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Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing preferentially affects enhancer regions

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Original Article Topics:	Asthma and Lower Airway Disease
News and Views Topics:	
Keywords:	epigenetics, asthma, pediatrics, epidemiology
Abstract:	**Background:** Childhood asthma is a result of a complex interaction of genetic and environmental components causing epigenetic and immune dysregulation, airway inflammation and impaired lung function. Although different microarray based EWAS studies have been conducted, the impact of epigenetic regulation in asthma development is still widely unknown. We have therefore applied unbiased whole genome bisulfite sequencing (WGBS) to characterize global DNA-methylation profiles of asthmatic children compared to healthy controls. **Methods:** Peripheral blood samples of 40 asthmatic and 42 control children aged 5-15 years from three birth cohorts were sequenced together with paired cord blood samples. Identified differentially methylated regions (DMRs) were categorized in genotype-associated, cell-type-dependent, or prenatally-primed. Network analysis and subsequent natural language processing of DMR-associated genes was complemented by targeted analysis of functional translation of epigenetic regulation on the transcriptional and protein level. **Results:** In total, 158 DMRs were identified in asthmatic children compared to controls of which 37% were related to the eosinophil content. A global hypomethylation was identified affecting predominantly enhancer regions and regulating key immune genes such as *IL4, IL5RA*, and *EPX.* These DMRs were confirmed in n=267 samples and could be linked to aberrant gene expression. Out of the 158 DMRs identified in the established phenotype, 56 were perturbed already at birth and linked, at least in part, to prenatal influences such as tobacco smoke exposure or phthalate exposure. **Conclusion:** This is the first epigenetic study based on whole genome sequencing to identify marked dysregulation of enhancer regions as a hallmark of childhood asthma.

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Point-by-point response

Date: 21st December 2022
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Title of Article (revised): Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing preferentially affects enhancer regions
(Originally submitted title: Genome wide DNA-methylation sequencing identifies massive enhancer reprogramming in childhood asthma)
Name of the Corresponding Author: Prof. Dr. Irina Lehmann
Email Address of the Corresponding Author: irina.lehmann@bih-charite.de

Dear Zuzana Diamant,

Hereby, we would like to resubmit our revised manuscript. We thank the reviewers once again for their valuable comments and for their critical examination of our manuscript. We hope that we have now been able to fully address all points of criticism.

In the following, please find the specific responses to the issues raised by the reviewers.

Response to Reviewer #1:

MINOR COMMENTS

Comment 1: *For Mann-Whitney U test, it is useful to calculate the effect size. Glass rank bi-serial correlation coefficient (rg) is the appropriate method of obtaining effect size for Mann-Whitney U test.*

Reply 1:

The Glass rank biserial correlation coefficient is recommended for calculating the effect size for Mann-Whitney U (MWU) tests with an ordinal and a two-level nominal variable. In our case, the MWU was applied to metric variables (methylation beta-values or cellular composition) and a two-level nominal variable. Therefore, we report the point biserial correlation coefficient r_{bp} instead of r_g.

Figure 2C and Figure S5B were amended accordingly, as was Table S8 and the corresponding section in the main text:

"We observed an enhanced eosinophil frequency in the blood of asthmatic children (Mann-Whitney U test: Z=3.42, r_{pb}= 0.32, p= .017, Table S8), but not for the remaining cell types, i.e. B cells, T cells, monocytes, NK cells or neutrophils."

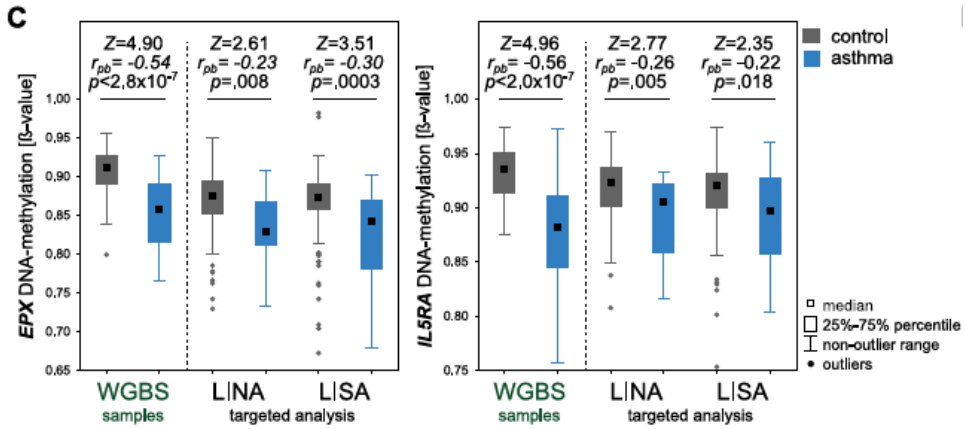

Figure 2 (C) DNA-methylation difference between asthmatic children and controls of the WGBS samples (asthma n=40, controls n=42), LINA study (asthma n=19, controls n=108) and LISA study (asthma n=25, controls n=115) for DMRs related to EPX and IL5RA as determined by sequencing or MassARRAY, respectively (p-value from Mann-Whitney U-test, rpb: point biserial correlation coefficient).

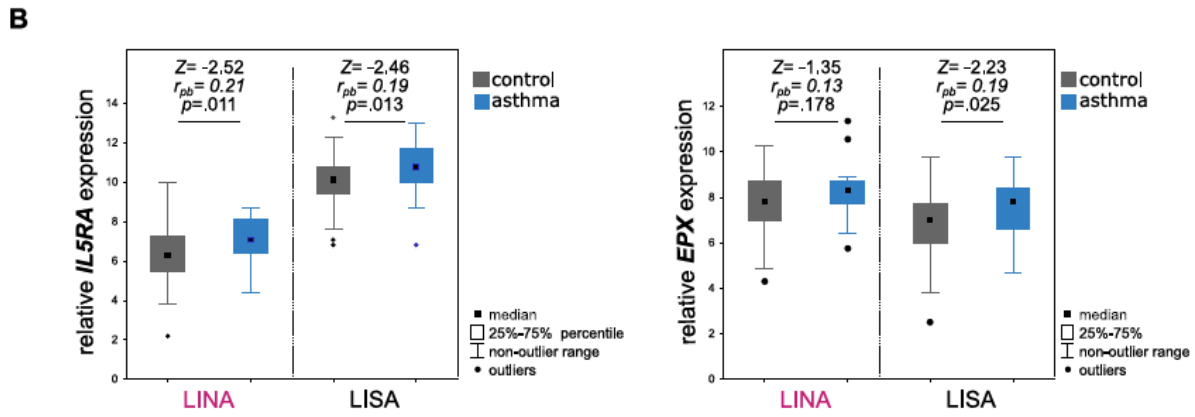

Figure S5 (B) Relative gene expression of IL5RA and EPX in asthmatic children and controls participated in LINA (asthma n=19, controls n=107) or LISA (IL5RA: asthma n=25, controls n=115, EPX: asthma n=25, controls n=113). p-value from Mann-Whitney U-test, rpb: point biserial correlation coefficient.

Excerpt of Table S8 indicating Mann Whitney U-test statistics comparing controls (n=42) vs. asthma (n=40) samples.

Cell type	control vs. asthma		
	Z	*p*-value	r_{pb} correlation coefficient
B cell	-0,37	0,715	0,057
NK	-1,03	0,316	0,056
T cell	1,1	0,273	-0,133
monocytes	-0,09	0,93	-0,012
neutrophils	-0,29	0,771	0,018
eosinophils	-3,43	0,017	0,317

Response to Reviewer #2:

Authors have adequately addressed the comments in the revised version of the manuscript. I have no further comments.

Reply 1:

We thank the reviewer for the time and effort taken to reevaluate our manuscript.

Response to Reviewer #3:

The authors have significantly improved their paper based on the extensive comments of the reviewers. There remain some limitation, but these have now adequately been addressed and discussed. I have no major comments.

MINOR COMMENTS

Comment 1: *Add the reference of SNPscore to the paper*

Reply 1:

We added the corresponding reference (The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. Nature, 578: 82–93 (2020). DOI 10.1038/s41586-020-1969-6).

Comment 2: *IN table 1, child's sex, there is a type : Female should read 50 % but now states 0 %*

Reply 2:

We thank the reviewer for pointing this out. We corrected the typo.

Comment 3: *Part of the Supplemental table is still partly printed and appears to be out of page bounds (i.e. Table S3, from page PDF 183 onwards). Please verify this in the final version.*

Reply 3:

All supplementary tables were provided as an Excel file. Unfortunately, they were still compiled in the pdf document, which made them partly unreadable. We apologize for this inconvenience.

Sincerely,

Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing preferentially affects enhancer regions

Short title: The epigenetic landscape of asthma.

Loreen Thürmann[1#], Matthias Klös[1#], Sebastian D. Mackowiak[2#], Matthias Bieg[2#], Tobias Bauer[3], Naveed Ishaque[2], Marey Messingschlager[1], Carl Herrmann[4], Stefan Röder[5], Mario Bauer[5], Sascha Schäuble[6,7], Erik Faessler[6], Udo Hahn[6] , Dieter Weichenhan[8], Oliver Mücke[8], Christoph Plass[8,9], Michael Borte[10], Erika von Mutius[9,11,12] , Gabriele I. Stangl[13], Roger Lauener[14], Anne M. Karvonen[15], Amandine Divaret-Chauveau[16,17,18], Josef Riedler[19], Joachim Heinrich[9,20,21], Marie Standl[9,21], Andrea von Berg[22], Beate Schaaf[23], Gunda Herberth[5], Michael Kabesch[24#], Roland Eils[2,4,9#], Saskia Trump[1#], Irina Lehmann[1,9#*]

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#equal contribution

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Conflict of Interest Declaration:

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All other authors declare no conflict of interest.

ABSTRACT

Background: Childhood asthma is a result of a complex interaction of genetic and environmental components causing epigenetic and immune dysregulation, airway inflammation and impaired lung function. Although different microarray based EWAS studies have been conducted, the impact of epigenetic regulation in asthma development is still widely unknown. We have therefore applied unbiased whole genome bisulfite sequencing (WGBS) to characterize global DNA-methylation profiles of asthmatic children compared to healthy controls.

Methods: Peripheral blood samples of 40 asthmatic and 42 control children aged 5-15 years from three birth cohorts were sequenced together with paired cord blood samples. Identified differentially methylated regions (DMRs) were categorized in genotype-associated, cell-type-dependent, or prenatally-primed. Network analysis and subsequent natural language processing of DMR-associated genes was complemented by targeted analysis of functional translation of epigenetic regulation on the transcriptional and protein level.

Results: In total, 158 DMRs were identified in asthmatic children compared to controls of which 37% were related to the eosinophil content. A global hypomethylation was identified affecting predominantly enhancer regions and regulating key immune genes such as *IL4, IL5RA*, and *EPX*. These DMRs were confirmed in n=267 samples and could be linked to aberrant gene expression. Out of the 158 DMRs identified in the established phenotype, 56 were perturbed already at birth and linked, at least in part, to prenatal influences such as tobacco smoke exposure or phthalate exposure.

Conclusion: This is the first epigenetic study based on whole genome sequencing to identify marked dysregulation of enhancer regions as a hallmark of childhood asthma.

Key words:

asthma, cord blood, DNA-methylation, prenatal exposure

INTRODUCTION

Asthma is the most common chronic inflammatory disease in childhood. With an estimated prevalence of asthma ranging from 2.6% to 30.5%[1] varying according to the age and origin of the children, childhood asthma is a major health concern worldwide. Over the last decades, the prevalence of childhood asthma increased in a majority of countries worldwide, which has been mainly attributed to an interaction of genetic predisposition with a changing environment and a Westernized lifestyle[1,2]. Although the etiology of pediatric asthma remains incompletely understood, its origin is thought to be found early in life[3]. There is a larger number of studies supporting the notion that asthma-related immune alterations are already established during the prenatal development phase when the maturation of the immune system begins[4]. Although the molecular mechanisms initiating and maintaining these aberrant immune functions are largely unknown, epigenetic mechanisms are thought to play a central role in not only mediating the adverse effects of an intrauterine environment but also in preserving the established asthma-promoting phenotype[4]. However, the knowledge of asthma-related epigenetic modifications is limited and no genome-wide studies at a single base-pair resolution are available. So far, DNA-methylation changes in asthma, have been described based on target-specific analyses or on DNA-methylation microarrays[5-9] covering 27,000-850,000 CpG sites of the approximately 28 million CpGs of the human genome.

To date, several childhood asthma-associated DNA-methylation changes at single CpG sites located in immune regulatory genes such as *ALOX12*, *IL13*, and *RUNX3*, or genes involved in arachidonic acid metabolism, T cell differentiation, and IgE production, have been described in whole blood samples[7,10]. In addition, more than 100 differentially methylated sites were identified by array-based epigenome-wide association studies (EWAS) on respiratory cells, such as buccal cells or epithelial cells of the nasopharynx, amongst others CpGs in the close vicinity of established asthma-associated genes, such as *ZFPM1*, *NLRP3*, *IFNGR2*, *NTRK1*, or

ALOX15[11-13]. However, all of the current EWAS on asthma are biased by the pre-selection of CpG sites covered by the commercially available DNA-methylation arrays.

The genomic localization of DNA-methylation changes is critical for their functional impact on gene expression and associated relevance to the disease phenotype. Perturbations in regulatory regions, and in particular enhancers regulating multiple genes, are assumed to drive disease progression[14]. Enhancers are not commonly in close vicinity of their target gene, but rather may be located several thousands of base pairs away[15]. Although previous studies of asthma-associated DNA-methylation changes provided valuable information on CpG sites potentially contributing to disease etiology and suggested an enhancer-centric epigenetic dysregulation[9], a plethora of enhancer elements have since been identified that are not covered by DNA-methylation arrays and thus have previously escaped analysis. Even with the advanced EPIC array only 7% of distal and 27% of proximal ENCODE regulatory elements, and less than 4% of all CpGs of the genome are represented[16].

As a consequence of this limited genomic coverage of previous methylation array studies only little is known about enhancer dysregulation in childhood asthma. To overcome this knowledge gap, this study used a different approach and determined the unbiased global DNA-methylation profile at a single-base pair resolution by applying whole-genome bisulfite sequencing (WGBS). Whole blood samples of 40 asthmatic children from three independent prospective birth cohorts were compared to 42 sex- and age-matched controls. It is well known that the methylation of adjacent CpG sites is mutually dependent[17] and regional changes in DNA-methylation are assumed to be functionally more relevant than single CpG positions[18]. Thus, we determined differentially methylated regions (DMRs) rather than reporting methylation changes at single CpG positions and subsequently confirmed our findings by targeted methylation analyses in larger number of cases that included subjects from two of the three cohorts. The comprehensive assessment of the genomic distribution of the DMRs was complemented by elucidating the functional consequences of aberrant DNA-methylation

associated with key immune modulating genes. To this end, cord blood - available for a subset of the children - provided the opportunity to assess potential prenatal priming of the DNA-methylation changes identified in asthmatic children.

METHODS

Detailed information can be found in the Online Supplement.

Study characteristics

This study comprises data and samples derived from the three different birth cohorts LINA[19], LISA[20], and PASTURE[21]. A detailed cohort description can be found in the Online Supplement. Participation in all three cohort studies was voluntary and written informed consent was given by the parents or children if applicable. The studies were approved by their respective ethics committees (LINA: 046-2006, 160-2008, 160b/2008, EK-BR-02/13-1, 169/13ff, 150/14ff, LISA: 398-12-05112012, PASTURE: 02046, 9/11-E1/651-2002, 415-E401/4-2007).

Asthma outcome

Asthma was defined based on the confirmative answer to the question: "Has a physician-diagnosed your child with asthma during the last 12 months (=current asthma)?" asked in the parent-reported questionnaires at the time-point when blood samples were obtained for DNA-methylation analysis.

Sample selection

From each of the three cohorts, cases and controls were randomly selected to derive a balanced selection of children diagnosed with asthma and of age- and sex-matched controls. As a prerequisite a sufficient quantitative and qualitative amount of genomic DNA had to be available. For the asthma group only children with a physician-made asthma diagnosis at the time of WGBS analysis were selected. For the control group, children were chosen who never reported wheezing symptoms, obstructive bronchitis, asthma, rhinitis or atopic dermatitis. A total of 40 children aged five to 15 years of age with a current asthma diagnosis and 42 age-

and sex-matched controls were selected for WGBS analysis. An overview of the selected samples is provided in Table S1.

For 48 children investigated at the time of an established asthma phenotype paired cord blood DNA samples were available (n=23 asthma, n=25 controls; Table S1) and also subjected to whole genome bisulfite sequencing.

Whole-genome bisulfite sequencing (WGBS)

To assess quantitative DNA-methylation information at single base pair resolution, whole blood genomic DNA samples from 82 children of the three cohorts and 48 matched cord blood samples available from LINA and PASTURE (Table S1, Table S2) were subjected to WGBS (see Online Supplement for details) as previously described[22]. All samples showed bisulfite conversion rates >99%.

Pre-processing of WBGS data

Sequencing data for each sample was input to the one touch pipeline[23] and processed using bwa v0.6.1.[24] and methylCtools v1.0.0[25] resulting in tab separated output files containing CpG position, number of reads with a methylated cytosine at this position, total number of reads covering the CpG and a *snp score*[26], which is the estimated probability of the CpG to be a SNP. CpGs were removed from the whole cohort if at least one of the 82 samples had a *snp score* of 0.25 or greater.

Determination of asthma-associated DMRs

Asthma-related DMRs were determined by a three-step procedure (i-iii). (i) DMRs were defined as at least three consecutive differentially methylated CpG sites between asthmatics (n=40) and controls (n=42). DMRs were called by two independent algorithms, a DMR calling strategy, which was applied in the latest meta-analysis on childhood asthma using 450k array data[8]. For

our WGBS data we used DSS version v2.12.0[27], and metilene version v0.2-6[28] as DMR calling tools. For DSS we used a Wald-test *p*-value threshold of .01 to mark a CpG as differentially methylated. The minimum DMR length was set to 50 bp, the maximum distance between two CpGs was set to 100 bp and the fraction of differentially methylated CpGs was set to minimum 0.3. Metilene uses circular binary segmentation followed by two dimensional Kolmogorov–Smirnov test (2D-KS test) and a DMR was considered significant if the obtained *q*-value was less than 0.05. Only chromosomes 1-22 were included in the analysis, while sex chromosomes were omitted. DSS adopts a highly appropriate beta binomial model for modelling DNA-methylation from WGBS count data but does not provide significance testing nor multiple testing correction of the identified DMRs. On the other hand, metilene offers the ability to perform multiple testing correction for the identified DMRs. Given the different approaches and features adopted by these two tools, we deemed their overlap to be highly conservative, thereby reducing potential false positives. (ii) To reduce the likelihood of false-positive DMR calls, we kept only the metilene DMRs that overlapped at least by 1 bp with the DMRs from DSS. The overlap was determined by using *intersectBed* from Bedtools version 2.24.0[29]. (iii) Concordant DMRs were tested for significance in each of the three cohorts LINA, LISA, and PASTURE by a factorial ANOVA using R version 4.0.2[30]. Log transformed β-values with a pseudo count of 0.006 of all differentially methylated CpGs within a DMR were modelled by using the disease condition asthma/control and the CpG position within a DMR. If the Bonferroni adjusted *p*-values in each of the three cohorts were $p<.05$ then a DMR was considered as significantly differentially methylated and retained for further analysis.

Overlap with previous asthma-associated EWAS

Previous asthma-associated EWAS studies in the PubMed database were identified by the search term: ("asthma" OR "wheeze") AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip") AND "blood"

(query data 27.10.2022). This search retrieved 68 publications, from which two reviews, one RCT and one systematic review were excluded. After manual curation 22 EWAS studies (including meta-analyses) remained that reported DNA-methylation changes in blood related to asthma or lung function (Figure S1A). DNA-methylation changes described in these manuscripts were related to the DMRs observed in our study.

Gene annotation and definition of enhancer and promoter DMRs

Genomic annotation of DMRs to the nearest transcription start site (TSS) from Gencode v19 gene models in human genome version hg19 was obtained by using the 'closest' module from Bedtools. Promoter regions were defined as 2 kb up- and downstream of the TSS. DMRs overlapping with at least 1 bp were categorized as promoter DMRs. DMRs were defined as enhancer DMRs, if their genomic location intersected at least 1 bp with GeneHancer[31], ENCODE[32], or ROADMAP[33] enhancer regions, or with an active histone mark as previously identified in LINA children according to Bauer *et al.*[22] (Table S3). Predicted target genes of enhancer DMRs were identified by using GeneHancer.

DMR classification

All asthma-related DMRs were classified into different categories: (i) genotype-/non-genotype-associated, (ii) cell-type-dependent, (iii) already present in cord blood. Asthma-related DMRs already present in cord blood were overlapped with previous EWAS studies investigating prenatal factors that affect DNA-methylation (see Online Supplement for details and Figure S1B).

According to previous works[19,22], a DMR was categorized as genotype-associated (gDMR) whenever a significant correlation between the methylation value of the DMR and any SNP in a +/-5 kb window around the DMR was determined (see Online Supplement for details). Likewise, DMRs with no significant association to methylation quantitative trait loci (meQTLs)

were classified as a non-genotype associated DMR (ngDMR). All meQTL SNPs were checked against the EMBL GWAS catalogue[34] (Query date: 01.11.2022) for previous associations to any phenotypic outcomes including asthma.

