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Nasal DNA methylation differentiates severe from non-severe asthma in African-American children

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Abstract

Background: Asthma is highly heterogeneous and severity evaluation is key to asthma management. DNA methylation (DNAm) contributes to asthma pathogenesis. This study aimed to identify nasal epithelial DNAm differences between severe and non-severe asthmatic children and evaluate the impact of environmental exposures.

Methods: Thirty-three non-severe and 22 severe asthmatic African-American children were included in an epigenome-wide association study. Genome-wide nasal epithelial DNAm and gene expression were measured. CpG sites associated with asthma severity and environmental

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Authors' Contributions

HJ conceived the study in discussion with XZ and JBM. TZ drafted the manuscript with the help of HJ, XZ, XC and JBM. XZ performed array processing and statistical analysis, XC performed HOMER and RELI analysis in discussion with MTW, APB assisted XZ in computational analysis. TWG and GKKH contributed to identification and recruitment of cohort participants. JBM manages GSEP and ESS cohorts, and provided RNA-seq and methylation data from GSEP. All authors have approved the final version of this manuscript. Part of this study has been published as an abstract (DOI:https://doi.org/10.1016/j.jaci.2019.12.087).

Competing interests

Dr. Zhu, Dr. Zhang, Dr. Chen, Dr. Brown and Dr. Weirauch have nothing to disclose. Dr. Guilbert reports personal fees from American Board of Pediatrics; Pediatric Pulmonary Subboard, personal fees from GSK, personal fees from TEVA, personal fees from Novartis, grants and personal fees from Astra-Zeneca, grants and personal fees from Sanofi/Regeneron, grants from NIH, and other from UpToDate outside the submitted work. Dr. Khurana Hershey reports grants from NIH, grants from CHMC CTC (Adare) during the conduct of the study. Dr. Ji reports grants from National Institute of Health, grants from American Lung Association, during the conduct of the study.

exposures and predictive of severe asthma were identified. DNAm was correlated with gene expression. Enrichment for transcription factor (TF) binding sites or histone modifications surrounding DNAm differences were determined.

Results: We identified 816 differentially methylated CpG positions (DMPs) and 10 differentially methylated regions (DMRs) associated with asthma severity. Three DMPs exhibited discriminatory ability for severe asthma. Intriguingly, six DMPs were simultaneously associated with asthma, allergic asthma, total IgE, environmental IgE, and FeNO in an independent cohort of children. 27 DMPs were associated with traffic-related air pollution or secondhand smoke. DNAm at 22 DMPs were altered by diesel particles or allergen in human bronchial epithelial cells. DNAm levels at 39 DMPs were correlated with mRNA expression. Proximal to 816 DMPs, three histone marks and several TFs involved in asthma pathogenesis were enriched.

Conclusions: Significant differences in nasal epithelial DNAm were observed between non-severe and severe asthma in African-American children, a subset of which may be useful to predict disease severity. These CpG sites are subject to the influences of environmental exposures and may regulate gene expression.

Keywords

asthma severity; DNA methylation; epigenetics; environmental exposures

INTRODUCTION

Asthma is the most common non-communicable airway disorder of children in the US, and it has a substantial impact on quality of life [1]. According to National Health Interview Survey (NHIS), it is estimated that the prevalence of asthma in children was 8.3% in 2016 [2]. However, asthma is still not controlled well in children. Zahran HS *et al* reported that in 2013 and 2016, 54% of asthmatic children experienced at least one asthma attack, 71.1% had routine care visits, 4.7% were hospitalized, and 16.7% had an ED/UC visit associated with an asthma attack in the US [2]. Meanwhile, asthma is highly heterogeneous with a wide range of severity. Different severity levels require different treatments and have different prognoses [3]. Therefore, it is critical to regularly evaluate the severity of asthma to adjust the therapeutic plan and predict the risk. Presently, symptom scores, lung function, bronchial provocation test (BPT), EOS% in induced sputum and blood, FeNO, and IgE in blood are commonly used to evaluate severity and assess how well the asthma is being controlled [4,5]. However, due to the invasiveness of some of these methods, they have limitations in pediatric practice, making an accurate assessment of asthma severity more difficult in children. A non-invasive method with high sensitivity and specificity is thus desired to facilitate the evaluation of asthma severity in pediatric practice.

Accumulating evidence suggests that DNA methylation (DNAm) contributes substantially to the pathogenesis of asthma and allergy [6-8]. Significant differences in DNA methylation between asthma and non-asthma were observed in both humans [9-12] and animals [13]. DNAm is highly tissue- and cell-type specific. Lower airway epithelium is considered the best sample type to study asthma. However, it is invasive, costly, and dangerous to collect, particularly in children and during asthma attacks. In contrast, nasal epithelium collection is

easy, non-invasive, and more acceptable to the parents and children. Recently several studies confirmed that nasal epithelium is a biologically relevant proxy and useful surrogate for lower airway epithelium in asthma [14-16]. In addition to aiding asthma diagnosis, we also showed that DNA methylation can predict the response to steroid treatment in hospitalized children [17]. Despite recent progress, the role of DNAm in asthma severity is not well studied and DNAm markers associated with the severity of asthma symptoms remain unknown.

