

DR ALBERTA L WANG (Orcid ID : 0000-0002-2151-4823)

DR JUAN CARLOS CELEDON (Orcid ID : 0000-0002-6139-5320)

Article type : Original Article-Asthma and Rhinitis

DNA methylation is associated with inhaled corticosteroid response in persistent childhood asthmatics

Alberta L. Wang, MD^{a,b}, Olena Gruzieva, MD, PhD^c, Weiliang Qiu, PhD^a, Simon Kebede Merid, MSc^c, Juan C. Celedón, MD, DrPH^d, Benjamin A. Raby, MD^{a,e,f}, Cilla Söderhäll, PhD^{g,h}, Dawn L. DeMeo, MD^{a,e}, Scott T. Weiss, MD^a, Erik Melén, MD, PhD^{c,i}, Kelan G. Tantisira, MD^{a,e}

Running head: Association between DNA methylation and inhaled corticosteroid response in pediatric asthmatics

^aChanning Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

^bDivision of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

^cInstitute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; and Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden

^dDivision of Pediatric Pulmonary Medicine, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

^eDivision of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

^fDivision of Pulmonary Medicine, Boston Children's Hospital, Harvard Medical School, Boston MA

^gDepartment of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden,

^hDepartment of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden;

ⁱSachs' Children's Hospital, Stockholm, Sweden

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.13447

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Corresponding author: Kelan G. Tantisira, Channing Division of Network Medicine, Brigham and Women's Hospital, 181 Longwood Avenue, Boston, MA 02115; phone: 617-525-0863, e-mail: rekgt@channing.harvard.edu

Funding: NIH T32 AI007306, T32 HL007427, RC2 HL101543, R01 HL127332, R01 HL129935, U01 HL65899, P01 HL132825. BAMSE: The Swedish Heart-Lung Foundation, The Swedish Research Council, Stockholm County Council (ALF), MeDALL (Mechanisms of the Development of ALLergy; European Union grant agreement No. 261357) and the Swedish foundation for strategic research (SSF) (RBc08-0027).

ABSTRACT

BACKGROUND: Response to inhaled corticosteroids is highly variable, and the association between DNA methylation and treatment response is not known.

OBJECTIVE: To examine the association between peripheral blood DNA methylation and inhaled corticosteroid response in children with persistent asthma.

METHODS: Epigenome-wide DNA methylation was analyzed in individuals on inhaled corticosteroids in three independent and ethnically diverse cohorts--Childhood Asthma Management Program (CAMP); Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE); and Genetic Epidemiology of Asthma in Costa Rica Study (GACRS). Treatment response was evaluated using two definitions, the absence of emergency department visits and/or hospitalizations and the absence oral corticosteroid use while on inhaled corticosteroid therapy. CpG sites meeting nominal significance ($P < 0.05$) for each outcome were combined in a three-cohort meta-analysis with adjustment for multiple testing. DNA methylation was correlated with gene expression using Pearson and partial correlations.

RESULTS: In 154 subjects from CAMP, 72 from BAMSE, and 168 from GACRS, relative hypomethylation of cg00066816 (171 bases upstream of *IL12B*) was associated with the absence of emergency department visits and/or hospitalizations ($Q = 0.03$) in all cohorts and lower *IL12B* expression ($\rho = 0.34$, $P = 0.01$) in BAMSE. Relative hypermethylation of cg04256470 (688 bases upstream of *CORT*) was associated with the absence of oral corticosteroid use ($Q = 0.04$) in all cohorts and higher *CORT* expression ($\rho = 0.20$, $P = 0.045$) in CAMP.

CONCLUSION & CLINICAL RELEVANCE: Differential DNA methylation of *IL12B* and *CORT* are associated with inhaled corticosteroid treatment response in persistent childhood asthmatics. Pharmaco-methylation can identify novel markers of treatment sensitivity in asthma.

Clinical trial registration: Childhood Asthma Management Program (CAMP) Phases I (Trial), II (CAMPCS), III (CAMPCS/2), and IV (CAMPCS/3) NCT00000575

ABBREVIATIONS

Inhaled corticosteroid (ICS)

Oral corticosteroid (OCS)

Childhood Asthma Management Program (CAMP)

Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE)

Genetic Epidemiology of Asthma in Costa Rica Study (GACRS)

Institutional review boards (IRBs)

Emergency department (ED)

False discovery rate (FDR)

Interleukin (IL)

T helper (T_H)

INTRODUCTION

Asthma continues to be the leading cause of emergency room visits, hospitalizations, and school absenteeism in children[1]. Inhaled corticosteroids (ICS) are the most effective long-term medication for asthma, yet up to one-third of patients have a poor response to treatment[2]. Several genes have been associated with ICS treatment response, but none so far have been translated into reliable prognostic tools or therapeutic targets[3, 4].

Epigenetics can play a role in drug sensitivity and resistance[5-10]. DNA methylation is an epigenetic mechanism that regulates both the activation and silencing of DNA transcription. Although DNA methylation has been implicated as a modifier of both asthma susceptibility and severity, the role of

DNA methylation as a modifier of ICS treatment response in asthma has not been studied[11-13]. *In vitro* studies have demonstrated that site-specific CpG methylation regulates dexamethasone sensitivity and resistance in human endothelial cells, supporting a role for DNA methylation in ICS pharmacogenetics[14]. A small pilot study examined DNA methylation patterns in 33 pediatric asthmatics during asthma exacerbations and detected an association between methylation of the *OTX2* promoter in nasal epithelial cells and oral corticosteroid (OCS) response[15]. However, this association was not significant after adjustment for multiple testing, and the DNA methylation mark was not associated with *OTX2* expression[15]. Given the known complexity of ICS responsiveness in asthma, studies of substantially large sample size are needed to confidently explore a pharmacogenetic hypothesis.

To address this, we now explore the relationship between DNA methylation and ICS response in children with persistent asthma. DNA methylation was studied in three independent and ethnically diverse cohorts--the Childhood Asthma Management Program (CAMP); the Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE); and the Genetic Epidemiology of Asthma in Costa Rica Study (GACRS). We further characterized the potential functional role of DNA methylation by correlation with gene expression.

METHODS

Study populations

Epigenome-wide DNA methylation analysis was performed in three independent cohorts--CAMP, BAMSE, and GACRS. A total of 394 subjects were included, 154 from CAMP, 72 from BAMSE, and 168 from GACRS. CAMP was a four-year randomized, placebo-controlled trial of inhaled treatments for mild-to-moderate persistent childhood asthma in North America[16]. In CAMP, asthma was defined as having at least one of the following findings for at least 6 months in the prior year: 1)

asthma symptoms at least two times per week, 2) at least two usages per week of an inhaled bronchodilator, 3) daily asthma medication. Written informed consent was provided by all parents, with subject assent, and the institutional review boards (IRBs) of the Brigham and Women's Hospital and each of the CAMP study centers approved the study (1999P0015492). BAMSE was a prospective, population-based cohort study on the risk factors for childhood asthma and allergic diseases in Stockholm, Sweden[17]. In BAMSE, asthma was defined as having a positive history to two of the following three criteria: 1) doctor's diagnosis of asthma, 2) asthma medication use in the past 12 months, 3) asthma symptoms (i.e., wheezing, chest tightness, dyspnea) in the past 12 months. BAMSE was approved by the Regional Ethical Review Board of the Karolinska Institutet (dnr 02-420) and written informed consent was obtained from the parents of all participating children. The GACRS was a cross-sectional study focused on an isolated Hispanic population with high asthma prevalence in the Central Valley of Costa Rica[18]. In GACRS, asthma was defined as having a history of physician-diagnosed asthma and at least two respiratory symptoms or a history of asthma attacks in the previous year. Written parental consent and written subject assent was obtained for all GACRS participants. This study was approved by the IRBs of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (2000P001130). Details on the study populations can be found in the online data supplement.

Inhaled corticosteroid response

The primary outcome was the absence of severe exacerbations while on ICS treatment, as defined by the absence of asthma-related emergency department (ED) visits and/or hospitalizations in the prior year. This outcome was measured in all three cohorts. The secondary outcome was the absence of asthma-related OCS use in the prior year. The secondary outcome was measured in the CAMP and GACRS cohorts but not in the BAMSE cohort.

DNA methylation

Peripheral blood DNA methylation analysis was performed using the Infinium HumanMethylation27 BeadChip assay (Illumina, San Diego, CA) in CAMP and the Infinium HumanMethylation450 BeadChip assay (Illumina, San Diego, CA) in BAMSE and GACRS. Standard quality control processing was performed in each cohort and described in the online data supplement. R software (version 3.4.0) was used for data analyses. In CAMP, the percentages of white blood cell counts measured from whole blood were used for adjustment of white blood cell composition in subsequent statistical models. The reference-based Houseman method was used to estimate the composition of white blood cells in BAMSE and GACRS[19].

Gene expression

The association between DNA methylation and gene expression was investigated for the statistically significant CpG sites with the same direction of effect in the CAMP and BAMSE cohorts. Gene expression data from CAMP and BAMSE are described in prior publications and in the online data supplement[20, 21]. Only subjects with both DNA methylation and gene expression data were analyzed. In CAMP, 109 subjects had both DNA methylation and gene expression data, and in BAMSE, 58 subjects had both DNA methylation and gene expression data.