To determine whether the asthma-related DMRs were already differentially methylated at the time of birth, WGBS-based DNA-methylation data of matched cord blood samples were analysed (n=48, Table S1, Table S2). Whenever a DMR was significantly differentially methylated at the time of birth as determined by factorial ANOVA followed by a multiple test correction (Bonferroni-corrected $p<.05$, corresponding to a nominal $p<.00032$ separately in all three cohorts), the corresponding DMR was classified as a cord blood asthma-DMR already present at the time of birth.

To identify which cord blood DMRs were associated with a prenatal influencing factor, previously published array- or WGBS-based EWAS conducted with cord blood samples were evaluated (see Figure S1B and Online Supplement for details). This included studies on maternal smoking during pregnancy, maternal mental health, maternal disease such as diabetes and atopy, maternal BMI and diet, or environmental exposures. Whenever a CpG or region previously associated with a prenatal influencing factor overlapped with at least 1 bp with a cord blood DMR in our data set, this DMR was considered to be associated with this prenatal influencing factor.

Cell-type dependency

The frequency of the main blood cell types (T cells, B cells, NK cells, monocytes, neutrophils, eosinophils) was estimated by deconvolution of the WGBS data using *EpiDish*[35].

Next, the cell-type dependency of DMRs was determined using adjusted multiple regression models with the mean DNA-methylation of the DMR as the dependent variable and the main blood cell-type estimates as the independent variables (confounder: child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at

birth, growing up on a farm). DMRs significantly (Bonferroni-corrected $p<.05$, corresponding to a nominal $p<.00032$) associated to a specific blood cell type were classified as cell-type-dependent (see Online Supplement for details).

Enhancer-, pathway- and TFBS motif enrichment

We used Fisher's exact test in R to test if asthma-related DMRs were enriched for enhancer elements (Table S3) when comparing them with all other methylated regions in the genome that have similar characteristics as our DMRs but are not called as such (see Online Supplement for details).

For gene enrichment analysis the genomic positions of asthma-related DMRs were subjected to GREAT (Genomic Regions Enrichment of Annotations Tool) version 3.3.0 analysis tool[36] setting "whole genome" as background and a significance level of $\alpha<.05$.

The MEME-ChIP tool implemented in the MEME Suite version 5.4.1 (Motif-based sequence analysis tools)[37] was used to identify transcription factor binding site (TFBS) based on the HOCOMOCOv11 core HUMAN database including *de novo* motifs within the asthma-related DMRs. DMRs were elongated by 20 bp at the start and at the end to ensure an intersection with motif sequences. Only motifs with a length of four to fifteen nucleotides were considered. Motif enrichment with an *E*-value<.05 (estimate of the statistical significance of each motif) was considered significant.

Network analysis and Natural Language Processing

For network and module analysis of DMR associated genes including all enhancer DMR target genes or genes closest to the next TSS (n=435 genes) were subjected to Cytoscape analysis version 3.8.2[38]. The Reactome Functional Interaction (FI) plugin version 8.0.4 (released Feb 2022) was used to determine network patterns of common and predicted interactions as estimated via Naïve Bayes Classifier excluding linker genes. Cluster FI network was applied to

identify cluster of genes (=modules)[39]. Subsequently, a pathway enrichment analysis (significance cut-off: FDR<0.01) was performed using the databases CellMap, Reactome, KEGG, NCI PID, Panther and BioCarta for each module.

To identify genes in the network, previously associated with asthma-related outcomes, natural language processing (NLP, see Online Supplement for details) was applied. In brief, mentions of genes and gene products were searched in the PubMed and PubMed Central open access literature databases and additionally filtered by the following terms “asthma”, “asthmatic”, “asthmatics”, “wheeze”, “bronchial hyperreactivity”, “airway hyperreactivity”, “bronchial hyperresponsiveness”, or “hyperreactive airway disease”.

Targeted analyses: DNA-methylation, transcription, and protein measurement

Targeted analyses were performed in a larger sample set obtained from the 6-8 years old LINA children and the 15-years old LISA children from the Leipzig study centre. No further PASTURE samples were available for these analyses. All available samples from LINA and LISA fulfilling these two criteria were included: (i) samples from children diagnosed with asthma by a physician and (ii) control samples that never reported wheezing symptoms, obstructive bronchitis, or asthma, however they could have developed atopic dermatitis or rhinitis. An overview of the selected samples for these analyses is provided in Table S1 and Table 1B.

Targeted DNA-methylation analysis was performed for a set of selected DMRs in n=127 LINA and n=140 LISA samples using the Sequenom´s MassARRAY platform (San Diego, CA, USA, Table S4 for primer sequences, Figure S2) as previously described[22].

Functional translation of methylation changes for selected genomic regions was determined by RNA and protein expression analyses of the associated genes. Whole blood samples for transcriptional analyses were collected at the same time as blood samples for DNA-methylation analyses. RNA expression data were obtained for *EPX*, *IL4,* and *IL5RA* for n=126 LINA and

n=140 LISA samples by qPCR on the Biomark HD system as previously described[22] (see Table S5 for primer sequences).

Within the LINA study phytohaemagglutinin (PHA)-stimulated IL-4 concentrations obtained from a whole blood assay were available. IL-4 concentrations were measured by cytometric bead array (BD CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) as previously described[40].

Detailed information can be found in the Online Supplement.

Statistics

WGBS samples

To determine potential differences in the study characteristics between asthmatic and control children a Fisher's exact- test or Mann-Whitney *U*-test were applied. As confounding factors in the models analysing WGBS-data the child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up on a farm and cell composition were included.

Targeted analyses

To test whether there were differences between asthmatic and control children of the LINA and LISA cohorts with respect to the child's age and sex, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up on a farm, or the presence of rhinitis or atopic dermatitis in the child, Fisher's exact- test or Mann-Whitney-*U* test were applied.

A Mann-Whitney-*U* test was used to determine if there were significant differences in DNA-methylation and transcription between groups. Spearman correlation was used to determine the association between DNA-methylation, relative gene expression, or protein concentration. Correlation coefficients are reported as effect size measures (point biserial (r_{pb}) for Mann-

Whitney U and Spearman's rho ρ). The selection of confounders associated with asthma or affecting DNA-methylation patterns was based on *a priori* knowledge. The child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth were introduced as confounding factors in all models.

Confounder adjusted logistic regression analyses were applied to compare the DNA-methylation and relative gene expression of asthmatic and control children. Confounder adjusted mediation analyses were performed using the *PROCESS* macro version v3.4[41] for SPSS. Statistical significance of the indirect effect was determined by bootstrapping as implemented in the *PROCESS* macro version 3.4[41]. Bias-corrected 95% confidence intervals were derived from the distribution of bootstrap estimates of the indirect effect from random resampling of 5,000 samples. Only for non-dichotomous independent variables a standardized indirect effect was calculated. Effect sizes of regression analyses are either provided as unstandardized *b*, standardized β, or as odds ratio (OR).

Statistical analyses were performed using STATISTICA for Windows Version 12.0/13.0 (Statsoft Inc. Europe, Hamburg, Germany), IBM SPSS Statistics for Windows Version 25 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) or R version 4.0.2[30]. *P*-values $\leq .05$ were considered significant.

RESULTS

Genome-wide DNA-hypomethylation in childhood asthma

To evaluate epigenetic alteration in the global DNA-methylation pattern of asthmatic children at single base-pair-resolution, we performed WGBS and subsequent DMR calling of whole blood samples from n=82 children participating in the LINA, LISA, or PASTURE cohort (Figure 1, Table S1). In total, samples from n=40 asthmatic children were compared to n=42 age-matched controls without an asthma history or other respiratory symptoms (Table 1A). High quality WGBS data were derived with a mean genome coverage of 56.3x (Table S2A). To retain highly confident asthma-related DMRs for downstream analyses, a multiple-step DMR-calling approach was utilized (Figure S3). Using these two independent DMR-calling algorithms, DSS and metilene, 1,021 and 758 DMRs were determined, respectively. DMRs overlapping between these two approaches (n=385) were subjected to factorial ANOVA analysis to assess whether significant DNA-methylation differences could be observed separately in each of the three cohorts and were in the same direction. Only these concordant DMRs (n=158 out of n=385) were retained for further assessment (Figure S3, see Table S6A for asthma-related DMR list). These 158 asthma-related DMRs were distributed over all autosomes (Figure 2A) and had a read coverage of 31.5x in average (Table S2B). Unsupervised cluster analysis of these derived 158 DMRs resulted in a clear separation between asthmatics and control children (Figure S4A). The vast majority of the asthma-related DMRs were hypomethylated in asthmatic children (Figure 2A), while only two hypermethylated DMRs located in the *TET3 (ten-eleven translocation 3 or tet methylcytosine dioxygenase 3)* gene and the long coding RNA *AL645608.1* were identified. In line with previous asthma EWAS studies our DMRs overlapped with several CpG sites or DMRs identified based on array approaches (see Table S7 for overlap and references and Figure S1A for evaluated EWAS studies).

Genetic and cell type composition influences on asthma-related DMRs

Since the level of DNA-methylation can be strongly dependent on the genotype or the cell type composition, asthma-related DMRs were categorized according to cell type-dependency and genotype-association (gDMRs). Based on this categorization, 38 out of the 158 DMRs were associated with the genetic background (24.1%), while the remaining 120 DMRs (75.9%) were classified as non-genotype associated DMRs (ngDMRs). A total of 465 meQTLs were identified in relation to the 38 gDMRs, of which none has been previously described as an asthma risk factor in genome-wide association studies (Table S6B). However, including all phenotypic traits of the GWAS catalogue, we found 14 DMRs associated with at least one trait. For eight of these DMRs, the trait showed a loose phenotypic association with asthma (Table S6B) including lung function (rs645601 and rs7700998). Five SNPs were associated to counts of different blood cell types with SNPs rs4328821 and rs7646596 upstream of the *RPN1*-DMR associating to the eosinophil count. Additionally, rs12699415 related to the *MAD1L1*-DMR was linked to idiopathic pulmonary fibrosis[34].

We observed an enhanced eosinophil frequency in the blood of asthmatic children (Mann-Whitney *U* test: Z=3.42, r_{pb}=0.32, p= .017, Table S8), but not for the remaining cell types, i.e. B cells, T cells, monocytes, NK cells or neutrophils. We applied adjusted multiple regression analyses to test whether different cell type frequencies have an impact on the DNA-methylation level of the determined DMRs. To this end, 37% of the asthma-DMRs (58 DMRs) were associated with the eosinophil proportion and only three DMRs in total to B cells, T cells, monocytes, NK cells or neutrophils (Table S6A). However, even after accounting for these cell types in the adjusted multiple regression models, asthma was still a significant contributor of the DNA-methylation status for all cell-type-dependent DMRs (Table S9).

Altered DNA-methylation pattern associates with perturbed immune regulation

To elucidate the relevance of the asthma-related aberrant DNA-methylation profile, a pathway enrichment analysis was performed. Besides a strong enrichment in the asthma pathway, we

found classical immune system-related pathways enriched, such as IL-5- known to be crucial for asthma pathophysiology[42,43] (Figure 2B, Table S10). To ensure that the DNA-methylation differences observed in the small number of sequenced samples can be reproduced in larger sample numbers, targeted analyses were performed in further samples (n=267) including six to eight-years-old LINA children (n=127) and 15-years-old LISA adolescents (n=140, Table S1, Table 1B). Here, we focused on DMRs that are likely to influence aberrant immune gene expression driving asthma onset. Therefore, the DNA-methylation of two prototypical DMRs (Figure S4B) linked to genes of the asthma pathway (eosinophil peroxidase, *EPX*) - the pathway with the strongest enrichment - and the IL-5 signalling pathway (*IL5RA*) (Figure 2B) known to promote severe atopic asthma associated with eosinophilia[42], was measured in the larger sample set using a targeted DNA-methylation assay. Significant hypomethylation of these DMRs located in the sixth exon of *EPX*, and in the *IL5RA* promoter, could be confirmed in meta-analysis combining samples of the LINA and LISA cohort (adj. OR/95% CI *EPX*: 0.87/0.81-0.94, p=.0004; *IL5RA:* 0.83/0.73-0.94, p=.003, n=223 controls vs. n=44 asthmatics, Figure 2C,D) using logistic regression adjusted for the child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth. Furthermore, for both DMRs a negative correlation with the relative gene expression of the associated genes *EPX* was observed (ρ= -0.40, p=1.4x10^{-11}, n=264) and *IL5RA* (ρ= -0.32, p=1.4x10^{-7}, n=266, Figure S5A). In line, expression of *EPX* and *IL5RA* is not only increased in asthmatic children (Figure S5B) but is also associated with an increased risk for asthma during childhood (relative expression *EPX*: adj. OR/95% CI: 1.44/1.09-1.91, p=.010, n=220 controls vs. n=44 asthmatics, *IL5RA:* adj. OR/95% CI: 1.59/1.19-2.13, p=.002, n=222 controls vs. n=44 asthmatics, Figure 2D).

DNA-methylation changes in asthma affect regulatory hubs

The identified DMRs showed enrichment for 20 binding motifs related to different transcription factors previously associated with asthma including the Th2 master regulator GATA3[44] (Table S11). Additionally, two third of the DMRs were located in genomic regulatory elements, 74% of the DMRs intersecting with enhancers, and 1% with promoters (Figure 3A). In particular, the DMR enrichment in enhancer regions was highly significant (OR/95% CI: 5.83/4.05-8.53, $p<4.0x10^{-26}$). Among the DMRs overlapping with a ROADMAP enhancer active in specific blood cells (Table S3), 17 DMRs overlapped with a T helper cell-type specific enhancer including a hypomethylated enhancer DMR associated with the mTORC1 scaffolding protein coding gene *RPTOR* (Table S6A).

One of those hypomethylated enhancer regions showed an enhancer specific ENCODE histone modification profile and a ChiA-PET interaction to the *IL4* promoter (Figure 3B). Although IL4 is one of the key regulators in allergic diseases including asthma, the relevance of this particular enhancer region associated to IL-4 expression has not been addressed so far. We confirmed the asthma-related DNA-hypomethylation of this *IL4* enhancer in the meta-analysis combining the two cohorts LINA and LISA (adj.OR/95% CI: 0.83/0.74-0.94, p=.002, n=223 controls vs. n=44 asthmatics). In addition, in the LINA cohort, where IL-4 protein concentration measurements were available (Table S1), the *IL4* enhancer DNA-methylation was associated with *IL4* transcription (ρ= -.35, p=.0001) and PHA-stimulated IL-4 protein concentrations (ρ= -.31, p=0.0009, Figure 3C). In line, two confounder adjusted mediation models were applied to evaluate the relevance of this hypomethylated *IL4* enhancer region in asthma: The first model showed a significant indirect effect of *IL4* enhancer DNA-methylation on IL-4 protein concentration via *IL4* transcription as a mediator (β/95% CI: -0.07/ -0.14- -0.03, Figure 3D), whereas the direct effect was not significant (b/95% CI: -0.92/ -3.79- 1.95, p= .525). Second, the asthma phenotype contributed to an increase in IL-4 protein concentration in asthmatics again solely indirectly via the DNA-methylation changes of this *IL4* enhancer and *IL4*

transcription as mediators (Figure S6, indirect effect: *b*/95% CI: 0.05/ 0.01- 0.13; direct effect *b*/95% CI: 0.23/ -0.26- 0.73, *p*=.352).

Genes affected by DNA-methylation changes are functionally connected

To elucidate whether DMR associated genes (n=435 genes, Table S6A) were functionally connected, these genes were subjected to network analysis based on established protein-protein interactions with a subsequent pathway enrichment of the derived network modules. The resulting network consisted of 102 genes in thirteen distinct modules. These modules were related, among others, to immune response and inflammation, cilium assembly and general gene regulation, and to Jak-STAT signalling (Figure 4, Table S12). The vast majority of the network genes (97 out of 102 genes) were targets of differentially methylated enhancers. Our NLP analysis revealed that 33.3% of these enhancer target genes such as the central transcription factors of the immune system *RELA* (NFκB subunit encoding gene)*, GATA2,* and *ZFPM1,* the Th2 cytokine *IL4,* or the mTOR complex 1 scaffold protein *RPTOR* have previously been described in the literature in association with asthma (red genes in Figure 4, Table S13A). In addition, we identified novel genes not yet associated to asthma, such as the A-kinase anchoring protein-9 (*AKAP9*). *ANKAP9* is prominently expressed in T cells and involved in immune synapse formation[45]. Among the proteins interacting with ANKAP9 for its proper function are TUBGCP2/TUBGCP6, for which we also observed an enhancer DMR[46].

Prenatal priming for asthma

To discriminate between DMRs that are a consequence of the disease from those predisposing an individual, we subjected matched cord blood samples (n=23 asthmatics vs. n=25 controls) to WGBS and assessed whether the methylation changes of the 158 asthma-related DMRs were already present at time of birth (Figure 5A). 35% (56/158 DMRs) of the DMRs identified in the established asthma phenotype were already significantly differentially methylated in cord blood

samples (Table S6C). Most of the cord blood DMRs were again located in enhancers (43 out of 56), 39% (n=22/56 DMRs) were gDMRs including those already identified in GWAS as a risk factor for lung dysfunction and idiopathic pulmonary fibrosis[34] (Table S6C). For 22 out of the 56 cord blood DMRs, we found an overlap with previous EWAS studies investigating the impact of a variety of different prenatal factors on DNA-methylation (Figure S1B). These factors included exposure to tobacco smoke, to air pollution or to environmental chemicals such as phthalates or lead, maternal diet-related metabolites as well as factors related to maternal health like gestational diabetes or preeclampsia (Figure 5B, Table 13B). When focusing our network analysis on cord blood DMR associated genes the network was comprised of several members of the LFA-1 signaling pathway (Figure 5C). Next to *ITGAL* coding for one of the subunits of LFA-1 (=CD11a), also the LFA-1 ligand ICAM-1, and the co-chaperones *ANKAP9*, *TUBGC2/6* were among the target genes of DMRs already observed in cord blood.

DISCUSSION

To characterize the complete genome-wide DNA-methylation pattern in childhood asthma, this study determined the DNA-methylation profile of 40 asthmatic and 42 control children by utilizing WGBS followed by calling of differentially methylated regions (DMRs) and discriminating between genotype-, and non-genotype-associated as well as cell-type-dependent, or -independent DNA-methylation changes. In total, 158 regions were found to be differentially methylated in childhood asthma, all hypomethylated except for two, which includes a hypermethylated enhancer region for *TET*. Since TET proteins initiate DNA-demethylation, this DMR might be directly related to the global DNA-methylation aberrations observed in asthma. Whether this DMR in asthma is an initiating event or a compensatory mechanism remains to be elucidated in follow-up studies. The predominant global hypomethylation suggests a pronounced epigenetic activation affecting a variety of immune-related genes associated with asthma development and exacerbation. Here, with this first EWAS using a genome-wide sequencing approach and thus not relying on pre-selected CpGs as performed in previous asthma EWASs, we show that this epigenetic activation primarily affects enhancer elements indicating that a predominant enhancer activation underlies the exacerbated immune response characteristic of childhood asthma[47]. The tight connectivity of these epigenetically dysregulated asthma genes is evident in our inferred interaction network. A comprehensive search of the current scientific literature by NLP analytics revealed that while almost 34% of the enhancer target genes have already been associated with asthma or asthma-related terms, several of the enhancer-DMRs have not yet been discussed in the context of asthma. Most of the asthma-DMRs were enriched for multiple TFBS indicating multiple regulatory effects of the epigenetically perturbed regions. Most of the transcription factors binding to these DMR-enriched TFBS motifs are known to be associated with asthma, such as GATA3[44], NFACT1[48], IRF-1[49], GATA-6[50], STAT2[51], THB[52], or EGR1[53], and even possess a master regulatory capacity of Th2 differentiation[54,55].

LFA-1 is mainly known for its role in T cell adhesion and Th1 effector polarization. However, a recent report shows that LFA-1 and its ligand ICAM-1 are expressed on group 2 innate immune cells (ILC2). ILC2 are able to induce eosinophilic lung injury and are elevated in the blood of asthmatics compared to healthy controls[56]. Knock-down of LFA-1 or ICAM-1 both attenuated airway hyperresponsiveness, reduced airway inflammation and decreased lung ILC2 accumulation in mouse models of allergic asthma[57]. As such the observed cord blood DNA-hypomethylation of several regions involved in the LFA-1 signalling cascade might predispose children to a higher risk of allergic asthma.

The vast majority of DMRs was not associated with a meQTL indicating that mainly other than genetic factors contribute to the observed aberrant DNA-methylation in childhood asthma. About one third (35%) of the asthma-related DMRs were already found in cord blood. A variety of environmental insults experienced during the highly susceptible prenatal developmental phase - mostly related to maternal lifestyle factors during pregnancy - have been associated with an increased asthma risk of the child. A comparison to previous EWAS studies revealed that 22 of the asthma-related DMRs already identified in cord blood, including 17 differentially methylated enhancers, overlapped with DNA-methylation changes described in association to prenatal asthma risk factors (for references refer to Table S13B). Among others, these factors included maternal exposure to tobacco smoke or environmental chemicals as well as maternal health (e.g. gestational diabetes, preeclampsia). Although more studies are necessary to investigate whether these regions of persistent differential DNA-methylation are missing links between an adverse intrauterine environment and childhood asthma development, it is prudent to reduce these adverse exposures during vulnerable periods.

