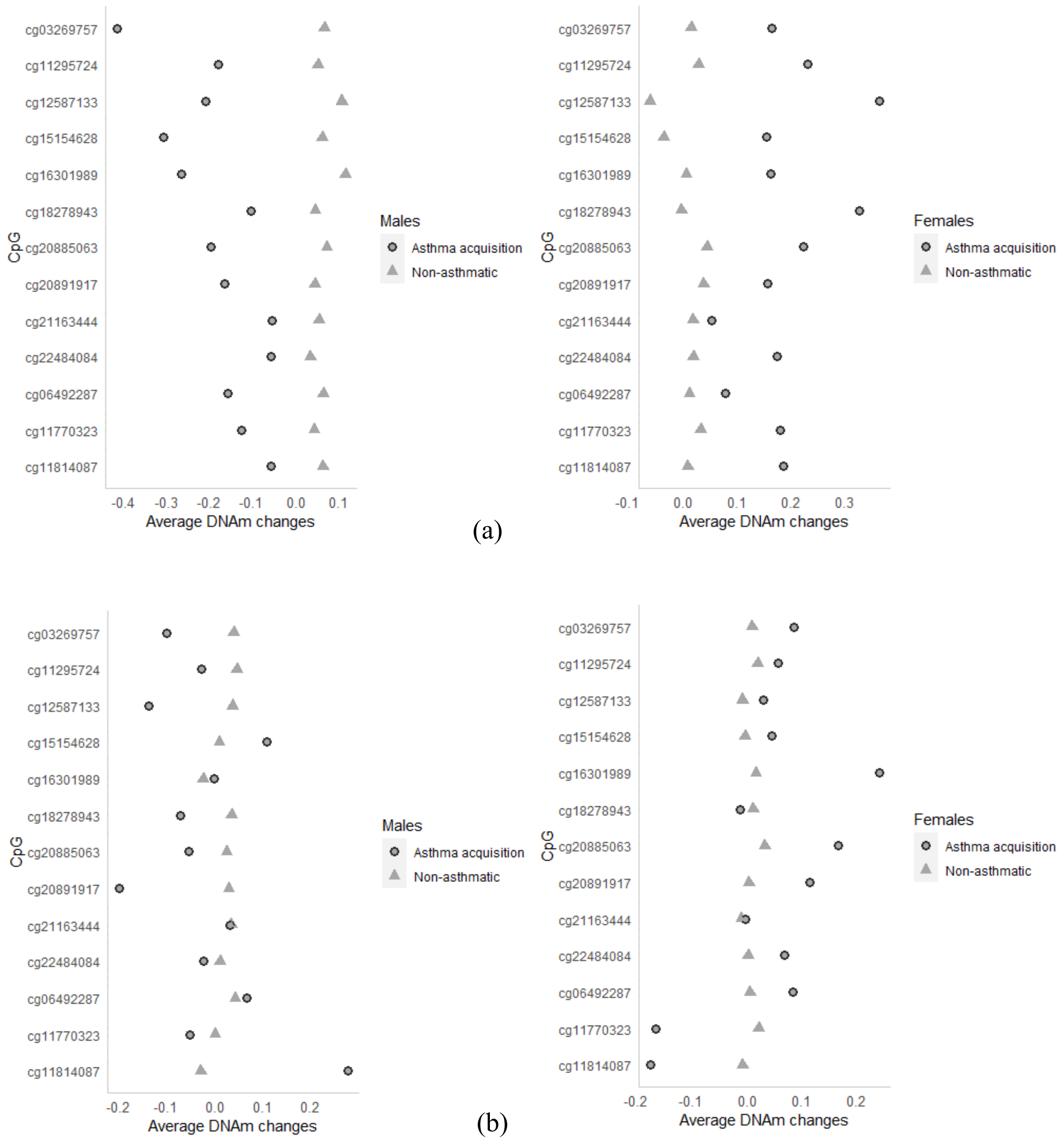

Figure 2: Scatter plots showing average DNAm changes from pre-adolescence to late-(ALSPAC)/post-(IOWBC) adolescence, stratified by sex. (a) Average DNAm changes between 10 and 18 years in IOWBC. (b) Average DNAm changes between 7 and 15 or 17 years in the ALSPAC cohort. In both panels, left figure is for males, and right for females.

Sex-specific associations of asthma acquisition with changes in DNA methylation during adolescence

Rutu Patel, MPH, MBBS[1]*, Farnaz Solatikia, MPH, MS[1,2]*, Hongmei Zhang, PhD, MS[1]**, Alemayehu Wolde, MS[2], Latha Kadalayil PhD[3], Wilfried Karmaus, MD, Dr.med., MPH[1], Susan Ewart, DVM, PhD, DACVIM[4], Ryan Arathimos, PhD[5,6,7,8], Caroline Relton, PhD[5,6,7], Susan Ring, PhD, BSc[5,6,7], A. John Henderson[6], MD, S. Hasan Arshad, MBBS, DM, FRCP [9,10,11], John W Holloway, PhD[3,11]

[1]Division of Epidemiology, Biostatistics and Environmental Health, School of Public Health, University of Memphis, Memphis, TN, USA.

[2]Department of Mathematical Sciences, University of Memphis, Memphis, TN, USA.

[3]Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK

[4]College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.

[5]MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK.

[6]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK.

[7]National Institute for Health Research Bristol Biomedical Research Centre, University of Bristol and University Hospitals Bristol NHS Foundation Trust, Bristol, UK.

[8]Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK

[9]Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.

[10]David Hide Asthma and Allergy Research Centre, Isle of Wight, UK.

[11]NIHR Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, UK

*: equal contribution, **: corresponding author

Corresponding author: Dr. Hongmei Zhang

Phone: 901.678.4707

Email: hzhang6@memphis.edu

Fax:901.678.1715

Office: 224 Robison Hall, 3825 DeSoto Avenue, Memphis, TN 38152-0001

Supplemental Material

S1. Quality control and pre-processing genome-wide DNA methylation data

A standard salting out procedure was used to extract DNA from peripheral blood samples collected at ages 10 and 18 years [1]. DNA concentration was determined by PicoGreen dsDNA quantitation (Molecular Probes, INC. OR, USA) or Qubit (Thermofisher, MA, USA). One microgram of DNA was bisulfite-treated for cytosine to thymine conversion using the EZ 96-DNA Methylation Kit (Zymo Research, Irvine, CA, USA) for each sample. Arrays were processed using a standard protocol with multiple identical control samples assigned to each bisulfite conversion batch to assess assay variability. Methylation level at >484,000 and >850,000 CpG sites was assessed using Infinium HumanMethylation450 BeadChips or Infinium MethylationEPIC BeadChips (Illumina, Inc., San Diego, CA), respectively. Probes not reaching a detection p-value of 10^{-16} in at least 95% of samples were excluded, and the same criterion was applied to the exclusion of a sample. CpGs on the sex chromosomes were excluded from analyses to avoid bias.