Statistical analyses

For each outcome, multivariable linear regression models were first generated in the CAMP DNA methylation dataset using the iCheck package (**Figure 1**)[22]. CAMP was used as the discovery cohort because it was the most phenotypically well characterized and had both ICS response outcomes. Next, the BAMSE and GACRS datasets were restricted to the CpG sites that met nominal significance in CAMP ($P < 0.05$) for each outcome and multivariable linear regression modeling was performed. A sequential analysis was performed because CAMP by design interrogated less CpG sites

than BAMSE or GACRS. Prior to model building, DNA methylation beta values were converted to M values to correct for heteroscedasticity[23]. The models are described in detail in the online data supplement. The CpG sites that met nominal significance in each cohort were combined in a meta-analysis using Fisher's method for each respective outcome, and correction for multiple testing was performed by controlling the false discovery rate (FDR)[24].

For the statistically significant CpG sites on meta-analyses, normalized DNA methylation M values were correlated with $\log_2$ transformed and normalized gene expression using Pearson correlation. In addition, partial correlations between gene expression and DNA methylation controlling for the respective ICS treatment response outcome were performed. The partial correlation models are described in the online data supplement.

To determine if the effect of differential methylation on asthma exacerbation was specific to subjects on ICS treatment, we performed multivariable linear regression interaction modeling. Interaction models were generated for only the CpG sites that correlated with gene expression. These models were generated in CAMP because unlike BAMSE and GACRS, CAMP has DNA methylation data for subjects on ICS and placebo treatment (**Supplemental table 1**), and the primary interest was the interaction between treatment group and the respective asthma exacerbation outcome. Each model controlled for age, sex, batch effect, treatment group, and white blood cell count.

RESULTS

The mean age was 9.8 ($\pm$ 2.0) years in CAMP, 8.3 ($\pm$ 0.4) years in BAMSE, and 9.2 ($\pm$ 2.0) years in GACRS (**Table 1**). All three cohorts were predominantly male, consistent with other childhood asthma cohorts. In CAMP, BAMSE, and GACRS, 89.0%, 59.7%, and 23.8% of the respective subjects did not have ED visits and/or hospitalizations in the prior year while on ICS therapy. In

CAMP and GACRS, 54.4% and 35.7% of the respective subjects did not have OCS use in the prior year while on ICS therapy.

Absence of severe exacerbations on ICS treatment

For the primary outcome of the absence of severe exacerbations, 1236 CpG sites in CAMP, 1167 CpG sites in BAMSE, and 71 CpG sites in GACRS were differentially methylated (nominal $P < 0.05$). On meta-analysis of the three cohorts, 42 CpG sites had the same direction of effect (**Supplemental table 2**), and one CpG site was significant after FDR-adjustment (**Table 2**). Relative hypomethylation of cg00066816 was associated with the absence of ED visits and/or hospitalizations in the past year (standardized coefficient in CAMP -3.101, BAMSE -2.953, and GACRS -2.310; $Q = 0.03$) (**Table 2, Supplemental figure 1**). Interaction analysis demonstrated that the association between hypomethylation of cg00066816 and the absence of severe exacerbations was specific to the subjects on ICS (standardized coefficient -3.051, $P = 0.002$) (**Figure 2A**).

Absence of OCS use on ICS treatment

For the secondary outcome of the absence of OCS use, 1685 CpG sites in CAMP and 133 CpG sites in GACRS were differentially methylated (nominal $P < 0.05$). The BAMSE cohort did not have data on OCS use. On meta-analysis of the CAMP and GACRS cohorts, 62 CpG sites had the same direction of effect (**Supplemental table 3**), and 13 CpG sites were significant after FDR-adjustment (**Table 2, Supplemental figure 2**). Relative hypermethylation of cg04256470 was associated with the absence of OCS use while on ICS (standardized coefficient in CAMP 3.620 and GACRS 2.329; $Q = 0.04$), and this effect was specific to the subjects on ICS treatment on interaction analysis (standardized coefficient 2.322, $P = 0.02$) (**Figure 2B**).

Functional annotation of the identified CpG sites

The CpG site associated with severe exacerbations (cg00066816) maps to two genes, the interleukin 12B gene (*IL12B*) and the long non-coding RNA *LOC285626* on chromosome five. Cg00066816 is located 171 bases upstream of the *IL12B* transcription start site. Relative hypomethylation of cg00066816 was associated with lower *IL12B* expression in BAMSE ($\rho = 0.34$, $P = 0.01$) (**Figure 3A**). No association between cg00066816 methylation and *IL12B* expression was detected in CAMP.

Of the 13 CpG sites significantly associated with the absence of OCS use while on ICS treatment, only one CpG site (cg04256470) was associated with gene expression. Cg04256470 was positively correlated with *CORT* expression ($\rho = 0.20$, $P = 0.045$) in CAMP (**Figure 3B**). Only a small number of subjects in CAMP had gene expression cell type composition data available. Therefore, after adjustment for cell type composition, the partial correlation analysis was underpowered and not statistically significant (**Supplemental figure 3**). Cg04256470 methylation was not associated with gene expression in BAMSE. Cg04256470 is located 688 bases upstream of the *CENPS-CORT* transcription start site on chromosome one. The *CENPS-CORT* locus has read-through transcription of the centromere protein S (*CENPS*) gene and cortistatin (*CORT*) gene, and alternative splicing produces transcript variants of the genes.

DISCUSSION

We found DNA methylation patterns to be associated with response to ICS treatment in three independent and ethnically diverse cohorts of children with mild-to-moderate persistent asthma. Response to ICS was investigated using two definitions, the absence of severe exacerbations as measured by the absence of asthma-related ED visits and/or hospitalizations and the absence of OCS use for asthma while on ICS treatment. Hypomethylation of cg00066816 (*IL12B*) and hypermethylation of cg04256470 (*CORT*) were associated with response to ICS using the severe

exacerbation and OCS use definitions, respectively. In addition, hypomethylation of cg00066816 was associated with lower *IL12B* expression, and hypermethylation of cg04256470 was associated with higher *CORT* expression. *IL12B* is involved in asthma pathogenesis and *CORT* regulates endogenous corticosteroids[25, 26]. Our results provide novel evidence on the association of pharmacomethylation with ICS treatment resistance and response. The pharmacoeypigenetic aspect of these associations is further highlighted by the interaction analyses demonstrating differential DNA methylation in the ICS but not the placebo treatment groups (**Figure 2**).

Hypomethylation of *IL12B* was associated with the absence of severe exacerbations and decreased *IL12B* expression among children with asthma on ICS. *IL12B* encodes the common beta subunit of interleukin (IL) 12 and IL-23. Depletion of IL-12 attenuates T helper (T_H) 1 immune response and enhances T_H2 antigen-induced airway hyperresponsiveness and pulmonary eosinophilia[27, 28]. In bronchial biopsies, asthmatics sensitive to prednisone have upregulation of IL-12 and downregulation of IL-13 expression, whereas prednisone-resistant asthmatics have no change in expression of either cytokine[29]. These findings in lung tissue contrast with our findings in peripheral blood, and this difference may be due to the effects of different steroid concentrations in the lung versus the periphery after different routes of glucocorticoid exposure (oral versus inhaled), in addition to baseline differences in tissue specific gene expression. IL-23 regulates T_H17 differentiation, and stimulation with IL-23 *in vitro* protects human lung fibroblasts and endothelial cells from dexamethasone induced apoptosis[30]. IL-23 also weakens dexamethasone-induced inhibition of peripheral blood mononuclear cell proliferation[31]. Therefore, IL-23 may have a role in corticosteroid resistance, and the reduction of IL-23 expression via hypomethylation of the *IL12B* subunit may be a mechanism for corticosteroid sensitivity.

Hypermethylation of *CORT* was associated with the absence of OCS use while on ICS and increased *CORT* expression. *CORT* encodes the neuropeptide cortistatin, which has high structural homology to somatostatin[25, 26]. Cortistatin regulates the hypothalamic-pituitary-adrenal axis and exerts anti-inflammatory actions[25, 26]. In the human immune system, cortistatin production is upregulated in activated monocytes, macrophages, and dendritic cells, along with expression of its receptor *sst₂*, suggesting an autocrine regulatory pathway[32]. Cortistatin is also expressed by peripheral neurons, which in turn may have a paracrine role in regulating immune cells[32]. In a mouse model of allogeneic skin transplantation, cortistatin treatment increased the numbers of regulatory T cells and prolonged survival of transplanted skin grafts[33]. In experimental models of sepsis, cortistatin inhibited the release of local and systemic inflammatory cytokines (*TNF α* , *IL-6*, *IL-1 β* , *IFN γ* , *IL-12*) and chemokines (*MIP-2*, *RANTES*), increased production of anti-inflammatory *IL-10*, and protected against mortality in endotoxemic mice[34]. Finally, cortistatin deficient mice are in a systemically immunosuppressed state with elevated basal levels of the glucocorticoid corticosterone and increased susceptibility to infection[35]. Our data suggest that regulation of cortistatin by DNA methylation may modulate ICS sensitivity in childhood asthmatics.

In nasal epithelial cells, hypomethylation of *OTX2* has been nominally associated with response to OCS in pediatric asthmatics during exacerbations, and interestingly, in the GACRS cohort, relative hypomethylation of *OTX2* in peripheral blood was nominally associated the absence of OCS use while on ICS therapy (standardized coefficient -2.120, $P = 0.04$)[15]. Hence, differential methylation of *OTX2* is a potential marker of both OCS and ICS response, and larger studies with tissue specific analyses are needed to confirm this association. In a prior study, differential methylation of the glucocorticoid receptor gene (*NR3C1*) was associated with dexamethasone sensitivity in endothelial cells, but we did not find an association of *NR3C1* methylation with ICS response in peripheral blood[14]. In addition, differential methylation of genetic loci previously associated with asthma exacerbations on ICS treatment (*ST13*, *FCER2*, *P2RX7*, *CMTR1*) were not detected[36]. This absence of association may be due to phenotypic heterogeneity of the measured outcomes or a lack of

methylation quantitative trait association at these genetic loci. Further research is needed to determine the association between genetic variation and quantitative changes in DNA methylation in asthma pharmacogenomics.