In this study, we aimed to identify differences in DNAm in nasal epithelium between severe and non-severe asthmatic children, and explore how well these differences could predict asthma severity in children. We also evaluated the impact of environmental exposures known to increase asthma severity, such as secondhand smoke (SHS) and air pollution, on these markers. Furthermore, we examined the impact of these DNA methylation markers on gene regulation and proposed possible mechanisms based on predicted changes in chromatin modification and binding of transcription factors.

METHODS

A full description of the methods used in this study is provided in the Online Supplement Materials.

Study populations and definitions

The Exposure Sibling Study (ESS) and the Genomics of Secondhand-Smoke Exposure in Pediatric Asthma Study (GSEP) were approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (CCHMC). Written informed consent was obtained as previously described [12]. Among the asthmatics, asthma severity was defined by symptom frequency using previously validated respiratory symptom score (maximum respiratory symptom score, maxRSS) [18]. Severe asthma was defined as a symptom score of 3 or 4, while non-severe asthma was defined as a symptom score of 0, 1 or 2. Allergic children were defined as having a positive doctor's diagnosis of allergy. Elemental carbon attributable to traffic (ECAT), approximately 95% of which are diesel exhaust particles (DEP) [19], herein referred to as traffic-related air pollution (TRAP), was estimated using previously established methods [20]. To determine the association between DNAm and asthma severity, we combined 29 asthmatics from ESS and 33 asthmatics from GSEP. Nasal cells were obtained and DNA and RNA were obtained as previously described [12]. See Supplemental Methods for additional details.

Illumina bead array processing

Genomic DNA from the nasal cells was bisulfite treated and assayed by the Illumina Infinium HumanMethylation450 BeadChip (Illumina) (29 ESS participants) and by the Illumina EPIC array (33 GSEP participants). After quality assessment [12], the samples that passed the quality control check were included in the analysis. Array data of ESS were previously deposited to GEO (GSE109446). Data of GSEP have been deposited to GEO (GSE161485). See Supplemental Methods for additional details.

Statistical Analysis

Prior to statistical analysis, data quality and distributions were examined. We used T and Fisher's exact tests in SAS 9.4 to compare the demographics and characteristics. DMPs were identified using the R package 'limma'. DMPs were further analyzed using the Python library "comb-p" to identify differential methylation regions (DMRs). The univariate logistic regression and stepwise model selection were used to generate a predictive model for asthma severity, which were conducted by SAS 9.4. The empirical p values and fold of enrichment for overlapping DMPs between this study and the previous study were calculated by resampling CpGs without associations. See Supplemental Methods for additional details.

Functional genomics analysis

The correlation between gene expression and DNAm was identified by calculating the Pearson correlation coefficient. HOMER [21] / Cis-BP [22] and RELI [23] analyses were used to search for enriched TF binding site motifs and ChIP-seq datasets, respectively, as previously described [24]. See Supplemental Methods for additional details.

RESULTS

Demographics and characteristics of asthmatic children.

Subjects included in the current study consisted of 29 children from ESS and 33 from GSEP; 7 children from GSEP without maxRSS were excluded (Supplement Table S1 and Figure 1). Among the 33 children from GSEP, SHS data were missing for 3 of them (Supplement Table S1), leading to 1 severe asthmatic child without SHS data (Table 1). No significant differences in age, sex, SHS exposure and current ECAT were observed between participants from ESS and participants from GSEP. More asthmatics from GSEP were allergic (p=0.035) (Supplement Table S1). When children with non-severe and severe asthma were compared, no differences in age, sex, SHS, current ECAT, or allergy were observed (Table 1). According to self-reports, 98% of the subjects were African-American.

DNAm is associated with asthma severity in children.

After removing CpG sites associated with common genetic variations [12], 816 differentially methylated positions (DMPs) associated with asthma severity were identified ($p<0.05$ absolute difference in beta ≥ 0.05, Supplementary Table S2). Of the 816, 237 were located in CpG islands, 325 were in open sea regions, and 254 were in shore and shelf regions (Figure 2 and Table 2), with 136 residing within 1500bp upstream of the transcription start site (Supplementary Table S2). When examining the direction of DNAm changes, we found that compared with non-severe asthma, 441 DMPs had reduced while 375 had increased DNA methylation in severe asthmatics (Table 2). The genomic distribution of DMPs between hypomethylated and hypermethylated CpG sites significantly differed ($p<0.001$), with more hypermethylated CpG sites located in shore and shelf regions, while more hypomethylated sites were located in islands.

Among the 816 DMPs associated with asthma severity, 498 DMPs were annotated to 398 genes, while 318 DMPs had no known gene annotation (Supplementary Table S2). In 59 genes, we observed multiple DMPs close together within a relatively small region

(Supplementary Table S2), suggesting that these DMPs form differentially methylated regions (DMRs). Indeed, our DMR analysis revealed 10 regions (at least 3 DMPs spanning 2000 base pairs) that are significantly different between non-severe and severe asthmatics (Supplementary Table S2).

Subsequently, pathway analysis was performed to explore the enriched pathways among genes with DMPs. Sixteen significantly enriched pathways were identified (Supplementary Figure S1). The significantly enriched pathways associated with asthma include Signaling by Rho Family GTPases [25], Gαs Signaling [26], Eicosanoid Signaling [27], Cardiac β-adrenergic Signaling [28], and CREB Signaling in Neurons [29].