DNAm data were preprocessed using the CPACOR pipeline [2]. Specifically, DNAm intensity data were quantile-normalized using the R package, *minfi* [3]. Beta values were calculated representing proportions of intensity of methylated (M) over the sum of methylated and unmethylated (U) probes ($\beta = M / [c + M + U]$, where c is a constant to prevent zero in the denominator if $M + U$ is too small). Beta values close to 0 or 1 tend to suffer from severe heteroscedasticity. Therefore base-2 logit transformed beta values (denoted as M values) were used in analyses [4].

Principal components (PCs) inferred based on control probes to represent latent chip-to-chip and technical variations were inferred. Since DNAm data were from two different platforms (450K and EPIC), PCs were determined based on DNAm at 195 control probes shared between the platforms; the top 15 control probe PCs were used to represent latent batch factors [2]. These 15 PCs were included in subsequent analyses. Probes that contained single nucleotide polymorphisms (SNPs) within 10 base pairs of a targeted CpG site with a minor allele in more than 0.7% subjects (corresponding to at least 10 subjects in IOWBC with n = 1,456) were excluded due to their potential influence on DNAm measurement. After preprocessing, a total of 439,635 CpGs in common between the two platforms were included in the analyses.

S2. Genome-wide RNA-seq gene expression data generation

Gene expression levels from peripheral blood samples collected at 26 years from the IoW cohort was determined using paired-end (2*75 bp) RNA sequencing with the Illumina Tru-Seq Stranded mRNA Library Preparation Kit with IDT for Illumina Unique Dual Index (UDI) barcode primers following the manufacturer's recommendations. All samples were sequenced twice using the identical protocol and for each sample the output from both runs were combined. FASTQC were run to assess the quality of the FASTQ files (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were mapped against Human Genome (GRch37 version 75) using HISAT2 (v2.1.0) aligner [5]. The alignment files, produced in the Sequence Alignment Map (SAM) format, were converted into the Binary Alignment Map (BAM) format using SAMtools (v1.3.1) [6]. HTseq (v0.11.1) was used to count the number of reads mapped to each gene in the same reference genome used for alignment [7]. Normalized read count FPKM (Fragments Per Kilobase of transcript per Million mapped reads) were calculated using the countToFPKM package (https://github.com/AAlhendi1707/countToFPKM) and their log transformed values were used for data analysis.

S3. Screening asthma-associated CpG sites

An R package, *ttScreening*, was implemented to screen for CpGs whose methylation (in M values) was associated with asthma cross-sectionally [8]. This method utilizes training and testing data in robust linear regressions with surrogate variables included to adjust for unknown (latent) factors. Screening was performed separately for each sex at ages 10 and 18 years. Subjects with DNAm and asthma data at one or both ages were included in the screening. The minimum frequency of selecting an informative CpG sites was set at 50%, i.e., a CpG site gained statistical significance in at least 50% of the randomly selected training and testing data set pairs. CpGs that passed the screening at either age for one or both sexes were considered to be potentially associated with asthma and were included as candidate CpGs in subsequent analyses. This approach of screening was cross-sectional and focused on asthma status rather than asthma transition, to avoid data double dipping, i.e., avoiding using the same or a very similar model in screening as well as in final data analyses with the same data.

S4. The replication cohort, ALSPAC

DNAm in the ALSPAC cohort was assessed using the Infinium HumanMethylation450 BeadChip. DNAm data on 1,018 offspring in the ALSPAC cohort were available at ages 7 and 15 or 17 years [9]. The pre-processing of DNAm was performed by correcting for batch effects using the *minfi* package [3] and removing CpGs with detection p-value ≥0.01. Samples were flagged that contained sex-mismatch based on X-chromosome methylation. Estimated cell type proportions of CD4+ T cells, natural killer cells, neutrophil, B cells, monocytes, and granulocytes cells were used in the analyses to adjust for cell heterogeneity. DNAm changes between pre- and post-adolescence were calculated in a similar way to that in IOWBC, except that PCs were not utilized in the ALSPAC cohort for batch effect adjustment.

Asthma acquisition was defined as no asthma at age 7 but asthma at age 15 years. Identical logistic regression models were used with comparable covariates (as those in IOWBC) available in ALSPAC, including maternal asthma, sex, birth weight, socio-economic status, and atopy status at 7 years, and relative changes of height and BMI from 7 to 15 or 17 years.

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Supplemental Table 1: Results of CpG sites that passed screening. “Frequency” is the frequency of selecting a CpG site based on 100 randomly chosen training and testing data. CpGs with a frequency of 50 and more are included in the table. “$\text{Coef}_{\text{asthma}}$” reflects the effect of asthma and “p_{asthma}” is the associated p-value. We adjusted for batch effects in the screening process and the batch variable represents different batches by which DNA methylation was measured.

Age 10 Female

CpG	Frequency	$\text{Coef}_{\text{asthma}}$	$\text{Coef}_{\text{batch}}$	P_{asthma}	P_{batch}
cg2533143	76	0.447377	0.169589	2.49E-06	0.087365
cg0809231	57	-0.29112	0.099644	3.38E-05	0.177629
cg2118523	51	0.427722	-0.0659	0.000151	0.580256
cg2206846	60	-0.29445	0.592191	1.18E-05	6.50E-14
cg0405587	55	0.360233	1.446003	2.61E-05	2.99E-32
cg2084003	52	0.31917	0.934488	4.11E-05	3.51E-21
cg1785116	73	-0.36101	0.357198	1.30E-05	6.45E-05
cg0350398	60	0.358672	1.331627	3.38E-05	5.98E-29
cg0303087	69	0.344976	0.404608	3.27E-06	6.05E-07
cg2199240	53	0.323739	0.88341	8.56E-05	4.09E-18
cg2109556	55	0.354243	0.893372	6.27E-05	1.06E-16
cg2007098	51	0.367637	0.513139	4.05E-05	2.39E-07
cg2403058	54	0.346869	0.167238	0.0001	0.076864
cg0512419	50	-0.38323	0.934518	7.98E-05	1.36E-15
cg1448011	64	0.391627	-0.34825	1.89E-06	7.57E-05
cg1644921	58	-0.42341	0.715569	6.51E-05	2.49E-09
cg0703745	52	-0.38069	1.037257	0.000234	2.01E-16
cg0729899	57	0.284279	0.973611	3.11E-05	2.04E-26
cg2591291	50	0.28097	0.351626	6.82E-05	5.57E-06
cg2071595	62	-0.31716	0.350184	1.08E-05	7.97E-06
cg0872828	50	0.273311	0.608093	6.92E-05	7.26E-14
cg0405133	55	-0.39382	0.014578	5.73E-06	0.871928
cg2661165	55	0.336989	0.551394	2.07E-05	7.66E-10
cg0092650	56	0.273283	1.331253	0.000229	3.16E-34
cg0568258	50	0.894524	0.146532	2.56E-05	0.510833
cg1056183	51	0.340971	0.192399	2.76E-05	0.025451
cg1980821	62	0.536805	0.425467	1.71E-05	0.001417
cg2553292	56	-0.54257	-0.71184	3.72E-05	9.23E-07
cg0296338	52	0.537687	1.203842	4.75E-05	1.82E-14
cg1318667	53	-1.03582	-1.50242	1.11E-05	1.16E-08
cg1630758	54	-4.0993	2.219087	5.49E-06	0.019454
cg1944715	82	-3.82058	0.391963	6.54E-09	0.55715
cg0045984	58	0.440901	1.255816	2.71E-05	4.24E-21
cg0133067	67	-2.8867	-1.4078	2.82E-06	0.029404
cg1625515	53	-0.41167	0.173212	4.69E-05	0.104788
cg1034987	51	-1.11264	-0.49751	1.98E-05	0.070172
cg0359553	67	-2.54524	-1.65332	3.07E-07	0.001549
cg1136144	63	-0.30076	1.625247	4.23E-05	7.11E-43
cg0944690	54	-0.33478	0.16986	2.30E-05	0.04197
cg0075201	74	-2.22482	0.595272	3.67E-07	0.188088
cg1221368	52	-0.47836	1.037266	6.65E-05	2.07E-13