In this study, we demonstrate the first association between DNA methylation and response to ICS treatment in asthma across three independent populations of different ethnicities. Pediatric cohorts with drug treatment response outcomes and DNA methylation are few and with small sample sizes. The discovery of CpG sites in this study was limited by the small cohort sizes, but we were adequately powered to identify novel CpG sites by meta-analyzing our results across cohorts. The variation in the rate of ED visits and/or hospitalizations and OCS use across the cohorts may reflect health care system differences and/or compliance, which are factors that may have influenced our results. Nonetheless, BAMSE and GACRS have successfully been used as replication cohorts for CAMP[37, 38]. CAMP and BAMSE are white cohorts whereas GACRS is a Hispanic cohort. Racial differences in DNA methylation may explain the low numbers of replicated CpG sites in GACRS[39]. However, the inclusion of replication in GACRS increases the global generalizability of our results. The number of CpG sites investigated was limited by the use of the Illumina 27k methylation array in CAMP, which is enriched at CpG island promoters, and replication was performed in the corresponding subset of CpGs from BAMSE and GACRS. Therefore, CpGs involved in ICS response located outside of CpG island promoters may have been missed, and additional DNA methylation marks would likely have been found had more CpG sites been examined using newer technologies. Future studies interrogating a larger number of genome-wide CpGs are needed to identify the full extent of how DNA methylation is associated with ICS response.

DNA methylation and gene expression are tissue specific, and peripheral blood DNA methylation and gene expression was examined because it is non-invasive and asthma inflammation and ICS absorption are detected systemically. Cell type deconvolution was performed to reduce false-positive

results due to cell type heterogeneity in whole blood[40]. Each identified CpG site was located far upstream of the respective gene, suggesting that methylation may be acting on an upstream gene modifier or that collaborating epigenetic modifications, such as histone modifications, may be contributing to the regulation of gene expression of both *IL12B* and *CORT*[41-46]. The statistically significant CpG sites each mapped to more than one gene, but there is little known about these additional genes (*LOC285626* and *CENPS*), including no known association with asthma. Gene expression data were collected after DNA methylation data in both CAMP and BAMSE, which may explain why the associations between DNA methylation and gene expression were not replicated across both cohorts. Lastly, as with most pharmacogenomic studies, we were not able to determine if DNA methylation preceded or resulted from ICS treatment. There is evidence that high-dose systemic glucocorticoids can induce DNA methylation changes in the buccal mucosal and peripheral blood, but it is not known if ICS exposure can mediate systemic changes in DNA methylation[47, 48].

Nonetheless, our results demonstrate that DNA methylation is a marker of treatment response in subjects on ICS. Future longitudinal studies are needed to understand the temporality and underlying mechanisms linking DNA methylation and ICS response.

There is an urgent need to understand the biologic basis for ICS response and resistance in order to develop new biomarkers and therapies for ICS resistant asthmatics. We identified DNA methylation marks in *IL12B* and *CORT* to be consistently associated with ICS response in persistent childhood asthmatics across three independent cohorts. Pharmaco-methylation is a novel approach in asthma research with promising applications across medicine. Our findings demonstrate its potential to identify treatment sensitivity in asthmatics. Epigenetic therapeutics that selectively target DNA methylation have been shown to increase the effectiveness of existing treatments in the field of cancer biology, and these therapeutics may have applications in other fields and diseases, including asthma[7, 8, 10]. Further research into the identified DNA methylation marks may lead to the discovery of epigenetically-mediated pathways of drug resistance and novel epigenetic therapeutics to increase sensitivity to ICS treatment.

DATA AVAILABILITY STATEMENT

Phenotypes for the CAMP cohort are available through the EVE Asthma Genetics Consortium (dbGaP Study Accession: phs001156.v2.p1). In addition, phenotype and DNA methylation data for CAMP and GACRS are available through the NHLBI Trans-Omics for Precision Medicine (TOPMed)[49]. Gene expression data for CAMP is available through the Asthma BioRepository for Integrative Genomic Exploration (Asthma BRIDGE)[20]. DNA methylation data for BAMSE are available through the European Genome-phenome Archive (EGA Study Accession: EGAS00001002746).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ACKNOWLEDGEMENTS

The BAMSE team would like to thank Professors Jean Bousquet and Josep Maria Antó for coordinating the MeDALL project and Professor Charles Auffray and his team for generating the transcriptomics data in BAMSE.

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Figure 1 Legend. DNA methylation analysis of inhaled corticosteroid response in the CAMP^{*}, BAMSE[†], and GACRS[‡]

^{*}Childhood Asthma Management Program

[†]Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology

[‡]Genetic Epidemiology of Asthma in Costa Rica Study

[§]The number of differentially methylated CpG sites for each outcome that met nominal $P < 0.05$ are shown.

^{||}Oral corticosteroid

^{**}The absence of oral corticosteroid use outcome was not measured in BAMSE

^{††}False discovery rate

Figure 2 Legend. DNA methylation is a pharmacoepigenetic marker of inhaled corticosteroid treatment response^{**†}

a) Cg00066816 hypomethylation was associated with the absence of severe exacerbations only in subjects on inhaled corticosteroid treatment compared to placebo (standardized coefficient -3.051, $P = 0.002$). b) Cg04256470 hypermethylation was associated with the absence of oral corticosteroid only in subjects on inhaled corticosteroid treatment compared to placebo (standardized coefficient 2.322, $P = 0.02$).

^{*}The analyses were performed using DNA methylation M values. β values are displayed for easier biologic interpretability.

[†]Interaction analyses was performed only in CAMP because BAMSE and GACRS did not have DNA methylation data available on subjects on inhaled corticosteroids and placebo.

Figure 3 Legend. DNA methylation is associated with gene expression in the BAMSE and CAMP cohorts

a) Partial correlation between DNA methylation and gene expression controlling for emergency department visits and/or hospitalizations in the prior year while on inhaled corticosteroid therapy ($\rho = 0.34$, $P = 0.01$). b) Partial correlation between DNA methylation and gene expression controlling for oral corticosteroid use in the prior year while on inhaled corticosteroid therapy ($\rho = 0.20$, $P = 0.045$).

Table 1. Demographics for the subjects on inhaled corticosteroids in the CAMP^{*}, BAMSE[†], and GACRS[‡] cohorts

	CAMP (n = 154)	BAMSE (n = 72)	GACRS (n = 168)
Age (mean ± SD)	9.8 (± 2.0)	8.3 (± 0.4)	9.2 (± 2.0)
Age at asthma onset (median ± IQR)	2.0 (± 3.0)	4.0 (± 2.7)	2.0 (± 3.2)
Gender			
Female	64 (41.6%)	26 (36.1%)	69 (41.1%)
Male	90 (58.4%)	46 (63.9%)	99 (58.9%)
ED [§] visit and/or hospitalization in the past year			
No	137 (89.0%)	43 (59.7%)	40 (23.8%)
Yes	17 (11.0%)	29 (40.3%)	128 (76.2%)
Oral corticosteroid use in the past year			
No	80 (54.4%)	-	60 (35.7%)
Yes	67 (45.6%)	-	108 (64.3%)
History of atopy			
No	113 (73.4%)	25 (34.7%)	18 (14.2%)
Yes	41 (26.6%)	47 (65.3%)	109 (85.8%)
History of parental smoking			
No	101 (66.0%)	63 (87.5%)	132 (79.5%)
Yes	52 (34.0%)	9 (12.5%)	34 (20.5%)
History of parental asthma			
No	78 (53.1%)	41 (60.3%)	90 (53.9%)
Yes	69 (46.9%)	27 (39.7%)	77 (46.1%)
IgE sensitization ^{**}	133 (86.4%)	52 (72.2%)	123 (73.7%)

^{*}Childhood Asthma Management Program

[†]Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology

[‡]Genetic Epidemiology of Asthma in Costa Rica Study

[§]Emergency department

^{||}In BAMSE, history of parental smoking was defined as positive if the mother smoked at least one cigarette per day at the time of the baseline questionnaire and/or smoked at least one cigarette per day at any point of time during the pregnancy.

^{**}In CAMP, IgE sensitization was defined as any positive aeroallergen skin prick test. In BAMSE and GACRS, IgE sensitization was defined as any positive aeroallergen and/or food specific IgE (≥ 0.35 kU/L).