This study has to be seen in the light of some limitations. The sample size of whole-genome sequencing approaches seems to be low when compared to previous EWAS using less cost-intensive array based epigenetic profiling methods[5,8,58], however, in comparison to previous

WGBS studies[59-62] we included a considerable higher number of samples. In addition, the enrichment of the DMRs in the asthma pathway, the overlap between the DMR-associated genes with known asthma genes such as *IL4*, *EPX*, *IL5RA* and *ZFPM1* as identified by NLP, in conjunction with the overlap of previously reported CpG sites (e.g. *ACOT7*, *DEGS2*, *EPX* and *GATA2*) of asthma EWAS support the validity of the applied strategy to determine asthma-related DMRs. Although we confirmed the differential DNA-methylation of selected DMRs and their influence on associated target gene expression that are likely to contribute to asthma pathology in a larger sample set, further studies are needed to show whether the DMRs observed in our study can be replicated in independent cohorts and to determine the effect of the identified DMRs on the transcriptome. In addition, since more than half of the asthmatic children reported rhinitis or atopic dermatitis in their life, we cannot exclude that the observed asthma-related DMRs might also be influenced by other atopic diseases such as rhinitis or atopic dermatitis.

The whole blood-based sequencing of DNA-methylation might be seen as a further limitation. To overcome this problem, the proportion of the different cell populations was determined by a deconvolution approach and the DMRs annotated with respect to their cell-type dependency. The deconvolution approach might have led to misclassification or underrepresentation of minor cell types. However, we were able to annotate the small population of eosinophils and to show a significant difference in the eosinophil count between children with asthma and controls without respiratory disease. For a global overview of aberrant DNA-methylation changes and an unbiased interpretation of EWAS[63], we deem the here utilized approach more appropriate, i.e., not to adjust for cell-type composition beforehand, but rather to determine all DMRs and subsequently annotate them as cell-type-dependent or genotype-associated.

To our best knowledge, this is the first study evaluating the children's methylome at single base-pair resolution – including the comprehensive information on the genetic background - using repeatedly collected samples of the same individual. We were able to confirm our findings in a larger sample set of two cohorts and showed functional translation to the transcriptional and

protein level for selected DMRs. We identified global DNA-methylation changes particularly affecting enhancers, which likely contribute to an altered gene expression of key immune genes involved in asthma pathology. Most of the immune system-related epigenetic alterations including the hypomethylated *IL-4* enhancer, or the *IL5RA* promoter are not present in cord blood, supporting the notion that they are developed during the shift of the immune response toward a Th2 reactivity contributing to the development of an atopic asthma phenotype. Although most of the cord blood DMRs are not directly related to the immune dysfunction characterizing the asthmatic phenotype, these regions related to genes involved in LFA-1 signaling. In light of the emerging role of LFA-1 in ILC2 modulated allergic asthma, these cord blood DNA-methylation changes might be involved in predisposing children to a higher risk for asthma development. Future studies will show if these regions have the ability to predict high-risk children.

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Author contribution

IL, RE, MKa, and ST provided project leadership.

AvB, BS, JH, MB, SR, EvM, JR, ADC, RL, MKa, AMK, IL, GH were involved in the recruitment and field work of the cohorts.

GH provided cytokine data.

MB provided the RNA transcription data.

SDM, MK, MB, TB, and CH performed the DMR calling and DMR annotation.

MK, LT, DW, OM, and CP performed or guided targeted methylation analyses.

MK, SDM, MM, MB, NI, TB, CH, ST, GS, and LT performed or supervised data analysis.

SS, EF, UH, MK and ST performed or evaluated NLP analysis.

LT, ST, MK, and IL wrote the manuscript.

All authors were involved in the discussion and contributed to the final manuscript.

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Figure captions

Figure 1: Study design. Blood samples derived from asthmatics or control children of the three cohorts were subjected to WGBS to determine asthma-related DMRs. DMRs comparing asthmatic and control children were determined by the two independent DMR-calling algorithms DSS and metilene. Asthma-related DMRs were subsequently analysed.

DMR = differentially methylated region, WGBS = whole-genome bisulfite sequencing, LINA = lifestyle and environmental factors and their influence on newborns allergy risk, LISA = influences of lifestyle-related factors on the immune system and the development of allergies in childhood, PASTURE = Protection Against Allergy: Study in Rural Environments

[1] based on available cord blood sample of LINA and PASTURE

[2] based on available whole blood samples of LINA and LISA

[3] based on available plasma samples of LINA

Figure 2: DMR distribution and down-stream analyses. (A) Circos plot represents the distribution of the 158 differentially methylated regions (DMRs) identified in asthmatic children vs. controls across all autosomes. The outer circle shows the 22 autosomes. The bars in the inner circle represent the DMRs and their chromosomal location. Hypermethylated DMRs are indicated as red bars, hypomethylated DMRs in blue. The height of each bar indicates the DNA-methylation differences between asthmatics and controls. **(B)** KEGG pathway enrichment for all asthma-DMRs based on their genomic location. **(C)** DNA-methylation difference between asthmatic children and controls of the WGBS samples (asthma n=40, controls n=42), LINA study (asthma n=19, controls n=108) and LISA study (asthma n=25, controls n=115) for DMRs related to *EPX* and *IL5RA* as determined by sequencing or MassARRAY, respectively (*p*-value from Mann-Whitney U-test, r_{pb}:

point biserial correlation coefficient). **(D)** Association of *EPX* and *IL5RA* DNA-methylation (black whiskers) and transcription (magenta whiskers) to asthma outcome in meta-analysis combining LINA and the LISA study (DNA-methylation: asthma n=44, controls n=223, *EPX* transcription: asthma n=44, controls n=220, *IL5RA* transcription: n=44 asthma n=222 controls). Given are ORs with +/-95% CIs from logistic regression adjusted for child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth using ln-transformed DNA-methylation values.

Figure 3: Genomic location of asthma-related DMRs and functional translation of *IL4* enhancer hypomethylation. (A) Pie chart represents the proportional distribution of the genomic regions affected by asthma-related DMRs. **(B)** Genomic location of the *IL4* DMR and the genomic region analysed by MassARRAY in the UCSC genome browser[64]. **(C)** Scatterplots show the association of *IL4* DNA-methylation to *IL4* transcription (n=112) and IL-4 protein concentration (n=115) and the association of IL-4 protein concentration to *IL4* transcription (n=111) in six-years-old children of the LINA study. Correlation coefficient (ρ) and p-value from Spearman correlation. **(D)** Mediation analysis for the relationship of *IL4* enhancer DNA-methylation, *IL4* transcription, and IL-4 protein concentration of six-years-old children of LINA (n=111). Model was adjusted for child's sex, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth. IL-4 protein concentrations were determined after PHA-stimulation. Protein and DNA-methylation data were ln-transformed before analysis. Effect sizes for indirect path is given as standardized β-values with +/-95% CIs. Significance determined by bias-corrected bootstrapping.

MA = MassARRAY, DMR = differentially methylated region

Figure 4: Network module analysis of asthma-DMR associated genes. Shown are all asthma-DMR associated genes, which show a predicted or experimentally based interaction. Only modules with more than one connection are shown. Target genes of enhancers affected by a DMR are highlighted by blue outline circles. Genes related to asthma or similar terms as determined by the natural language processing tool are indicated in red font. Module nomenclature is based on subsequent pathway enrichment analysis (Table S12).

Figure 5: Cord blood asthma-DMRs. (A) Matched cord blood samples were subjected to WGBS to determine the DNA-methylation level of the asthma-related DMRs at time of birth in control children compared to those children who later developed asthma. **(B)** Pie charts represent the portion of genotype-, and non-genotype-associated DMRs (g/ngDMRs) and those DMRs, which were already differentially methylated in cord blood samples (=cord blood DMRs). The table lists all prenatal influencing factors that have previously been associated with CpGs included in the n=56 cord blood asthma-related DMRs (see Table S6C for EWAS references and cord blood DMR list). Genes highlighted in red font were described with asthma as identified by natural language processing (see Table S13B for references). #ngDMRs indicated with light blue and gDMR with dark blue background. *Enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annotation was available the closest TSS gene is given. **(C)** Network module analysis for cord blood DMR associated genes (left panel) and for genes associated to DMRs only present in asthma phenotype (right panel). Only modules with more than one connection are shown. Module nomenclature is based on network of Figure 4.

PFOS = perfluorooctane sulfonic acid, NLP = natural language processing

Figure S1: Literature search for overlap of DMRs with previous EWAS. (A) Workflow summarizes the EWAS studies investigated asthma-related outcomes, which were used for the overlap with n=158 asthma-related DMRs. **(B)** Workflow summarizes the EWAS studies investigated prenatal influencing factors, which were used for the overlap with n=56 cord blood asthma-related DMRs.

Figure S2: Quality control of the MassARRAY amplicons. Graphs show DNA-methylation values derived by MassARRAY measurements of standard samples (0%, 20%, 40%, 60%, 80%, and 100% methylated genomic DNA) representing the mean DNA-methylation of the MassARRAY amplicon for **(A)** *IL5RA* (including 7 CpGs), **(B)** *EPX* (including 9 CpGs) and **(C)** *IL4* (including 6 CpGs) DMR (given are mean ±SD of two replicates and r^2 from linear regression).

Figure S3: Study workflow. DMRs comparing asthmatics and control children of three cohorts were called by two independent algorithms (DSS, metilene) and concordant DMRs were subjected to multiple test corrected factorial ANOVA analysis to determine asthma-related DMRs. These 158 DMRs were subsequently analysed and categorized based on their dependency on the genotype, the cell type composition or their presence also in cord blood.

DMR = differentially methylated region, ng/gDMR = non-genotype/genotype-associated DMRs

Figure S4: Characteristics of asthma-related DMRs. (A) Cluster analysis with ß-methylation values of the 158 asthma-related DMRs. **(B)** CoMET plot of the validated *EPX* and *IL5RA* DMR. Plots show WGBS derived β-methylation values of asthmatics vs. control children on top (n=40 vs. 42, mean +/-SEM) followed by *p*-values from logistic regression analysis (asthma phenotype ~

β-values) and UCSC tracks (ENSEMBL genes, CpG islands, DNAse-sensitive regions and SNPs) as well as the DNA-methylation correlation matrix for all CpG sites within the DMRs.

Figure S5: Epigenetic regulation of *IL5RA* and *EPX* transcription in asthma. (A) Correlation between *IL5RA* and *EPX* DNA-methylation to *IL5RA*/*EPX* transcription of children of the LINA (magenta) and LISA (black) study. Correlation coefficient ρ and *p*-value from Spearman correlation. **(B)** Relative gene expression of *IL5RA* and *EPX* in asthmatic children and controls participated in LINA (asthma n=19, controls n=107) or LISA (*IL5RA*: asthma n=25, controls n=115, *EPX:* asthma n=25, controls n=113). *p*-value from Mann-Whitney U-test, r_{pb}: point biserial correlation coefficient.

Figure S6: IL-4 protein and *IL4* enhancer hypomethylation in asthma. Illustrated is the mediation model adjusted for child's sex, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth. Effect sizes for the indirect path is given as standardized *β*-values with +/-95% bias-corrected bootstrap CIs (n=111, significant as determined by bootstrap CI). The analysis is shown for the LINA subcohort with available PHA-stimulated IL-4 protein concentrations. Protein and DNA-methylation data were ln-transformed before analysis.

Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing preferentially affects enhancer regions

Short title: The epigenetic landscape of asthma.

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ABSTRACT

Background: Childhood asthma is a result of a complex interaction of genetic and environmental components causing epigenetic and immune dysregulation, airway inflammation and impaired lung function. Although different microarray based EWAS studies have been conducted, the impact of epigenetic regulation in asthma development is still widely unknown. We have therefore applied unbiased whole genome bisulfite sequencing (WGBS) to characterize global DNA-methylation profiles of asthmatic children compared to healthy controls.

Methods: Peripheral blood samples of 40 asthmatic and 42 control children aged 5-15 years from three birth cohorts were sequenced together with paired cord blood samples. Identified differentially methylated regions (DMRs) were categorized in genotype-associated, cell-type-dependent, or prenatally-primed. Network analysis and subsequent natural language processing of DMR-associated genes was complemented by targeted analysis of functional translation of epigenetic regulation on the transcriptional and protein level.

Results: In total, 158 DMRs were identified in asthmatic children compared to controls of which 37% were related to the eosinophil content. A global hypomethylation was identified affecting predominantly enhancer regions and regulating key immune genes such as *IL4, IL5RA*, and *EPX*. These DMRs were confirmed in n=267 samples and could be linked to aberrant gene expression. Out of the 158 DMRs identified in the established phenotype, 56 were perturbed already at birth and linked, at least in part, to prenatal influences such as tobacco smoke exposure or phthalate exposure.

Conclusion: This is the first epigenetic study based on whole genome sequencing to identify marked dysregulation of enhancer regions as a hallmark of childhood asthma.

Key words:

asthma, cord blood, DNA-methylation, prenatal exposure

INTRODUCTION

Asthma is the most common chronic inflammatory disease in childhood. With an estimated prevalence of asthma ranging from 2.6% to 30.5%[1] varying according to the age and origin of the children, childhood asthma is a major health concern worldwide. Over the last decades, the prevalence of childhood asthma increased in a majority of countries worldwide, which has been mainly attributed to an interaction of genetic predisposition with a changing environment and a Westernized lifestyle[1,2]. Although the etiology of pediatric asthma remains incompletely understood, its origin is thought to be found early in life[3]. There is a larger number of studies supporting the notion that asthma-related immune alterations are already established during the prenatal development phase when the maturation of the immune system begins[4]. Although the molecular mechanisms initiating and maintaining these aberrant immune functions are largely unknown, epigenetic mechanisms are thought to play a central role in not only mediating the adverse effects of an intrauterine environment but also in preserving the established asthma-promoting phenotype[4]. However, the knowledge of asthma-related epigenetic modifications is limited and no genome-wide studies at a single base-pair resolution are available. So far, DNA-methylation changes in asthma, have been described based on target-specific analyses or on DNA-methylation microarrays[5-9] covering 27,000-850,000 CpG sites of the approximately 28 million CpGs of the human genome.

To date, several childhood asthma-associated DNA-methylation changes at single CpG sites located in immune regulatory genes such as *ALOX12*, *IL13*, and *RUNX3*, or genes involved in arachidonic acid metabolism, T cell differentiation, and IgE production, have been described in whole blood samples[7,10]. In addition, more than 100 differentially methylated sites were identified by array-based epigenome-wide association studies (EWAS) on respiratory cells, such as buccal cells or epithelial cells of the nasopharynx, amongst others CpGs in the close vicinity of established asthma-associated genes, such as *ZFPM1*, *NLRP3*, *IFNGR2*, *NTRK1*, or

ALOX15[11-13]. However, all of the current EWAS on asthma are biased by the pre-selection of CpG sites covered by the commercially available DNA-methylation arrays.

The genomic localization of DNA-methylation changes is critical for their functional impact on gene expression and associated relevance to the disease phenotype. Perturbations in regulatory regions, and in particular enhancers regulating multiple genes, are assumed to drive disease progression[14]. Enhancers are not commonly in close vicinity of their target gene, but rather may be located several thousands of base pairs away[15]. Although previous studies of asthma-associated DNA-methylation changes provided valuable information on CpG sites potentially contributing to disease etiology and suggested an enhancer-centric epigenetic dysregulation[9], a plethora of enhancer elements have since been identified that are not covered by DNA-methylation arrays and thus have previously escaped analysis. Even with the advanced EPIC array only 7% of distal and 27% of proximal ENCODE regulatory elements, and less than 4% of all CpGs of the genome are represented[16].

As a consequence of this limited genomic coverage of previous methylation array studies only little is known about enhancer dysregulation in childhood asthma. To overcome this knowledge gap, this study used a different approach and determined the unbiased global DNA-methylation profile at a single-base pair resolution by applying whole-genome bisulfite sequencing (WGBS). Whole blood samples of 40 asthmatic children from three independent prospective birth cohorts were compared to 42 sex- and age-matched controls. It is well known that the methylation of adjacent CpG sites is mutually dependent[17] and regional changes in DNA-methylation are assumed to be functionally more relevant than single CpG positions[18]. Thus, we determined differentially methylated regions (DMRs) rather than reporting methylation changes at single CpG positions and subsequently confirmed our findings by targeted methylation analyses in larger number of cases that included subjects from two of the three cohorts. The comprehensive assessment of the genomic distribution of the DMRs was complemented by elucidating the functional consequences of aberrant DNA-methylation

associated with key immune modulating genes. To this end, cord blood - available for a subset of the children - provided the opportunity to assess potential prenatal priming of the DNA-methylation changes identified in asthmatic children.

METHODS

Detailed information can be found in the Online Supplement.

Study characteristics

This study comprises data and samples derived from the three different birth cohorts LINA[19], LISA[20], and PASTURE[21]. A detailed cohort description can be found in the Online Supplement. Participation in all three cohort studies was voluntary and written informed consent was given by the parents or children if applicable. The studies were approved by their respective ethics committees (LINA: 046-2006, 160-2008, 160b/2008, EK-BR-02/13-1, 169/13ff, 150/14ff, LISA: 398-12-05112012, PASTURE: 02046, 9/11-E1/651-2002, 415-E401/4-2007).

Asthma outcome

Asthma was defined based on the confirmative answer to the question: "Has a physician-diagnosed your child with asthma during the last 12 months (=current asthma)?" asked in the parent-reported questionnaires at the time-point when blood samples were obtained for DNA-methylation analysis.

Sample selection

From each of the three cohorts, cases and controls were randomly selected to derive a balanced selection of children diagnosed with asthma and of age- and sex-matched controls. As a prerequisite a sufficient quantitative and qualitative amount of genomic DNA had to be available. For the asthma group only children with a physician-made asthma diagnosis at the time of WGBS analysis were selected. For the control group, children were chosen who never reported wheezing symptoms, obstructive bronchitis, asthma, rhinitis or atopic dermatitis. A total of 40 children aged five to 15 years of age with a current asthma diagnosis and 42 age-

and sex-matched controls were selected for WGBS analysis. An overview of the selected samples is provided in Table S1.

For 48 children investigated at the time of an established asthma phenotype paired cord blood DNA samples were available (n=23 asthma, n=25 controls; Table S1) and also subjected to whole genome bisulfite sequencing.

Whole-genome bisulfite sequencing (WGBS)

To assess quantitative DNA-methylation information at single base pair resolution, whole blood genomic DNA samples from 82 children of the three cohorts and 48 matched cord blood samples available from LINA and PASTURE (Table S1, Table S2) were subjected to WGBS (see Online Supplement for details) as previously described[22]. All samples showed bisulfite conversion rates >99%.

Pre-processing of WBGS data

Sequencing data for each sample was input to the one touch pipeline[23] and processed using bwa v0.6.1.[24] and methylCtools v1.0.0[25] resulting in tab separated output files containing CpG position, number of reads with a methylated cytosine at this position, total number of reads covering the CpG and a *snp score*[26], which is the estimated probability of the CpG to be a SNP. CpGs were removed from the whole cohort if at least one of the 82 samples had a *snp score* of 0.25 or greater.

Determination of asthma-associated DMRs

Asthma-related DMRs were determined by a three-step procedure (i-iii). (i) DMRs were defined as at least three consecutive differentially methylated CpG sites between asthmatics (n=40) and controls (n=42). DMRs were called by two independent algorithms, a DMR calling strategy, which was applied in the latest meta-analysis on childhood asthma using 450k array data[8]. For

our WGBS data we used DSS version v2.12.0[27], and metilene version v0.2-6[28] as DMR calling tools. For DSS we used a Wald-test *p*-value threshold of .01 to mark a CpG as differentially methylated. The minimum DMR length was set to 50 bp, the maximum distance between two CpGs was set to 100 bp and the fraction of differentially methylated CpGs was set to minimum 0.3. Metilene uses circular binary segmentation followed by two dimensional Kolmogorov–Smirnov test (2D-KS test) and a DMR was considered significant if the obtained *q*-value was less than 0.05. Only chromosomes 1-22 were included in the analysis, while sex chromosomes were omitted. DSS adopts a highly appropriate beta binomial model for modelling DNA-methylation from WGBS count data but does not provide significance testing nor multiple testing correction of the identified DMRs. On the other hand, metilene offers the ability to perform multiple testing correction for the identified DMRs. Given the different approaches and features adopted by these two tools, we deemed their overlap to be highly conservative, thereby reducing potential false positives. (ii) To reduce the likelihood of false-positive DMR calls, we kept only the metilene DMRs that overlapped at least by 1 bp with the DMRs from DSS. The overlap was determined by using *intersectBed* from Bedtools version 2.24.0[29]. (iii) Concordant DMRs were tested for significance in each of the three cohorts LINA, LISA, and PASTURE by a factorial ANOVA using R version 4.0.2[30]. Log transformed β-values with a pseudo count of 0.006 of all differentially methylated CpGs within a DMR were modelled by using the disease condition asthma/control and the CpG position within a DMR. If the Bonferroni adjusted *p*-values in each of the three cohorts were $p<.05$ then a DMR was considered as significantly differentially methylated and retained for further analysis.