cg1224543	63	-0.38224	-0.62643	1.10E-05	2.20E-10
cg1712580	50	0.514999	1.367136	6.34E-05	6.55E-18
cg0518401	52	-0.58201	1.157031	0.000123	3.84E-11
cg0972173	62	-1.23891	-1.69883	4.27E-06	1.43E-08

Age 18 Female

CpG	Frequency	Coef$_{\text{asthma}}$	Coef$_{\text{batch}}$	P$_{\text{asthma}}$	P$_{\text{batch}}$
cg0191999	52	0.128502	0.791633	0.000123	1.08E-06
cg2026373	58	-0.26957	0.309714	1.51E-05	0.291615
cg1347563	51	-0.12412	0.255558	0.00011	0.093107
cg1627489	51	0.099569	0.325036	2.05E-05	0.003457
cg2680433	70	-0.12563	-0.08267	1.70E-06	0.501895
cg2162718	65	-0.2036	0.235659	7.41E-07	0.221569
cg0412498	50	0.314541	-0.66464	0.000191	0.096803
cg0523552	56	0.112004	0.18901	7.85E-05	0.159355
cg0849878	69	-0.13414	0.278106	4.15E-05	0.072726
cg1759351	50	-0.14951	0.53652	8.06E-05	0.003018
cg0533134	51	0.073431	0.39167	0.00015	2.74E-05
cg1640505	53	-0.73057	0.712838	0.00013	0.430255
cg1408460	51	-0.22373	-0.20799	0.000118	0.449438
cg1265171	56	0.125271	-0.29756	4.51E-05	0.040964
cg1498354	70	-0.21829	0.496746	1.02E-06	0.018239
cg1754792	70	0.093811	0.207197	1.45E-06	0.023911
cg0991255	59	0.100349	0.167257	7.05E-05	0.161735
cg1865565	54	-0.26186	-0.25997	0.000255	0.443663
cg2149847	58	-0.13147	-0.0942	2.26E-05	0.519596
cg0391554	57	-0.11649	0.081549	0.000115	0.568188
cg2159748	58	0.233701	0.746125	7.05E-05	0.007685
cg2746915	62	-0.15395	-0.17697	3.12E-05	0.310657
cg0578228	59	0.115487	-0.17844	0.000187	0.223412
cg0633380	58	0.124635	0.438473	8.51E-07	0.000265
cg0700685	60	-0.19394	-0.87386	3.06E-05	8.94E-05
cg2449161	74	-0.23769	0.281088	6.37E-07	0.20892
cg2482367	70	-0.16695	0.288477	1.75E-06	0.079124
cg0970578	70	-0.25888	0.350026	6.33E-07	0.150937
cg0106946	59	-0.15135	-0.14626	5.26E-06	0.349424
cg1846010	67	-0.15494	-0.05125	1.26E-05	0.759032
cg0619637	50	0.083316	0.777704	0.000132	9.59E-13
cg2436896	64	-0.23601	0.456403	1.10E-05	0.071852
cg0655975	50	-0.12395	-0.16707	8.09E-05	0.261519
cg0571047	79	-0.18799	-0.03221	6.48E-06	0.869453
cg0313176	55	-0.16437	-0.06505	5.62E-05	0.735705
cg0421785	55	-0.18017	0.311851	2.87E-06	0.085326
cg0947075	70	0.094184	0.605839	8.18E-06	4.18E-09
cg0280199	57	0.11953	0.602027	1.94E-05	7.87E-06
cg1383568	60	-0.24684	-0.22278	4.47E-06	0.378432
cg0607062	52	-0.17746	0.520816	2.09E-05	0.008537
cg1379258	65	-0.23768	0.499392	1.18E-06	0.030157
cg0613071	50	0.188224	0.077771	0.000261	0.750177
cg1740453	60	0.105776	0.494215	6.63E-06	1.16E-05
cg1662735	58	-0.2261	0.288055	2.12E-06	0.198565
cg0933250	50	-0.18689	0.247092	6.13E-05	0.26253

cg0088137	56	-0.14595	0.274315	2.48E-05	0.093746
cg2088506	58	-0.2583	0.150845	8.01E-06	0.579431
cg0109710	51	-0.13979	0.108781	2.62E-05	0.487996
cg1665819	72	-0.25531	0.388234	1.19E-06	0.115875
cg0948947	58	-0.17075	0.56702	4.98E-05	0.004667
cg1426677	55	0.113142	0.522463	1.38E-05	2.87E-05
cg0021328	58	-0.19377	0.682097	1.94E-05	0.001592
cg0575379	65	0.104559	0.412927	3.05E-06	0.000111
cg2397855	52	-0.20188	0.610467	0.00023	0.019387
cg2393909	61	0.088493	0.926449	2.39E-05	5.36E-18
cg2129188	54	0.270503	-0.75996	0.000277	0.031896
cg0190157	51	-0.2014	0.627137	3.70E-05	0.0069
cg0343760	52	-0.18806	-0.22047	1.47E-05	0.281237
cg1453946	52	-0.13064	0.186561	0.000164	0.256073
cg2261625	62	-0.12207	0.465948	3.91E-05	0.001007
cg0426407	57	0.136903	0.822224	1.12E-05	5.67E-08
cg1855084	60	-0.22345	0.295708	4.14E-05	0.251003
cg0161475	61	-0.22576	0.254549	3.29E-06	0.264506
cg0970806	50	0.298306	1.04242	4.14E-05	0.002644
cg1070417	60	-0.24003	0.121415	7.79E-06	0.630691
cg0959719	59	-0.21493	-0.78265	4.62E-06	0.000457
cg0025940	64	0.102377	0.372734	1.47E-06	0.000228
cg0518018	50	-0.13973	0.108876	0.000244	0.546323
cg1353479	56	0.084494	0.483044	2.23E-06	2.46E-08
cg0879939	67	-0.15697	-0.61085	4.09E-07	3.48E-05
cg0038139	58	-0.21175	0.400225	1.08E-05	0.078219
cg0590401	55	0.09098	0.520269	3.11E-05	8.66E-07
cg0686620	53	-0.11776	-0.5258	4.49E-05	0.000144
cg0772424	63	0.11882	0.191743	3.28E-05	0.156265
cg0400460	62	-0.19059	0.766405	2.33E-05	0.00037
cg1455471	64	-0.18512	-0.0141	2.62E-05	0.945837
cg1461387	65	-0.19268	0.202986	1.39E-06	0.27857
cg0188189	58	0.180121	0.543922	5.19E-05	0.010168
cg1751903	50	-0.18022	0.284698	1.39E-05	0.145773
cg1316743	52	0.182251	0.277384	3.75E-06	0.134811
cg0639141	60	-0.1911	-0.11777	1.49E-05	0.571011
cg2467876	67	-0.09795	-0.15161	1.74E-05	0.158981
cg1138996	58	0.098463	0.317521	0.000104	0.00861
cg0959664	62	-0.22314	0.605538	2.62E-06	0.007012
cg0500033	71	-0.18002	0.43759	1.60E-06	0.013433
cg0790865	58	-0.22495	0.173045	2.04E-05	0.48701
cg0816579	54	-0.13764	0.109289	6.20E-06	0.445511
cg0224426	53	-0.24017	0.888739	0.000244	0.004488
cg0267483	53	0.116824	0.520249	0.000118	0.000349
cg0299922	51	0.111894	0.558218	2.22E-05	1.09E-05
cg1210569	54	-0.22913	0.488386	5.78E-05	0.070471
cg0695896	56	-0.18692	-0.08038	0.000113	0.725523
cg0807780	50	-0.24041	0.245788	3.66E-05	0.371485
cg1395100	59	-0.13984	0.242296	1.79E-05	0.115576
cg1834219	52	0.292897	1.275395	0.000328	0.001101
cg0877694	53	-0.17039	0.178989	1.61E-05	0.336508
cg1622580	60	-0.1999	-0.41395	1.22E-06	0.032841