Table 2. Differentially methylated CpG sites associated with inhaled corticosteroid response in the CAMP, BAMSE, and GACRS cohorts*

CpG	Gene(s)	CAMP standardized coefficient	BAMSE standardized coefficient	GACRS standardized coefficient	CAMP <i>P</i> value (nominal)	BAMSE <i>P</i> value (nominal)	GACRS <i>P</i> value (nominal)	Meta-analysis Q value (FDR-adjusted)
Absence of severe exacerbations^{†,‡}:								
cg00066816	<i>IL12B</i>	-3.101	-2.953	-2.310	0.002	0.003	0.021	0.028
Absence of asthma-related oral corticosteroid use[§]:								
cg00557354	<i>ARHGEF7</i>	-3.490	-	-2.238	0.001	-	0.027	0.035
cg04256470	<i>CORT, CENPS</i>	3.620	-	2.329	< 0.001	-	0.021	0.035
cg09495977	<i>HTRA3</i>	-2.420	-	-3.466	0.017	-	0.001	0.035
cg12333095	<i>ANKRD13A</i>	-3.485	-	-2.336	0.001	-	0.021	0.035
cg13818573	<i>C1QL1</i>	-3.596	-	-2.163	< 0.001	-	0.032	0.035
cg21589280	<i>DDAH1</i>	-3.063	-	-2.786	0.003	-	0.006	0.035
cg03080985	<i>SH3BGR2</i>	-3.077	-	-2.458	0.003	-	0.015	0.039
cg04330449	<i>NEUROG1</i>	-2.646	-	-2.921	0.009	-	0.004	0.039
cg05307923	<i>ADARB2</i>	-2.577	-	-2.959	0.011	-	0.004	0.039
cg08724517	<i>MAP9</i>	2.951	-	2.653	0.004	-	0.009	0.039
cg11665562	<i>PSMC1</i>	-3.250	-	-2.275	0.001	-	0.024	0.039
cg14269514	<i>OAZ3, MRPL9</i>	-3.112	-	-2.246	0.002	-	0.026	0.043
cg24322623	<i>MYOD1</i>	-2.964	-	-2.386	0.004	-	0.018	0.044

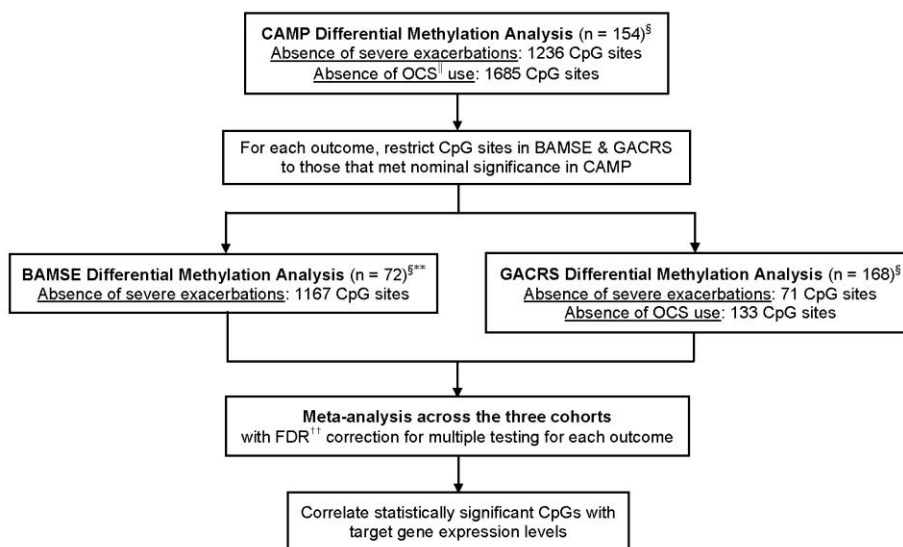
*The binary outcome of interest was the absence of exacerbations. The presence of exacerbations was the reference level, measure respectively as severe exacerbations or oral corticosteroid use. All models controlled for age, sex, batch effect, and white blood cell composition.

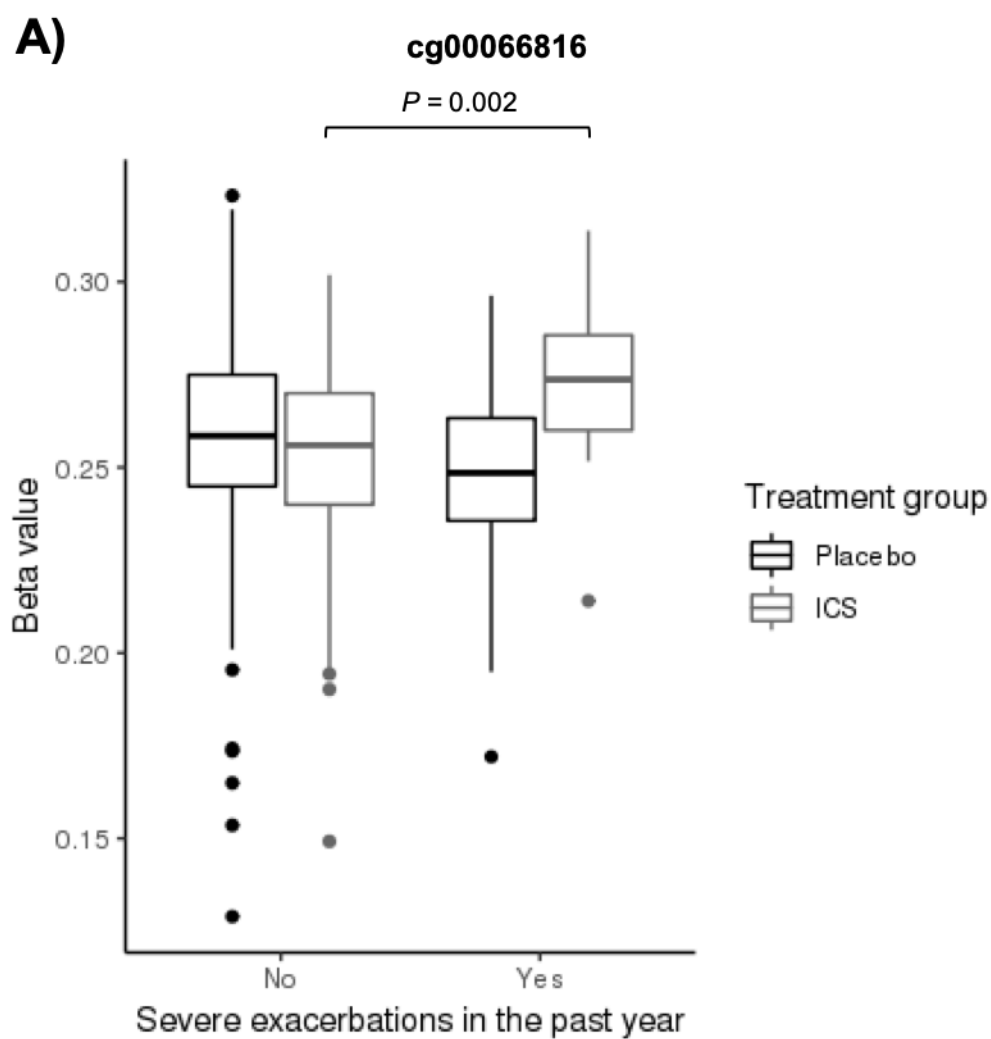
[†]CAMP analysis for the absence of asthma-related emergency department visits and/or hospitalizations included adjustment for history of atopy

[‡]BAMSE analysis for the absence of asthma-related emergency department visits and/or hospitalizations included adjustment for history of atopy and IgE sensitization

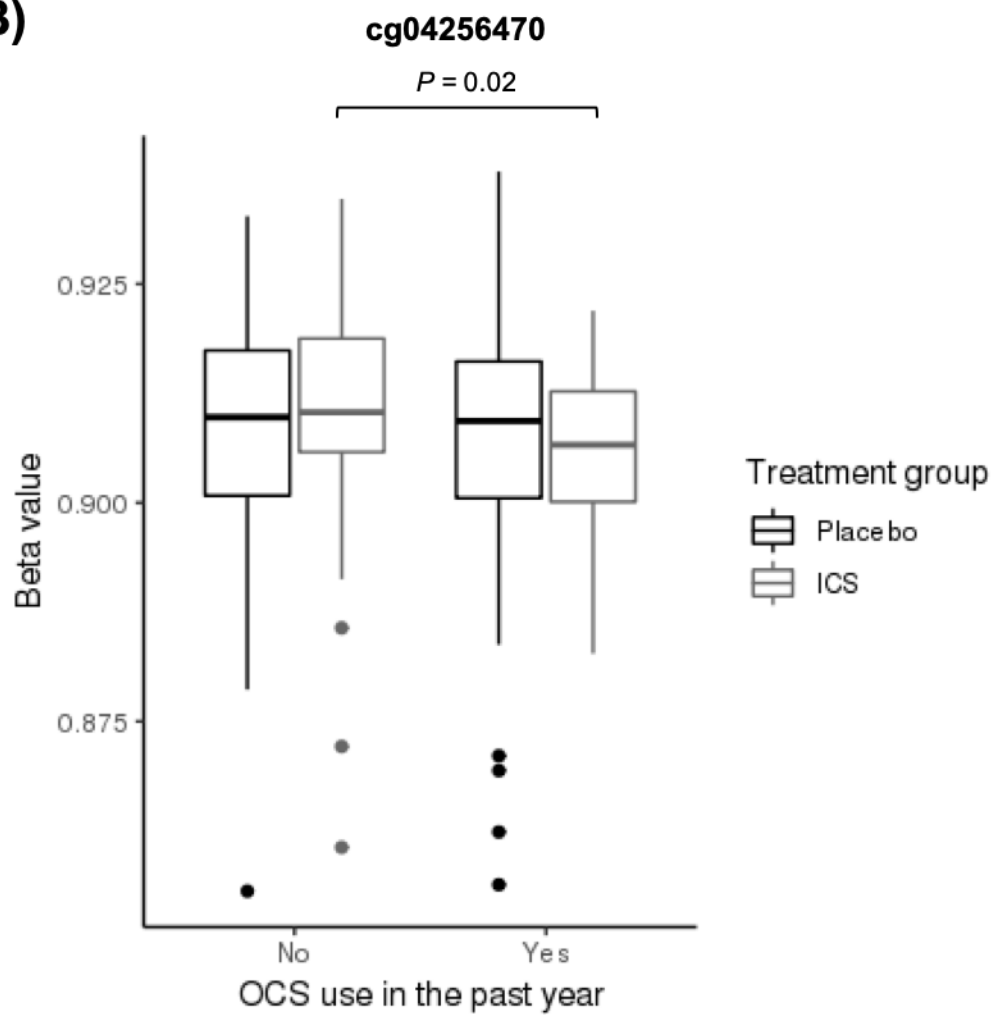
[§]CAMP analysis for the absence of asthma-related oral corticosteroid use included adjustment for history of parental asthma

