Epigenome-wide DNA methylation association study of circulating IgE levels identifies novel targets for asthma

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Summary

Background Identifying novel epigenetic signatures associated with serum immunoglobulin E (IgE) may improve our understanding of molecular mechanisms underlying asthma and IgE-mediated diseases.

Methods We performed an epigenome-wide association study using whole blood from Framingham Heart Study (FHS; n = 3,471, 46% females) participants and validated results using the Childhood Asthma Management Program (CAMP; n = 674, 39% females) and the Genetic Epidemiology of Asthma in Costa Rica Study (CRA; n = 787, 41% females). Using the closest gene to each IgE-associated CpG, we highlighted biologically plausible pathways underlying IgE regulation and analyzed the transcription patterns linked to IgE-associated CpGs (expression quantitative trait methylation loci; eQTMs). Using prior UK Biobank summary data from genome-wide association studies of asthma and allergy, we performed Mendelian randomization (MR) for causal inference testing using the IgE-associated CpGs from FHS with methylation quantitative trait loci (mQTLs) as instrumental variables.

Findings We identified 490 statistically significant differentially methylated CpGs associated with IgE in FHS, of which 193 (39.3%) replicated in CAMP and CRA (FDR < 0.05). Gene ontology analysis revealed enrichment in pathways related to transcription factor binding, asthma, and other immunological processes. eQTM analysis identified 124 *cis*-eQTMs for 106 expressed genes (FDR < 0.05). MR in combination with drug-target analysis revealed *CTSB* and *USP20* as putatively causal regulators of IgE levels (Bonferroni adjusted P < 7.94E-04) that can be explored as potential therapeutic targets.

Interpretation By integrating eQTM and MR analyses in general and clinical asthma populations, our findings provide a deeper understanding of the multidimensional inter-relations of DNA methylation, gene expression, and IgE levels.

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Keywords: EWAS; DNA methylation; IgE; Asthma; RNA-Sequencing; Mendelian randomization; Lung; eQTM; Drug targets

Abbreviations: immunoglobulin E, IgE; epigenome-wide association study, EWAS; Framingham Heart Study, FHS; Childhood Asthma Management Program, CAMP; Genetic Epidemiology of Asthma in Costa Rica Study, CRA; expression quantitative trait methylation loci, eQTMs; Mendelian randomization, MR; methylation quantitative trait loci, mQTLs; experimentally-derived Functional element Overlap analysis of ReGions from EWAS, eFORGE

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Research in context

Evidence before this study

Several molecular studies of asthma and related conditions have been conducted in the last few decades. The latest efforts are focusing on DNA methylation, which is one of the most widely studied epigenetic marks and can serve as an informative biomarker for asthma susceptibility. Many of the previous epigenome-wide DNA methylation association studies report on the relations of gene expression or DNA methylation to asthma and allergic rhinitis. A few of them have studied IgE, however, none used Mendelian randomization (MR) to infer causal relations between IgE-associated methylation in conjunction with gene expression and asthma or allergic disease. We searched PubMed for studies using different combinations of the search terms: asthma AND IgE AND DNA methylation AND gene expression (yielded 18 studies) OR asthma AND IgE AND DNA methylation AND transcriptome (yielded 6 studies) OR asthma AND IgE AND mendelian randomization (yielded 8 studies) OR asthma AND IgE AND gene expression AND mendelian randomization (yielded 1 study) OR asthma AND IgE AND DNA methylation AND Mendelian randomization (no studies) OR asthma AND IgE AND DNA methylation and gene expression AND mendelian randomization (no studies). None of the identified studies performed a multidimensional data driven analysis integrating epigenetic and transcriptomic as well as genetic information. Seven of the eight studies that applied mendelian randomization were limited to studying genetic variants for asthma and other IgE-mediated diseases.

Added value of this study

Our robust, extensive, and multidimensional analytical strategy leverages a discovery and replication approach using multiple omic datasets from three different cohorts and fills a crucial knowledge gap by integrating not only epigenetic and transcriptomic but also genetic information providing additional insight into causal pathways and gene regulatory mechanisms of IgE. In addition to 124 IgE-associated *cis*-CpG-transcript pairs, we identified associations with 12 druggable candidate gene targets that had at least one approved drug for treatment of asthma or for other respiratory conditions. Of those, *CTSB* and *USP20* can be explored as potential therapeutic targets.

Implications of all the available evidence

Our data-driven multifaceted study builds upon prior knowledge of IgE regulation by providing a deeper understanding of the relationship between DNA methylation, gene expression, and circulating IgE levels. To date, none of the previously published epigenetic studies of IgE integrated both epigenetics and transcriptomics with Mendelian randomization analyses in the general population and in people with asthma. By integrating these multiple perspectives in combination with druggable gene target analysis, our findings can inform future functional studies, suggest new avenues for novel pharmaco-epigenetic interventions and potential epigenetic-based drug therapies for asthma and other IgE-mediated diseases.

Introduction

Immunoglobulin E (IgE) is a class of antibodies produced by the immune system usually in response to allergen exposures.[1] In addition to providing immune protection, IgE is a key mediator in the pathogenesis of allergic diseases, most notably asthma, atopic dermatitis, and allergic rhinitis.[2–6] Thus, it is critical to better understand the molecular mechanisms underlying the regulation of IgE to facilitate improved drug therapies for the prevention and treatment of IgE-associated diseases.

In recent years, there have been numerous efforts to examine if and how epigenetic mechanisms contribute to complex disease pathology.[7–9] Several epigenome-wide association studies (EWASes) have identified associations between serum IgE and DNA methylation at CpG sites located in genes that are either known or hypothesized to be involved in allergic inflammatory responses, asthma, and other immune system diseases.[10–13]

Despite the increasing number of CpGs identified in recent IgE EWAS, there are no studies to date that have examined the association between IgE-associated DNA methylation and gene expression (i.e., expression quantitative trait methylation loci; eQTMs). The majority of previous EWAS findings focused on asthma and rhinitis, not on IgE.[11,12,14,15] Additionally, there are no studies that used Mendelian randomization (MR) to infer causal relations between IgE-associated methylation and diseases such as asthma, allergy, or related conditions. We designed a study that integrates EWAS, eQTM and MR analyses to conduct a robust DNA methylation analysis of IgE.

To that end, we first performed an EWAS of IgE in 3471 Framingham Heart Study (FHS) participants to identify significant differentially methylated CpGs associated with IgE in a general population. We replicated our results using two independent external studies (the Genetic Epidemiology of Asthma in Costa Rica Study (CRA) and the Childhood Asthma Management Program (CAMP)). Second, we conducted two-sample MR testing using prior UK Biobank genome-wide association study (GWAS) data[16] to determine the direction of effect and infer causal relations between DNA methylation and asthma. Third, we analyzed associations between IgE-associated CpGs and expressed gene transcripts. Last, using established bioinformatic approaches, we highlighted biologically plausible pathways and drug targets underlying the regulation of IgE.