cg0602175	50	0.186385	0.528445	0.000204	0.026933
cg1106452	71	0.087713	0.309391	1.71E-06	0.000383
cg2438065	56	-0.13786	0.157948	1.74E-05	0.296697
cg1208230	51	-0.1671	0.321969	9.88E-05	0.113513
cg1696342	61	0.191317	1.070219	8.24E-05	5.17E-06
cg1588905	50	-0.19319	0.172223	0.000103	0.464323
cg0275281	51	-0.16999	0.673222	4.28E-05	0.000694
cg2165086	66	0.106725	-0.06344	1.96E-06	0.546663
cg2002565	59	0.204988	0.561847	5.75E-06	0.008654
cg0507809	51	-0.36221	0.197137	4.85E-05	0.639577
cg0268811	54	0.131662	0.521966	5.46E-05	0.000816
cg1887938	56	-0.21603	-0.2404	6.24E-06	0.285426
cg0010710	59	0.1088	0.405079	0.000262	0.004447
cg2021725	56	0.093911	0.312996	9.51E-06	0.001906
cg1177032	67	-0.17173	0.044751	1.10E-06	0.786361
cg2625207	51	-0.15638	0.117456	7.80E-05	0.530231
cg0626658	64	-0.18525	-0.33638	4.78E-05	0.118773
cg1927298	52	-0.09178	-0.27155	0.000919	0.039822
cg0512419	54	-0.27407	0.176716	8.07E-05	0.590749
cg1930145	54	0.108051	0.421408	0.000106	0.001559
cg0325338	62	-0.19771	0.228188	6.50E-06	0.268886
cg1347133	50	0.184327	0.229748	8.45E-05	0.300396
cg0171574	54	0.141126	-0.62087	0.000252	0.000784
cg1992870	65	-0.22795	0.721242	1.10E-05	0.003407
cg2547909	63	-0.2161	0.163779	1.09E-05	0.478919
cg0265608	52	-0.16792	0.463044	0.000221	0.032327
cg1212094	75	-0.18525	-0.49787	1.89E-06	0.006738
cg0945109	52	-0.09739	0.241288	1.53E-05	0.023656
cg1907150	61	-0.21339	0.138963	8.23E-06	0.537162
cg0435955	79	-0.36578	1.470251	6.49E-07	2.73E-05
cg2307208	61	0.166445	0.442185	7.20E-06	0.011793
cg0372733	58	-0.15413	0.009825	1.61E-05	0.953452
cg0240242	53	-0.17251	0.339512	1.60E-05	0.072371
cg2215066	52	-0.23016	0.202444	3.81E-05	0.442911
cg1636214	64	-0.26727	0.634652	3.44E-06	0.019578
cg0294185	57	-0.14236	0.492228	2.93E-05	0.002415
cg0213835	57	0.095201	0.096269	7.39E-06	0.335623
cg1207409	66	-0.20584	0.768594	3.21E-06	0.000261
cg0484704	60	-0.1909	-0.09732	2.21E-05	0.64639
cg0976480	52	-0.1788	0.550024	7.83E-05	0.010675
cg1696255	85	-0.28767	0.130781	4.69E-08	0.593641
cg2736597	54	-0.13488	-0.45946	0.000154	0.006859
cg2122438	60	0.145176	0.985977	4.62E-06	2.28E-10
cg1375318	65	-0.1671	0.202997	1.19E-05	0.258919
cg1581744	64	-0.16169	0.254545	1.05E-05	0.141397
cg0214827	51	-0.1527	0.031207	5.28E-05	0.861096
cg0550228	60	-0.13572	0.046189	4.59E-06	0.739932
cg0924748	63	-0.18416	0.280088	4.66E-06	0.13911
cg0794808	81	-0.20928	0.480544	5.45E-08	0.007778
cg1977526	51	-0.1822	-0.38211	5.71E-05	0.074859
cg0435140	57	-0.13657	0.261715	7.89E-05	0.110364
cg0357099	56	0.202523	0.590142	3.85E-05	0.011574