Nasal DNA methylation levels predict asthma severity in children.

We explored the predictive value of DNAm for asthma severity. Out of the 816 DMPs associated with asthma severity, 39 showed discriminant power (AUC>0.75 in uni-variable logistic regression, Supplementary Table S2). Stepwise model selection with the 39 sites further narrowed the model down to three DMPs (cg06654369, cg07463541, and cg19551589). When combined, these three sites predicted asthma severity with an area under the ROC curve of 0.9683 (Figure 3, predicted probability cutoff=0.68, sensitivity=0.86, specificity=0.97). cg07463541 was associated with *PCDHA2*, while cg19551589 was associated with *UCKL1*; the roles of both genes in the regulation of asthma is unclear. These results suggest a potential value of DNAm in predicting asthma severity.

CpG sites associated with asthma severity are associated with asthma status and clinical features of asthma in an independent cohort of children.

We further explored whether the 816 nasal DMPs associated with asthma severity were associated with asthma status and other asthma-related features from another nasal epithelial EWAS study in children [30]. We identified numerous DMPs associated with asthma status (n=50, p=0.002, 1.6 fold enrichment), allergic asthma (n=46, p=0.039, 1.4 fold), bronchodilator response (BDR) (n=38, p=0.029, 1.4 fold), environment IgE (n=48, p=0.004, 1.5 fold), total IgE (n=59, p=0.009, 1.4 fold), FeNO (n=85, p=0.011, 1.3 fold), and FEV/FVC z-score (n=51, p=0.008, 1.4 fold) in Project Viva participants (n=547) (Supplementary Table S3). In clinical practice, IgE and FeNO are commonly used parameters to diagnosis or phenotype asthma and are highly associated with allergy in asthma [31]. Six DMPs were associated with asthma severity and asthma status, allergic asthma, total IgE, environment IgE, and FeNO (Supplementary Table S3). Although the roles of genes annotated to these six DMPs (*TMEM51, WDR25, HIPK3 and KLF11*) in asthma remain unclear, these data support the involvement of identified DMPs in regulation of asthma severity.

Asthma-severity associated DMPs are associated with exposures to TRAP and SHS in children, and responsive to allergen and diesel particles in HBECs.

Traffic-related air pollution (TRAP) and secondhand smoke (SHS) exposure are risk factors of asthma, particularly in early life [12,32,33]. Among 816 DMPs associated with asthma severity, 11 DMPs also associated with TRAP exposure (11 fold enrichment, p<0.001) (Supplementary Table S2). Meanwhile, 16 DMPs associated with SHS exposure (16 fold

enrichment, p<0.001) (Supplementary Table S2). As a significant contributor to particulate matter in TRAP exposure, DEP is known to exacerbate asthma phenotypes. Exposure to HDM is also known to cause allergic asthma. Our previous study identified CpG sites with DNAm changes following DEP and HDM challenges in human bronchial epithelial cells (HBECs) [24]. Interestingly, 13 out of the 816 CpG sites underwent changes in DNAm when HEBCs were exposed to DEP (2.6 fold enrichment, p=0.003) and 9 CpG sites underwent DNAm changes when exposed to HDM (2.3 fold enrichment, p=0.036) (Supplementary Table S4). Collectively, these data support that genes regulating asthma severity may be influenced by exposures through regulation of DNAm.

Asthma severity-associated DMPs correlate with gene expression and are located near TF binding sites and histone marks.

To evaluate the biological function of the 816 DMPs associated with asthma severity, we examined the correlation between DNAm and gene expression in the 17 asthmatic children from GSEP study who also had available RNA-seq data. Among these 17 asthmatics, 4 had severe asthma, 7 had mild-moderate and 6 had missing severity. The DNAm in 39 DMPs significantly correlated with mRNA in 38 genes (p<0.05, |r| ranges from 0.48-0.76, Supplementary Table S5). Twenty-three DMPs showed negative correlations and 16 showed positive correlations, suggesting a possible role of these DMPs in regulation of gene expression. Two DMPs (cg18415791 and cg03174521) were associated with the same gene, *OSBPL5*. Among these 38 genes, *ACOT7* [30], *CCR1* [34], *COL18A1* [35], *FGR* [36], *FAM13A* [37], and *PTGER4* [38] have previously been linked to asthma pathogenesis. No significant correlations were found in the CpG sites annotated to *PCDHA2* and *UCKL1*.

To better understand the potential functions of DMPs associated with asthma severity in children, bioinformatic analyses were used to identify possible transcription factor (TF) binding site and histone marks around all 816 DMPs. Using HOMER [21] and human TF binding site motifs contained in the Cis-BP database [22], we identified enriched binding sites for several TFs within a 200bp window containing identified CpG sites (Figure 4A and Supplementary Table S6), including TFs known to be involved in lung development and morphogenesis (FOXA1), linked to cytokine production and response to bacterial/viral infection (AP-1). The same TFs were also identified in a RELI analysis, which leverages available ChIP-seq datasets in cells and tissues for TFs (Figure 4B and Supplementary Table S6). Binding sites for TFs known to be highly associated with asthma, such as AP-1 (FOS, JUN, and FOSL2) [39], GATA1 [40], RUNX1 [41], GATA3 [42], and SMAD3 [43], were enriched in the RELI analysis. A similar RELI analysis for histone marks showed significant enrichment for H3K9me3, H3K4me1, and H3K27me3 histone marks (Figure 4C and Supplementary Table S6). H3K4me1 is an active histone mark associated with gene enhancers [44], and H3K9me3 and H3K27me3 are repressive histone marks often found at promoters [45]. In conclusion, our data indicate that CpG sites associated with asthma severity may function to regulate binding of particular TFs and histone modifiers, which in turn regulate asthma severity in children.