Overlap with previous asthma-associated EWAS

Previous asthma-associated EWAS studies in the PubMed database were identified by the search term: ("asthma" OR "wheeze") AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip") AND "blood"

(query data 27.10.2022). This search retrieved 68 publications, from which two reviews, one RCT and one systematic review were excluded. After manual curation 22 EWAS studies (including meta-analyses) remained that reported DNA-methylation changes in blood related to asthma or lung function (Figure S1A). DNA-methylation changes described in these manuscripts were related to the DMRs observed in our study.

Gene annotation and definition of enhancer and promoter DMRs

Genomic annotation of DMRs to the nearest transcription start site (TSS) from Gencode v19 gene models in human genome version hg19 was obtained by using the 'closest' module from Bedtools. Promoter regions were defined as 2 kb up- and downstream of the TSS. DMRs overlapping with at least 1 bp were categorized as promoter DMRs. DMRs were defined as enhancer DMRs, if their genomic location intersected at least 1 bp with GeneHancer[31], ENCODE[32], or ROADMAP[33] enhancer regions, or with an active histone mark as previously identified in LINA children according to Bauer *et al.*[22] (Table S3). Predicted target genes of enhancer DMRs were identified by using GeneHancer.

DMR classification

All asthma-related DMRs were classified into different categories: (i) genotype-/non-genotype-associated, (ii) cell-type-dependent, (iii) already present in cord blood. Asthma-related DMRs already present in cord blood were overlapped with previous EWAS studies investigating prenatal factors that affect DNA-methylation (see Online Supplement for details and Figure S1B).

According to previous works[19,22], a DMR was categorized as genotype-associated (gDMR) whenever a significant correlation between the methylation value of the DMR and any SNP in a +/-5 kb window around the DMR was determined (see Online Supplement for details). Likewise, DMRs with no significant association to methylation quantitative trait loci (meQTLs)

were classified as a non-genotype associated DMR (ngDMR). All meQTL SNPs were checked against the EMBL GWAS catalogue[34] (Query date: 01.11.2022) for previous associations to any phenotypic outcomes including asthma.

To determine whether the asthma-related DMRs were already differentially methylated at the time of birth, WGBS-based DNA-methylation data of matched cord blood samples were analysed (n=48, Table S1, Table S2). Whenever a DMR was significantly differentially methylated at the time of birth as determined by factorial ANOVA followed by a multiple test correction (Bonferroni-corrected $p<.05$, corresponding to a nominal $p<.00032$ separately in all three cohorts), the corresponding DMR was classified as a cord blood asthma-DMR already present at the time of birth.

To identify which cord blood DMRs were associated with a prenatal influencing factor, previously published array- or WGBS-based EWAS conducted with cord blood samples were evaluated (see Figure S1B and Online Supplement for details). This included studies on maternal smoking during pregnancy, maternal mental health, maternal disease such as diabetes and atopy, maternal BMI and diet, or environmental exposures. Whenever a CpG or region previously associated with a prenatal influencing factor overlapped with at least 1 bp with a cord blood DMR in our data set, this DMR was considered to be associated with this prenatal influencing factor.

Cell-type dependency

The frequency of the main blood cell types (T cells, B cells, NK cells, monocytes, neutrophils, eosinophils) was estimated by deconvolution of the WGBS data using *EpiDish*[35].

Next, the cell-type dependency of DMRs was determined using adjusted multiple regression models with the mean DNA-methylation of the DMR as the dependent variable and the main blood cell-type estimates as the independent variables (confounder: child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at

birth, growing up on a farm). DMRs significantly (Bonferroni-corrected $p<.05$, corresponding to a nominal $p<.00032$) associated to a specific blood cell type were classified as cell-type-dependent (see Online Supplement for details).

Enhancer-, pathway- and TFBS motif enrichment

We used Fisher's exact test in R to test if asthma-related DMRs were enriched for enhancer elements (Table S3) when comparing them with all other methylated regions in the genome that have similar characteristics as our DMRs but are not called as such (see Online Supplement for details).

For gene enrichment analysis the genomic positions of asthma-related DMRs were subjected to GREAT (Genomic Regions Enrichment of Annotations Tool) version 3.3.0 analysis tool[36] setting "whole genome" as background and a significance level of $\alpha<.05$.

The MEME-ChIP tool implemented in the MEME Suite version 5.4.1 (Motif-based sequence analysis tools)[37] was used to identify transcription factor binding site (TFBS) based on the HOCOMOCOv11 core HUMAN database including *de novo* motifs within the asthma-related DMRs. DMRs were elongated by 20 bp at the start and at the end to ensure an intersection with motif sequences. Only motifs with a length of four to fifteen nucleotides were considered. Motif enrichment with an *E*-value<.05 (estimate of the statistical significance of each motif) was considered significant.

Network analysis and Natural Language Processing

For network and module analysis of DMR associated genes including all enhancer DMR target genes or genes closest to the next TSS (n=435 genes) were subjected to Cytoscape analysis version 3.8.2[38]. The Reactome Functional Interaction (FI) plugin version 8.0.4 (released Feb 2022) was used to determine network patterns of common and predicted interactions as estimated via Naïve Bayes Classifier excluding linker genes. Cluster FI network was applied to

identify cluster of genes (=modules)[39]. Subsequently, a pathway enrichment analysis (significance cut-off: FDR<0.01) was performed using the databases CellMap, Reactome, KEGG, NCI PID, Panther and BioCarta for each module.

To identify genes in the network, previously associated with asthma-related outcomes, natural language processing (NLP, see Online Supplement for details) was applied. In brief, mentions of genes and gene products were searched in the PubMed and PubMed Central open access literature databases and additionally filtered by the following terms “asthma”, “asthmatic”, “asthmatics”, “wheeze”, “bronchial hyperreactivity”, “airway hyperreactivity”, “bronchial hyperresponsiveness”, or “hyperreactive airway disease”.

Targeted analyses: DNA-methylation, transcription, and protein measurement

Targeted analyses were performed in a larger sample set obtained from the 6-8 years old LINA children and the 15-years old LISA children from the Leipzig study centre. No further PASTURE samples were available for these analyses. All available samples from LINA and LISA fulfilling these two criteria were included: (i) samples from children diagnosed with asthma by a physician and (ii) control samples that never reported wheezing symptoms, obstructive bronchitis, or asthma, however they could have developed atopic dermatitis or rhinitis. An overview of the selected samples for these analyses is provided in Table S1 and Table 1B.

Targeted DNA-methylation analysis was performed for a set of selected DMRs in n=127 LINA and n=140 LISA samples using the Sequenom´s MassARRAY platform (San Diego, CA, USA, Table S4 for primer sequences, Figure S2) as previously described[22].

Functional translation of methylation changes for selected genomic regions was determined by RNA and protein expression analyses of the associated genes. Whole blood samples for transcriptional analyses were collected at the same time as blood samples for DNA-methylation analyses. RNA expression data were obtained for *EPX*, *IL4,* and *IL5RA* for n=126 LINA and

n=140 LISA samples by qPCR on the Biomark HD system as previously described[22] (see Table S5 for primer sequences).

Within the LINA study phytohaemagglutinin (PHA)-stimulated IL-4 concentrations obtained from a whole blood assay were available. IL-4 concentrations were measured by cytometric bead array (BD CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) as previously described[40].

Detailed information can be found in the Online Supplement.

Statistics

WGBS samples

To determine potential differences in the study characteristics between asthmatic and control children a Fisher's exact- test or Mann-Whitney *U*-test were applied. As confounding factors in the models analysing WGBS-data the child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up on a farm and cell composition were included.

Targeted analyses

To test whether there were differences between asthmatic and control children of the LINA and LISA cohorts with respect to the child's age and sex, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up on a farm, or the presence of rhinitis or atopic dermatitis in the child, Fisher's exact- test or Mann-Whitney-*U* test were applied.

A Mann-Whitney-*U* test was used to determine if there were significant differences in DNA-methylation and transcription between groups. Spearman correlation was used to determine the association between DNA-methylation, relative gene expression, or protein concentration. Correlation coefficients are reported as effect size measures (point biserial (r_{pb}) for Mann-

Whitney U and Spearman's rho ρ). The selection of confounders associated with asthma or affecting DNA-methylation patterns was based on *a priori* knowledge. The child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth were introduced as confounding factors in all models.

Confounder adjusted logistic regression analyses were applied to compare the DNA-methylation and relative gene expression of asthmatic and control children. Confounder adjusted mediation analyses were performed using the *PROCESS* macro version v3.4[41] for SPSS. Statistical significance of the indirect effect was determined by bootstrapping as implemented in the *PROCESS* macro version 3.4[41]. Bias-corrected 95% confidence intervals were derived from the distribution of bootstrap estimates of the indirect effect from random resampling of 5,000 samples. Only for non-dichotomous independent variables a standardized indirect effect was calculated. Effect sizes of regression analyses are either provided as unstandardized *b*, standardized β, or as odds ratio (OR).

Statistical analyses were performed using STATISTICA for Windows Version 12.0/13.0 (Statsoft Inc. Europe, Hamburg, Germany), IBM SPSS Statistics for Windows Version 25 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) or R version 4.0.2[30]. *P*-values $\leq .05$ were considered significant.

RESULTS

Genome-wide DNA-hypomethylation in childhood asthma

To evaluate epigenetic alteration in the global DNA-methylation pattern of asthmatic children at single base-pair-resolution, we performed WGBS and subsequent DMR calling of whole blood samples from n=82 children participating in the LINA, LISA, or PASTURE cohort (Figure 1, Table S1). In total, samples from n=40 asthmatic children were compared to n=42 age-matched controls without an asthma history or other respiratory symptoms (Table 1A). High quality WGBS data were derived with a mean genome coverage of 56.3x (Table S2A). To retain highly confident asthma-related DMRs for downstream analyses, a multiple-step DMR-calling approach was utilized (Figure S3). Using these two independent DMR-calling algorithms, DSS and metilene, 1,021 and 758 DMRs were determined, respectively. DMRs overlapping between these two approaches (n=385) were subjected to factorial ANOVA analysis to assess whether significant DNA-methylation differences could be observed separately in each of the three cohorts and were in the same direction. Only these concordant DMRs (n=158 out of n=385) were retained for further assessment (Figure S3, see Table S6A for asthma-related DMR list). These 158 asthma-related DMRs were distributed over all autosomes (Figure 2A) and had a read coverage of 31.5x in average (Table S2B). Unsupervised cluster analysis of these derived 158 DMRs resulted in a clear separation between asthmatics and control children (Figure S4A). The vast majority of the asthma-related DMRs were hypomethylated in asthmatic children (Figure 2A), while only two hypermethylated DMRs located in the *TET3 (ten-eleven translocation 3 or tet methylcytosine dioxygenase 3)* gene and the long coding RNA *AL645608.1* were identified. In line with previous asthma EWAS studies our DMRs overlapped with several CpG sites or DMRs identified based on array approaches (see Table S7 for overlap and references and Figure S1A for evaluated EWAS studies).

Genetic and cell type composition influences on asthma-related DMRs

Since the level of DNA-methylation can be strongly dependent on the genotype or the cell type composition, asthma-related DMRs were categorized according to cell type-dependency and genotype-association (gDMRs). Based on this categorization, 38 out of the 158 DMRs were associated with the genetic background (24.1%), while the remaining 120 DMRs (75.9%) were classified as non-genotype associated DMRs (ngDMRs). A total of 465 meQTLs were identified in relation to the 38 gDMRs, of which none has been previously described as an asthma risk factor in genome-wide association studies (Table S6B). However, including all phenotypic traits of the GWAS catalogue, we found 14 DMRs associated with at least one trait. For eight of these DMRs, the trait showed a loose phenotypic association with asthma (Table S6B) including lung function (rs645601 and rs7700998). Five SNPs were associated to counts of different blood cell types with SNPs rs4328821 and rs7646596 upstream of the *RPN1*-DMR associating to the eosinophil count. Additionally, rs12699415 related to the *MAD1L1*-DMR was linked to idiopathic pulmonary fibrosis[34].

We observed an enhanced eosinophil frequency in the blood of asthmatic children (Mann-Whitney *U* test: Z=3.42, r_{pb}=0.32, p= .017, Table S8), but not for the remaining cell types, i.e. B cells, T cells, monocytes, NK cells or neutrophils. We applied adjusted multiple regression analyses to test whether different cell type frequencies have an impact on the DNA-methylation level of the determined DMRs. To this end, 37% of the asthma-DMRs (58 DMRs) were associated with the eosinophil proportion and only three DMRs in total to B cells, T cells, monocytes, NK cells or neutrophils (Table S6A). However, even after accounting for these cell types in the adjusted multiple regression models, asthma was still a significant contributor of the DNA-methylation status for all cell-type-dependent DMRs (Table S9).

Altered DNA-methylation pattern associates with perturbed immune regulation

To elucidate the relevance of the asthma-related aberrant DNA-methylation profile, a pathway enrichment analysis was performed. Besides a strong enrichment in the asthma pathway, we

found classical immune system-related pathways enriched, such as IL-5- known to be crucial for asthma pathophysiology[42,43] (Figure 2B, Table S10). To ensure that the DNA-methylation differences observed in the small number of sequenced samples can be reproduced in larger sample numbers, targeted analyses were performed in further samples (n=267) including six to eight-years-old LINA children (n=127) and 15-years-old LISA adolescents (n=140, Table S1, Table 1B). Here, we focused on DMRs that are likely to influence aberrant immune gene expression driving asthma onset. Therefore, the DNA-methylation of two prototypical DMRs (Figure S4B) linked to genes of the asthma pathway (eosinophil peroxidase, *EPX*) - the pathway with the strongest enrichment - and the IL-5 signalling pathway (*IL5RA*) (Figure 2B) known to promote severe atopic asthma associated with eosinophilia[42], was measured in the larger sample set using a targeted DNA-methylation assay. Significant hypomethylation of these DMRs located in the sixth exon of *EPX*, and in the *IL5RA* promoter, could be confirmed in meta-analysis combining samples of the LINA and LISA cohort (adj. OR/95% CI *EPX*: 0.87/0.81-0.94, p=.0004; *IL5RA:* 0.83/0.73-0.94, p=.003, n=223 controls vs. n=44 asthmatics, Figure 2C,D) using logistic regression adjusted for the child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth. Furthermore, for both DMRs a negative correlation with the relative gene expression of the associated genes *EPX* was observed (ρ= -0.40, p=1.4x10^{-11}, n=264) and *IL5RA* (ρ= -0.32, p=1.4x10^{-7}, n=266, Figure S5A). In line, expression of *EPX* and *IL5RA* is not only increased in asthmatic children (Figure S5B) but is also associated with an increased risk for asthma during childhood (relative expression *EPX*: adj. OR/95% CI: 1.44/1.09-1.91, p=.010, n=220 controls vs. n=44 asthmatics, *IL5RA:* adj. OR/95% CI: 1.59/1.19-2.13, p=.002, n=222 controls vs. n=44 asthmatics, Figure 2D).

DNA-methylation changes in asthma affect regulatory hubs

The identified DMRs showed enrichment for 20 binding motifs related to different transcription factors previously associated with asthma including the Th2 master regulator GATA3[44] (Table S11). Additionally, two third of the DMRs were located in genomic regulatory elements, 74% of the DMRs intersecting with enhancers, and 1% with promoters (Figure 3A). In particular, the DMR enrichment in enhancer regions was highly significant (OR/95% CI: 5.83/4.05-8.53, $p<4.0x10^{-26}$). Among the DMRs overlapping with a ROADMAP enhancer active in specific blood cells (Table S3), 17 DMRs overlapped with a T helper cell-type specific enhancer including a hypomethylated enhancer DMR associated with the mTORC1 scaffolding protein coding gene *RPTOR* (Table S6A).

One of those hypomethylated enhancer regions showed an enhancer specific ENCODE histone modification profile and a ChiA-PET interaction to the *IL4* promoter (Figure 3B). Although IL4 is one of the key regulators in allergic diseases including asthma, the relevance of this particular enhancer region associated to IL-4 expression has not been addressed so far. We confirmed the asthma-related DNA-hypomethylation of this *IL4* enhancer in the meta-analysis combining the two cohorts LINA and LISA (adj.OR/95% CI: 0.83/0.74-0.94, p=.002, n=223 controls vs. n=44 asthmatics). In addition, in the LINA cohort, where IL-4 protein concentration measurements were available (Table S1), the *IL4* enhancer DNA-methylation was associated with *IL4* transcription (ρ= -.35, p=.0001) and PHA-stimulated IL-4 protein concentrations (ρ= -.31, p=0.0009, Figure 3C). In line, two confounder adjusted mediation models were applied to evaluate the relevance of this hypomethylated *IL4* enhancer region in asthma: The first model showed a significant indirect effect of *IL4* enhancer DNA-methylation on IL-4 protein concentration via *IL4* transcription as a mediator (β/95% CI: -0.07/ -0.14- -0.03, Figure 3D), whereas the direct effect was not significant (b/95% CI: -0.92/ -3.79- 1.95, p= .525). Second, the asthma phenotype contributed to an increase in IL-4 protein concentration in asthmatics again solely indirectly via the DNA-methylation changes of this *IL4* enhancer and *IL4*

transcription as mediators (Figure S6, indirect effect: *b*/95% CI: 0.05/ 0.01- 0.13; direct effect *b*/95% CI: 0.23/ -0.26- 0.73, *p*=.352).

Genes affected by DNA-methylation changes are functionally connected

To elucidate whether DMR associated genes (n=435 genes, Table S6A) were functionally connected, these genes were subjected to network analysis based on established protein-protein interactions with a subsequent pathway enrichment of the derived network modules. The resulting network consisted of 102 genes in thirteen distinct modules. These modules were related, among others, to immune response and inflammation, cilium assembly and general gene regulation, and to Jak-STAT signalling (Figure 4, Table S12). The vast majority of the network genes (97 out of 102 genes) were targets of differentially methylated enhancers. Our NLP analysis revealed that 33.3% of these enhancer target genes such as the central transcription factors of the immune system *RELA* (NFκB subunit encoding gene)*, GATA2,* and *ZFPM1,* the Th2 cytokine *IL4,* or the mTOR complex 1 scaffold protein *RPTOR* have previously been described in the literature in association with asthma (red genes in Figure 4, Table S13A). In addition, we identified novel genes not yet associated to asthma, such as the A-kinase anchoring protein-9 (*AKAP9*). *ANKAP9* is prominently expressed in T cells and involved in immune synapse formation[45]. Among the proteins interacting with ANKAP9 for its proper function are TUBGCP2/TUBGCP6, for which we also observed an enhancer DMR[46].

Prenatal priming for asthma

To discriminate between DMRs that are a consequence of the disease from those predisposing an individual, we subjected matched cord blood samples (n=23 asthmatics vs. n=25 controls) to WGBS and assessed whether the methylation changes of the 158 asthma-related DMRs were already present at time of birth (Figure 5A). 35% (56/158 DMRs) of the DMRs identified in the established asthma phenotype were already significantly differentially methylated in cord blood

samples (Table S6C). Most of the cord blood DMRs were again located in enhancers (43 out of 56), 39% (n=22/56 DMRs) were gDMRs including those already identified in GWAS as a risk factor for lung dysfunction and idiopathic pulmonary fibrosis[34] (Table S6C). For 22 out of the 56 cord blood DMRs, we found an overlap with previous EWAS studies investigating the impact of a variety of different prenatal factors on DNA-methylation (Figure S1B). These factors included exposure to tobacco smoke, to air pollution or to environmental chemicals such as phthalates or lead, maternal diet-related metabolites as well as factors related to maternal health like gestational diabetes or preeclampsia (Figure 5B, Table 13B). When focusing our network analysis on cord blood DMR associated genes the network was comprised of several members of the LFA-1 signaling pathway (Figure 5C). Next to *ITGAL* coding for one of the subunits of LFA-1 (=CD11a), also the LFA-1 ligand ICAM-1, and the co-chaperones *ANKAP9*, *TUBGC2/6* were among the target genes of DMRs already observed in cord blood.

DISCUSSION

To characterize the complete genome-wide DNA-methylation pattern in childhood asthma, this study determined the DNA-methylation profile of 40 asthmatic and 42 control children by utilizing WGBS followed by calling of differentially methylated regions (DMRs) and discriminating between genotype-, and non-genotype-associated as well as cell-type-dependent, or -independent DNA-methylation changes. In total, 158 regions were found to be differentially methylated in childhood asthma, all hypomethylated except for two, which includes a hypermethylated enhancer region for *TET*. Since TET proteins initiate DNA-demethylation, this DMR might be directly related to the global DNA-methylation aberrations observed in asthma. Whether this DMR in asthma is an initiating event or a compensatory mechanism remains to be elucidated in follow-up studies. The predominant global hypomethylation suggests a pronounced epigenetic activation affecting a variety of immune-related genes associated with asthma development and exacerbation. Here, with this first EWAS using a genome-wide sequencing approach and thus not relying on pre-selected CpGs as performed in previous asthma EWASs, we show that this epigenetic activation primarily affects enhancer elements indicating that a predominant enhancer activation underlies the exacerbated immune response characteristic of childhood asthma[47]. The tight connectivity of these epigenetically dysregulated asthma genes is evident in our inferred interaction network. A comprehensive search of the current scientific literature by NLP analytics revealed that while almost 34% of the enhancer target genes have already been associated with asthma or asthma-related terms, several of the enhancer-DMRs have not yet been discussed in the context of asthma. Most of the asthma-DMRs were enriched for multiple TFBS indicating multiple regulatory effects of the epigenetically perturbed regions. Most of the transcription factors binding to these DMR-enriched TFBS motifs are known to be associated with asthma, such as GATA3[44], NFACT1[48], IRF-1[49], GATA-6[50], STAT2[51], THB[52], or EGR1[53], and even possess a master regulatory capacity of Th2 differentiation[54,55].