cg1462438	51	-0.17511	0.333494	6.70E-05	0.108978
cg1517296	55	-0.16294	-0.54265	9.43E-06	0.001912
cg0266877	53	0.094032	0.615123	0.00014	3.07E-07
cg1594339	63	-0.24843	-0.3997	5.16E-05	0.168598
cg0332181	66	0.079295	0.164361	2.14E-05	0.062567
cg1666054	64	0.144838	0.807626	1.37E-06	2.72E-08
cg0403385	56	-0.14943	0.009445	2.19E-05	0.954598
cg2187180	55	-0.14563	0.422964	2.61E-05	0.010104
cg0712471	72	-0.19277	0.371949	2.53E-07	0.034012
cg0717786	56	-0.13838	0.161286	1.16E-05	0.278154
cg0066793	62	0.238873	0.855663	0.000157	0.004552
cg0664878	54	-0.22482	0.24995	2.37E-05	0.319355
cg2560530	64	-0.24301	0.56595	1.04E-06	0.015779
cg0529661	61	0.081072	0.673215	4.98E-05	1.14E-11
cg2015110	58	-0.16911	0.090098	8.23E-06	0.613587
cg1571096	69	-0.26152	0.491606	5.33E-06	0.069646
cg2299617	55	-0.15985	0.638682	4.62E-05	0.000659
cg1704151	61	-0.17152	0.444965	2.67E-05	0.021582
cg2467338	61	0.144465	0.325756	8.20E-06	0.033352
cg2663337	57	0.101496	-0.04554	1.31E-05	0.677881
cg0407921	53	0.093182	0.717963	1.78E-05	2.29E-11
cg1728320	69	-0.28871	0.327946	2.41E-05	0.309411
cg1197025	67	-0.29601	0.859036	8.01E-07	0.002447
cg1181408	57	0.110184	0.857829	1.16E-05	5.64E-12
cg1394888	51	-0.14493	0.180502	3.63E-05	0.276248
cg0154931	62	-0.25573	1.037221	2.02E-05	0.000297
cg1923216	53	-0.22428	0.595706	6.89E-05	0.026016
cg0682419	59	-0.18528	-0.24821	3.29E-06	0.185178
cg2067396	50	-0.13528	0.178383	4.08E-05	0.252275
cg0336489	66	-0.08633	0.155396	1.56E-05	0.099671
cg0344830	60	-0.25358	0.318718	8.52E-05	0.296606
cg1131093	71	-0.23333	0.011848	2.09E-06	0.95903
cg2333866	56	0.157134	0.717371	1.10E-05	2.84E-05
cg0942365	54	-0.241	0.552313	2.77E-05	0.042545
cg2258898	78	-0.23675	0.559704	1.73E-08	0.004434
cg1416590	57	0.122216	0.409004	9.00E-06	0.001775
cg2522436	60	-0.14381	-0.28275	2.32E-06	0.048666
cg1009293	54	-0.1142	0.610502	0.000113	1.88E-05
cg1570063	53	-0.18772	-0.26904	2.84E-05	0.20408
cg2208316	56	-0.13321	-0.08213	4.65E-05	0.59487
cg0649680	59	0.118163	0.783257	7.06E-06	1.17E-09
cg0083270	63	-0.22451	-0.4323	2.33E-05	0.084928
cg1188159	77	-0.23844	0.607224	1.55E-07	0.004516
cg0018346	62	-0.14041	0.703691	6.28E-05	3.07E-05
cg1408005	65	0.150447	0.608269	1.95E-06	5.53E-05
cg1866645	70	-0.24532	0.012969	1.17E-06	0.956263
cg0947324	51	-0.14999	0.315286	3.23E-05	0.064947
cg2432602	57	-0.12987	0.936504	6.55E-05	4.31E-09
cg1640945	70	-0.32496	0.267375	6.26E-07	0.381216
cg0899249	58	-0.19222	0.368756	1.82E-06	0.051818
cg0582508	59	-0.16719	0.423678	3.71E-05	0.027538
cg0034441	57	-0.21967	0.823018	1.43E-05	0.00065

cg1834883	67	-0.26122	1.103264	1.67E-05	0.000144
cg0384084	54	-0.13834	0.884184	6.60E-05	1.59E-07
cg1859372	67	-0.19842	0.756707	1.03E-05	0.000421
cg0006815	50	-0.17272	-0.64841	2.24E-05	0.000849
cg2424941	72	0.162006	0.451019	1.58E-06	0.004696
cg2106775	70	-0.1431	0.041472	2.57E-05	0.795863
cg0937753	63	-0.33899	-0.69042	3.57E-06	0.045295
cg1015952	52	-0.21263	0.652776	2.60E-05	0.006585
cg2527042	50	-0.22353	0.009387	4.24E-05	0.970943
cg0851045	59	0.121006	0.501351	5.28E-05	0.000463
cg0734417	54	-0.14803	-0.18896	0.000117	0.298888
cg0010070	70	-0.22805	0.704203	4.74E-08	0.000351
cg0213371	62	-0.20023	0.002558	1.58E-06	0.989563
cg2123931	55	-0.17406	0.308709	1.05E-05	0.097659
cg1932805	66	-0.15185	0.976412	1.66E-05	1.36E-08
cg0924188	65	-0.18701	0.184356	3.38E-06	0.329731
cg2593964	50	-0.18241	0.203891	4.61E-05	0.335068
cg2642382	56	-0.16616	-0.1926	1.18E-05	0.281178
cg2353430	52	-0.12867	0.487157	0.000122	0.002321
cg2595052	73	-0.24047	0.999423	1.27E-06	2.48E-05
cg1832486	55	-0.09015	0.488589	3.59E-05	3.51E-06
cg0597981	52	-1.4935	-1.83046	2.45E-05	0.273274
cg0041407	57	-0.14477	0.446692	3.09E-06	0.002394
cg0450493	71	-0.32326	1.404281	2.19E-06	1.72E-05
cg0464292	58	-0.36512	0.023575	5.85E-05	0.95618
cg2518690	52	0.067979	0.409659	9.37E-05	1.20E-06
cg1169781	52	-1.82193	-2.67166	4.06E-05	0.202998
cg1619638	73	-0.94591	3.943278	3.08E-06	4.69E-05
cg0103749	50	-0.16142	1.564182	8.71E-05	4.03E-14
cg1305452	57	-0.35821	0.475515	8.48E-06	0.209646
cg0498368	58	-0.45797	-0.40754	1.39E-05	0.411772
cg2122072	53	-0.56459	0.547234	2.48E-05	0.386331
cg0373838	62	-0.31918	1.152627	2.98E-06	0.00039
cg1064125	51	-0.13244	0.802096	5.34E-06	1.47E-08
cg1169912	50	-0.45623	0.575906	7.05E-05	0.288803
cg0592897	56	-0.3707	0.192014	0.000135	0.676051
cg2686549	55	-0.20779	0.809165	2.24E-05	0.000545
cg2729842	59	-0.44766	0.577198	1.75E-05	0.240545
cg0159842	52	-0.40782	0.688814	6.02E-05	0.152226
cg0530071	76	-0.47623	0.202271	3.55E-07	0.643595
cg2224727	57	-0.5901	-1.26283	0.000324	0.105463
cg1359604	52	-0.54483	-1.22819	2.48E-05	0.044673
cg0039069	51	-0.30834	1.297571	2.23E-05	0.000191
cg0924980	64	-0.47974	0.962612	5.55E-06	0.053375
cg1014287	56	-0.3203	0.952071	1.65E-05	0.006994
cg0093756	56	-0.35768	0.008436	6.92E-05	0.98415
cg1533445	62	-0.216	-1.02527	6.08E-05	7.41E-05
cg1126658	59	-0.25368	2.725382	1.59E-05	2.14E-19
cg0151390	53	-0.94077	0.006277	0.000183	0.995791