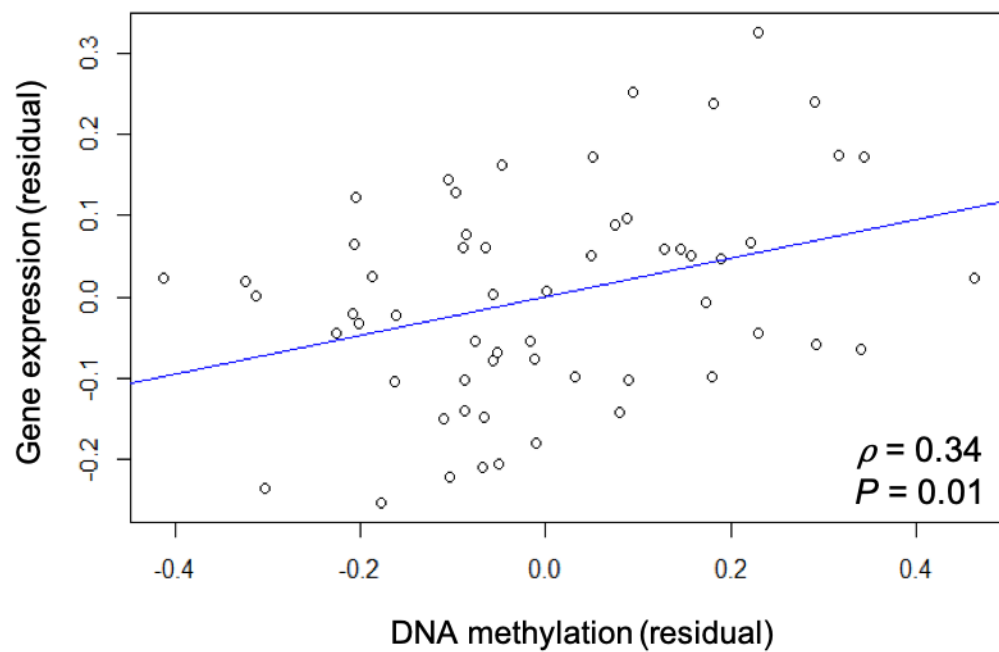
Figure 1. DNA methylation analysis of inhaled corticosteroid response in the CAMP[§], BAMSE[†], and GACRS[‡]

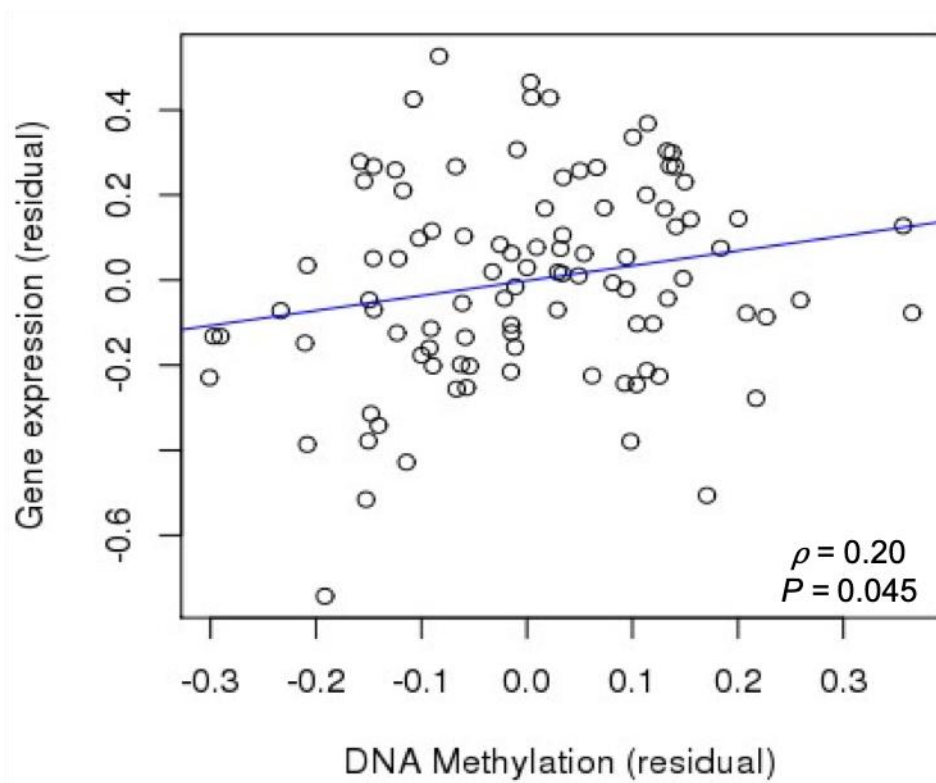




B)







DNA methylation is associated with inhaled corticosteroid response in persistent childhood asthmatics

Alberta L. Wang, MD, Olena Gruzieva, MD, PhD, Weiliang Qiu, PhD, Simon Kebede Merid, MSc, Juan C. Celedón, MD, DrPH, Benjamin A. Raby, MD, Cilla Söderhäll, PhD, Dawn L. DeMeo, MD, Scott T. Weiss, MD, Erik Melén, MD, PhD, Kelan G. Tantisira, MD

Online Data Supplement

SUPPLEMENTAL METHODS

Study populations

Childhood Asthma Management Program (CAMP)

CAMP was a four-year randomized, placebo-controlled trial of inhaled treatments for mild-to-moderate persistent childhood asthma in North America[1]. From December 1993 to September 1995, 1041 children between the ages 5 to 12 years were randomized to receive inhaled budesonide, nedocromil, or placebo for a mean 4.3 years, with an additional 13 years of observational follow-up. DNA methylation analysis was performed in 554 subjects, of whom 163 subjects received inhaled budesonide and 223 subjects received placebo. All 163 subjects on inhaled budesonide were of non-Hispanic white ethnicity, and 154 subjects remained after removal of sex mismatches and outliers. For the primary analysis, the 154 subjects on inhaled budesonide were utilized. For the interaction analysis, both the subjects on inhaled budesonide and subjects on placebo were utilized; 222 subjects on placebo passed quality control.

Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE)

BAMSE was a prospective, population-based cohort study on the risk factors for childhood asthma and allergic diseases in Stockholm, Sweden[2]. Between February 1994 and November 1996, 4,089 children were recruited from all eligible newborns living in a catchment area in central and northwestern Stockholm, Sweden. The parents of the children completed questionnaires at baseline and when the children were 1, 2, 4, 8, 12, and 16 years of age. Epigenome-wide DNA methylation was measured in 472 white children at age eight years[3]. After removal of five sex outliers and eight poor quality arrays, DNA methylation data was available for 72 eight-year-old subjects on inhaled corticosteroid (ICS) for asthma.

Genetic Epidemiology of Asthma in Costa Rica Study (GACRS)

The GACRS focused on an isolated Hispanic population with high asthma prevalence in the Central Valley of Costa Rica[4]. Children with asthma and Central Valley ancestry were eligible to participate. From February 2001 to March 2005, 439 children ages 6 to 14 years enrolled in 95 schools in Costa Rica were recruited via questionnaires sent to their parents. DNA methylation analysis was performed in 367 subjects, of whom 168 subjects were on ICS treatment.

DNA methylation

CAMP

Whole blood was collected in the first year of CAMP, and DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). DNA was then bisulfite converted with the EZ-96 DNA Methylation Kit (Zymo Research, Irvine, CA) followed by PCR amplification. DNA was then processed using the Infinium HumanMethylation27 BeadChip assay (Illumina, San Diego, CA), which measured 27,578 CpG sites across the genome. CpG sites that resided on sex chromosomes, SNPs, indels, or repeats were removed, leaving 17,078 CpG sites. Quantile normalization was performed, and the percentages of white blood cell counts measured from whole blood were used for adjustment of white blood cell composition in subsequent statistical models.

BAMSE

DNA from peripheral blood collected at age eight years was processed using the Infinium HumanMethylation450 BeadChip assay (Illumina, San Diego, CA), which measured 485,512 CpG sites across the genome[5]. Samples with a call rate < 99%, color balance > 3, low staining efficiency, poor extension efficiency, poor hybridization performance, low stripping efficiency after extension, and poor bisulfite conversion were excluded. Probes on SNPs or sex chromosomes and probes with non-optimal binding were excluded, which left 438,713 CpG sites. Signal correction and normalization was performed using the wateRmelon package[6]. The composition of white blood cells was estimated

through the reference-based Houseman method using the estimateCellCounts function in the minfi package[7, 8].