Discussion

In this study, DNAm and RNA expression in nasal epithelium of asthmatic African-American children were analyzed at a genome-wide scale. We found that 816 DMPs and 10 DMRs were associated with asthma severity. Three DMPs showed promising ability to distinguish severe from non-severe asthma. Six DMPs were found to be associated with asthma, allergy asthma, total IgE, environment IgE, and FeNO simultaneously in an independent cohort of children [30]. We also found that DMPs associated with asthma severity are associated with TRAP and SHS among asthmatic children. Additionally, some of these sites underwent DNAm changes following DEP and HDM exposures in HBECs [24]. To evaluate the possible biological function of these DNAm changes, we found that DNAm levels at 39 DMPs significantly correlated with corresponding changes in mRNA levels. There is significant enrichment of three regulatory histone marks and a number of binding sites associated with TFs known to be involved in asthma pathogenesis around these regions. Taken together, these results indicate that asthma severity may be regulated by DNAm in children and provide evidence supporting DNAm as an interface between environment factors and asthma severity. These DMPs (especially cg06654369, cg07463541, and cg19551589) could potentially be used to assess or predict asthma severity in children.

DNAm plays an essential role in the pathogenesis of many diseases, including asthma and allergy [6,7,9,10,46]. To date, lower airway biopsies are the best available sample type to study asthma-related biological processes in humans. However, it is invasive and difficult to collect, particularly in children and during asthma attacks. Additionally, DNAm is highly tissue- and cell-type specific. Recent studies showed that the methylation patterns of peripheral blood mononuclear cells and airway epithelial cells were not well correlated in asthma [14,47] and other lung diseases [48]. Nevertheless, accumulating evidence indicates that the nasal epithelium is a biologically relevant proxy for the lower airway epithelium in asthma and are better biomarkers for asthma prediction [14,15,47]. Several studies [10,14,15,30,49], consistent with our previous studies [11,12], identified significant DNAm differences in nasal epithelium between asthmatic and non-asthmatic children. Compared to other sample collection techniques, nasal epithelium collection is easy, non-invasive, and more acceptable to the parents and children. Nasal epithelium DNAm analysis represents a promising test in asthma, especially for children. However, no previous studies have yet identified DNAm variation associated with the severity of childhood asthma.

In this study, the role of nasal epithelium DNAm in asthma severity (defined as symptom frequency) was explored in AA children. Then, 816 DMPs and 10 DMRs associated with asthma severity were identified. Sixteen pathways were significantly enriched among the 398 genes associated with these DMPs. Fifty-nine genes have multiple DMPs (≥2). Several of these genes, including *LTB4R2* [50], *DPP10* [51], *IL17RA* [52], *CYFIP2* [53], *DNAH5* [54], *MRPL28* [55], and *PTPRN2* [56], are critical in different aspects of asthma. Among genes associated with the DMRs, *LTB4R2* is essential for leukotriene B4 (LTB4) synthesis. LTB4 levels in exhaled breath condensate (EBC) significantly correlated with asthma severity in children [57]. The leukotriene system is a critical pharmacological target of asthma [58]. Additionally, polymorphisms of *DPP10* were associated with asthma and related traits [59]. Zhang Y *et al* showed that HDM-induced AHR and airway inflammation in *DPP10* mutant

mice were more severe than in wild-type mice [51], providing *in vivo* evidence supporting the protective role of *DPP10* in regulation of asthma severity. Among the 816 DMPs, we also found that three DMPs had the greatest values in the prediction of asthma severity in children. High sensitivity and specificity are always desired in predictive/classification models. Our logistic regression is still a probability model, and future studies will be needed to further suggest applicable cutoff values for these three DMPs to maximize sensitivity and specificity in asthma severity classification. Additional studies are also needed to elucidate the role of genes associated with these three DMPs in asthma.

We further sought to identify DMPs associated with asthma severity that were also associated with asthma and asthma-related phenotypes in previous studies [30]. Since IgE and FeNO are critical markers for asthma phenotype assessment in clinical practices, we were excited to find six DMPs (annotated to *TMEM51*, *WDR25*, *HIPK3*, and *KLF11*) simultaneously associated with asthma severity in this study, and asthma status, allergy asthma, total IgE, environment IgE, and FeNO from a recent EWAS study using nasal DNA from children [30]. KLF11 belongs to the Sp1-like zinc finger transcription factor family, which is essential in inflammation [60] and cell growth and differentiation [61]. However, the role of KLF11 in asthma is still unclear. Collectively, the role of these genes in regulation of asthma severity needs further investigation.

It is well-known that both air pollution and SHS exposure are significant risk factors for asthma [62]. By examining DMPs associated with both asthma severity and TRAP or SHS, we identified two genes (*RBMS1* and *CPEB1*) that are possibly important in mediating the impact of exposures on asthma severity. Additionally, it is reported that HDM and DEP exposures play critical roles in the pathogenesis and exacerbation of asthma [63]. Our findings that some of the CpG sites associated with asthma severity underwent DNAm changes following exposures to DEP or HDM in HBECs support that DNAm mediates the effects of environmental exposures on the severity of childhood asthma.