Age 10 Male

CpG	Frequency	Coef$_{\text{asthma}}$	Coef$_{\text{batch}}$	P$_{\text{asthma}}$	P$_{\text{batch}}$

cg2067807	51	-0.18765	0.461832	0.000326	4.10E-10
cg0012309	57	-0.19383	0.708067	9.96E-05	6.64E-21
cg2414319	59	-0.20395	0.949922	3.69E-05	5.71E-32
cg1850140	76	-0.25867	1.083831	2.37E-05	1.08E-28
cg0559972	60	0.154322	0.670415	3.23E-05	2.00E-29
cg0767337	50	-0.22545	0.670569	0.000114	3.66E-15
cg0888510	82	0.229256	1.190168	2.09E-06	4.81E-44
cg1515462	50	-0.28028	0.670571	0.000278	7.37E-10
cg1926903	61	0.252973	0.656774	0.000103	1.94E-12
cg2416563	62	0.197224	0.798737	2.57E-05	3.23E-27
cg2309015	61	-0.2756	0.221754	3.35E-05	0.012932
cg2746915	60	-0.20474	0.277968	3.69E-05	3.98E-05
cg0316537	59	0.208718	0.307291	6.43E-05	1.71E-05
cg0721471	52	0.188421	0.246856	0.000153	0.00027
cg0285081	69	0.191192	0.428921	2.14E-05	1.74E-11
cg1873017	56	0.171581	0.556236	7.40E-05	5.12E-18
cg2322845	63	-0.13886	0.470049	4.37E-05	3.76E-20
cg0197369	69	-0.25818	0.723974	2.34E-05	4.78E-16
cg0130934	51	0.116595	0.771269	0.000296	6.52E-42
cg0655975	55	-0.17221	0.623033	6.59E-05	2.04E-21
cg2281634	51	-0.19303	0.831089	0.000169	3.69E-25
cg0241566	54	-0.21028	0.477803	8.18E-05	2.34E-10
cg0869868	60	-0.21726	0.611987	0.00016	1.79E-13
cg0179839	74	-0.23182	0.212516	1.17E-05	0.002795
cg0194264	57	-0.2203	0.889144	0.000135	1.86E-23
cg0826450	67	0.233655	0.62053	7.49E-05	2.73E-13
cg0297067	58	-0.22712	0.868716	0.000114	4.63E-22
cg0613071	53	0.288241	0.511423	0.000232	2.24E-06
cg1662735	50	-0.26036	0.681517	0.000296	3.01E-11
cg0860312	58	-0.17842	0.823354	0.000188	1.96E-27
cg0247017	60	-0.23702	0.507624	7.97E-05	1.77E-09
cg2611835	56	0.149625	0.486613	0.000108	1.87E-17
cg1182360	53	-0.22102	0.21047	6.70E-05	0.004876
cg0573664	58	-0.28304	0.644769	6.49E-05	1.23E-10
cg1548149	50	0.126522	0.628152	0.000148	5.57E-31
cg1461634	54	0.140911	0.561173	0.000108	1.63E-23
cg1665819	54	-0.24662	0.759261	0.000121	6.98E-16
cg2707266	56	0.172179	0.636432	0.000165	3.04E-20
cg2099715	62	0.149763	0.278472	1.00E-05	3.79E-09
cg1258713	55	0.231561	0.793408	0.000142	2.31E-18
cg0233834	60	-0.2525	0.652151	0.000142	6.10E-12
cg0407708	86	-0.33749	0.666959	7.70E-07	4.34E-12
cg0549030	62	0.154915	0.414077	7.60E-05	2.10E-13
cg0663346	72	-0.29871	0.394863	3.44E-06	6.55E-06
cg1855084	57	-0.34744	0.848742	0.000128	4.50E-11
cg0257143	54	0.191508	0.338594	0.000188	1.74E-06
cg0833299	54	-0.20531	1.280756	0.000195	1.74E-40
cg0140489	59	-0.11192	0.784307	5.33E-05	4.06E-51
cg2314744	59	-0.32608	1.125084	2.49E-05	9.94E-22
cg1437589	57	-0.26326	0.596742	5.50E-05	1.01E-10
cg2031595	69	-0.26057	0.78947	2.14E-05	3.61E-18
cg1070417	55	-0.26596	0.553091	0.00023	4.08E-08

cg1323576	76	0.139558	0.619289	1.08E-05	8.52E-33
cg1422621	57	-0.25744	0.577764	3.46E-05	5.11E-11
cg2723000	50	0.120459	0.475582	0.000216	1.12E-21
cg1100824	58	0.264354	0.518116	0.000102	4.54E-08
cg1251877	50	-0.1128	0.718878	5.90E-05	1.37E-45
cg0532291	57	0.247114	0.577531	0.000145	3.30E-10
cg1638615	54	-0.25622	0.40935	0.000128	8.40E-06
cg0478528	65	0.270564	0.920965	2.74E-05	3.86E-21
cg1861961	54	0.194432	0.388685	8.85E-05	1.95E-08
cg2039060	76	-0.68141	1.048625	4.44E-06	2.75E-07
cg1516720	72	0.15079	0.3303	2.27E-05	5.21E-11
cg1586769	52	-0.20025	-0.02076	0.000655	0.792406
cg0888993	62	-0.20692	0.31594	0.000114	1.71E-05
cg2335038	61	0.191621	0.38629	7.24E-05	1.03E-08
cg1229626	50	0.10858	0.78308	0.000425	6.15E-45
cg1943493	51	-0.19092	0.836564	0.000301	2.48E-24
cg0247569	60	-0.24379	0.832074	0.000106	8.35E-19
cg0284989	54	0.15592	0.814694	8.31E-05	7.99E-35
cg0673644	58	-0.17958	0.720058	0.000161	7.57E-23
cg2311758	59	-0.1961	0.334513	7.65E-05	1.01E-06
cg0790865	52	-0.25048	0.540907	0.000203	1.02E-08
cg0749918	83	-0.60528	0.174437	1.10E-06	0.2879
cg1546224	58	0.214882	1.034706	0.000199	6.33E-29
cg1672572	66	-0.14814	0.81153	1.81E-05	2.50E-41
cg2203485	60	0.185306	1.374938	2.11E-05	1.53E-57
cg1827894	79	-0.26335	0.495366	2.82E-06	2.99E-10
cg0709850	50	-0.29266	1.04226	0.000405	2.26E-17
cg2150798	57	0.151945	0.334277	0.000194	5.14E-09
cg0317350	57	0.325648	0.147951	0.000128	0.193758
cg1538440	60	0.265789	0.388608	5.04E-05	1.48E-05
cg0161307	53	-0.31141	0.845067	0.000103	2.59E-13
cg2089191	50	-0.22507	0.476387	0.000209	2.00E-08
cg1358762	53	-0.20717	0.877775	0.00022	5.92E-24
cg0690686	61	-0.19521	0.719728	3.85E-05	4.53E-23
cg0264884	50	-0.16765	1.178549	0.000233	5.78E-46
cg1037254	54	-0.20851	0.406411	0.00043	7.52E-07
cg0866456	68	0.177158	0.606601	1.65E-05	3.30E-22
cg1177032	63	-0.21247	0.310275	4.10E-05	1.20E-05
cg1160895	73	0.136787	0.516311	1.73E-05	1.99E-25
cg2612636	52	0.133162	0.945915	0.000589	8.20E-43
cg2050310	74	0.086615	0.595344	2.98E-06	6.65E-59
cg0158858	51	0.482781	0.534434	0.000443	0.00413
cg1139302	51	-0.23036	0.637135	0.00032	3.88E-12
cg1129572	64	-0.18546	0.626686	3.24E-05	1.27E-20
cg0343354	58	-0.28357	0.069396	8.16E-05	0.470343
cg0493353	61	-0.22266	0.839618	4.67E-05	1.96E-23
cg2501970	51	-0.27207	0.457061	0.000164	4.28E-06
cg1836811	77	-0.27718	0.601077	3.87E-06	1.77E-12
cg1497824	63	-0.24455	0.186525	4.73E-05	0.020805
cg2371669	55	-0.20163	0.405807	0.000129	3.28E-08
cg0546181	51	0.229036	0.430434	4.68E-05	3.74E-08
cg0090215	51	0.297491	0.355584	0.000149	0.000839