LFA-1 is mainly known for its role in T cell adhesion and Th1 effector polarization. However, a recent report shows that LFA-1 and its ligand ICAM-1 are expressed on group 2 innate immune cells (ILC2). ILC2 are able to induce eosinophilic lung injury and are elevated in the blood of asthmatics compared to healthy controls[56]. Knock-down of LFA-1 or ICAM-1 both attenuated airway hyperresponsiveness, reduced airway inflammation and decreased lung ILC2 accumulation in mouse models of allergic asthma[57]. As such the observed cord blood DNA-hypomethylation of several regions involved in the LFA-1 signalling cascade might predispose children to a higher risk of allergic asthma.

The vast majority of DMRs was not associated with a meQTL indicating that mainly other than genetic factors contribute to the observed aberrant DNA-methylation in childhood asthma. About one third (35%) of the asthma-related DMRs were already found in cord blood. A variety of environmental insults experienced during the highly susceptible prenatal developmental phase - mostly related to maternal lifestyle factors during pregnancy - have been associated with an increased asthma risk of the child. A comparison to previous EWAS studies revealed that 22 of the asthma-related DMRs already identified in cord blood, including 17 differentially methylated enhancers, overlapped with DNA-methylation changes described in association to prenatal asthma risk factors (for references refer to Table S13B). Among others, these factors included maternal exposure to tobacco smoke or environmental chemicals as well as maternal health (e.g. gestational diabetes, preeclampsia). Although more studies are necessary to investigate whether these regions of persistent differential DNA-methylation are missing links between an adverse intrauterine environment and childhood asthma development, it is prudent to reduce these adverse exposures during vulnerable periods.

This study has to be seen in the light of some limitations. The sample size of whole-genome sequencing approaches seems to be low when compared to previous EWAS using less cost-intensive array based epigenetic profiling methods[5,8,58], however, in comparison to previous

WGBS studies[59-62] we included a considerable higher number of samples. In addition, the enrichment of the DMRs in the asthma pathway, the overlap between the DMR-associated genes with known asthma genes such as *IL4*, *EPX*, *IL5RA* and *ZFPM1* as identified by NLP, in conjunction with the overlap of previously reported CpG sites (e.g. *ACOT7*, *DEGS2*, *EPX* and *GATA2*) of asthma EWAS support the validity of the applied strategy to determine asthma-related DMRs. Although we confirmed the differential DNA-methylation of selected DMRs and their influence on associated target gene expression that are likely to contribute to asthma pathology in a larger sample set, further studies are needed to show whether the DMRs observed in our study can be replicated in independent cohorts and to determine the effect of the identified DMRs on the transcriptome. In addition, since more than half of the asthmatic children reported rhinitis or atopic dermatitis in their life, we cannot exclude that the observed asthma-related DMRs might also be influenced by other atopic diseases such as rhinitis or atopic dermatitis.

The whole blood-based sequencing of DNA-methylation might be seen as a further limitation. To overcome this problem, the proportion of the different cell populations was determined by a deconvolution approach and the DMRs annotated with respect to their cell-type dependency. The deconvolution approach might have led to misclassification or underrepresentation of minor cell types. However, we were able to annotate the small population of eosinophils and to show a significant difference in the eosinophil count between children with asthma and controls without respiratory disease. For a global overview of aberrant DNA-methylation changes and an unbiased interpretation of EWAS[63], we deem the here utilized approach more appropriate, i.e., not to adjust for cell-type composition beforehand, but rather to determine all DMRs and subsequently annotate them as cell-type-dependent or genotype-associated.

To our best knowledge, this is the first study evaluating the children's methylome at single base-pair resolution – including the comprehensive information on the genetic background - using repeatedly collected samples of the same individual. We were able to confirm our findings in a larger sample set of two cohorts and showed functional translation to the transcriptional and

protein level for selected DMRs. We identified global DNA-methylation changes particularly affecting enhancers, which likely contribute to an altered gene expression of key immune genes involved in asthma pathology. Most of the immune system-related epigenetic alterations including the hypomethylated *IL-4* enhancer, or the *IL5RA* promoter are not present in cord blood, supporting the notion that they are developed during the shift of the immune response toward a Th2 reactivity contributing to the development of an atopic asthma phenotype. Although most of the cord blood DMRs are not directly related to the immune dysfunction characterizing the asthmatic phenotype, these regions related to genes involved in LFA-1 signaling. In light of the emerging role of LFA-1 in ILC2 modulated allergic asthma, these cord blood DNA-methylation changes might be involved in predisposing children to a higher risk for asthma development. Future studies will show if these regions have the ability to predict high-risk children.

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Author contribution

IL, RE, MKa, and ST provided project leadership.

AvB, BS, JH, MB, SR, EvM, JR, ADC, RL, MKa, AMK, IL, GH were involved in the recruitment and field work of the cohorts.

GH provided cytokine data.

MB provided the RNA transcription data.

SDM, MK, MB, TB, and CH performed the DMR calling and DMR annotation.

MK, LT, DW, OM, and CP performed or guided targeted methylation analyses.

MK, SDM, MM, MB, NI, TB, CH, ST, GS, and LT performed or supervised data analysis.

SS, EF, UH, MK and ST performed or evaluated NLP analysis.

LT, ST, MK, and IL wrote the manuscript.

All authors were involved in the discussion and contributed to the final manuscript.

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Figure captions

Figure 1: Study design. Blood samples derived from asthmatics or control children of the three cohorts were subjected to WGBS to determine asthma-related DMRs. DMRs comparing asthmatic and control children were determined by the two independent DMR-calling algorithms DSS and metilene. Asthma-related DMRs were subsequently analysed.

DMR = differentially methylated region, WGBS = whole-genome bisulfite sequencing, LINA = lifestyle and environmental factors and their influence on newborns allergy risk, LISA = influences of lifestyle-related factors on the immune system and the development of allergies in childhood, PASTURE = Protection Against Allergy: Study in Rural Environments

[1] based on available cord blood sample of LINA and PASTURE

[2] based on available whole blood samples of LINA and LISA

[3] based on available plasma samples of LINA

Figure 2: DMR distribution and down-stream analyses. (A) Circos plot represents the distribution of the 158 differentially methylated regions (DMRs) identified in asthmatic children vs. controls across all autosomes. The outer circle shows the 22 autosomes. The bars in the inner circle represent the DMRs and their chromosomal location. Hypermethylated DMRs are indicated as red bars, hypomethylated DMRs in blue. The height of each bar indicates the DNA-methylation differences between asthmatics and controls. **(B)** KEGG pathway enrichment for all asthma-DMRs based on their genomic location. **(C)** DNA-methylation difference between asthmatic children and controls of the WGBS samples (asthma n=40, controls n=42), LINA study (asthma n=19, controls n=108) and LISA study (asthma n=25, controls n=115) for DMRs related to *EPX* and *IL5RA* as determined by sequencing or MassARRAY, respectively (*p*-value from Mann-Whitney U-test, r_{pb}:

point biserial correlation coefficient). **(D)** Association of *EPX* and *IL5RA* DNA-methylation (black whiskers) and transcription (magenta whiskers) to asthma outcome in meta-analysis combining LINA and the LISA study (DNA-methylation: asthma n=44, controls n=223, *EPX* transcription: asthma n=44, controls n=220, *IL5RA* transcription: n=44 asthma n=222 controls). Given are ORs with +/-95% CIs from logistic regression adjusted for child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth using ln-transformed DNA-methylation values.

Figure 3: Genomic location of asthma-related DMRs and functional translation of *IL4* enhancer hypomethylation. (A) Pie chart represents the proportional distribution of the genomic regions affected by asthma-related DMRs. **(B)** Genomic location of the *IL4* DMR and the genomic region analysed by MassARRAY in the UCSC genome browser[64]. **(C)** Scatterplots show the association of *IL4* DNA-methylation to *IL4* transcription (n=112) and IL-4 protein concentration (n=115) and the association of IL-4 protein concentration to *IL4* transcription (n=111) in six-years-old children of the LINA study. Correlation coefficient (ρ) and p-value from Spearman correlation. **(D)** Mediation analysis for the relationship of *IL4* enhancer DNA-methylation, *IL4* transcription, and IL-4 protein concentration of six-years-old children of LINA (n=111). Model was adjusted for child's sex, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth. IL-4 protein concentrations were determined after PHA-stimulation. Protein and DNA-methylation data were ln-transformed before analysis. Effect sizes for indirect path is given as standardized β-values with +/-95% CIs. Significance determined by bias-corrected bootstrapping.

MA = MassARRAY, DMR = differentially methylated region

Figure 4: Network module analysis of asthma-DMR associated genes. Shown are all asthma-DMR associated genes, which show a predicted or experimentally based interaction. Only modules with more than one connection are shown. Target genes of enhancers affected by a DMR are highlighted by blue outline circles. Genes related to asthma or similar terms as determined by the natural language processing tool are indicated in red font. Module nomenclature is based on subsequent pathway enrichment analysis (Table S12).

Figure 5: Cord blood asthma-DMRs. (A) Matched cord blood samples were subjected to WGBS to determine the DNA-methylation level of the asthma-related DMRs at time of birth in control children compared to those children who later developed asthma. **(B)** Pie charts represent the portion of genotype-, and non-genotype-associated DMRs (g/ngDMRs) and those DMRs, which were already differentially methylated in cord blood samples (=cord blood DMRs). The table lists all prenatal influencing factors that have previously been associated with CpGs included in the n=56 cord blood asthma-related DMRs (see Table S6C for EWAS references and cord blood DMR list). Genes highlighted in red font were described with asthma as identified by natural language processing (see Table S13B for references). #ngDMRs indicated with light blue and gDMR with dark blue background. *Enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annotation was available the closest TSS gene is given. **(C)** Network module analysis for cord blood DMR associated genes (left panel) and for genes associated to DMRs only present in asthma phenotype (right panel). Only modules with more than one connection are shown. Module nomenclature is based on network of Figure 4.

PFOS = perfluorooctane sulfonic acid, NLP = natural language processing

Figure S1: Literature search for overlap of DMRs with previous EWAS. (A) Workflow summarizes the EWAS studies investigated asthma-related outcomes, which were used for the overlap with n=158 asthma-related DMRs. **(B)** Workflow summarizes the EWAS studies investigated prenatal influencing factors, which were used for the overlap with n=56 cord blood asthma-related DMRs.

Figure S2: Quality control of the MassARRAY amplicons. Graphs show DNA-methylation values derived by MassARRAY measurements of standard samples (0%, 20%, 40%, 60%, 80%, and 100% methylated genomic DNA) representing the mean DNA-methylation of the MassARRAY amplicon for **(A)** *IL5RA* (including 7 CpGs), **(B)** *EPX* (including 9 CpGs) and **(C)** *IL4* (including 6 CpGs) DMR (given are mean ±SD of two replicates and r^2 from linear regression).

Figure S3: Study workflow. DMRs comparing asthmatics and control children of three cohorts were called by two independent algorithms (DSS, metilene) and concordant DMRs were subjected to multiple test corrected factorial ANOVA analysis to determine asthma-related DMRs. These 158 DMRs were subsequently analysed and categorized based on their dependency on the genotype, the cell type composition or their presence also in cord blood.
DMR = differentially methylated region, ng/gDMR = non-genotype/genotype-associated DMRs

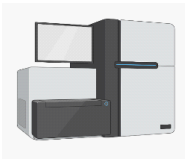
Figure S4: Characteristics of asthma-related DMRs. (A) Cluster analysis with ß-methylation values of the 158 asthma-related DMRs. **(B)** CoMET plot of the validated *EPX* and *IL5RA* DMR. Plots show WGBS derived β-methylation values of asthmatics vs. control children on top (n=40 vs. 42, mean +/-SEM) followed by *p*-values from logistic regression analysis (asthma phenotype ~

β-values) and UCSC tracks (ENSEMBL genes, CpG islands, DNAse-sensitive regions and SNPs) as well as the DNA-methylation correlation matrix for all CpG sites within the DMRs.

Figure S5: Epigenetic regulation of *IL5RA* and *EPX* transcription in asthma. (A) Correlation between *IL5RA* and *EPX* DNA-methylation to *IL5RA*/*EPX* transcription of children of the LINA (magenta) and LISA (black) study. Correlation coefficient ρ and p-value from Spearman correlation~~Indicated is Spearman correlation~~. **(B)** Relative gene expression of *IL5RA* and *EPX* in asthmatic children and controls participated in LINA (asthma n=19, controls n=107) or LISA (*IL5RA*: asthma n=25, controls n=115, *EPX:* asthma n=25, controls n=113). p-value from Mann-Whitney U-test, r_{pb}: point biserial correlation coefficient.

Figure S6: IL-4 protein and *IL4* enhancer hypomethylation in asthma. Illustrated is the mediation model adjusted for child's sex, prenatal tobacco smoke exposure, family history of atopy, parental school education and maternal age at birth. Effect sizes for the indirect path is given as standardized β-values with +/-95% bias-corrected bootstrap CIs (n=111, significant as determined by bootstrap CI). The analysis is shown for the LINA subcohort with available PHA-stimulated IL-4 protein concentrations. Protein and DNA-methylation data were ln-transformed before analysis.

Figure 1

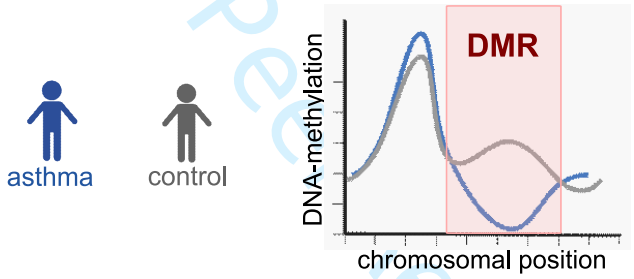


Whole-genome bisulfite sequencing (WGBS)

asthma vs. control

cohort	LINA		LISA		PASTURE		**total WGBS samples**	
age [years]	5-8		15		6		5-15	
blood samples	n=12	n=13	n=14	n=15	n=14	n=14	n=40	n=42

Calling of differentially methylated regions (DMRs)



Down-stream analyses of asthma-related DMRs

DMR categorization

- genotype-associated
- cell-type associated
- present in cord blood[1]

Enrichment analyses

- genomic regulatory elements
- signaling pathways
- network-modules

Functional translation

- RNA expression[2]
- protein concentration[3]

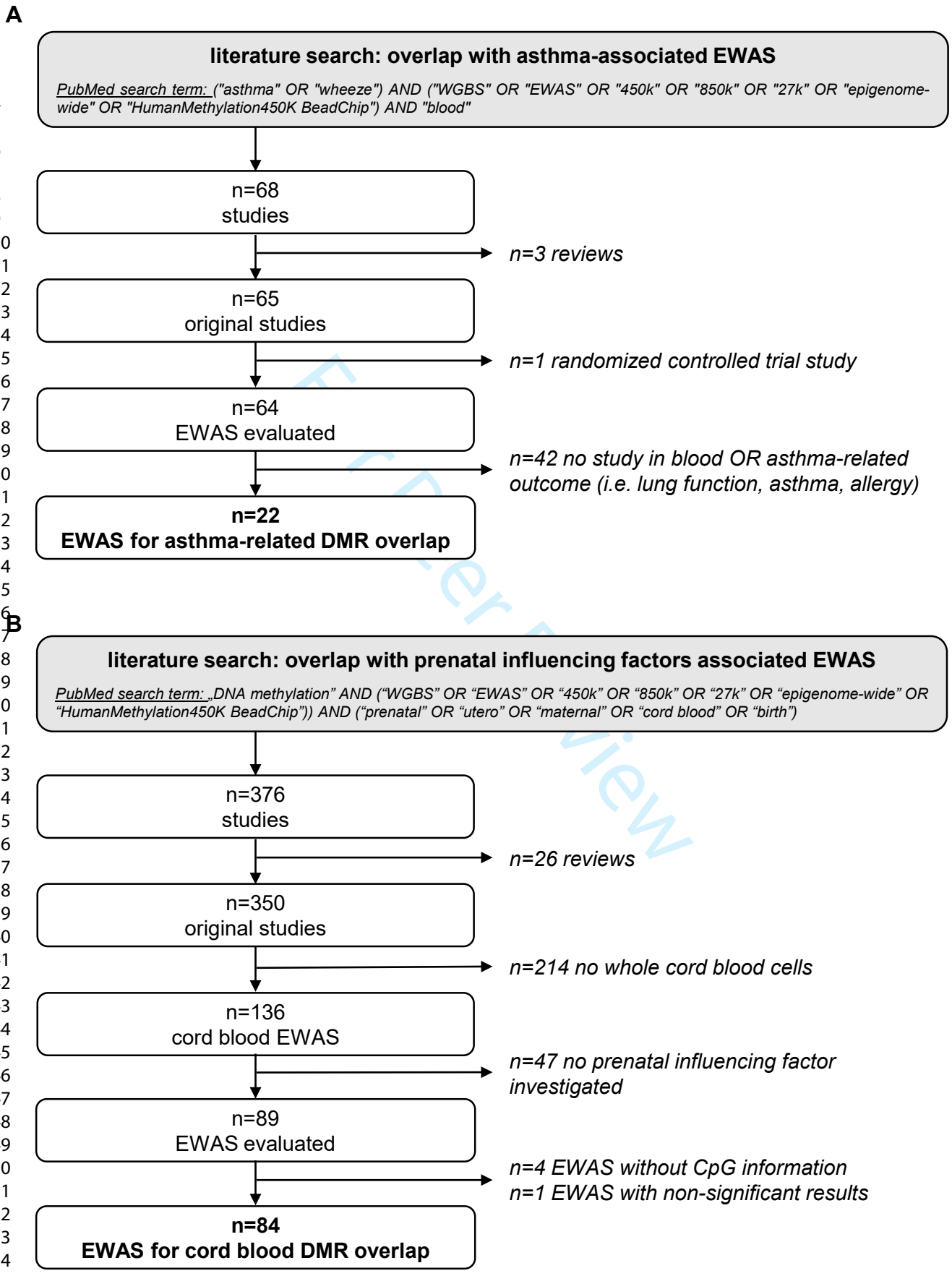
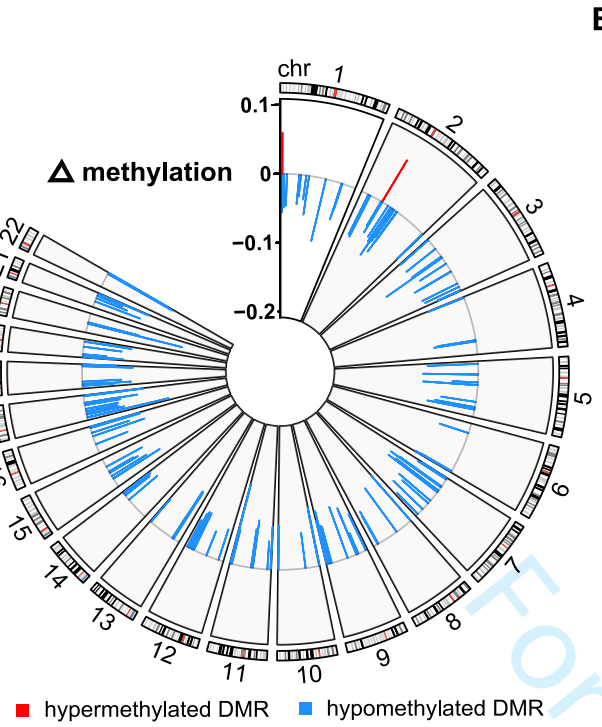
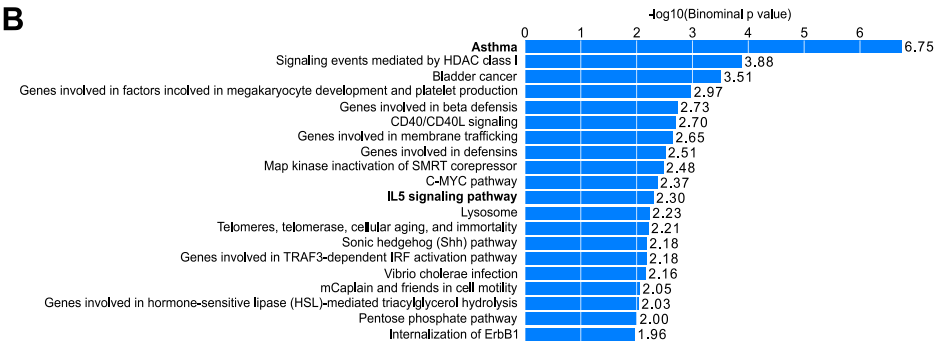
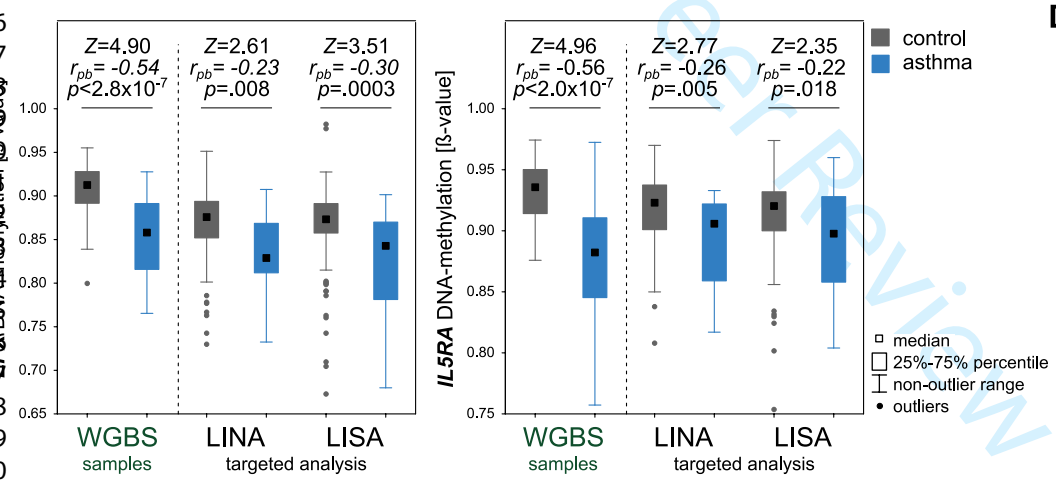