Methods

This study was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE, Supplementary Information STROBE checklist table) reporting guidelines.[17]

FHS methods

Study population

A flowchart of the study design is displayed in Fig. 1. FHS is a community-based study that began in 1948 and now has three generations of participants.[18–20] The study sample consisted of 3471 individuals/adults from the FHS Offspring (n = 2082) and Third Generation (n = 1389) cohorts, in whom IgE levels and DNA methylation were measured from the blood samples. The study protocol received approval by the Institutional Review Board at Boston University Medical Center (Boston, MA, Protocol number: H33525). All study participants gave their informed consent for genetic research.

DNA methylation and its association with IgE levels

Using HumanMethylation450 BeadChips (Illumina, USA), genome-wide DNA methylation data was measured on FHS Offspring and Third Generation participants. For details on data quality control and filtering, see the Methods section in the Supplementary Information. A linear mixed model implemented in the *lmekin()* R package[21] was used to analyze associations between serum $\log_{10}$ IgE concentration (IU/mL) as the predictor and DNA methylation betas as the outcome after adjusting for age, sex, cigarette smoking status, pack-years smoked, technical covariates including batch effects, Houseman method[22] imputed cell types (including CD8+ T-lymphocytes, CD4+ T-lymphocytes, natural killer cells, B-lymphocytes, monocytes, and neutrophils), and family structure. A secondary analysis was conducted after adjusting for eosinophils since eosinophil count is considered as residing in the causal pathway of IgE.[6] To account for multiple testing, the

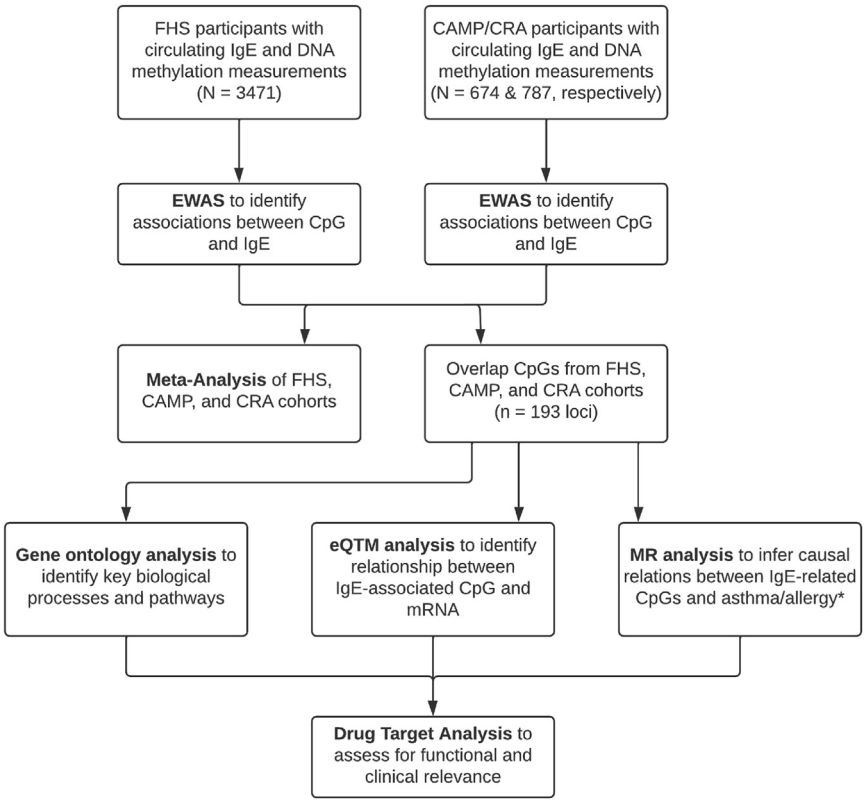

Fig. 1: Flowchart of overall study design in discovery and replication cohorts. The study design shows the cohorts, the sample sizes and shared CpG loci. For these analyses, cohorts included the Framingham Heart Study (FHS), The Childhood Asthma Management Program (CAMP) and The Genetic Epidemiology of Asthma in Costa Rica Study (CRA) that were utilized for EWAS and meta-analysis. The overlapping CpGs between all three cohorts were subjected to pathway, eQTM, MR, Drug-target and eFORGE analyses for functional and clinical relevance. EWAS, epigenome-wide association study; eQTM, expression-methylation quantitative trait loci; MR, mendelian randomization; eFORGE, experimentally-derived Functional element Overlap analysis of ReGions from EWAS.

Benjamini and Hochberg method of False Discovery Rate (FDR) was used.[23]

Association between DNA methylation and gene expression

Association tests of DNA methylation and absolute counts of gene expression data as assessed by RNA sequencing were performed in 1045 FHS participants from the Third Generation after adjusting for age, sex, complete blood cell components (WBC, RBC, platelets, neutrophils, lymphocytes, monocytes, and basophils), and technical covariates.[24] Benjamini and Hochberg method was used to account for multiple testing.[23] A secondary post-hoc analysis was also conducted adjusting for eosinophils since eosinophil count is considered as residing in the causal pathway of IgE.[6] We used *cis*-CpG-mRNA pairs defined as a CpG residing ±500 kB of the transcription start site of the corresponding gene encoding the mRNA (*cis*-eQTM), in which the CpG is in association with IgE in EWAS. A liftOver procedure to transform the data from the hg19 to hg38 genome build was conducted using the liftOver Bioconductor package in R.[25]

CAMP and CRA methods

Study populations

CAMP is a multi-center, double blind randomized clinical trial in 1041 children of age 5–12 years with mild-to-moderate asthma[26] from December 1993 to September 1995. In this analysis, we included 674 children who had IgE measurements and DNA methylation data. The collection of peripheral blood for DNA extraction and total serum IgE occurred during the closeout visits in 1999[27,28]; results of the follow-up phase have been previously published.[27,29–32] CAMP was approved by the Institutional Review Boards of Brigham and Women's Hospital (BWH, Protocol number: 1999-P-001549/29), the Partners Human Research Committee at BWH (PHRC; Protocol number: 2002P000331, ClinicalTrials.gov Identifier: NCT00000575) and other participating centers. Written informed consent was obtained from parents of participants and assent was obtained from children.

CRA is a cross sectional study of 1165 children 6–14 years of age between 2001 and 2008 with mild-to-moderate asthma.[33,34] This study includes 787 children with IgE measurements and DNA methylation data. The study was approved by the Partners Human Research Committee at BWH (Boston, MA; Protocol number: 2000-P-001130/55) and the Hospital Nacional de Niños (San José, Costa Rica).