GACRS

Peripheral blood DNA was also processed using the Infinium HumanMethylation450 BeadChip assay (Illumina, San Diego, CA). Normal-exponential out-of-band background correction and beta-mixture quantile normalization were performed. CpG sites with a median detection *P*-value > 0.05, non-CpG methylation probes, and probes that resided on SNPs were removed, leaving 360,478 CpG sites. The composition of white blood cells was estimated using the referenced-based Houseman method[7].

Gene expression

CAMP

All CAMP subjects were invited to participate in ancillary sample collection for DNA gene expression, and gene expression was measured from whole blood samples in 448 subjects using the Illumina HumanHT-12 v4 Expression BeadChip Kit (Illumina, San Diego, CA). These samples are contained within the publicly available Asthma BioRepository for Integrative Genomic Exploration (Asthma BRIDGE) dataset[9]. In CAMP, 109 out of 154 subjects had both DNA methylation and gene expression data.

BAMSE

Gene expression data in BAMSE were generated at age 16 years through the MeDALL Project[10]. Whole blood was collected in PAXGene tubes, and RNA was extracted using PAXgene Blood RNA Kit (QIAGEN, Courtaboeuf, France) and assessed for quality. Gene expression data were obtained using Affymetrix GeneChip HTA 2.0 (Affymetrix, Inc., Santa Clara, CA). Automated cell counts were

obtained by flow cytometry performed at the Karolinska University Laboratory in Stockholm, Sweden. In BAMSE, 58 out of 72 subjects had both DNA methylation and gene expression data.

Statistical Analyses

The association between ICS response and differential DNA methylation was investigated by performing multivariate linear regression using the iCheck package[11]. History of atopy, parental asthma, and parental smoking were selected *a priori* as potential confounders in all three cohorts, and the association between each exposure and outcome was examined using univariate logistic regression. Serum vitamin D level, IgE level, and eosinophil count were additional potential confounders examined in CAMP and GACRS that were not available in BAMSE. The potential confounders that were statistically significant on univariate analyses were included in the multivariate linear regression model for each outcome. In addition, all models were adjusted for age, sex, batch effect, and white blood cell composition. In the CAMP analyses, clinic site was included to control for residual confounding, and in BAMSE, batch effect for DNA methylation and gene expression were corrected using ComBat in the sva package[12].

Meta-analyses were performed using the metap package, and multiple testing was adjusted for using the number of CpG sites from the CAMP cohort to control the false discovery rate (FDR)[13, 14]. The FDb.InfiniumMethylation.hg19 package was used to annotate the DNA methylation probes to the Genome Reference Consortium Human Build 37[15].

In the partial correlation analyses, DNA methylation was adjusted for the same covariates described in the multivariate linear regression models above, and gene expression was adjusted for age, sex, and batch effect. In BAMSE, gene expression analysis was also adjusted for cell type. Cell type was only available in a subset (n = 60) of CAMP gene expression data. A sensitivity analysis of the partial

correlation adjusting for gene expression cell type composition was performed in this subset of subjects in CAMP.

Supplemental Table 1. Demographics for the subjects on inhaled corticosteroids and placebo included in the interaction analyses in CAMP

	ICS (n = 154)	Placebo (n = 222)
Age (mean $\pm$ SD)	9.8 ($\pm$ 2.0)	9.7 ($\pm$ 2.1)
Age at asthma onset (median $\pm$ IQR)	2.0 ($\pm$ 3.0)	2.0 ($\pm$ 3.0)
Gender		
Female	64 (41.6%)	88 (39.6%)
Male	90 (58.4%)	134 (60.4%)
ED visit and/or hospitalization in the past year		
No	137 (89.0%)	177 (79.7%)
Yes	17 (11.0%)	45 (20.3%)
Oral corticosteroid use in the past year		
No	80 (54.4%)	77 (36.8%)
Yes	67 (45.6%)	132 (63.2%)
History of atopy		
No	113 (73.4%)	162 (73.0%)
Yes	41 (26.6%)	60 (27.0%)
History of parental smoking		
No	101 (66.0%)	129 (58.1%)
Yes	52 (34.0%)	93 (41.9%)
History of parental asthma		
No	78 (53.1%)	127 (61.4%)
Yes	69 (46.9%)	80 (38.6%)
IgE sensitization	133 (86.4%)	187 (84.2%)

Supplemental Table 2. Differentially methylated CpG sites associated with the absence of severe exacerbations in the past year in persistent childhood asthmatics on inhaled corticosteroids from the CAMP, BAMSE, and GACRS cohorts

CpG	Gene(s)	CAMP standardized coefficient	CAMP P value (nominal)	BAMSE standardized coefficient	BAMSE P value (nominal)	GACRS standardized coefficient	GACRS P value (nominal)	Meta-analysis P value (nominal)	Meta-analysis Q value (FDR-adjusted)
cg00066816	<i>IL12B</i>	-3.1012	0.0023	-2.9528	0.0031	-2.3097	0.0221	0.0000	0.0280
cg24088229	<i>SMU1</i>	3.0275	0.0030	-2.3635	0.0181	2.1027	0.0370	0.0002	0.1212
cg02831294	<i>CALML3</i>	2.3270	0.0215	-2.5496	0.0108	2.0563	0.0413	0.0008	0.1621
cg10219037	<i>C11orf1, FDXACB1</i>	2.7815	0.0062	-0.1932	0.8468	2.9588	0.0035	0.0013	0.1621
cg11037424	<i>PRPF6</i>	-3.0626	0.0026	-1.4183	0.1561	-1.9757	0.0499	0.0014	0.1621
cg26540127	<i>ACTR3</i>	-2.6202	0.0098	1.8207	0.0687	-2.2638	0.0249	0.0012	0.1621
cg07210490	<i>SMARCD1</i>	-3.1795	0.0018	-0.9159	0.3597	-2.0588	0.0411	0.0018	0.1643
cg10523019	<i>RHBDD1</i>	-2.8751	0.0047	-0.0087	0.9931	-2.7832	0.0060	0.0019	0.1643
cg09299381	<i>DNAL4</i>	-2.6273	0.0096	0.4279	0.6687	-2.8108	0.0055	0.0023	0.1751
cg06825166	<i>OPALIN</i>	2.4921	0.0139	0.4053	0.6852	2.8982	0.0043	0.0025	0.1839
cg07443748	<i>CCT8L2</i>	2.3014	0.0229	0.7109	0.4772	2.8141	0.0055	0.0035	0.2148
cg24692716	<i>C6orf192</i>	-2.3136	0.0222	0.3149	0.7529	-2.8350	0.0052	0.0047	0.2508
cg16310717	<i>CCND2</i>	2.0181	0.0456	1.1411	0.2538	2.7203	0.0072	0.0045	0.2508
cg23213688	<i>C1orf124, EXOC8</i>	-2.8001	0.0059	-0.1611	0.8720	-2.3914	0.0179	0.0049	0.2522
cg21989500	<i>NDUFAB1</i>	-2.7258	0.0073	-0.3089	0.7574	-2.3441	0.0203	0.0057	0.2527
cg27210766	<i>BUB1</i>	-2.0232	0.0450	-1.0432	0.2968	-2.6598	0.0086	0.0059	0.2527
cg12997958	<i>FLII</i>	2.2132	0.0286	0.9923	0.3210	2.5260	0.0125	0.0059	0.2527
cg12940430	<i>SRF</i>	2.1697	0.0318	-0.2388	0.8113	2.8203	0.0054	0.0069	0.2607
cg03201604	<i>NAAA</i>	-2.2796	0.0242	1.0457	0.2957	-2.3530	0.0198	0.0070	0.2607
cg17169998	<i>MLC1</i>	-2.6267	0.0096	0.5123	0.6084	-2.2945	0.0230	0.0067	0.2607
cg14712964	<i>BRPF1</i>	-2.6152	0.0099	-0.8301	0.4065	-2.0901	0.0381	0.0074	0.2704
cg08211091	<i>NAT8L</i>	2.4808	0.0143	-0.3435	0.7313	2.4140	0.0169	0.0083	0.2934
cg08293367	<i>YPEL1</i>	-2.4944	0.0138	0.3883	0.6978	-2.3079	0.0222	0.0097	0.3234
cg10908369	<i>CNRIP1</i>	-2.0596	0.0414	1.3898	0.1646	-2.1137	0.0360	0.0108	0.3501
cg14849423	<i>MIMT1, PEG3, ZIM2</i>	2.3046	0.0227	0.9591	0.3375	2.1404	0.0338	0.0112	0.3533
cg21452766	<i>CTH</i>	-2.4012	0.0177	-0.4170	0.6767	-2.2182	0.0279	0.0137	0.3858
cg08223748	<i>MEF2C</i>	-2.2724	0.0246	-0.3868	0.6989	-2.3106	0.0221	0.0152	0.3921
cg21589280	<i>DDAH1</i>	-2.2705	0.0248	-0.1052	0.9163	-2.4172	0.0167	0.0151	0.3921
cg07536847	<i>PAX7</i>	-2.4271	0.0165	0.2829	0.7772	-2.1408	0.0338	0.0168	0.4153