We also explored possible mechanisms through which DNAm regulates gene expression and asthma severity. Through HOMER and RELI analyses, predicted and experimentally determined binding sites for several TFs that play critical roles in asthma, particularly AP-1 (FOS, JUN, and FOSL2), FOXA1 and RUNX1, were enriched in regions near CpGs associated with asthma severity. Moreover, our data identified enrichment for three regulatory histone marks associated with functional gene regulatory elements around CpG sites associated with asthma severity. Meanwhile, we found that DNAm levels of 39 DMPs significantly correlated with mRNA levels in children with RNA-seq data available. Additionally, it was reported that histone modifications were essential for the pathogenesis and progression of asthma [64,65], which regulates gene function together with or independent of DNAm. Therefore, DNAm may influence both TF binding and histone modifications to regulate gene expression and contribute to asthma severity in children. Further studies need to be performed to explore these interactions.

There were several strengths in our study. Firstly, both DNA methylation modification and gene expression in nasal epithelium from the same individual were measured. Thus, correlations between DNA methylation changes and gene expression were determined to

infer the functional impact of DNAm on gene expression. Secondly, computations analysis leveraging existing omics data was used to infer potential mechanisms through which asthma-severity associated-DNAm variation regulate gene expression. Lastly, data from relevant EWAS studies were obtained to support the relevance of our findings. While asthma severity and allergic co-morbidity were defined by symptom frequency scores rather than lung function, frequency of exacerbations, or skin prick tests, these scores have been validated[18]. Although we didn't have access to a cohort that exactly matches with our discovery cohort to replicate our findings, we were able to find validation in published studies. Data on asthma and allergy medication use was not available; this is a limitation of these datasets. Additionally, due to our limited sample size, nominal p values were used in combination with effect sizes to enhance statistical powers. Some of the analyses were performed post-hoc, such as the correlations between DNAm and RNA-seq. These put our study at a higher risk of false positive findings. Future sufficiently powered studies will be needed to study the relationships between DNAm, gene expression and asthma severity. Our findings are from participants that primarily self-reported as African American. This, on one hand, reduced confounding factors in our analysis; however, on the other hand, findings from this cohort need to be replicated in other races to make them more generalizable. Nevertheless, the association of a subset of CpG sites with clinical features relevant to asthma severity in an independent cohort of children support the involvement of these CpG sites in regulation of asthma severity. Though further investigation is warranted, our study, for the first time, showed that DNA methylation was associated with asthma severity in children, demonstrating its potential value in clinical practice and suggesting possible mechanisms that regulate gene expression in severe asthma.

Conclusions

In summary, we observed significant differences in DNAm between non-severe and severe asthmatic children, a subset of which showed promising ability to distinguish severe asthma from non-severe asthma. A small but significant portion of the CpGs associated with disease severity were associated with clinical features of asthma in an independent cohort of children, which further supports the involvement of these identified CpGs in regulation of asthma severity. Several CpG sites were also associated with asthma-related exposures, suggesting that they may play a role in mediating the impact of exposures on disease severity. These DNAm variations correlate with changes in gene expression and are predicted to modify the binding of transcription factors known to be involved in asthma. A group of asthma severity-associated genes were identified, providing novel candidate genes to be studied *in vivo* in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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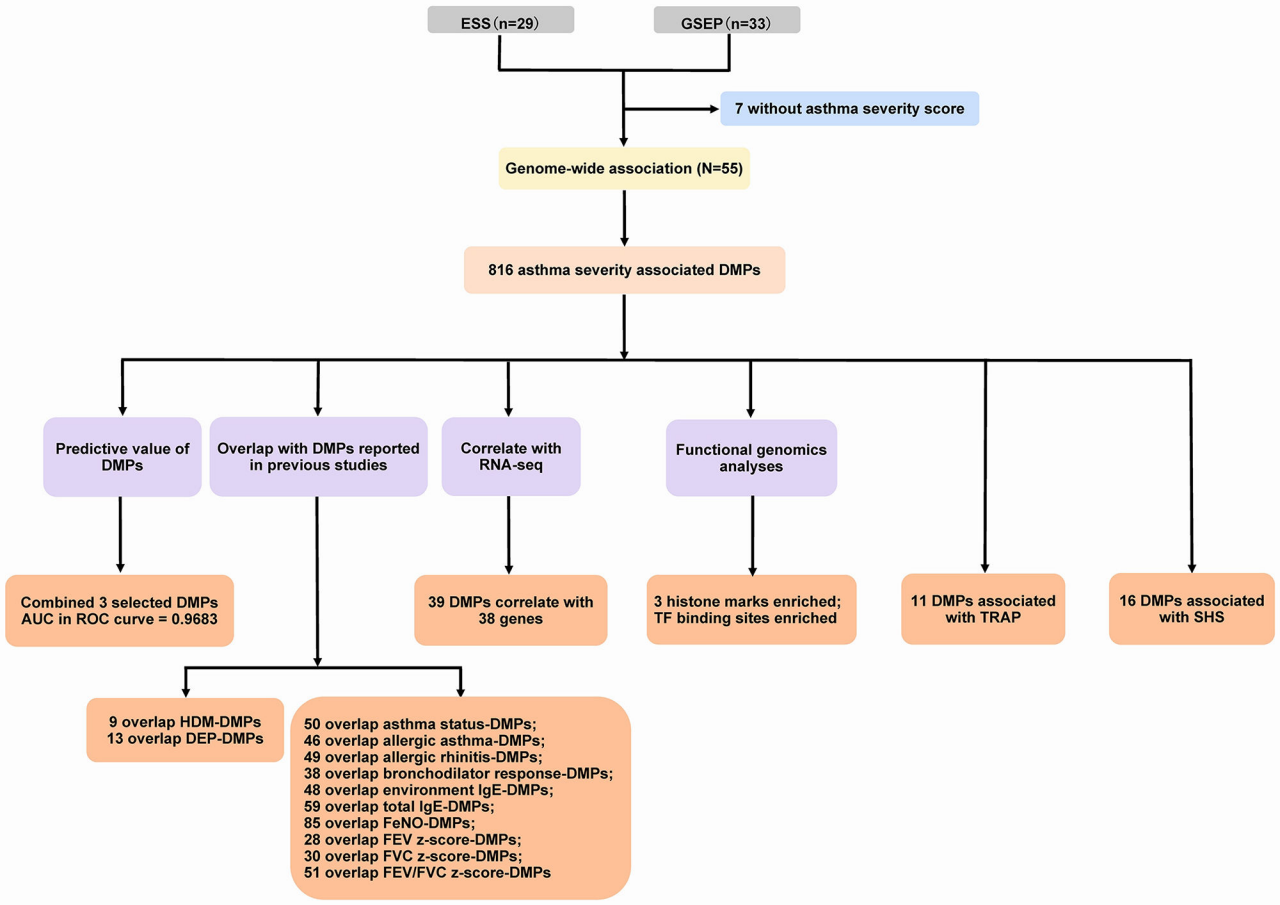