DNA methylation and its association with IgE levels

Genome-wide DNA methylation data was generated as part of the NHLBI Trans-Omics for Precision Medicine (TOPMed) consortium using the standard protocol for the Human Methylation 850K EPIC microarray BeadChips (Illumina, USA), as implemented at the University of Southern California Methylation Characterization Center. For details on data quality control and filtering, see the Methods section in the Supplementary Information. A multivariable linear regression model implemented in the *limma*[35] package was used to analyze single CpG site associations between $\log_{10}$IgE (IU/mL) as the predictor and DNA methylation as the outcome. We used *DMRCate*[36] with default settings to identify differentially methylated regions (DMRs) associated with $\log_{10}$IgE levels. The resulting regions overlapping in the same direction of effect between CAMP and CRA were subjected to the Genomic Regions Enrichment of Annotations Tool (GREAT) web server for region-based enrichment analysis.[37,38] Stouffer's FDR of 5% was chosen as a threshold for multiple testing corrections which is a summary transformation based on z-scores of the individual CpG FDRs.[39] We additionally applied a threshold of ≥5 CpGs within the associated region to present the most robust set of regions. All models were adjusted for age at blood draw, sex, smoke exposure, maternal asthma, first two surrogate variables for batch variable sample plate and estimated cell type fractions (CD8+ T-lymphocytes, CD4+ T-lymphocytes, natural killer cells, B-lymphocytes, monocytes, and neutrophils). We additionally adjusted for self-reported race (African Americans, Whites, Hispanics, Others) in CAMP. Since treatment with inhaled corticosteroids (ICS) is the main therapy used for the management of moderate to severe asthma and allergic diseases, as a sensitivity check, analyses were also evaluated after adjusting for other potential confounders like asthma severity measured by hospitalization visits and medication use measured by ICS use in CRA and CAMP. All analyses were subjected to multiple testing corrections and performed in R using R[40]/Bioconductor.[25]

Mendelian randomization and in-silico analyses for functional and clinical relevance

Identified genes were mapped to pathways, disease terms and gene-sets for functional network visualization using R/Bioconductor package *clusterProfiler*.[41] Experimentally-derived Functional element Overlap analysis of ReGions from EWAS[42] (eFORGE) tool was used to explore enrichment for cell and tissue type specific histone methylation or chromatin states using data from the Roadmap Epigenetics Consortium. To further understand the enrichment results from eFORGE,[43,44] we performed pathway analysis on the probes that showed tissue-specific enrichment signal for lung and blood using gProfiler R package.[45] An inverse variance weighted (IVW) meta-analysis was conducted using the metagen() function in the R package Meta[46] on overlapping IgE associations. We chose a random-effects model because of the heterogeneity in the population demographics of the FHS (mean age = 57.9 years) in comparison with CRA and CAMP (mean

ages = 12.9 years and 9.3 years respectively). $P < 1 \times 10^{-7}$ was chosen as a Bonferroni threshold to account for the large number of tests used for the CpGs (0.05/number of tests ~450,000). Two-sample MR was used to test if DNA methylation drives asthma/allergy (i.e., SNPs → CpG → asthma/allergy) and identify putatively causal CpGs for both asthma and allergic diseases using the *MRbase* package in R[47] (details in Supplementary Information). Estimated associations and effect sizes between SNPs as instrumental variables and asthma and allergic diseases were based on published UK Biobank GWAS of asthma and allergic disease phenotypes (hay fever, allergic rhinitis, or eczema).[16] We explored approved or experimental drugs targeting the overlapping set of CpGs among FHS, CAMP, and CRA using the *rDGIdb* R package,[48] an R wrapper for The Drug Gene Interaction Database; and CLUE app in the ConnectivityMap suite from the Broad Institute.[49]

Role of the funding source

The funders had no role in study design, data collection, analyses, or interpretation of the data and results presented in this study.

Population	FHS	CAMP	CRA
Number, n	3471	674	787
Age (y), mean (SD)	57.9 (13.4)	12.9 (2.1)	9.3 (1.9)
Female, n (%)	1874 (46)	266 (39)	320 (41)
Log_{10}IgE (IU/mL, SD)	1.52 (0.58)	2.3 (0.6)	2.5 (0.7)
Presence of Asthma, n (%)	201 (5.8)	674 (100)	787 (100)
BMI (mean kg/height in m^2, SD)	27.4 (5.2)	21.5 (4.7)	18.4 (3.8)
Smoking, n (%)			
Tobacco exposure	NA	265 (39.3)	240 (30.5)
Current smoker	420 (12.1)	NA	NA
Former smoker	1413 (40.7)	NA	NA
Race, n (%)			
Hispanic	NA	64 (9.5)	787 (100)
Caucasian white	3439 (99.1)	467 (69.3)	NA
African American	NA	85 (12.6)	NA
Other	32 (0.9)	58 (8.6)	
Lung Function			
FEV1-predicted, % (SD)	96.0 (14.7)	94.3 (14.2)	98.3 (17.0)
FVC-predicted, % (SD)	100.2 (13.0)	105.6 (12.8)	105.1 (16.2)
Ratio FEV1/FVC, mean (SD)	75.3 (7.2)	77.9 (8.9)	93.7 (8.3)

n, number of subjects; y, years; SD, standard deviation, BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; Tobacco exposure in CRA, Maternal smoking in Pregnancy or Smoking during infancy; Tobacco exposure in CAMP, Home exposure prior to enrollment (either mom or dad smoked).

Table 1: **Participant characteristics of the FHS, CAMP, and CRA cohorts.**

Results

EWAS of IgE levels

The clinical characteristics of the FHS, CAMP, and CRA participants are presented in Table 1 and in more detail in Supplementary Table S1. After quality control, a total of 415,318 CpGs were retained for further analysis[50] in the FHS cohort; 490 CpG sites were associated with total IgE concentration (at FDR threshold of 5%, Supplementary Table S2). A Q-Q plot (lambda 1.31) of the FHS EWAS results is provided in Supplementary Data S1a. After adjusting for eosinophil count, fewer significant loci were identified (25 CpGs, Supplementary Table S3). Eosinophil count is linked with IgE levels and was considered as residing in the causal pathway,[6] therefore the primary EWAS analysis did not adjust for it.

Among the 865,859 CpG sites measured on the 850K EPIC array platform, 785,352 sites in CAMP and 790,798 sites in CRA remained after quality control. Of those, we identified 14,223 IgE-associated CpG sites in CAMP and 21,801 IgE-associated CpGs in CRA; 10,426 CpGs overlapped between both cohorts mapping to 4195 unique genes (at FDR threshold of 5%, Supplementary Table S4). All shared CpG associations demonstrated the same direction of effect with 8397 CpGs hypo-methylated and 2029 CpGs hyper-methylated relative to increasing IgE levels in both cohorts. A Q-Q plot (lambdas: 1.13 for CAMP, 1.33 for CRA) for the EWAS results is provided in Supplementary Data S1b. We further identified 2408 and 3685 significant DMRs (Stouffer's FDR < 0.05) in CAMP and CRA respectively. Based on exact CpG site coordinates (chromosome, start, and end) and the same direction of effect, we identified 1271 hypo-methylated and 229 hypo-methylated regions overlapping between both cohorts that were enriched in mostly immune response and activation pathways (Supplementary Data S2). Interestingly apoptotic signaling and tissue remodeling were enriched for hypo-methylated regions whereas the aging biological process was specific to the hyper-methylated regions (Supplementary Data S2). At a threshold of ≥5 CpGs within the associated region, 214 highly significant and robust DMRs remained; 182 were hypo-methylated and 32 were hyper-methylated that overlapped between CAMP and CRA cohorts (Supplementary Table S5).