A
literature search: overlap with asthma-associated EWAS
PubMed search term: ("asthma" OR "wheeze") AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip") AND "blood"
n=68 studies
n=3 reviews
n=65 original studies
n=1 randomized controlled trial study
n=64 EWAS evaluated
n=42 no study in blood OR asthma-related outcome (i.e. lung function, asthma, allergy)
n=22 EWAS for asthma-related DMR overlap
B
literature search: overlap with prenatal influencing factors associated EWAS
PubMed search term: „DNA methylation" AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip")) AND ("prenatal" OR "utero" OR "maternal" OR "cord blood" OR "birth")
n=376 studies
n=26 reviews
n=350 original studies
n=214 no whole cord blood cells
n=136 cord blood EWAS
n=47 no prenatal influencing factor investigated
n=89 EWAS evaluated
n=4 EWAS without CpG information
n=1 EWAS with non-significant results
n=84 EWAS for cord blood DMR overlap

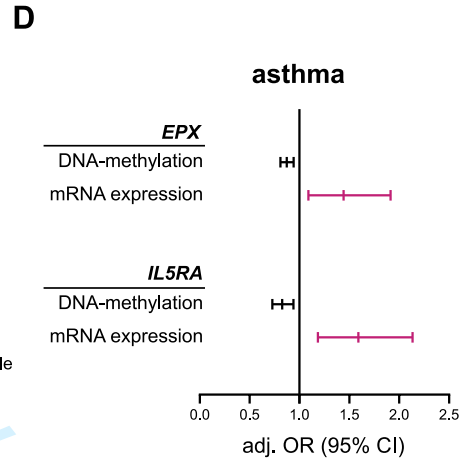
Figure 2





pathways	DMR associated genes in pathway	genes observed (total genes) in pathway
Asthma	***EPX***, *IL13, IL4*	3 (28)
Signaling events mediated by HDAC Class I	*GATA2, NCOR2, ZFPM1*	3 (64)
Bladder Cancer	*EGFR, MYC, THBS1, TYMP*	4 (42)
Genes involved in factors involved in megakaryocyte development and platelet production	*GATA2, PRKACB, RCOR1, ZFPM1*	4 (128)
Genes involved in beta defensins	*DEFB115*	1 (40)
CD40/CD40L signaling	*IL4, MYC, TRAF1, TRAF3*	4 (30)
Genes involved in membrane trafficking	*AP3B1, CHMP6, GJB6, TJP1*	4 (128)
Genes Involved in defensins	*DEFB115*	1 (49)
Map kinase Inactivation of SMRT corepressor	*EGFR, NCOR2*	2 (11)
C-MYC pathway	*KAT5, MYC, TAF9*	3 (25)
IL5 Signaling Pathway	*IL4,* ***IL5RA***	2 (10)
Lysosome	*AP3B1, CTSD, DNASE2B, LGMN*	4 (121)
Telomeres, telomerase, cellular aging, and immortality	*EGFR, MYC*	2 (18)
Sonic hedgehog (Shh) pathway	*DYRK1A, GLI2, PRKACB*	3 (16)
Genes involved in TRAF3-dependent IRF activation pathway	*DDX58, TRAF3*	2 (14)





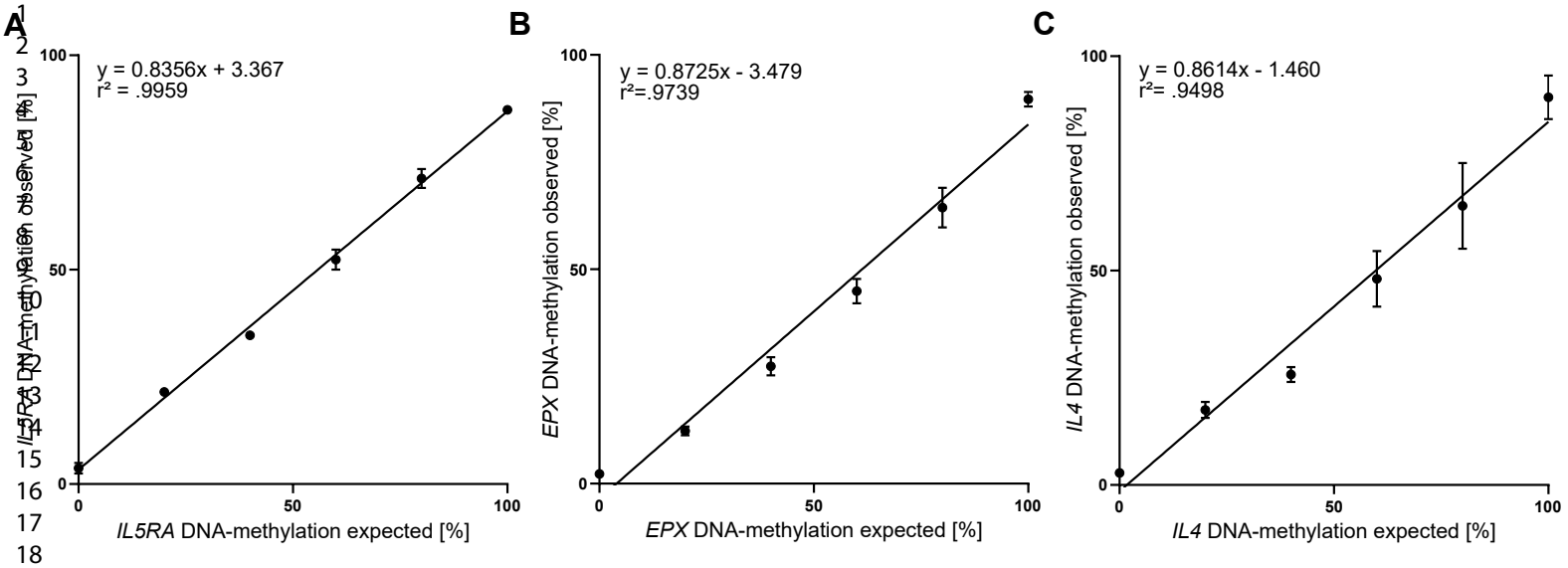
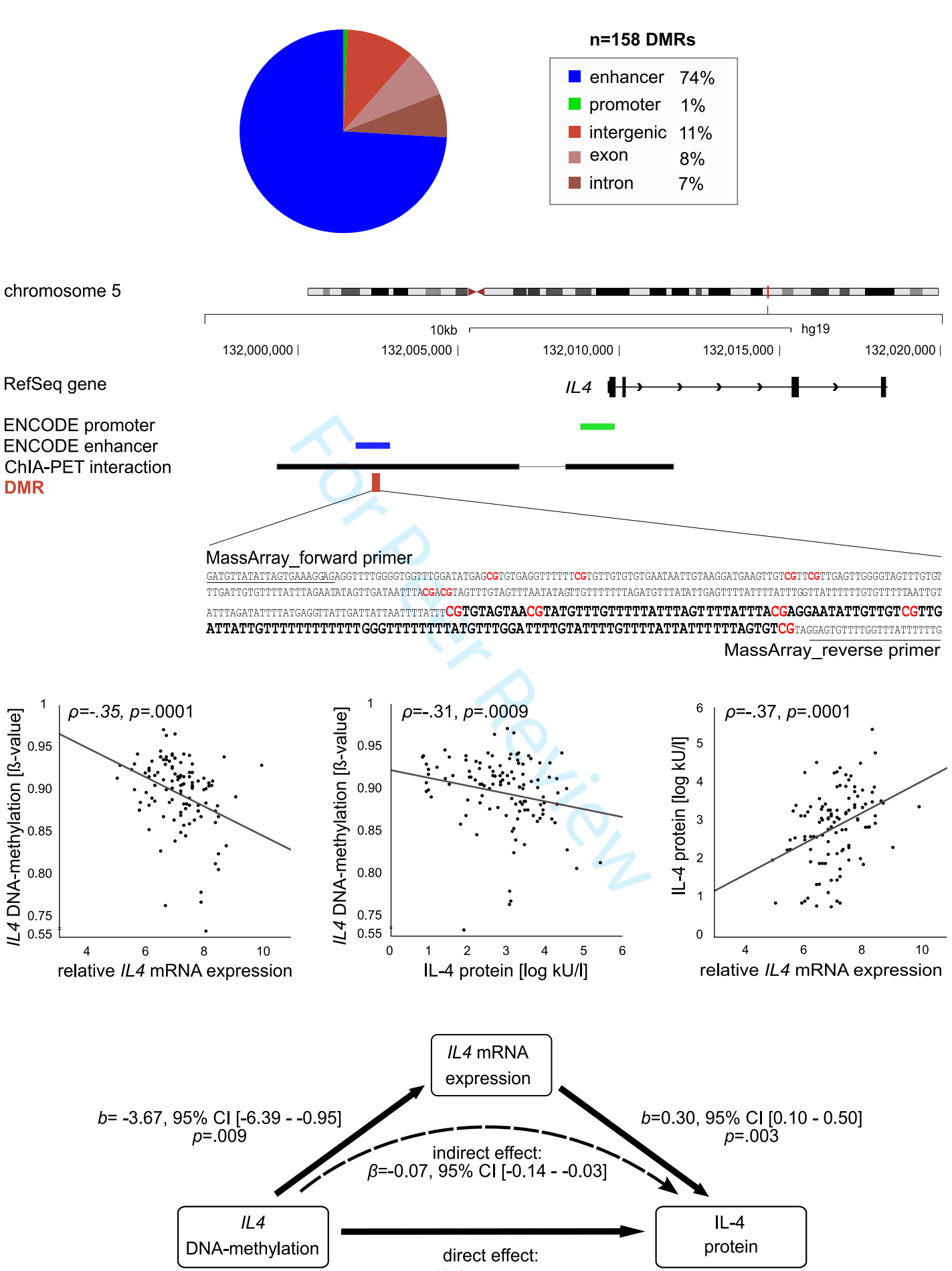


Figure 3



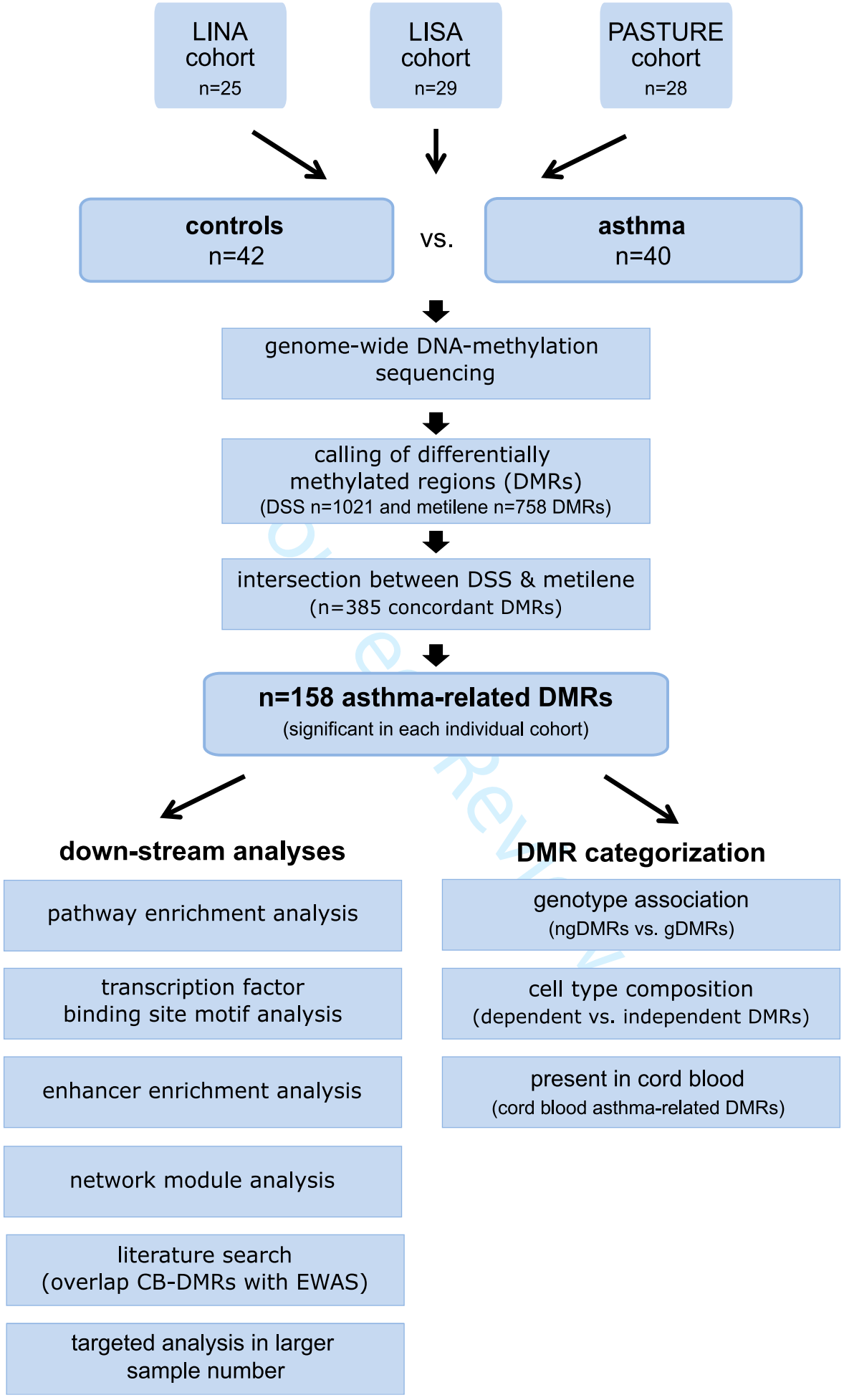
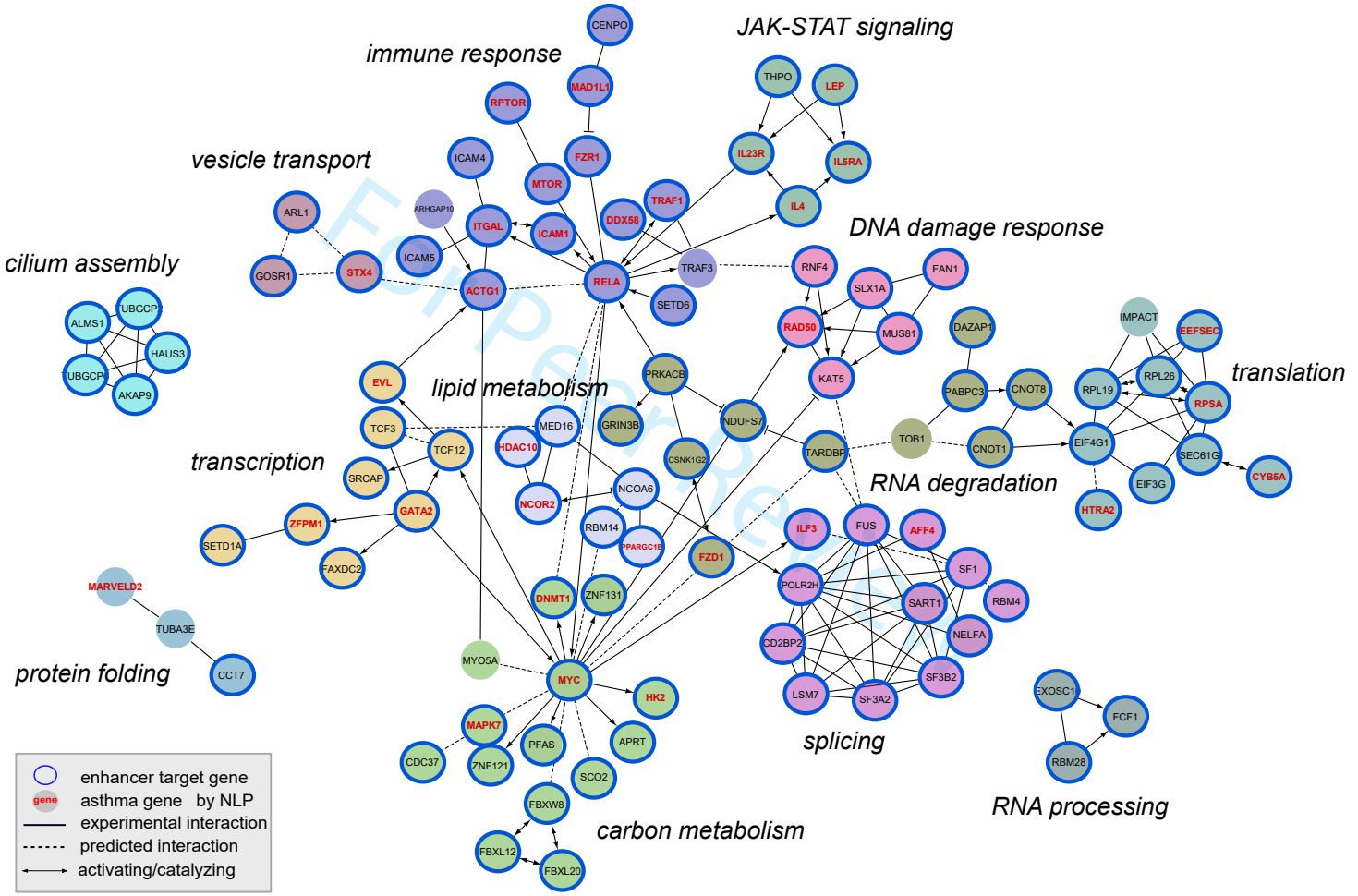

LINA cohort n=25
LISA cohort n=29
PASTURE cohort n=28
controls n=42
vs.
asthma n=40
genome-wide DNA-methylation sequencing
calling of differentially methylated regions (DMRs)
(DSS n=1021 and metilene n=758 DMRs)
intersection between DSS & metilene
(n=385 concordant DMRs)
n=158 asthma-related DMRs
(significant in each individual cohort)
down-stream analyses
pathway enrichment analysis
transcription factor binding site motif analysis
enhancer enrichment analysis
network module analysis
literature search (overlap CB-DMRs with EWAS)
targeted analysis in larger sample number
DMR categorization
genotype association
(ngDMRs vs. gDMRs)
cell type composition
(dependent vs. independent DMRs)
present in cord blood
(cord blood asthma-related DMRs)

Figure 4


immune response
JAK-STAT signaling
vesicle transport
cilium assembly
DNA damage response
lipid metabolism
translation
transcription
RNA degradation
protein folding
splicing
RNA processing
carbon metabolism
CENPO
MAD1L1
RPTOR
FZR1
ICAM4
MTOR
THPO
LEP
IL23R
IL5RA
IL4
TRAF1
DDX58
ARL1
ARHGAP10
ITGAL
ICAM1
ICAM5
GOSR1
STX4
ACTG1
RELA
TRAF3
SETD6
RNF4
SLX1A
FAN1
RAD50
MUS81
DAZAP1
KAT5
ALMS1
TUBGCP
HAUS3
AKAP9
EVL
PRKACB
TCF3
TCF12
MED16
HDAC10
GRIN3B
NDUFS7
CSNK1G2
TARDBP
TOB1
CNOT1
PABPC3
CNOT8
IMPACT
EEFSEC
RPL19
RPL26
RPSA
EIF4G1
SEC61G
EIF3G
CYB5A
HTRA2
SRCAP
GATA2
ZFPM1
SETD1A
FAXDC2
NCOR2
NCOA6
RBM14
PPARGC1B
FZD1
MARVELD2
TUBA3E
CCT7
DNMT1
ZNF131
ILF3
FUS
AFF4
POLR2H
SF1
SART1
RBM4
NELFA
CD2BP2
SF3B2
LSM7
SF3A2
MYO5A
MYC
HK2
MAPK7
CDC37
ZNF121
PFAS
APRT
SCO2
FBXW8
FBXL12
FBXL20
EXOSC1
FCF1
RBM28
enhancer target gene
gene
asthma gene by NLP
experimental interaction
predicted interaction
activating/catalyzing