Figure 1.

Flow chart of the study. In this study, data from 55 African-American asthmatic children from ESS and GSEP were analyzed. DMPs associated with asthma severity, environmental exposures and predictive of severe asthma were identified in nasal cells. The correlation between the DNAm levels and gene expression was then explored. Enrichment for transcription factors (TFs) binding sites and histone modifications surrounding DNAm differences were determined.

Abbreviations: ESS: Exposure Sibling Study; GSEP: Genomics of Secondhand-Smoke Exposure in Pediatric Asthma Study; DMPs: Differentially methylated CpG positions; AUC: Area under the curve; ROC: Receiver operating curve; TF: Transcription factor; HDM: House dust mite; DEP: Diesel exhaust particles; IgE: Immunoglobulin E; FeNO: Fractional exhaled nitric oxide; FEV: Forced expiratory volume; FVC: Forced vital capacity; TRAP: traffic-related air pollution; SHS: Secondhand smoke

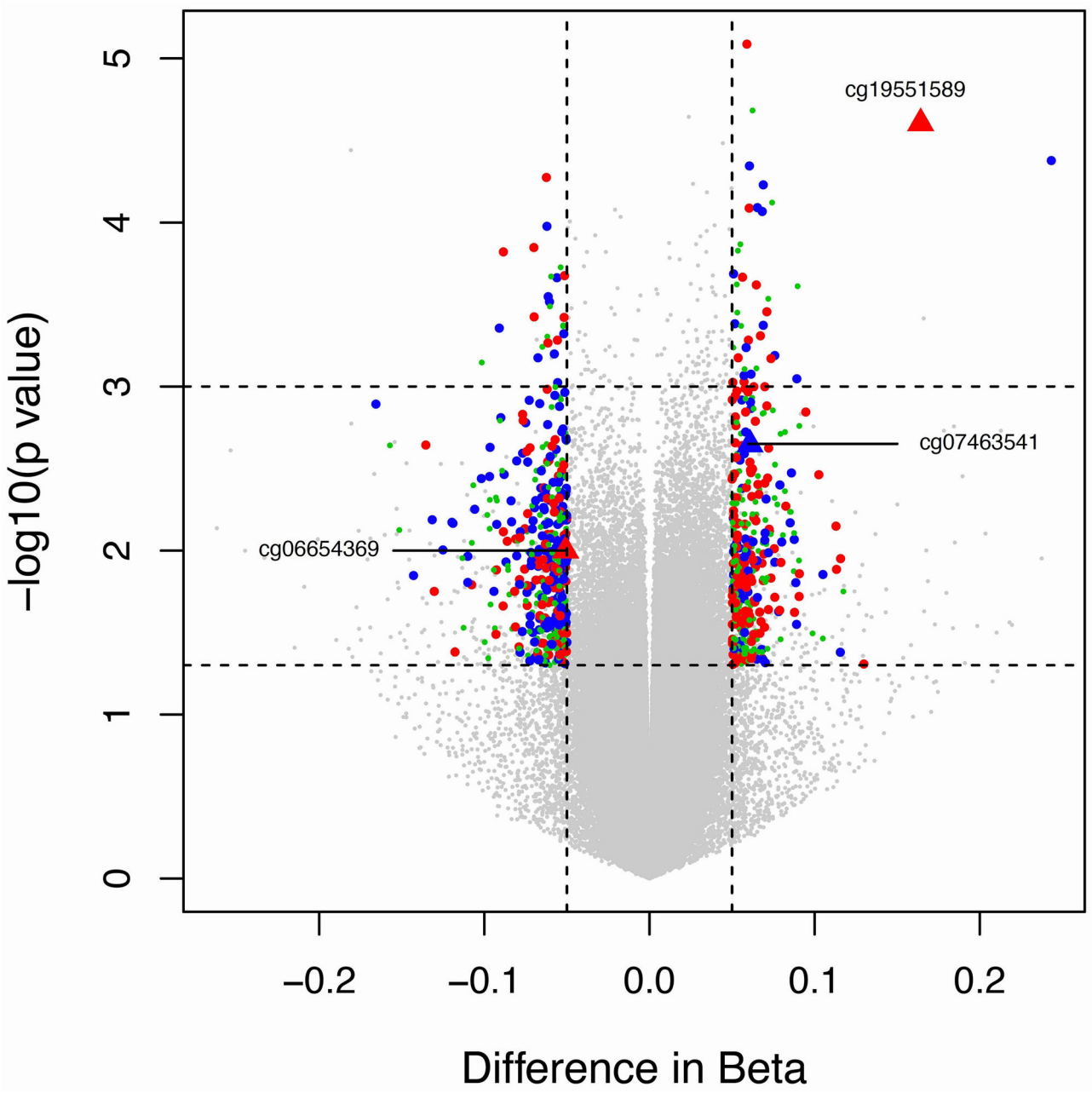

Figure 2.

DNAm is significantly different between non-severe and severe asthmatic children. Volcano plot of DMPs identified between non-severe and severe asthmatic children. X axis= severe minus non-severe. Significant DMPs are colored according to genomic locations. Blue: Island; Green: Open sea; Red: Shore and shelf. DMPs found in Figure 3 were labeled in triangles.

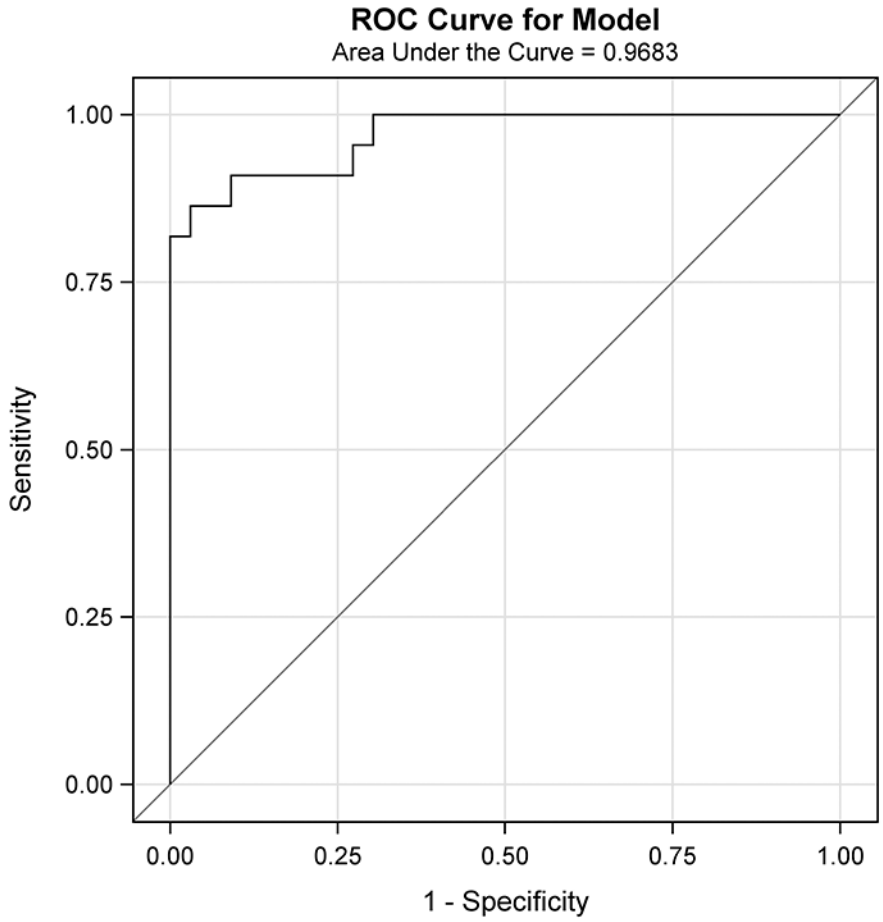

Figure 3.

ROC Curve of combined three DMPs (cg06654369, cg07463541, and cg19551589) in prediction of severe asthma in children. A probability model was used. AUC=0.9683 with predicted probability cutoff=0.68, sensitivity=0.86, specificity=0.97.