Replication

We investigated the genomic site-based overlap between the significant IgE-associated CpGs from FHS, CAMP, and CRA cohorts (Fig. 2). Of note, among the 490 FHS IgE-associated CpGs, 458 (93%) sites are included on the 850k platform. This number decreased to 439 among the tested sites for both CRA and CAMP. Therefore, 51 sites were not included on both platforms. Of note, we identified 193 CpGs[51] (43.9%) from the 439 CpG set (hypergeometric P = 4.2E-181) shared between the three cohorts with same direction of effect, demonstrating the robustness of replication findings. These included 185 hypo-methylated and eight hyper-methylated CpGs in relation to IgE (Supplementary Table S6); the top 20 based on significance in FHS are

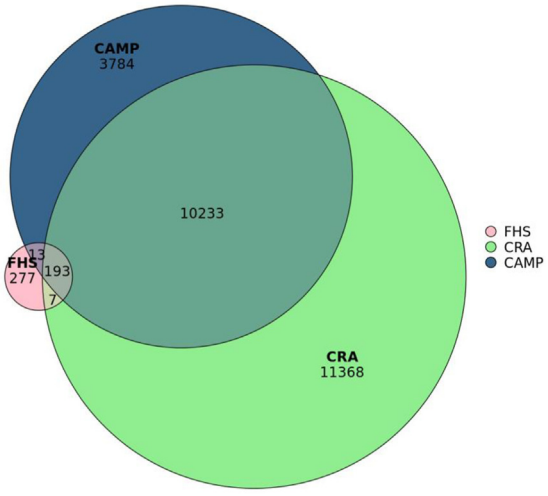

Fig. 2: Euler venn diagram showing the 193 shared DNA methylation CpG sites between FHS, CAMP, and CRA cohorts. These overlapping CpGs were significant in all three cohorts at an FDR threshold of 5%.

presented in Table 2; the top five CpGs were annotated to the genes *NDFIP2, ZFPM1, ACOT7,* and *KSR1*. Interestingly, 147 (76%) of these 193 shared CpGs were robust even after the Bonferroni correction for multiple testing in CAMP (0.05/785,352 total CpGs = 6.4×10^{-8}) and CRA (0.05/790,798 total CpGs = 6.3×10^{-8}) which provides strong evidence supporting the significant associations. Additionally, these shared associations remained robust after adjusting for potential confounders including hospitalization visits and inhaled steroid use in CRA (100% of 193 CpGs) and CAMP (99% of 193 CpGs).

Gene ontology and disease enrichment analyses highlight functional implications

The 193 overlapping CpGs between FHS, CRA, and CAMP mapped to 144 unique genes that were explored for gene ontology and disease enrichment, over-representation, and pathway analysis. The most enriched gene ontology terms (FDR < 0.05, Supplementary Table S7) included RNA polymerase II transcription factor binding (annotated genes: *HDAC1, SPI1, CREBBP, RERE, FBP1, ZFPM1*) and protein serine/threonine kinase activity (annotated genes: *SIK2, PRKCH, LTBP4, KSR1, PAK6, CAB39, CLK3, TNIK, PRKCZ, ENG, DAPK2*). The functions of the genes implicated in transcription factor binding are displayed in Table 3 eFORGE revealed enrichment of histone methylation (H3K36me3, H3K4me1, and H3K4me3) including in fetal thymus, lung, skin, and blood data (Supplementary Data S3, FDR < 0.01), corroborating potential impacts on transcription. We further performed pathway analysis on the probes that showed the enrichment in lung and blood data to understand the tissue-specific context of these findings. There was an overlap of 124 CpGs between blood (n = 159) and lung (n = 132) specific sites and both were enriched in KEGG pathways for Asthma and glycerophospholipid metabolism (Supplementary Table S8). However, among the genes mapped to asthma pathway, *IL4* was unique to blood suggesting that the DNA methylation associated with IgE levels may have systemic effects.

The most enriched terms from the gene-disease associations (Fig. 3, Supplementary Table S7) included allergic asthma (annotated genes: *EZR, MCC, IL13, SIGLEC8, IL4, IL5RA, INPP4A, EPX, SLC25A25, ZFPM1*), allergic rhinitis (annotated genes: *PVT1, IL13, HDAC1, IL4, LBR, NFIA, RERE, EPX, EZH2, MIR202*), and asthmatic pulmonary eosinophilia (annotated genes: *IL13, SIGLEC8, IL4, IL5RA, EPX*). Furthermore, the genes mapped to the hypo-methylated CpGs were specifically enriched for the asthma KEGG pathway (at FDR threshold of 1%), annotated genes: *PRG2, IL4, IL13, EPX*, Supplementary Table S7).

Given the young age and highly atopic nature of CAMP and CRA participants, secondary analyses were performed exclusively using these two cohorts. Based on a genome-wide threshold, there were 2865 CpGs annotated to 1546 unique genes overlapping between CRA and CAMP subjected to over-representation and pathway analyses. This analysis resulted in several clusters of functionally related and enriched biological processes including neutrophil degranulation, myeloid cell differentiation, regulation of hemopoiesis, and leukocyte differentiation (Supplementary Data S3 and Table S9). The phosphatidylinositol signaling system was the top KEGG pathway enriched specifically for the genes mapped to hypo-methylated sites (Supplementary Table S10).

Expression quantitative trait methylation and MR analysis

Analysis of the expression of genes associated with the 193 replicated IgE-associated CpGs (eQTMs) identified 124 *cis*-eQTMs (0.05/193 = Bonferroni adjusted P-value < 2.59×10^{-4}, Supplementary Table S11). These mapped to 84 unique CpG sites; the top 25 are shown in Table 4. The scatterplots for the top six CpG-transcript pairs are presented in Supplementary Data S5. Adjusting for eosinophil count revealed 32 *cis*-eQTMs (Bonferroni adjusted P-value < 2.59×10^{-4}) mapping to 18 unique CpGs (Supplementary Table S12). As noted above, the reduction in significant signals is expected when adjusting for eosinophils as they likely reside in the causal pathway for IgE.

We conducted secondary two-sample MR tests to infer causal relationship between IgE-related methylation and asthma. Among the 193 replicated IgE-associated CpGs, there were 63 suitable *cis*-mQTLs available for analysis. We identified three CpGs mapping to *CTSB*, *USP*20, and *CIRH1A* that were putatively causal for asthma (0.05/63 = Bonferroni adjusted P-

value < 7.94×10^{-4}) (Supplementary Table S13). Both *CTSB* and *USP20* were implicated as overlapping genes of interest in eQTM and MR testing.