cg1322291	53	0.132331	0.36081	0.000226	2.39E-12
cg1636214	65	-0.26805	0.96326	5.91E-05	2.04E-21
cg1461296	74	-0.29896	0.751029	2.90E-05	2.56E-13
cg2070414	50	1.457525	-1.27566	0.000427	0.022386
cg2359808	56	0.149893	0.432258	5.78E-05	1.88E-15
cg2484281	52	0.180557	0.85792	0.000327	5.09E-27
cg1634736	58	-0.2239	0.393096	9.39E-06	2.03E-08
cg1944829	66	-0.28666	0.290127	2.14E-05	0.001426
cg0985016	56	-0.27027	0.461183	0.000146	2.59E-06
cg1824508	56	0.185812	0.349222	4.34E-05	3.28E-08
cg2104982	51	-0.18981	0.376131	0.000334	3.12E-07
cg1448011	70	0.213061	0.418943	3.14E-05	4.65E-09
cg1342909	71	-0.18668	0.440854	1.88E-05	1.45E-12
cg0699625	50	-0.20482	0.843166	0.000254	1.19E-22
cg2107321	60	-0.24533	0.580868	0.000137	1.78E-10
cg0773247	54	-0.1461	0.750455	6.57E-05	1.01E-34
cg1659981	75	-0.24148	0.792295	8.84E-06	6.79E-22
cg0712471	66	-0.2514	0.539562	1.70E-05	6.21E-11
cg0058267	58	-0.22153	0.729688	5.53E-05	5.93E-19
cg1353173	56	-0.28201	0.628271	0.000232	5.19E-09
cg0234673	60	0.203514	0.386712	0.000206	3.68E-07
cg0869858	72	-0.29186	0.604311	2.61E-06	5.90E-12
cg1705661	74	0.194429	0.42342	1.69E-05	3.42E-11
cg1081615	56	0.148621	1.049109	0.000134	3.00E-48
cg0208683	53	0.165446	0.404417	0.0002	1.42E-10
cg2354845	61	0.162963	1.009398	7.88E-05	3.61E-43
cg1201288	62	-0.15479	0.810328	5.69E-05	5.55E-36
cg1803653	53	-0.19791	0.824739	8.06E-05	9.59E-26
cg2302963	54	0.124891	0.979	0.000188	8.34E-53
cg0317878	62	0.188835	1.070563	4.47E-05	1.81E-40
cg2436013	66	0.247892	0.297099	2.14E-05	0.000173
cg0407921	65	0.147319	0.446082	3.23E-05	1.58E-17
cg1432753	53	0.167956	0.13982	0.000238	0.023218
cg2766062	66	0.170457	0.54941	1.95E-05	4.61E-20
cg0756504	52	-0.23003	0.559937	0.000298	4.82E-10
cg1489726	52	0.15645	0.68033	0.000229	9.27E-25
cg0390950	56	-0.11873	0.481114	7.12E-05	5.01E-25
cg2086678	75	-0.22045	0.286216	7.62E-06	1.94E-05
cg2410850	71	-0.29127	0.449717	8.40E-06	5.54E-07
cg1131093	56	-0.2658	0.905892	0.000248	3.50E-17
cg1514656	50	-0.13974	0.819318	0.000234	6.87E-37
cg1899124	69	-0.18595	0.432157	6.89E-06	2.71E-13
cg2420050	63	-0.2254	0.697	3.03E-05	3.47E-18
cg1460940	51	0.15448	0.480115	0.000523	1.06E-13
cg0969478	67	0.247789	0.131366	8.06E-06	0.075672
cg2491382	59	-0.28236	0.339871	5.46E-05	0.000345
cg1877893	51	-0.25828	0.521013	0.000205	7.64E-08
cg1538458	57	-0.18469	0.375914	5.53E-05	4.50E-09
cg2294157	58	0.190114	0.365514	0.000147	1.49E-07
cg1779031	51	-0.21855	0.429231	0.000406	5.63E-07
cg0502230	53	-0.25865	0.485095	5.33E-05	4.96E-08
cg2038249	62	0.217485	0.36193	0.000235	8.65E-06

cg2077631	59	0.15592	0.913199	0.000118	4.41E-39
cg0048321	54	0.140457	0.442065	0.000195	1.23E-15
cg0296942	74	0.185271	0.44685	8.08E-06	6.69E-14
cg1778492	64	-0.22278	0.697561	3.22E-05	1.94E-18
cg0176125	52	0.244016	0.611974	3.75E-05	5.53E-13
cg2031743	58	-0.25565	0.480351	5.99E-05	5.77E-08
cg2116344	80	0.125066	0.432259	6.41E-07	5.94E-28
cg1846080	52	-0.24219	0.822893	0.000302	9.73E-17
cg0326975	57	-0.29607	0.638636	9.04E-05	1.83E-09
cg0966896	51	0.129336	0.41135	0.000285	3.05E-15
cg0401199	50	0.137344	0.564152	0.000195	3.34E-23
cg2530587	60	0.183699	0.484237	4.82E-05	1.07E-13
cg1461125	56	-0.23525	0.411204	0.000269	3.90E-06
cg0515760	52	0.17939	0.33272	0.000535	3.38E-06
cg0914784	83	-0.28995	0.532878	2.50E-06	5.65E-10
cg0890752	50	0.177221	0.669825	0.000332	2.02E-19
cg2748867	54	-0.32708	0.651314	0.000223	1.28E-07
cg0104383	62	-0.25719	0.669757	3.06E-05	5.26E-14
cg1744293	52	0.199861	0.134699	0.000703	0.090129
cg0937753	72	-0.43638	0.648015	2.64E-05	5.22E-06
cg1015952	69	-0.26067	0.867296	5.53E-05	3.08E-19
cg1258078	69	0.174382	0.403045	9.14E-06	6.79E-13
cg1563360	66	-0.29015	0.594653	3.23E-05	1.24E-09
cg0224553	51	-0.16343	0.774617	0.000214	4.15E-28
cg0213795	51	0.130041	0.822019	0.000121	8.79E-43
cg0924188	76	-0.23511	0.966154	7.52E-06	2.38E-30
cg1448053	51	-0.20772	0.762743	0.000222	1.95E-19
cg2393328	57	-0.21989	0.553091	0.00011	9.04E-12
cg2520362	50	-0.27799	0.706757	9.68E-05	4.06E-12
cg0535230	59	-0.67397	0.232259	5.39E-05	0.297289
cg2459732	51	-0.41873	0.60874	0.000203	7.81E-05
cg2248408	68	-0.21266	0.458611	6.09E-06	5.85E-12
cg0604411	51	-1.64688	0.639206	0.000345	0.300533
cg2391009	62	-0.44682	1.169815	0.000104	1.39E-12
cg0849326	53	0.255061	1.21237	0.00062	3.33E-25
cg2024221	52	-2.31846	2.8222	2.56E-05	0.00016
cg0640791	55	0.396893	1.032811	0.000266	2.77E-11
cg1597640	50	-0.12965	0.789244	7.91E-05	2.85E-42
cg0109738	50	-0.64914	1.21501	0.000175	4.04E-07
cg0704168	68	0.174259	0.284331	4.14E-05	1.18E-06
cg1453275	53	0.163151	0.899649	0.000436	4.36E-32
cg1372589	55	0.343886	0.425999	0.000145	0.000533
cg1426307	62	0.163841	0.345016	8.14E-05	3.25E-09
cg1611054	61	0.131487	0.244886	0.000271	9.66E-07
cg0484214	74	-1.10208	0.678813	4.93E-06	0.034774
cg1878073	54	-0.32714	-0.39714	1.21E-05	9.01E-05
cg1364418	50	-0.23131	0.593671	8.20E-05	1.69E-12
cg1221368	61	-0.32634	0.529893	6.66E-05	2.51E-06
cg0591867	51	-0.50984	0.431526	7.16E-05	0.012506
cg2668004	51	-0.92304	0.01147	6.36E-05	0.970278
cg0971780	66	-0.41527	0.744399	6.98E-05	2.62E-07
cg1050525	50	0.23443	0.211816	0.00017	0.011802