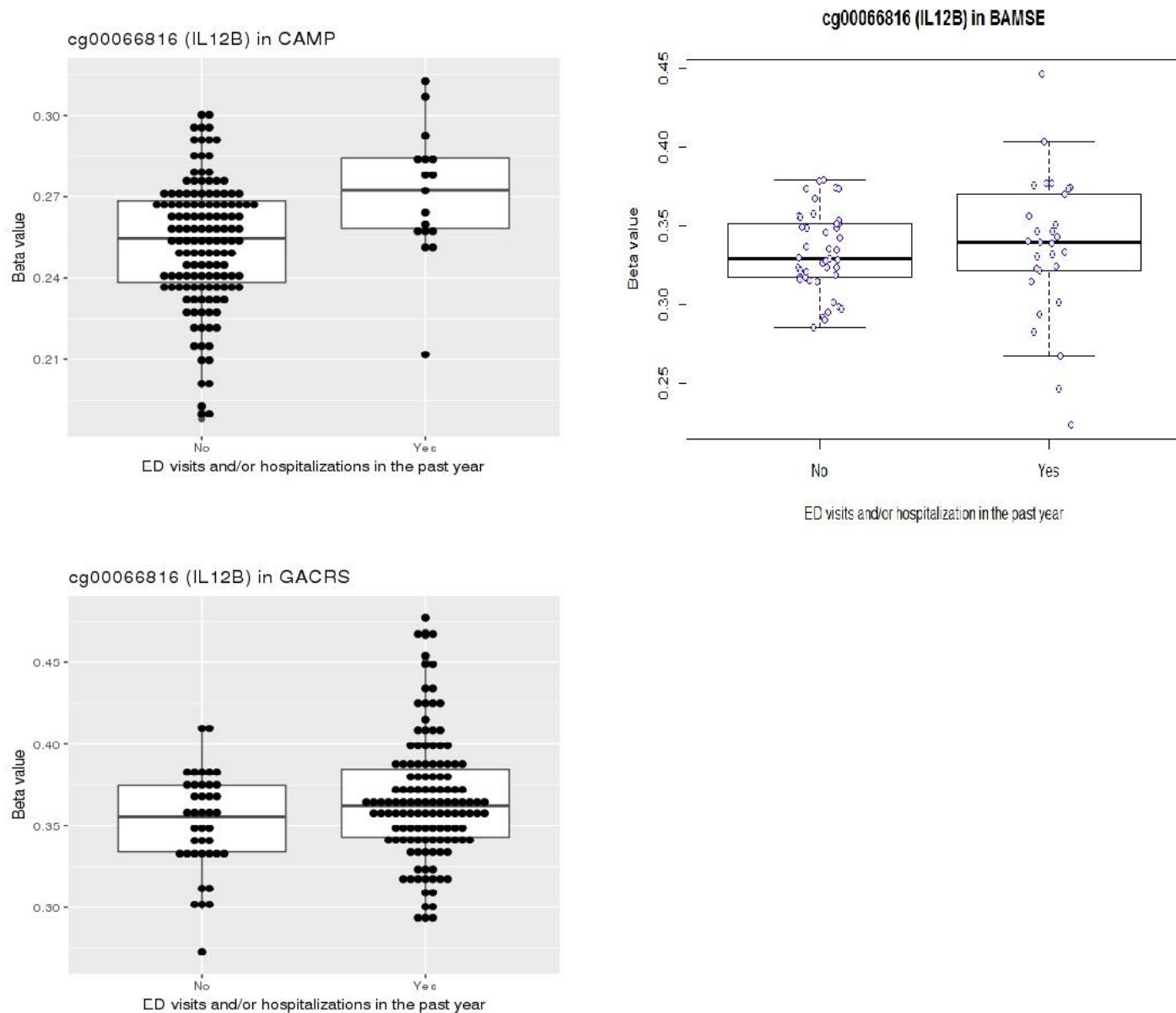
cg10002977	<i>SOD2</i>	2.0954	0.0380	-0.0250	0.9801	2.5091	0.0131	0.0184	0.4346
cg18579327	<i>CREB1</i>	-2.3218	0.0217	0.1725	0.8630	-2.2437	0.0262	0.0185	0.4346
cg26149738	<i>KCTD15</i>	2.0674	0.0406	0.4424	0.6582	2.3565	0.0196	0.0194	0.4451
cg24365867	<i>MRVI1</i>	2.2590	0.0255	-0.0946	0.9246	2.2860	0.0235	0.0203	0.4560
cg03843852	<i>PIGB</i>	-2.0006	0.0474	-0.2823	0.7777	-2.3860	0.0182	0.0235	0.4916
cg03139057	<i>DLL3</i>	-2.1451	0.0337	-0.6109	0.5413	-2.0243	0.0446	0.0272	0.5509
cg17409393	<i>ATP9A</i>	-2.3122	0.0223	0.1243	0.9011	-2.0703	0.0400	0.0269	0.5509
cg04314463	<i>PNKP</i>	-2.0076	0.0467	-0.3007	0.7637	-2.2013	0.0291	0.0327	0.6226
cg14269514	<i>OAZ3, MRPL9</i>	-2.0391	0.0434	0.6739	0.5004	-1.9872	0.0486	0.0331	0.6226
cg04316754	<i>MMP24</i>	2.2848	0.0239	0.0127	0.9899	1.9864	0.0486	0.0353	0.6427
cg05672265	<i>SLC20A1</i>	-2.1595	0.0326	-0.0230	0.9817	-2.0351	0.0434	0.0406	0.7271
cg19764436	<i>RTDR1, GNAZ</i>	-2.1279	0.0352	0.0000	1.0000	-1.9753	0.0499	0.0482	0.8512
cg20375554	<i>ARHGEF2</i>	-2.0075	0.0467	0.1196	0.9048	-2.0076	0.0463	0.0522	0.9090

Supplemental Table 3. Differentially methylated CpG sites associated with the absence of oral corticosteroid use in the past year in persistent childhood asthmatics on inhaled corticosteroids from the CAMP and GACRS cohorts

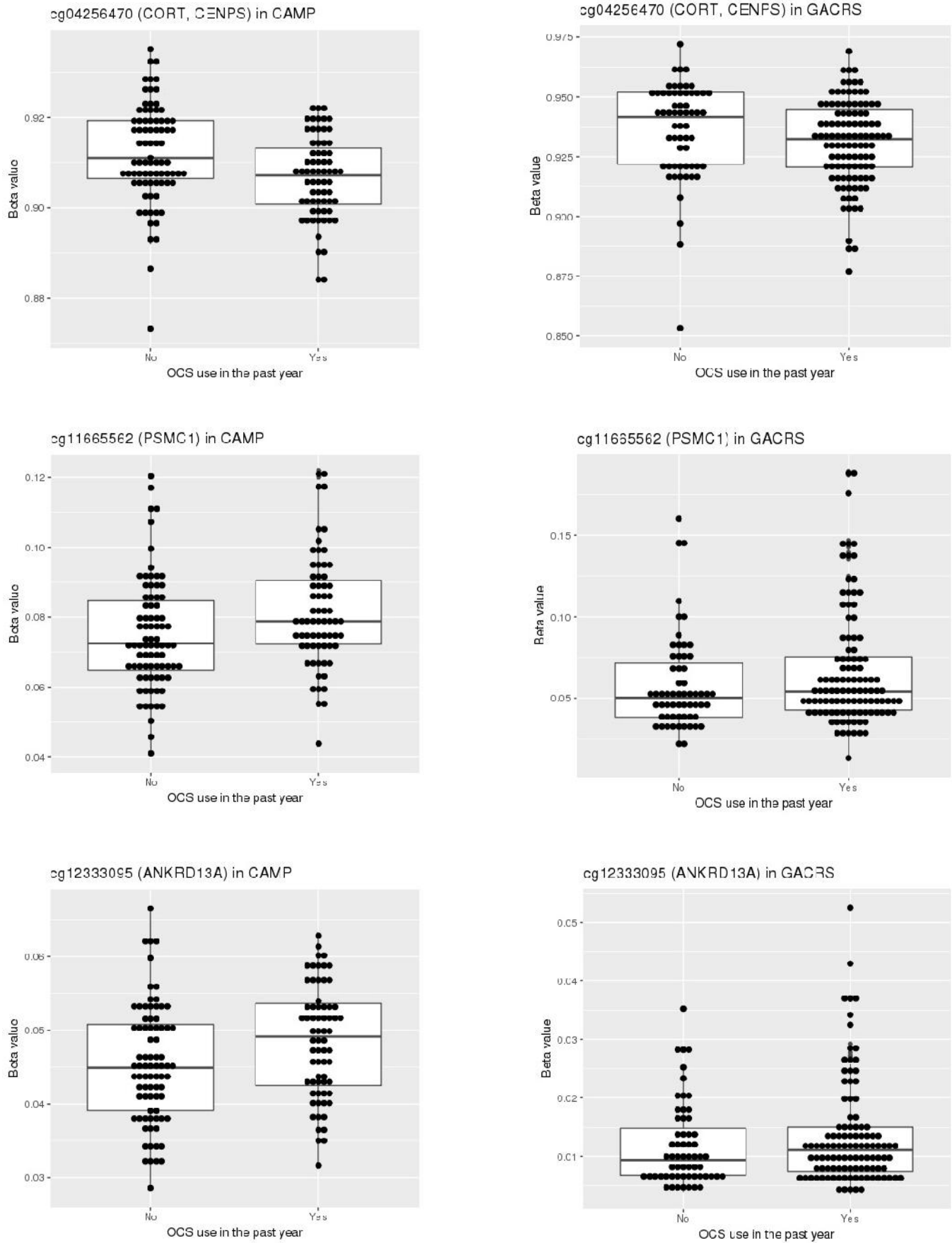
CpG	Gene(s)	CAMP standardized coefficient	CAMP P value (nominal)	GACRS standardized coefficient	GACRS P value (nominal)	Meta-analysis P value (nominal)	Meta-analysis Q value (FDR-adjusted)
cg04256470	<i>CORT, CENPS</i>	3.6199	0.0004	2.3292	0.0211	0.0001	0.0350
cg00557354	<i>ARHGEF7</i>	-3.4904	0.0007	-2.2379	0.0266	0.0002	0.0350
cg09495977	<i>HTRA3</i>	-2.4198	0.0170	-3.4661	0.0007	0.0001	0.0350
cg12333095	<i>ANKRD13A</i>	-3.4852	0.0007	-2.3365	0.0207	0.0002	0.0350
cg13818573	<i>C1QL1</i>	-3.5956	0.0005	-2.1628	0.0320	0.0002	0.0350
cg21589280	<i>DDAH1</i>	-3.0633	0.0027	-2.7863	0.0060	0.0002	0.0350
cg08724517	<i>MAP9</i>	2.9511	0.0038	2.6532	0.0088	0.0004	0.0389
cg03080985	<i>SH3BGRL2</i>	-3.0767	0.0026	-2.4580	0.0150	0.0004	0.0389
cg04330449	<i>NEUROG1</i>	-2.6462	0.0092	-2.9214	0.0040	0.0004	0.0389
cg05307923	<i>ADARB2</i>	-2.5771	0.0112	-2.9589	0.0035	0.0004	0.0389
cg11665562	<i>PSMC1</i>	-3.2496	0.0015	-2.2752	0.0242	0.0004	0.0389
cg14269514	<i>OAZ3, MRPL9</i>	-3.1126	0.0023	-2.2464	0.0260	0.0006	0.0434
cg24322623	<i>MYOD1</i>	-2.9644	0.0036	-2.3860	0.0182	0.0007	0.0439
cg25461905	<i>C1orf49</i>	2.9467	0.0038	2.2897	0.0233	0.0009	0.0510
cg11450001	<i>GABARAPL1</i>	-2.1750	0.0316	-3.0365	0.0028	0.0009	0.0510
cg08201421	<i>TNNC2</i>	2.6040	0.0104	2.5573	0.0114	0.0012	0.0531
cg25371182	<i>CTNS, SHPK</i>	2.7268	0.0073	2.4224	0.0165	0.0012	0.0531
cg06638012	<i>AFF4</i>	-2.4092	0.0175	-2.7006	0.0076	0.0013	0.0545
cg06825166	<i>OPALIN</i>	2.5295	0.0127	2.5227	0.0126	0.0016	0.0606
cg01994779	<i>ZNRF1</i>	2.1319	0.0350	2.8380	0.0051	0.0017	0.0611
cg19850163	<i>AGFG1</i>	-2.5084	0.0134	-2.4898	0.0138	0.0018	0.0611
cg13415434	<i>P4HTM, WDR6</i>	-2.6996	0.0079	-2.1764	0.0309	0.0023	0.0711
cg02144933	<i>AOX1</i>	-2.7101	0.0077	-2.0968	0.0375	0.0026	0.0768
cg27360282	<i>RUNX3</i>	2.0566	0.0419	2.7101	0.0074	0.0028	0.0780
cg19023700	<i>MKLN1</i>	-2.3597	0.0199	-2.4261	0.0163	0.0029	0.0797
cg14026602	<i>USP7</i>	-2.4963	0.0139	-2.2180	0.0279	0.0034	0.0815
cg03776464	<i>EPHA4</i>	-2.2450	0.0266	-2.4392	0.0158	0.0037	0.0861
cg27196745	<i>PTPRO</i>	-2.4832	0.0144	-2.1839	0.0304	0.0038	0.0870
cg21989500	<i>NDUFAB1</i>	-2.4825	0.0144	-2.1746	0.0311	0.0039	0.0876