Meta-analysis of IgE-associated methylation from FHS, CAMP, and CRA cohorts

Meta-analysis from all three cohorts revealed 1548 CpG sites ($P < 1.0 \times 10^{-7}$) considering random effects (Supplementary Table S14), while the meta-analysis of CAMP and CRA revealed 11,078 sites ($P < 1.0 \times 10^{-7}$) considering fixed effects at a genome-wide threshold. eFORGE for the top 1000 CpG site associations based on significance from the meta-analysis of all three cohorts also revealed enrichment of histone methylation (H3K36me3, H3K4me1 and H3K4me3) and enhancers in fetal tissues, thymus, intestine, pancreas, skin, lung, and blood data included in eFORGE (Supplementary Data S6, at FDR threshold of 1%).

Identification of druggable gene targets

Among the 144 genes annotated to the 193 replicated CpGs among the three cohorts, 35 mapped to 595 drug compounds from multiple drug database sources using *rDGIdb*. In comparison, ConnectivityMap identified 68 drug compounds mapping to 13 of the 144 genes. Twelve genes had drug targets identified by both methods; nine of those (*KCNH2, HDAC1, PRKCZ, IGF1R, CTSB, PRKCH, TNIK, IL4, IL5RA*) had at least one approved drug for treatment of asthma or candidate drug for other respiratory conditions. The findings are summarized in Supplementary Table S15.

Discussion

Epigenetic insights into atopy present an opportunity for innovation toward primary prevention of IgE-related diseases and asthma across the life course. As the rates of asthma and health care costs continue to increase each year,[52–55] it is imperative to explore the regulatory mechanisms underlying IgE production so that improved drug therapies and interventions can be developed. To that end, we performed an EWAS of IgE and identified 193 significant CpG loci that were shared among CAMP, CRA, and FHS participants.

Given the highly atopic nature of CAMP and CRA participants, these shared CpGs may represent a subset of CpGs that is differentially methylated in association with serum total IgE concentration in both atopic and non-atopic populations. The top five CpGs from the EWAS analysis annotated to the *NDFIP2, ZFPM1, ACOT7,* and *KSR1* genes have previously been linked to the pathogenesis of asthma or to other immune functions. For example, both *ZFPM1* (Zinc Finger Protein, FOG Family Member 1) and *ACOT7* (Acyl-CoA Thioesterase 7) are associated with asthma and have been implicated as top findings in previous EWAS of IgE.[9,12,56] *ZFPM1* down-regulates expression of interleukin 4

CGsite	CHR	MAPINFO	Gene	Island	RefGene Group	Results in FHS			Results in CAMP			Results in CRA		
						Estimate	95% CIs	FDR	Estimate	95% CIs	FDR	Estimate	95% CIs	FDR
cg09862509	1	181,069,576		OpenSea		-0.17	-0.22, -0.13	3.23E-10	-0.02	-0.02, -0.01	3.85E-11	-0.01	-0.01, -0.004	0.0009
cg11770323	13	80,066,032	*NDFIP2*	OpenSea	Body	-0.16	-0.20, -0.12	1.92E-07	-0.02	-0.02, -0.02	8.11E-18	-0.02	-0.03, -0.02	4.26E-12
cg04983687	16	88,558,223	*ZFPM1*	Island	Body	-0.17	-0.23, -0.12	2.76E-05	-0.06	-0.07, -0.05	3.94E-33	-0.05	-0.06, -0.04	4.53E-18
cg21220721	1	6,341,230	*ACOT7*	Island	Body	-0.18	-0.23, -0.12	2.76E-05	-0.06	-0.07, -0.05	3.94E-33	-0.04	-0.05, -0.03	3.95E-16
cg26423824	17	25,898,825	*KSR1*	OpenSea	5′UTR	-0.15	-0.20, -0.11	2.76E-05	-0.02	-0.02, -0.01	3.32E-11	-0.01	-0.01, -0.01	2.66E-08
cg06315149	1	2,036,398	*PRKCZ*	N_Shelf	5′UTR	-0.14	-0.19, -0.10	3.71E-05	-0.02	-0.03, -0.02	1.63E-17	-0.02	-0.02, -0.01	1.13E-08
cg12077460	8	8,702,053	*MFHAS1*	OpenSea	Body	-0.14	-0.18, -0.09	6.79E-05	-0.03	-0.01, -0.005	2.41E-19	-0.02	-0.01, -0.002	4.13E-10
cg03660377	16	368,048	*AXIN1*	OpenSea	Body	-0.14	-0.18, -0.09	6.79E-05	-0.01	-0.03, -0.02	0.0001	-0.005	-0.03, -0.01	0.01
cg13645296	15	64,275,810	*DAPK2*	OpenSea	Body	-0.13	-0.18, -0.09	8.17E-05	-0.03	-0.04, -0.03	1.62E-21	-0.03	-0.03, -0.02	1.22E-13
cg06957316	5	131,993,853	*IL13*	S_Shore	TSS200	-0.12	-0.17, -0.08	0.0001	-0.02	-0.02, -0.01	4.02E-13	-0.01	-0.02, -0.01	5.74E-09
cg16522484	14	95,942,049	*C14orf49*	OpenSea	1stExon	-0.14	-0.18. -0.09	0.0001	-0.01	-0.02, -0.01	1.79E-06	-0.01	-0.02, -0.01	2.11E-06
cg19928703	13	30,143,971	*SLC7A1*	OpenSea	5′UTR	-0.15	-0.20. -0.10	0.0001	-0.02	-0.02, -0.02	9.97E-22	-0.02	-0.02, -0.01	2.30E-12
cg18333787	19	930,871	*ARID3A*	N_Shore	Body	-0.15	-0.20. -0.10	0.0001	-0.02	0.02, -0.02	1.05E-20	-0.01	-0.02, -0.01	1.02E-10
cg12614529	4	154,269,418	*MND1*	S_Shelf	Body	-0.14	-0.18, -0.09	0.0002	-0.03	-0.04, -0.03	3.97E-23	-0.02	-0.03, -0.02	8.83E-10
cg02475695	16	616,220	*NHLRC4*	S_Shore	TSS1500	-0.13	-0.18, -0.09	0.0002	-0.02	-0.02, -0.01	1.36E-14	-0.01	-0.02, -0.01	5.95E-10
cg16409452	14	100,610,186	*EVL*	N_Shore	3′UTR	-0.15	-0.20. -0.10	0.0002	-0.02	-0.02, -0.01	2.08E-10	-0.01	-0.02, -0.01	1.09E-08
cg16597993	19	16,578,633	*EPS15L1*	N_Shelf	Body	-0.15	-0.20. -0.10	0.0002	-0.02	-0.02, -0.01	3.16E-20	-0.01	-0.02, -0.01	2.00E-09
cg14480531	17	55,700,565	*MSI2*	OpenSea	Body	-0.13	-0.17. -0.08	0.0002	-0.03	-0.03, -0.02	9.98E-26	-0.02	-0.02, -0.01	2.42E-09
cg13576859	9	97,403,129	*FBP1*	S_Shore	TSS1500	-0.12	-0.16. -0.08	0.0003	-0.03	-0.04, -0.03	4.62E-22	-0.02	-0.03, -0.02	6.52E-11
cg17374802	17	56,270,828	*EPX*	N_Shelf	Body	-0.14	-0.20. -0.09	0.0003	-0.03	-0.03, -0.02	5.90E-18	-0.02	-0.02, -0.01	1.19E-10
cg06866208	16	67,560,245		N_Shelf		-0.12	-0.17, -0.08	0.0004	-0.02	-0.02, -0.01	1.15E-16	-0.01	-0.02, -0.01	5.85E-08

CHR: chromosome; FDR: false discovery rate at 5%; the estimate column shows the effect estimates of all these CpGs and they are hypo-methylated in all three cohorts with increase in IgE levels.