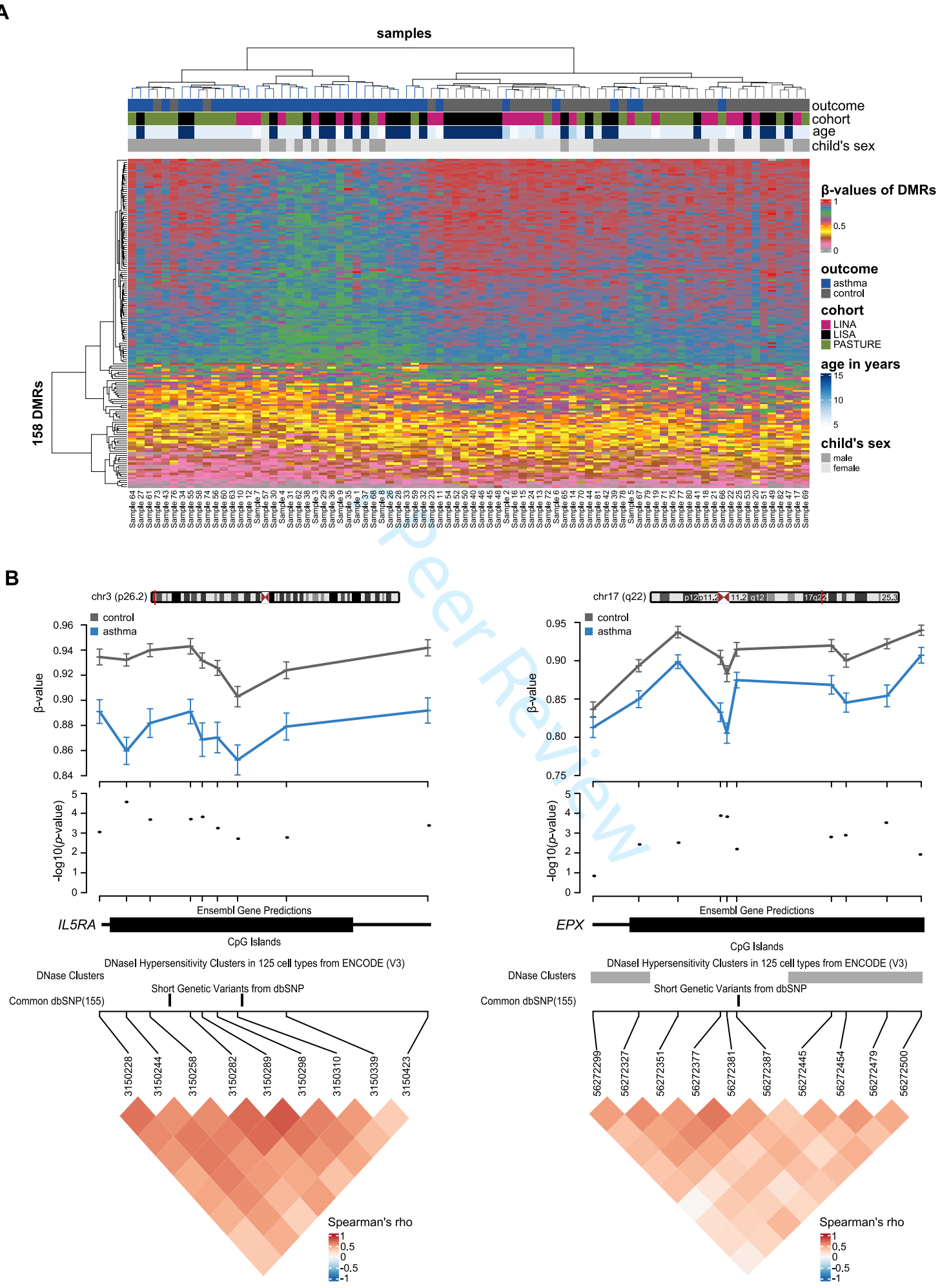
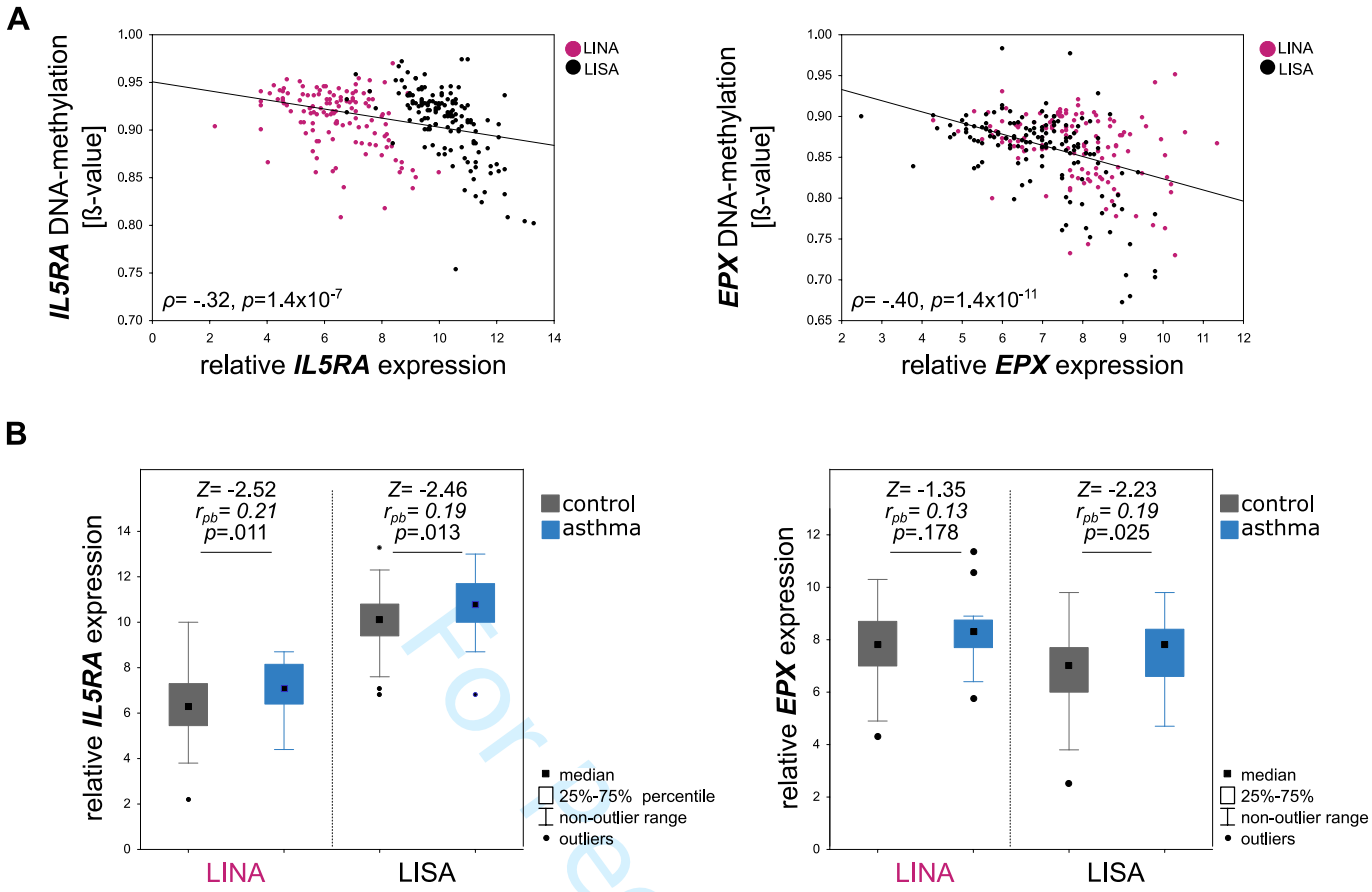
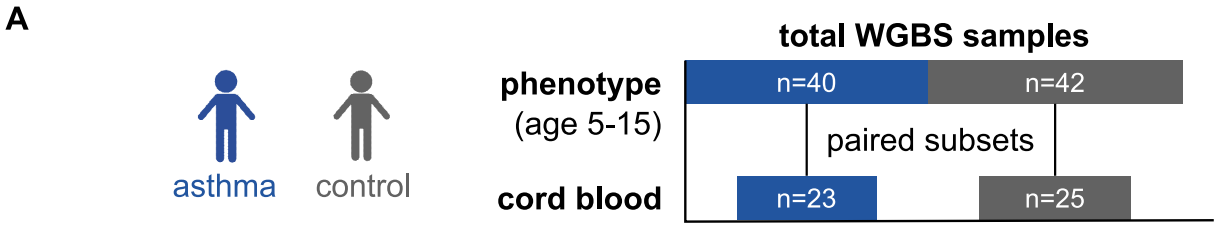
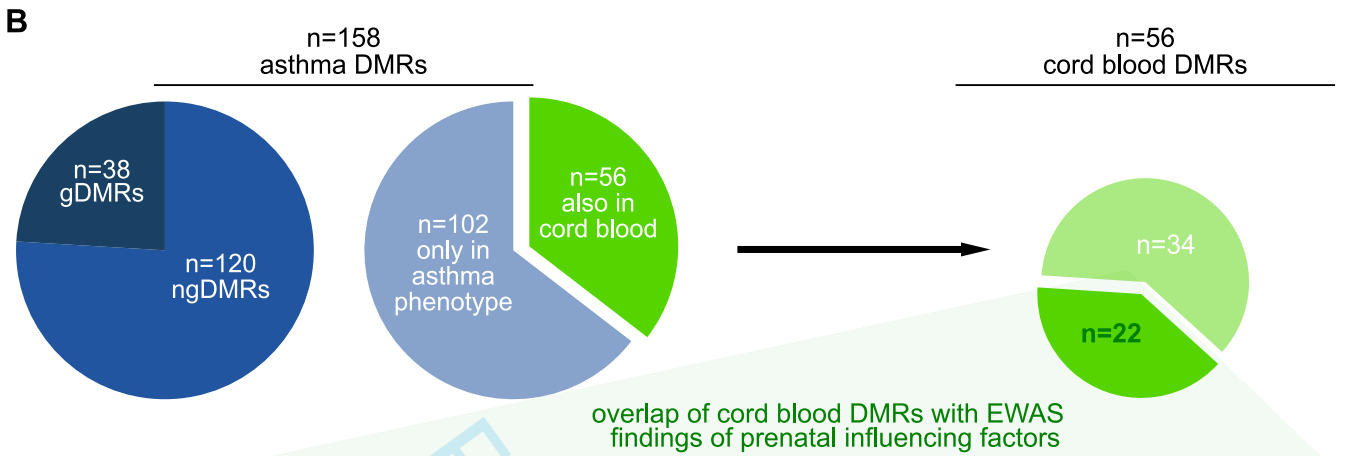


Fig. S5







overlap of cord blood DMRs with EWAS findings of prenatal influencing factors

	DMR#	associated genes*	prenatal influence
no enhancer	1	*AL645608.1*	lead
	2	*TFCP2L1*	maternal diabetis
	3	*WDR37*	maternal diabetis
	4	*IMPAD1*	S-adenosylhomocysteine
	5	*PDE1C*	tobacco smoke, maternal diabetis
enhancer	6	*AL359736.1*	lead
	7	*RP11-478C1.7;ZFYVE28;RNF4;NOP14-AS1;NELFA; POLN;HAUS3;RP11-317B7.3;C4orf48;MXD4*	air pollution
	8	*AC073046.25;TET3;MGC10955;ALMS1;CCT7;MOB1A; SLC4A5;HTRA2;DGUOK-AS1*	lead
	9	*KIF25;KIF25-AS1;FRMD1*	lead
	10	*GJB2;MIR4499*	lead, tobacco smoke
	11	*MIR4458HG;RP11-480D4.2;MTRR*	maternal diabetis
	12	*ACSF3;CBFA2T3;ZC3H18;GALNS;AC137932.1;PIEZO1; RP5-1142A6.9;ANKRD11;BANP;ZFPM1;MIR5189*	nPFOS
	13	*CTD-2542L18.1;GSE1*	preeclampsia, lead
	14	*RP11-62J1.4;RBM28;LEP*	tobacco smoke
	15	*OPCML*	tobacco smoke, folate
	16	*RP11-443B20.1;CENPO;ATAD2B;MFSD2B;UBXN2A*	maternal diabetis
	17	*ZNF426;ZNF846;ICAM1;ZNF121;DOCK6;ILF3;KRI1;CTC-325H20.4; DNMT1;ZNF561;ZNF699;FBXL12;ZNF559;CDC37;ZNF266;S1PR2; CTD-2369P2.8;ICAM4;ICAM5;EIF3G;RAVER1;PPAN;ZNF562*	lead
	18	*PANX2*	maternal diabetis, lead
	19	*CTD-2201E18.5;ZNF131*	nPFOS
	20	*TRMU*	phthalate, lead
	21	*GPC1;MIR149;ANKMY1*	tobacco smoke
	22	*LINC00960;RP11-803B1.3;LSP1P2*	tobacco smoke

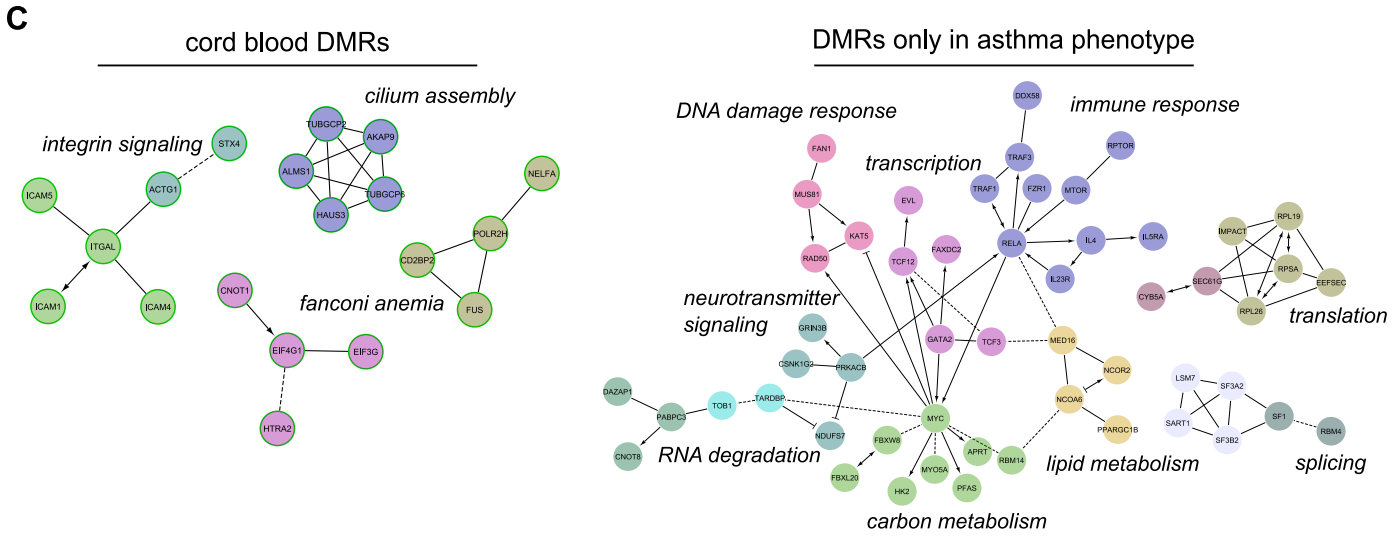


Fig. S6

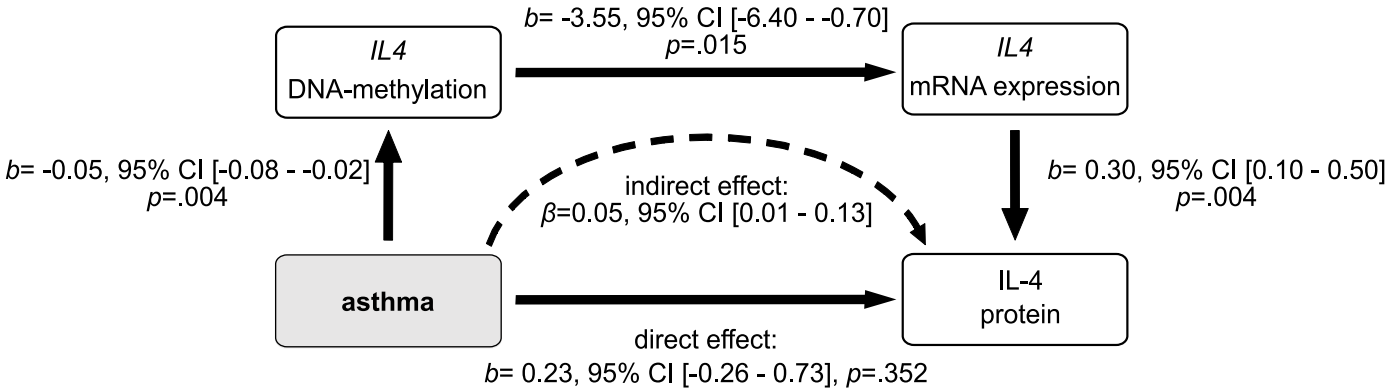


Table 1:

A) Characteristics of LINA, LISA and PASTURE subcohorts used for whole-genome bisulfite sequencing (WGBS).

	control n=42 n (%)	asthma n=40 n (%)	*p*-value	LINA n=25 n (%)	LISA n=29 n (%)	PASTURE n=28 n (%)
age [years]						
median	6.0	6.0	0.778#	6.0	15.0	6.0
LQ/UQ	6.0/15.0	6.0/15.0		5.0/6.0	15.0/15.0	6.0/6.0
child's sex						
female	21 (50.0)	15 (37.5)	0.275*	13 (48)	18 (62.1)	5 (17.9)
male	21 (50.0)	25 (62.5)		12 (52)	11 (37.9)	23 (82.1)
growing up on a farm						
no	38 (90.5)	34 (85.0)	0.514*	25 (100)	29 (100)	18 (64.3)
yes	4 (9.5)	6 (15.0)		0 (0.0)	0 (0)	10 (35.7)
prenatal tobacco smoke exposure						
no	40 (95.2)	35 (87.5)	0.259*	25 (100)	27 (93.1)	23 (82.1)
yes	2 (4.8)	5 (12.5)		0 (0.0)	2 (6.9)	5 (17.9)
maternal age at birth						
median	31.0	31.6	0.414#	30.8	30.0	31.5
LQ/UQ	29.0/33.0	28.9/35.0		28.3/37.5	28.0/32.0	29.6/34.4
parental education level						
low	2 (4.8)	1 (2.5)	0.029*	0 (0.0)	0 (0.0)	3 (10.7)
middle	9 (21.4)	19 (47.5)		4 (16.0)	13 (44.8)	11 (39.3)
high	31 (73.8)	20 (50.0)		21 (84.0)	16 (55.2)	14 (50.0)
family history of atopy						
no	22 (52.4)	8 (20)	0.003*	5 (20.0)	15 (51.7)	10 (35.7)
yes	20 (47.6)	32 (80)		20 (80.0)	14 (48.3)	18 (64.3)
phenotype						
control	n.a.	n.a.	n.a.**	13 (48)	15 (51.7)	14 (50.0)
asthma	n.a.	n.a.		12 (52)	14 (48.3)	14 (50.0)
rhinitis	0 (0.0)	15 (37.5)		5 (20.0)	5 (17.2)	5 (6.1)
atopic dermatitis	0 (0.0)	13 (32.5)		3 (12.0)	7 (24.1)	3 (3.7)

*from Fisher's exact test
#from Mann-Whitney U-test
**note that controls were selected from non-atopic children only
LQ = lower quartile, UQ = upper quartile

B) Characteristics of the LINA and LISA cohort considered in the targeted analysis.