cg08558873	<i>ZNF214, NLRP1</i>	-2.3955	0.0181	-2.2048	0.0288	0.0045	0.0967
cg17960516	<i>DOK7</i>	-2.3463	0.0206	-2.2483	0.0259	0.0045	0.0969
cg26195812	<i>DPYSL5</i>	-2.2064	0.0292	-2.3309	0.0210	0.0051	0.1057
cg13378388	<i>VEGFC</i>	2.0235	0.0452	2.4854	0.0139	0.0053	0.1071
cg07989514	<i>C14orf4</i>	2.3822	0.0188	2.1258	0.0350	0.0055	0.1071
cg23337382	<i>GPR125</i>	-2.2934	0.0235	-2.2200	0.0278	0.0054	0.1071
cg06938878	<i>CALCB</i>	-2.0127	0.0463	-2.4660	0.0147	0.0056	0.1087
cg08223748	<i>MEF2C</i>	-2.0319	0.0443	-2.4469	0.0155	0.0057	0.1087
cg02989940	<i>AHSP</i>	2.2419	0.0268	2.2370	0.0266	0.0059	0.1092
cg18858343	<i>UNC5B</i>	-1.9938	0.0484	-2.4710	0.0145	0.0058	0.1092
cg24692716	<i>C6orf192</i>	-2.1609	0.0327	-2.2876	0.0234	0.0063	0.1133
cg16016641	<i>C20orf4</i>	-1.9955	0.0482	-2.4105	0.0170	0.0067	0.1168
cg19167673	<i>PDGFB</i>	-2.3415	0.0208	-2.0502	0.0419	0.0070	0.1195
cg14732136	<i>KPNA2</i>	-2.0689	0.0407	-2.3166	0.0218	0.0071	0.1197
cg12997958	<i>FLII</i>	2.2118	0.0288	2.1145	0.0360	0.0082	0.1219
cg21122774	<i>SARDH</i>	2.2034	0.0294	2.1277	0.0348	0.0081	0.1219
cg03943081	<i>TCERG1L</i>	-2.1982	0.0298	-2.1754	0.0310	0.0074	0.1219
cg04461802	<i>GPR126</i>	-2.1676	0.0321	-2.1658	0.0318	0.0080	0.1219
cg09606564	<i>MFAP4</i>	-2.3504	0.0204	-1.9856	0.0487	0.0079	0.1219
cg11656547	<i>MAMDC2</i>	-2.3539	0.0202	-2.0088	0.0462	0.0074	0.1219
cg13603099	<i>RASSF4</i>	-2.1868	0.0307	-2.1503	0.0330	0.0080	0.1219
cg22994720	<i>CHRD12</i>	-2.1644	0.0324	-2.1848	0.0303	0.0078	0.1219
cg23489497	<i>GPN2</i>	-1.9822	0.0497	-2.3313	0.0209	0.0082	0.1219
cg02887841	<i>TBX1</i>	-2.1802	0.0312	-2.1235	0.0352	0.0086	0.1256
cg02305261	<i>KCNG1</i>	-2.0839	0.0393	-2.1945	0.0296	0.0090	0.1287
cg20798152	<i>CARTPT</i>	-2.1385	0.0345	-2.0710	0.0399	0.0104	0.1454
cg03169180	<i>NLGN2</i>	2.0503	0.0425	2.1461	0.0333	0.0107	0.1478
cg03059528	<i>RNF146</i>	-2.0312	0.0444	-2.0926	0.0379	0.0124	0.1669
cg08831701	<i>HELZ</i>	-2.1014	0.0377	-1.9990	0.0473	0.0130	0.1717
cg13179915	<i>KCNK7</i>	1.9861	0.0493	2.0453	0.0424	0.0150	0.1912
cg08907850	<i>IDI1</i>	-2.0381	0.0437	-1.9956	0.0476	0.0149	0.1912
cg11993754	<i>ERBB2</i>	-2.0401	0.0435	-1.9943	0.0478	0.0149	0.1912
cg13342120	<i>TRAFD1</i>	-1.9847	0.0494	-1.9916	0.0481	0.0167	0.2119

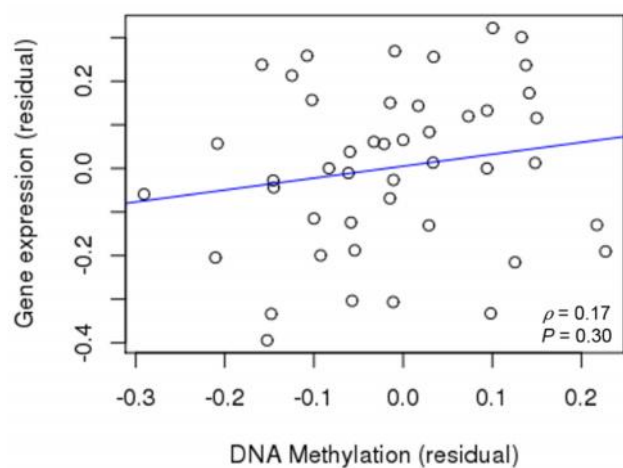
Supplemental Figure 1. Pediatric asthmatics on inhaled corticosteroids who have severe exacerbations requiring emergency department visits and/or hospitalizations have differential methylation of cg00066816 in the CAMP, BAMSE, and GACRS cohorts



Supplemental Figure 2. Representative plots of differential methylation in pediatric asthmatics on inhaled corticosteroids with and without oral corticosteroid use in the CAMP and GACRS cohorts



Supplemental Figure 3. Sensitivity analysis of the partial correlation between cg04256470 methylation and *CORT* expression in CAMP adjusting for oral corticosteroid use in the prior year*



*Both DNA methylation and gene expression were adjusted for cell type composition. Only a subset (n = 60) of CAMP subjects that had gene expression cell type composition data were included in this analysis.

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