Table 2: **Top 20 IgE-associated CpGs from the overlap between FHS, CAMP and CRA (FDR adjusted P-value < 0.05). The list is sorted by the significance in FHS.**

Gene	Function
HDAC1	catalyze acetyl group removal from lysine residues in histones and non-histone proteins
SPI1	encode an ETS-domain transcription factor that activates gene expression during myeloid and B-lymphoid cell development
CREBBP	involved in the transcriptional coactivation of many different transcription factors
RERE	acts as a transcriptional repressor during development; may play a role in the control of cell survival
FBP1	first identified as a DNA-binding protein that regulates c-Myc gene transcription through binding to the far upstream element
ZFPM1	transcription regulator that plays an essential role in erythroid and megakaryocytic cell differentiation

Table 3: **Genes implicated in transcription factor binding from gene ontology enrichment analysis using clusterProfiler Bioconductor R package (n = 6).**

(IL-4) and up-regulates expression of IFN-γ in CD4+ T cells, thereby reducing Th2 cell differentiation.[57] *ACOT7* contributes to the inflammatory response via production of arachidonic acid and prostaglandins.[10] *NDFIP2* (Nedd4 Family Interacting Protein 2), an activator of Nedd4 Family ubiquitin ligases, restricts the accumulation and function of effector T cells, thereby limiting inappropriate T-cell responses.[58] *KSR1* (Kinase Suppressor of Ras 1) is a scaffolding protein that contributes to T-cell response sensitivity by regulating mitogen-activated protein kinase (MAPK) activation and signaling in T cells.[59]

Subsequent gene ontology analysis revealed that many loci were involved in biological processes associated with transcription factor binding. We have previously identified DNA methylation of transcription factors in a lung tissue EWAS.[60,61] In this study, we identified *HDAC1, SPI1, CREBBP, RERE, FBP1,* and *ZFPM1* as the top genes implicated in transcription factor binding pathways. Of particular interest is the gluconeogenic enzyme *FBP1* (Fructose-Bisphosphatase 1) as it was recently shown to aggravate oxidative stress-induced apoptosis in bronchial epithelial cells by suppressing the nuclear factor erythroid-derived 2-related factor 2 (Nrf2) pathway, which is protective in asthmatic patients by maintaining the structure of the bronchial epithelial barrier.[62] *RERE* (Arginine-Glutamic Acid

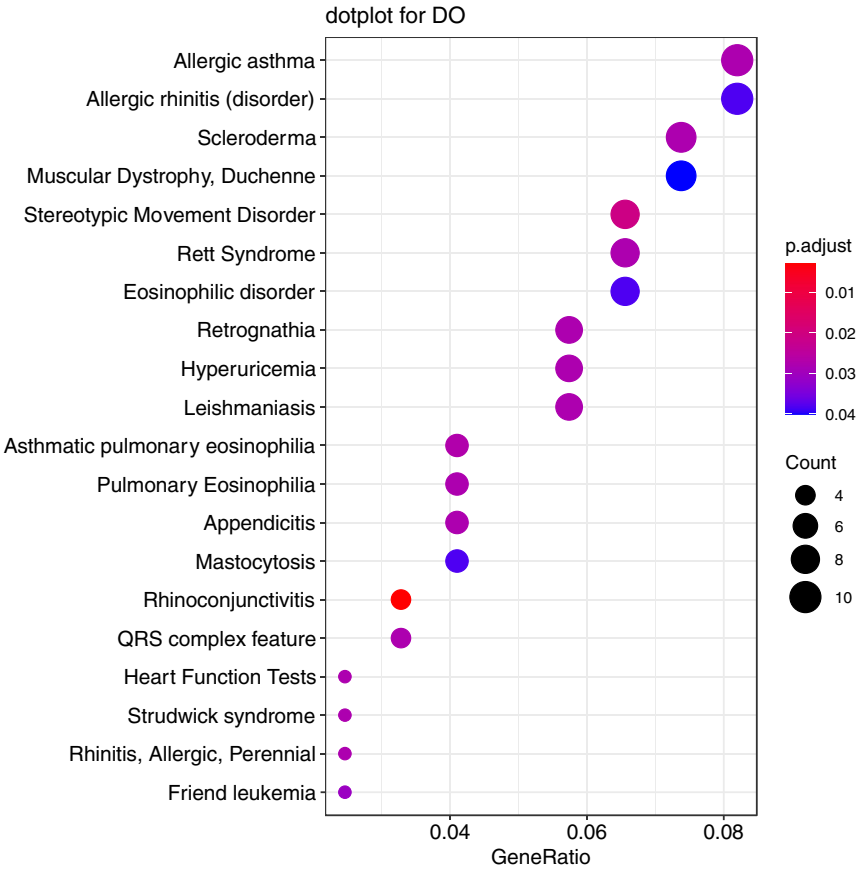

Fig. 3: Gene-disease associations for the overlapping set (n = 193 CpGs) between FHS, CRA and CAMP cohorts. The enriched terms are sorted based on the gene-overlap of the input set with the term set as represented by their size and the count key. The color key corresponds to the FDR adjusted P-value threshold of 5%.