cg2745648	56	0.159494	0.390927	0.000142	5.24E-11
cg0402391	59	-0.78528	0.173057	0.000339	0.555656
cg1779601	50	-0.18878	-0.33036	0.000292	4.30E-06
cg2617219	50	0.327485	0.189517	0.000191	0.107796
cg0119017	63	-0.2636	-0.50811	7.89E-05	4.55E-08
cg2457694	57	-0.76514	0.992501	0.000132	0.000269
cg1639543	55	0.196148	0.210035	0.00021	0.003371
cg0518401	52	-0.43239	0.9443	0.000142	3.28E-09
cg2653374	52	-2.12077	2.415689	8.72E-05	0.000974
cg2336397	55	-1.28684	2.467133	6.27E-05	3.52E-08
cg2259546	58	0.193562	0.378363	0.000131	7.70E-08
cg1244619	64	-0.28602	0.631866	0.000113	1.33E-09

Age 18 Male

CpG	Frequency	Coef$_{asthma}$	Coef$_{batch}$	Coef$_{batch2}$	P$_{asthma}$	P$_{batch}$	P$_{batch2}$
cg1615338	50	-0.07197	0.478624	-0.14961	3.26E-05	1.54E-19	5.38E-05
cg2161261	60	0.239181	0.702384	0.949393	3.22E-05	1.49E-05	1.89E-13
cg1948340	58	0.323488	0.60037	0.803372	3.58E-05	0.005817	1.93E-06
cg2505333	74	-0.19584	0.702493	0.087003	1.88E-06	2.28E-09	0.309714
cg1771429	57	0.205835	0.895016	0.70389	1.54E-05	8.81E-11	2.35E-11
cg0505112	74	-0.25315	0.366505	-0.12287	6.97E-06	0.018534	0.296829
cg2670962	55	-0.10468	0.225737	-0.35932	9.35E-06	0.000605	6.75E-12
cg2484208	50	-0.10577	0.562642	-0.15937	2.33E-05	2.89E-14	0.002612
cg0703107	57	-0.19051	0.798768	0.293259	2.77E-05	1.11E-09	0.002332
cg0677615	50	-0.25424	0.430996	0.140687	7.75E-05	0.015961	0.298394
cg2565162	70	-0.14868	1.14854	1.174768	3.68E-05	3.61E-24	1.52E-36
cg2038329	68	-0.17247	0.556059	-0.10441	7.35E-07	1.88E-08	0.149993
cg2121311	53	-0.29815	0.398007	-0.15717	4.67E-05	0.049744	0.306829
cg0899438	56	-0.22659	0.172274	0.067399	2.32E-05	0.243302	0.548049
cg0900428	52	-0.15026	0.644508	-0.10447	6.50E-05	2.82E-09	0.186888
cg0210970	56	-0.18249	-0.01503	0.004591	2.44E-05	0.899535	0.959569
cg2331896	50	0.133268	0.833523	0.490539	0.000285	2.52E-14	1.38E-09
cg1100108	50	-0.20758	0.944184	0.753469	2.34E-05	3.68E-11	5.01E-12
cg0338844	66	0.15472	0.474721	-0.05337	3.10E-06	4.17E-07	0.440484
cg1395543	59	-0.11256	0.297356	-0.36745	0.000144	0.00035	1.44E-08
cg2695089	54	-0.1092	0.463848	-1.33091	9.10E-06	7.55E-11	1.86E-66
cg1573305	50	-0.08623	0.009245	-0.67315	6.09E-05	0.87619	1.50E-34
cg0837528	53	-0.17187	1.528248	0.289276	1.47E-05	9.31E-32	0.00059
cg2717809	64	0.208441	0.690088	0.684973	4.79E-07	4.73E-09	1.02E-13
cg0649228	58	-0.10069	0.614196	0.10212	8.99E-06	4.82E-19	0.032123
cg2412035	50	0.243317	-0.02097	0.240943	9.63E-05	0.903161	0.067232
cg2022336	55	-0.16341	0.858364	0.345928	4.22E-05	2.89E-13	5.11E-05
cg0716123	67	-0.11059	0.069265	0.097372	4.13E-05	0.352039	0.086325
cg1538011	51	-0.23653	0.672961	0.484887	0.000209	0.000179	0.000378
cg2493672	60	-0.20884	0.454615	-0.11055	0.00011	0.002577	0.33053
cg0913300	51	-1.14059	0.556479	-0.75152	0.000126	0.498746	0.230432
cg1740369	53	0.178683	0.395707	0.481965	0.000264	0.003868	5.27E-06
cg0605651	50	0.262801	1.202148	1.389007	7.79E-05	4.47E-10	2.80E-19
cg0115951	58	-0.65531	0.866574	-0.34164	0.000133	0.068723	0.34416
cg2260622	53	-0.36812	2.508848	1.646287	8.44E-05	1.39E-18	8.54E-15
cg0229567	57	-0.6809	0.347333	1.36918	0.0001	0.472818	0.000252
cg1630198	52	-0.16553	0.842428	0.108884	0.000275	2.05E-10	0.256778

cg0485521	73	-0.68189	0.083662	-0.32633	4.96E-06	0.838128	0.295584
cg0353021	57	-0.33559	0.615966	-1.54751	7.91E-05	0.009251	1.29E-15
cg2590218	52	-0.85622	0.05983	0.332779	5.87E-05	0.918937	0.457184