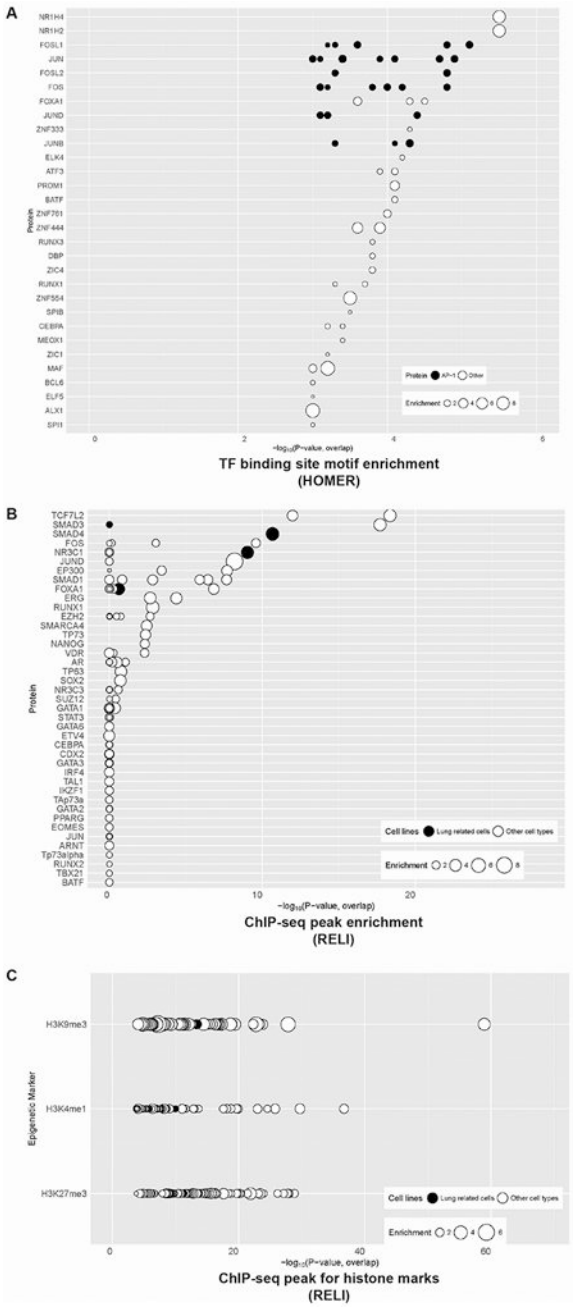

Figure 4.

Enriched regulatory protein binding events and histone marks within DMPs associated with asthma severity. (**A**) TF binding site motifs enriched within the DNA sequences flanking DMPs associated with asthma severity by HOMER. (**B**) ChIP-seq peaks enriched within the regions flanking DMPs associated with asthma severity. ChIP-seq peaks with at least one significant result (P<0.001) are shown. For figure panels in A and B, the Y-axis indicates regulatory proteins (e.g., TFs), in decreasing order of significance. The X-axis indicates the significance (-log P-value). The size of each circle indicates the fold-enrichment relative to background (see Methods). (**C**) ChIP-seq peaks for particular histone marks enriched within

the regions flanking DMPs associated with asthma severity. Histone marks with at least one significant result (P<0.01) are shown. The Y-axis indicates histone marks from experiments performed in a variety of cell types, in decreasing order of significance. The X-axis indicates the significance (-log P-value). The size of each circle indicates the fold-enrichment relative to background (see Methods).

Table 1.

Demographics and characteristics of subjects with non-severe and severe asthma

	Non-severe asthma (N=33)	Severe Asthma (N=22)	p
Age (years), [mean (SD)]	12.6 (2.4)	13.7 (3.0)	0.168
Sex			1.000
Male [n (%)]	16 (48.5)	10 (45.5)	
Female [n (%)]	17 (51.5)	12 (54.5)	
Race (self-reported)			1.000
African American [n (%)]	32 (96.9)	22 (100)	
Other [n (%)]	1 (3.1)	0 (0.0)	
SHS			*0.784
Yes [n (%)]	19 (57.6)	13 (59.1)	
No [n (%)]	14 (42.4)	8 (36.4)	
Missing [n (%)]	0 (0.0)	1 (4.5)	
Current ECAT			1.000
≥0.4 [n (%)]	11 (33.3)	7 (31.8)	
<0.4 [n (%)]	21 (66.7)	15 (68.2)	
Allergy			*0.346
Yes [n (%)]	20 (60.6)	17 (77.3)	
No [n (%)]	10 (30.3)	4 (18.2)	
Missing [n (%)]	3 (9.1)	1 (4.5)	
Allergy symptom score (median (IQR))	2 (1-2)	5 (5-7)	<0.001
ACT score [mean (SD)]	21.0 (3.0)	19.6 (3.3)	0.326

Note: Age is shown as mean (SD) and compared using t test; allergy symptom scores are compared using Wilcoxon rank sum test; other variables are shown as frequency (proportion) and compared using Fisher's exact tests.

*missing values were excluded from the Fisher's exact tests.

ESS: Exposure Sibling Study; GSEP: Genomics of Secondhand-Smoke Exposure in Pediatric Asthma Study; SHS: Secondhand smoke; ECAT: Elemental Carbon Attributable to Traffic; ACT: Asthma Control Test (only available for ESS participants)

Table 2.

Genomic distributions of DMPs between non-severe and severe asthmatic children (Severe minus Non-severe asthma).

	DMPs		
	DNAm decreased	**DNAm increased**	**p***
Island (Blue)	167 (38%)	70 (19%)	<0.001
Open sea (Green)	165 (37%)	160 (43%)	
Shore and shelf (Red)	109 (25%)	145 (39%)	
***Chi-square test**			