CpG	CHR	CpG_start	Tx_Start	Tx_Gene	DNAm_Gene	Beta	95% CI	P-value	Difference (kb)
cg25087851	11	60,856,445	60,850,933	*PTGDR2*	*GPR44*	−15.83	[−17.62, −14.04]	6.78E-59	−5512
cg10159529	3	3,110,846	3,066,326	*IL5RA*	*IL5RA*	−18.98	[−21.21, −16.75]	3.60E-55	−44520
cg02427831	19	51,458,683	51,450,847	*SIGLEC8*	*SIGLEC8*	−16.90	[−19.43, −14.38]	1.83E-36	−7836
cg25087851	11	60,856,445	60,841,806	*AP000777.3*	*GPR44*	−3.29	[−3.80, −2.77]	5.16E-33	−14639
cg18927901	6	36,270,266	36,697,826	*RAB44*	*PNPLA1*	−9.19	[−10.72, −7.65]	8.57E-30	427,560
cg20315954	17	15,233,987	15,229,777	*PMP22*	*PMP22*	−8.76	[−10.34, −7.18]	5.43E-26	−4210
cg13054523	17	83,097,977	83,104,255	*AC144831.1*		−6.85	[−8.11, −5.60]	2.20E-25	6278
cg16362140	5	10,708,605	10,564,070	*ANKRD33B*	*DAP*	−5.05	[−6.04, −4.07]	7.33E-23	−144,535
cg22503106	17	83,083,030	83,104,255	*AC144831.1*	*METRNL*	−4.42	[−5.28, −3.56]	8.32E-23	21,225
cg24678767	15	74,610,635	74,409,289	*SEMA7A*	*CLK3*	−7.59	[−9.08, −6.09]	2.56E-22	−201346
cg08077807	14	61,534,354	61,556,313	*LINC01303*	*PRKCH*	−7.70	[−9.22, −6.18]	3.98E-22	21,959
cg10555106	1	3,774,823	3,812,086	*CEP104*	*LOC388588*	−2.91	[−3.52, −2.31]	2.73E-20	37,263
cg20290167	17	83,082,848	83,104,255	*AC144831.1*	*METRNL*	−4.28	[−5.19, −3.36]	2.99E-19	21,407
cg19247032	21	42,366,618	42,311,667	*TFF3*	*TFF1*	−5.84	[−7.13, −4.54]	5.46E-18	−54951
cg18927901	6	36,270,266	36,737,050	*Z85996.1*	*PNPLA1*	−2.66	[−3.27, −2.06]	2.59E-17	466,784
cg13953978	9	129,838,509	129,827,290	*C9orf78*	*USP20*	−5.72	[−7.03, −4.41]	4.55E-17	−11219
cg10159529	3	3,110,846	3,039,033	*CNTN4-AS1*	*IL5RA*	−2.58	[−3.18, −1.99]	6.63E-17	−71813
cg21919729	8	11,861,858	11,842,524	*CTSB*	*CTSB*	−3.28	[−4.06, −2.50]	4.83E-16	−19334
cg27376514	17	17,155,108	17,200,995	*PLD6*	*MPRIP*	−4.28	[−5.30, −3.26]	7.33E-16	45,887
cg25689499	17	83,084,678	83,104,255	*AC144831.1*	*METRNL*	−4.51	[−5.61, −3.40]	3.57E-15	19,577
cg05480350	8	127,960,435	127,794,533	*PVT1*	*MIR1205;PVT1*	−3.75	[−4.71, −2.78]	6.33E-14	−165,902
cg06315149	1	2,104,959	2,549,920	*TNFRSF14-AS1*	*PRKCZ*	−3.82	[−4.84, −2.79]	5.95E-13	444,961
cg11770323	13	79,491,897	79,481,124	*NDFIP2*	*NDFIP2*	−3.19	[−4.08, −2.31]	2.34E-12	−10773
cg01942646	1	26,914,203	27,005,020	*TENT5B*	*NR0B2*	−2.81	[−3.59, −2.02]	4.20E-12	90,817
cg09212118	1	42,164,855	42,380,792	*RIMKLA*	*GUCA2A*	−2.31	[−2.97, −1.66]	7.43E-12	215,937

Table 4: **Top 25 *cis*-eQTMs non-adjusted for eosinophils from overlapping CpG set of FHS, CAMP, and CRA (n = 124 total). The difference is calculated as the distance from the CpG site upstream of the transcript (gene).**

Dipeptide Repeats) plays a role as a transcriptional repressor during development and was recently identified as a potentially new therapeutic target for asthma.[63] The transcription factor *SPI1* (Spi-1 Proto-Oncogene) is involved in activating gene expression during the development of T-cells, B-cells, dendritic cells, and monocytes, and has been identified as a therapeutic target for COPD.[64] As a component of the histone deacetylase complex, *HDAC1* (Histone Deacetylase 1) plays a critical role in epigenetic silencing. One study found elevated *HDAC1* expression in severe asthmatic patients as compared to healthy controls.[65] As such, HDAC inhibitors (HDACi) are increasingly recognized as therapeutic targets for asthma and other allergic diseases[66,67] to target immune and inflammatory pathways.[68] Overall, a deeper examination of the differential DNA methylation of transcription factors will facilitate a better understanding of the interplay of the environment, heritability, and immune biology on complex phenotypes such as asthma and allergic diseases.[56] One area for further research includes the modeling of both DNA methylation of transcription factors and their binding motifs using epigenetic extensions of gene regulatory network approaches.[69] It is known that such DNA methylation of CpG sites in genes encoding transcription factors can modulate transcription factor binding to DNA.[70,71] We do not have the ability, however, to evaluate DNA methylation in transcription factors; this should be a priority for future studies.

The disease enrichment analysis highlighted allergic asthma, allergic rhinitis, and asthmatic pulmonary eosinophilia. Genes that mapped to these diseases included interleukin *(IL)-4, IL-13*, and *EPX*. *IL-4* and *IL-13* are related cytokines known to play a role in the type II inflammatory response. One of the regulatory roles of *IL-4* is to activate B-cells and drive the process of immunoglobulin class switching to IgE.[72] *IL-13* is an effector cytokine, regulating smooth cell muscle contraction and mucus production in airway epithelium, such as that found in allergic asthma.[72] *IL13* is an important gene linked to other highly IgE dependent diseases like atopic dermatitis.[6] *EPX* (Eosinophil peroxidase) is a unique biomarker of airway eosinophilia, which is often found in cases of asthma.[73] Further, *PRG2* (pro eosinophil major basic protein)—which mapped to the hypo-methylated CpGs, was enriched for the asthma KEGG pathway, and was also identified by eQTM analysis—is associated with eosinophilia and therefore may play a role in the pathogenesis of asthma.[74] Notably, other disease terms related to neurodevelopmental, autoimmune, or skin disorders involve inflammatory pathways and an imbalance in the

dopaminergic and cholinergic system.[75] These pathways also affect lung disease outcomes and the respiratory muscles that maintain pulmonary function.[76]

One of the major challenges of large-scale epigenetic studies is to not only understand the molecular mechanisms, but also uncover genetic versus epigenetic influences and environmental drivers for disease risk. Recently, the largest multi-ethnic GWAS on total serum IgE identified several known allergic disease loci as well as new regions in chr11q13.5 and chr15q22.2.[77] However, this study provided limited power to detect small effects or large number of significant associations.[77] Our results of the MR analyses especially in conjunction with the eQTM findings provide further integrated evidence and support for putatively causal regulators of asthma. The eQTM component allowed us to identify the effects of the top loci on the expression of nearby transcripts. The top eQTM genes included *PTGDR2, IL5RA,* and *SIGLEC8*. While *PTGDR2* expression levels could be used to identify asthma patients,[78] targeting *SIGLEC8* expression may treat mast cell and eosinophil-driven asthma.[79,80] A longitudinal study of serum IgE levels and *IL5RA* expression identified associations with childhood asthma but not with persistent wheeze.[81,82] Targeting methylation life-course trajectories from early life to adulthood is another opportunity to epigenetically program IgE-mediated hypersensitivity during fetal development. For example, we have previously identified an association between several cord blood and mid-childhood DNA methylation marks and mid-childhood IgE concentration.[83] Some of those methylation marks mapped to the same genes as we report in our study here including *ACOT7, EPX, KSR1, ZFPM1, IL4, PRG2*.[83]

Few integrative studies have identified CpGs through overlapping expression and methylation QTLs, which do not yet have well-understood associations with asthma or immune functioning, and thus represent potential therapeutic targets.[10,84–86] Given the limited prior studies examining eQTMs in relation to asthma, our study provides additional insight into a gene regulatory mechanism of IgE.[14] In combination with MR testing using one of the largest and most comprehensive asthma GWAS, we identified *CTSB* and *USP20* as potential novel genes of interest. *CTSB* overexpression is often associated with immune cell infiltration and correlated with hypomethylation in different cancer subtypes and could represent a promising target for further assessment in relation to respiratory outcomes.[87,88] Likewise, *USP20* is a transcriptional activator and regulator of the Wnt/beta-catenin signaling pathway that has been implicated in several cancers but never in relation to asthma and merits more research.[89,90] The druggable target analysis further identified many compounds in use or under investigation in clinical trials and only proves crucial to enhance clinical relevance of our findings as targets of epigenetic interventions. For example, *HDAC1* regulates many pathways through epigenetic mechanisms and is the target for many drugs.[91] Bortezomib was annotated to *CTSB*, and its long-term use reduces allergen specific IgE in mice.[92] Quercetin is an aurora kinase inhibitor; its administration produces improvement in frequency of asthma attacks and was annotated to both *PRKCH* and *PRKCZ*.[93] Similarly, Tozasertib annotated to *PRKCZ* suppresses mast cell activation.[94] Hesperadin, a Th2 cytokine antagonist was annotated to *TNIK* and effectively reduces IgE production and eosinophil infiltration in allergic asthma lungs in mice.[95] Lastly, benralizumab annotated to *IL5RA* is an approved drug to prevent eosinophilic and severe asthma.[96,97] Considering these findings, identifying inexpensive, innovative, and convenient treatment options could benefit patients with IgE related diseases. Drug repurposing or repositioning combined with other 'omes' including gene expression, metabolomics, or proteomics to characterize small molecule biomarkers and electronic health records could be an effective approach in the coming years to mitigate the growing prevalence of asthma and allergic diseases.[98] Our approach of identifying drug-gene interactions is one such strategy that could prove cost effective especially for targeting drugs in related health outcomes, already approved by the Food and Drug administration (FDA) or under investigation in clinical trials.[99,100]

Although our study provided key novel insights, there are some limitations. First, the serum IgE concentration and blood DNA methylation was measured approximately six years apart in FHS participants. These time differences may weaken the observed association between IgE levels and methylation. We also acknowledge that CAMP and CRA have younger participants compared to FHS participants, which is a primarily healthy adult population and that could bolster the generalizability of the findings. Additionally, CRA is highly atopic in nature and may have predisposition to produce IgE in response to allergen exposure and parasitic infections, however the prevalence of parasitic infections in CRA is extremely low,[101] therefore we believe this would not have a huge impact on the findings. We also observed associations for a subset of CpG sites on EPIC array platform that were not present on the 450K platform. Therefore, only overlapping probes could be used for the analysis which may have contributed to differences in power to detect some CpG signals. Previously, it was reported that around 10% of the CpGs from the 450K array were underperforming and therefore were no longer included on the new EPIC array.[102] Hence, we think that this would add strength to our robust findings between both platforms. Further, our MR analysis had limitations. We did not have the genetic data for IgE available at the time of initial analyses, therefore for this study, the two outcomes used were asthma and allergic diseases leveraging the published UK Biobank GWAS data, which we hypothesized

would be clinically significant and relevant given that asthma and allergic disease are IgE-mediated hypersensitivities. Hence, the identified cis-mQTLs may not directly reflect the causal relationship between DNA methylation and asthma and could partly explain the underpowered MR findings. In addition, further investigations are needed to exclude the confounding due to horizontal pleiotropy associated with secondary traits or other mediators. We were unable to conduct these tests conclusively due to insufficient SNP numbers. Finally, we recognize presence of possible unmeasured confounders in these cohorts. While CAMP is an RCT of low dose ICS, we addressed this partially by showing that the IgE-DNA methylation associations remained significant even after adjusting for measures of asthma severity and ICS use in our primary EWAS analyses in CRA and CAMP and did not affect the shared CpG associations between the three cohorts. While the approaches implemented can account for part of this confounding, ultimately the use of rigorous pharmacoepidemiologic studies are required to delineate these effects more clearly. Realistically, confounding mechanisms can rarely be accounted for with certainty, however we hope that the measured covariates and potential confounders in these cohorts that we adjusted for are likely the best proxies of the true underlying confounding mechanisms.

In conclusion, our study builds upon prior knowledge of IgE regulation by providing a deeper understanding of the complex interplay among DNA methylation, gene expression, and circulating IgE levels across different populations. Moreover, our findings could be followed up with potential experimental and pharmacological validation in future studies. To date, none of the previously published EWAS of IgE have integrated both eQTM and MR analyses in the general population and in people with asthma. By integrating these multiple perspectives, our findings could not only serve as targets for novel pharmacoepigenetic interventions but also open new avenues for potential epigenetic-based drug therapies for asthma and other IgE-mediated diseases.

Contributors

D.L.D., D.L., G.T.C., S.T.W., G.O.C., D.V.D.B, P.K. were responsible for data collection, curation, conceptualization, funding acquisition, resources and supervision in this study. P.K., K.R., T.H., R.J., G.Y.L, H.B., D.H.L, J.G., C.Y., S.H. developed the methodology, performed the data processing, visualization and formal analysis. Writing - original draft, K.R., P.K. wrote the manuscript. D.L., D.L.D verified the underlying data, performed the review and provided critical feedback on the manuscript. All authors read and approved the final version of the manuscript. The TOPMed consortium facilitated the DNA methylation data generation using Infinium® MethylationEPIC 850K BeadChip.

Data sharing statement

All TOPMed data is person-sensitive, however it can be requested for access and can be made available through the TOPMed consortium after careful review and approval by the TOPMed Data Access Committee (https://topmed.nhlbi.nih.gov/). Participant consent and Data Use Limitations differs within and across TOPMed studies and should be requested individually. Additional documentation, such as of local IRB approval and/or letters of collaboration with the primary study PI(s) may be required. The FHS DNA methylation datasets analyzed in the current study are available at the database of Genotypes and Phenotypes (dbGaP) repository (phs000007. v32. p13) here: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v33.p14. The CAMP DNA methylation datasets analyzed in the current study are available at the dbGaP repository (phs001726. v2. p1) here: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001726.v2.p1. The CRA DNA methylation datasets analyzed in the current study are available at the dbGaP repository (phs000988. v5. p1) here: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000988.v5.p1.

All code for data processing and analyses are available via GitHub at: https://github.com/CDNMBioinformatics/IgE_eBioMedicine; https://github.com/robbyjo/GPU_eQTL.

Declaration of interests

DLD has received grants from Bayer and honoraria from Novartis. STW has received royalties from UpToDate and served on the Board of Histolix, a digital pathology company. Rest authors declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104758.

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