	LINA (n=127)			LISA (n=140)		
	control n=108 n (%)	**asthma n=19 n (%)**	***p*-value**	**control n=115 n (%)**	**asthma n=25 n (%)**	***p*-value**
age [years]						
median	7.0	6.9	0.553#	15.0	15.0	n.a.
LQ/UQ	7.0/7.0	7.0/7.0		15.0/15.0	15.0/15.0	
child's sex						
female	54 (50.0)	7 (36.8)	0.328*	73 (62.4)	11 (44.0)	0.117*
male	54 (50.0)	12 (63.2)		44 (37.6)	14 (56.0)	
growing up on a farm						
no	108 (100)	19 (100)	n.a.	117 (100)	25 (100)	n.a.
yes	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
prenatal tobacco smoke exposure						
no	95 (88.0)	19 (100)	0.214*	101 (87.8)	23 (92.0)	0.737*
yes	13 (12.0)	0 (0.0)		14 (12.2)	2 (8.0)	
maternal age at birth						
median	30.7	32.6	0.126#	29.5	29.0	0.746#
LQ/UQ	27.8/34.0	28.9/36.3		27.0/32.0	27.0/31.0	
parental education level						
low	1 (0.9)	0 (0.0)	0.522*	1 (0.9)	0 (0.0)	0.392*
middle	21 (19.4))	2 (10.5)		43 (37.4)	13 (52.0)	
high	86 (79.6)	17 (89.5)		71 (61.7)	12 (48.0)	
family history of atopy						
yes	72 (66.7)	15 (78.9)	0.423*	49 (42.6)	16 (64.0)	0.076*
no	36 (33.3)	4 (21.1)		66 (57.4)	9 (36.0)	
atopic phenotype						
rhinitis	7 (6.5)	7 (36.8)	0.170*	18 (15.7)	6 (24.0)	0.573*
atopic dermatitis	24 (22.2)	8 (42.1)		24 (20.9)	12 (48.0)	

*from Fisher's exact test
#from Mann-Whitney U-test
LQ = lower quartile, UQ = upper quartile

Supplementary Information

Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing preferentially affects enhancer regions

Short title: The epigenetic landscape of asthma.

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#equal contribution

Conflict of Interest Declaration: All authors declare no conflict of interest.

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METHODS

Cohort description

LINA cohort

For the prospective mother-child cohort LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) 629 mother-child pairs (622 mothers and 629 children; 7 twins) were recruited between March 2006 and December 2008 in Leipzig, Germany. Cord blood and whole blood samples from children were obtained annually during clinical visits until the age of six years and at the age of eight years. Standardized questionnaires assessing lifestyle factors, and children's disease outcomes were self-administered by the parents during pregnancy (34th week of gestation) and annually thereafter[1].

LISA cohort

This study is based on the 15-years follow-up of LISAplus (Influences of Lifestyle-related factors on the Immune System and the Development of Allergies in Childhood). LISA is a prospective birth cohort, for which 3,097 newborns were recruited during November 1997 and January 1999 at four different study centers in Germany (Munich, Leipzig, Wesel, and Bad Honnef). Blood samples were obtained at birth, age two, six, 10, and 15 during clinical visits. Note that no genomic DNA of cord blood samples was available for the LISA study. Standardized questionnaires were answered by the parents at each follow-up and complemented by questionnaires self-administered by the children at age 15[2].

The PASTURE cohort

This study used samples and data of the time of birth and the six-year follow-up of PASTURE (Protection Against Allergy: Study in Rural Environments). PASTURE is a prospective birth cohort study comprising participants from five European countries (Austria, Finland, France,

Germany, and Switzerland) including 1,133 mother-child pairs, which were recruited between August 2002 and March 2005[3]. Standardized questionnaires were answered by parents during pregnancy, at the time of birth, and every subsequent year until the age of six years. Cord blood and whole blood samples were collected at birth, age one, age 4.5 years and age six during clinical visits.

Genomic DNA extraction and bisulfite-conversion

Genomic DNA (gDNA) was isolated from whole blood and cord blood samples using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following manufactures instructions. For DNA-methylation analysis by MassARRAY and WGBS, gDNA was bisulfite converted according to the manufacturer´s protocol using the EZ DNA Methylation Kit (Zymo Research, Freiburg, Germany), the EZ DNA Methylation-Lightning Kit (Zymo Research, Freiburg, Germany), and the EpiTect Bisulfite Kit (Qiagen, Hilden, Germany), respectively.

Whole-genome bisulfite sequencing (WGBS)

Libraries were prepared using the TruSeq DNA Sample Prep Kit v2-Set A (Illumina Inc., San Diego, CA, USA) and EpiTect Il TruSeq DNA (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. Adapter-ligated libraries were bisulfite-treated and PCR-amplified. Whole-genome sequencing was performed on HiSeq2000 (three lanes, 101-bp paired-end) or Illumina HiSeq X Ten V2.5 (2 lanes,150 bp) using standard Illumina protocols and the 200-cycle TruSeq SBS Kit v3, and HiSeq X Ten Reagent Kit v2.5 (Illumina Inc., San Diego, CA, USA; Table S2). Reads were aligned against the phase II reference sequence of the 1000 genomes project including decoy sequences (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/phase2_reference_assembly_sequence/hs37d5.fa.gz). Since DNA sequences were bisulfite-converted, a special alignment protocol adapted for whole-genome bisulfite sequencing data was followed (using

methylCTools: https://github.com/hovestadt/methylCtools). In brief, the reference sequence was *in-silico* bisulfite converted. The same procedure was performed for the sequencing reads, but the original bases were stored. Afterward, the reads were aligned against the converted reference forward and reverse strands using BWA mem (version:0.7.8)[4]. Duplicate reads were removed using sambamba (version: 0.5.9)[5]. The aligned reads were back-transformed into their original state and methylation ratios were called per reference CpG site.

Definition of genotype-associated DMRs

Genotype-associated DMRs (gDMRs) were determined according to previous works[1,6]. Briefly, SNPs were called using Bis-SNP (version 0.81.2) applying default parameter settings[7]. For analysis, we considered all SNPs from dbSNP version 141[8]. *P*-value correction was performed using the Benjamini-Hochberg procedure with a 10% False Discovery Rate (FDR). Whenever a significant correlation (Spearman) of any SNP in a +/-5 kb window around the DMR to the mean DMR methylation was observed, the corresponding DMR was categorized as genotype-associated. Likewise, DMRs with no meQTL were classified as a non-genotype associated DMR (ngDMR).

Cell-type deconvolution from DNA-methylation

Blood cell type fractions within the convoluted methylation signals from whole blood were estimated using the *EpiDISH* R-package version 2.6.1[9-14]. Since EpiDISH uses as a reference data set originated from EPIC Array methylation data of sorted blood cell types (B cells, NK cells, $CD4^+$ T cells, $CD8^+$ T cells, monocytes, neutrophils, and eosinophils), WGBS methylation data were preprocessed prior to deconvolution. Briefly, WGBS methylation data were restricted to CG positions profiled on the EPIC Array. CG positions that were covered by less than ten reads or overlap with a SNP were removed. SNP calling on the WGBS data was performed using Bis-SNP software (version 0.81.2)[7]. The filtered methylation matrix was then

used to perform cell fraction estimation. Here, the function EpiDISH was used from the EpiDISH R-package where the filtered methylation matrix was assigned to the argument beta.m, and the reference matrix centDHSbloodDMC.m that was loaded from the EpiDISH R-package and assigned to the argument ref.m. As method for cell fraction estimation Robust Partial Correlations was used.

Definition of cell-type composition-dependent DMRs

Frequencies of blood cells including T cells, B cells, NK cells, monocytes, neutrophils, and eosinophils were estimated by cell-type deconvolution as aforementioned. Adjusted multiple regression models (confounder: child's sex, cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up on a farm) with the mean DNA-methylation of the DMR as the dependent variable and the specific cell type estimates ($CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, monocytes, neutrophils or eosinophils) as the independent variable was applied to assess cell-type dependent DMRs. Whenever an asthma-related DMR was significantly (Bonferroni-corrected $p<.05$, corresponding to a nominal $p<.00032$) associated with a specific blood cell type frequency, the corresponding DMR was categorized as a cell-type-dependent DMR.

Enhancer enrichment analysis of DMRs

To investigate the enrichment of known enhancers regions in DMRs against genomic background regions, we used Fisher's exact test implemented in the python scipy package. Genomic background regions were generated by merging CpGs that were at most 100 bp apart from each other. Further, regions that had less than three CpGs or overlapped with the DMRs were removed. The parameters chosen to create the background regions is similar to the parameters used for DMR calling and should give a reasonable set of background regions.

DNA-methylation analysis by MassARRAY

Bisulfite-converted DNA of six-, eight-year-old children of the LINA cohort and of 15-year-old children from the LISA cohort was PCR amplified using HotStarTag DNA Polymerase (Qiagen, Hilden, Germany) with the following cycling program: 95°C for 15 min, followed by 45 cycles at 94°C for 30 s, 52/56°C for 30 s, 72 °C for 1 min and a final elongation step at 72°C for 5 min (see Table S4 for primer sequences and primer-specific annealing temperature). The PCR product was *in vitro* transcribed, cleaved by RNase A using the EpiTyper T Complete Reagent Set (Agenea Bioscience, CA, USA), and subjected to MALDI-TOF mass spectrometry analysis to determine DNA-methylation levels. DNA-methylation standards (0%, 20%, 40%, 60%, 80%, 100% methylated genomic DNA) were used to control for potential PCR bias (Supplementary Fig. 2).

RNA isolation, cDNA synthesis, and qPCR

For transcriptional analysis of *EPX*, *IL4* and *IL5RA*, total RNA of the blood was extracted by PAXgene Blood RNA kit (Qiagen, Hilden, Germany) and reverse transcribed using the ImProm-II™ Reverse Transcription System (Promega, Mannheim, Germany) according to the manufacturer's instructions. Gene expression was measured on the Biomark HD system using Universal ProbeLibrary (UPL) hydrolysis probes (Roche Life Sciences, Germany) and 96.96 Dynamic Arrays (Fluidigm, San Francisco, CA, USA) as previously described (6) (see Table S5 for qPCR primer sequences). All reactions were performed in triplicates. Gene expression values were determined by the $2^{-\Delta\Delta CT}$ method with *GAPD*, *PGK1*, and *GUSB*, as reference genes and normalized to the lowest measured value.

Cytokine measurement

Heparinized whole blood of six year-old participants of the LINA study were used to measure IL-4 concentration in pg/ml as previously described[15]. Briefly, whole blood (500µl) was stimulated 4 h at 37°C with the mitogen phytohemagglutinin (PHA, 50 µg/ml; Sigma Aldrich, Hamburg, Germany) and then diluted 1:1 with RPMI1640 medium without supplements. Samples were centrifuged and the supernatant was measured by flow cytometry using cytometric bead array (BD CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) according to manufactures protocol. The detection limit was 3 pg/ml. Values below the limit of detection were set as half of detection limit.

Natural Language Processing of network genes

First, target gene names included in the network analysis were mapped to human NCBI Gene identifiers by symbol, name, or synonym. In case more than one NCBI Gene ID was identified, target gene names were matched to GeneRIF data[16], which are brief descriptions of gene functions frequently containing the respective gene name. Matches with GeneRIF were subsequently scored with Lucene's[17] BM25[18] implementation. The match with the highest score was used as the candidate target gene.

The BANNER[19] gene tagger was employed to identify any gene name mentioned in any publication listed in the PubMed and PubMed Central open access literature databases. The resulting document set was filtered by the terms “asthma”, “asthmatic”, “asthmatics”, “wheeze”, “bronchial hyperreactivity”, “airway hyperreactivity”, “bronchial hyperresponsiveness”, or “hyperreactive airway disease” co-occurring in the same sentence or the same paragraph with the already identified gene name (query date: November, 2022). Lastly, the mapped NCBI Gene IDs of the target genes were matched to the IDs extracted from the literature in the same manner as described above to filter the literature for sought genes.

Literature search of EWAS related to asthma or prenatal influencing factors

To identify previously published EWAS studies that investigated the effect of prenatal influencing factors or EWAS investigated asthma-related outcomes, we performed a literature search in PubMed.

Previous asthma-outcome associated EWAS studies in the PubMed database were identified by the search term: ("asthma" OR "wheeze") AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip") AND "blood" (query data 27.10.2022). This search retrieved n=68 publications, from which two reviews, one RCT and one systematic review were excluded. After manual curation n=22 EWAS studies (including meta-analyses) remained that reported DNA-methylation changes in blood related to asthma or lung function (Figure S1A).

Previous EWAS studies investigating prenatal influencing factors affecting DNA-methylation in cord blood in the PubMed database were identified by the search term: „DNA methylation" AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip")) AND ("prenatal" OR "utero" OR "maternal" OR "cord blood" OR "birth") (query date: 25.04.2022). Only EWAS studies that used array-based (Illumina's HumanMethylation 27/450/850k BeadChips) or sequencing approaches were considered. A total of n=376 studies were identified. Subsequently, these n=376 papers were independently screened by two researchers to select only original studies conducted with cord blood samples. Additionally, studies examining only prospective outcomes, or those that described solely sex-related DNA-methylation differences were excluded. This resulted in n=89 EWAS of which five studies were excluded, since no significant DNA-methylation changes were described, leaving n=84 EWAS for overlap analysis with cord-blood asthma-related DMRs (Supplementary Fig. 1B).

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Supplementary Information

~~Genome~~ Global hypomethylation in childhood asthma identified by genome-wide DNA-methylation sequencing ~~identifies massive~~preferentially affects enhancer ~~reprogramming in childhood asthma~~regions

Short title: The epigenetic landscape of asthma.

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METHODS

Cohort description

LINA cohort

For the prospective mother-child cohort LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) 629 mother-child pairs (622 mothers and 629 children; 7 twins) were recruited between March 2006 and December 2008 in Leipzig, Germany. Cord blood and whole blood samples from children were obtained annually during clinical visits until the age of six years and at the age of eight years. Standardized questionnaires assessing lifestyle factors, and children's disease outcomes were self-administered by the parents during pregnancy (34th week of gestation) and annually thereafter[1].

LISA cohort

This study is based on the 15-years follow-up of LISAplus (Influences of Lifestyle-related factors on the Immune System and the Development of Allergies in Childhood). LISA is a prospective birth cohort, for which 3,097 newborns were recruited during November 1997 and January 1999 at four different study centers in Germany (Munich, Leipzig, Wesel, and Bad Honnef). Blood samples were obtained at birth, age two, six, 10, and 15 during clinical visits. Note that no genomic DNA of cord blood samples was available for the LISA study. Standardized questionnaires were answered by the parents at each follow-up and complemented by questionnaires self-administered by the children at age 15[2].

The PASTURE cohort ~~– EFRAIM study group~~

This study used samples and data of the time of birth and the six-year follow-up of PASTURE (Protection Against Allergy: Study in Rural Environments~~)/EFRAIM.~~). PASTURE~~/EFRAIM~~ is a prospective birth cohort study comprising participants from five European countries

(Austria, Finland, France, Germany, and Switzerland) including 1,133 mother-child pairs, which were recruited between August 2002 and March 2005[3]. Standardized questionnaires were answered by parents during pregnancy, at the time of birth, and every subsequent year until the age of six years. Cord blood and whole blood samples were collected at birth, age one, age 4.5 years and age six during clinical visits.

Genomic DNA extraction and bisulfite-conversion

Genomic DNA (gDNA) was isolated from whole blood and cord blood samples using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following manufactures instructions. For DNA-methylation analysis by MassARRAY and WGBS, gDNA was bisulfite converted according to the manufacturer´s protocol using the EZ DNA Methylation Kit (Zymo Research, Freiburg, Germany), the EZ DNA Methylation-Lightning Kit (Zymo Research, Freiburg, Germany), and the EpiTect Bisulfite Kit (Qiagen, Hilden, Germany), respectively.

Whole-genome bisulfite sequencing (WGBS)

Libraries were prepared using the TruSeq DNA Sample Prep Kit v2-Set A (Illumina Inc., San Diego, CA, USA) and EpiTect Il TruSeq DNA (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. Adapter-ligated libraries were bisulfite-treated and PCR-amplified. Whole-genome sequencing was performed on HiSeq2000 (three lanes, 101-bp paired-end) or Illumina HiSeq X Ten V2.5 (2 lanes,150 bp) using standard Illumina protocols and the 200-cycle TruSeq SBS Kit v3, and HiSeq X Ten Reagent Kit v2.5 (Illumina Inc., San Diego, CA, USA; Table S2). Reads were aligned against the phase II reference sequence of the 1000 genomes project including decoy sequences (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/phase2_reference_assembly_sequence/hs37d5.fa.gz). Since DNA sequences were bisulfite-converted, a special alignment protocol adapted for whole-genome bisulfite sequencing data was followed (using

methylCTools: https://github.com/hovestadt/methylCtools). In brief, the reference sequence was *in-silico* bisulfite converted. The same procedure was performed for the sequencing reads, but the original bases were stored. Afterward, the reads were aligned against the converted reference forward and reverse strands using BWA mem (version:0.7.8)[4]. Duplicate reads were removed using sambamba (version: 0.5.9)[5]. The aligned reads were back-transformed into their original state and methylation ratios were called per reference CpG site.

Definition of genotype-associated DMRs

Genotype-associated DMRs (gDMRs) were determined according to previous works[1,6]. Briefly, SNPs were called using Bis-SNP (version 0.81.2) applying default parameter settings[7]. For analysis, we considered all SNPs from dbSNP version 141[8]. *P*-value correction was performed using the Benjamini-Hochberg procedure with a 10% False Discovery Rate (FDR). Whenever a significant correlation (Spearman) of any SNP in a +/-5 kb window around the DMR to the mean DMR methylation was observed, the corresponding DMR was categorized as genotype-associated. Likewise, DMRs with no meQTL were classified as a non-genotype associated DMR (ngDMR).

Cell-type deconvolution from DNA-methylation

Blood cell type fractions within the convoluted methylation signals from whole blood were estimated using the *EpiDISH* R-package version 2.6.1[9-14]. Since EpiDISH uses as a reference data set originated from EPIC Array methylation data of sorted blood cell types (B cells, NK cells, $CD4^+$ T cells, $CD8^+$ T cells, monocytes, neutrophils, and eosinophils), WGBS methylation data were preprocessed prior to deconvolution. Briefly, WGBS methylation data were restricted to CG positions profiled on the EPIC Array. CG positions that were covered by less than ten reads or overlap with a SNP were removed. SNP calling on the WGBS data was performed using Bis-SNP software (version 0.81.2)[7]. The filtered methylation matrix was then

used to perform cell fraction estimation. Here, the function EpiDISH was used from the EpiDISH R-package where the filtered methylation matrix was assigned to the argument beta.m, and the reference matrix centDHSbloodDMC.m that was loaded from the EpiDISH R-package and assigned to the argument ref.m. As method for cell fraction estimation Robust Partial Correlations was used.

Definition of cell-type composition-dependent DMRs

Frequencies of blood cells including T cells, B cells, NK cells, monocytes, neutrophils, and eosinophils were estimated by cell-type deconvolution as aforementioned. ~~To adjust the DMRs for the general cell-type heterogeneity and to account for a potential underlying shift in the granulocyte-to-lymphocyte ratio between asthmatics and controls, the proportions of NK-, T-, B cells, and monocytes, neutrophils, eosinophils were combined to lymphoid-, and myeloid cells, respectively [15].~~ Adjusted multiple regression models (confounder: ~~age,~~ child's sex, ~~recruitment location~~cohort, prenatal tobacco smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up on a farm) with the mean DNA-methylation of the DMR as the dependent variable and the ~~proportion of lymphoid cells~~specific cell type estimates ($CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, monocytes, neutrophils or eosinophils) as the independent variable was applied to assess cell-type dependent DMRs. ~~In a second model, the eosinophil count was included instead of the lymphoid/myeloid ratio.~~ Whenever an asthma-related DMR was significantly (Bonferroni-corrected $p<.05$, corresponding to a nominal $p<.00032$) associated with ~~eosinophil~~a specific blood cell type frequency ~~and the lymphoid/myeloid ratio (Bonferroni-corrected $p<0.00031$), the~~, the corresponding DMR was categorized as ~~an eosinophil, or~~ a cell-~~composition~~type-dependent DMR~~, respectively~~.

Enhancer enrichment analysis of DMRs

To investigate the enrichment of ~~DMRs within~~ known ~~enhancer~~enhancers regions~~, we applied the algorithm/R package Locus Overlap Analysis (LOLA) (version 1.16.0) [16]. LOLA is based on a Fisher's exact test, to check if the ratio of foreground features (~~ in ~~our case DMRs) that overlap with a list of regions of interest (ROIs) is enriched~~ DMRs against ~~the ratio of~~genomic background ~~features that overlap with a list of ROIs. We calculated~~regions, we used Fisher's exact test implemented in the ~~set of~~python scipy package.

Genomic background ~~features~~regions were generated by merging ~~CpG positions among the whole genome that have a distance of smaller or equal to~~ CpGs that were at most 100 bp~~. In a second step, we removed all merged regions~~ apart from ~~further analysis~~each other. Further, regions that ~~consist of~~had less than three ~~CpG sites.~~CpGs or overlapped with the DMRs were removed. The ~~definition of our~~ parameters chosen to create the background ~~set of features reflects~~regions is similar to the parameters used for DMR calling and ~~ensures that the foreground~~should give a reasonable set of ~~features is a subset of the~~ background ~~set of features~~regions.

DNA-methylation analysis by MassARRAY

Bisulfite-converted DNA of six-, eight-year-old children of the LINA cohort and of 15-year-old children from the LISA cohort was PCR amplified ~~(for primer sequences see Table S4)~~ using HotStarTag DNA Polymerase (Qiagen, Hilden, Germany) with the following cycling program: 95°C for 15 min, followed by 45 cycles at 94°C for 30 s, 52/56°C for 30 s, 72 °C for 1 min and a final elongation step at 72°C for 5 min (see Table S4 for primer sequences and primer-specific annealing temperature). The PCR product was *in vitro* transcribed, cleaved by RNase A using the EpiTyper T Complete Reagent Set (Agenea Bioscience, CA, USA), and subjected to MALDI-TOF mass spectrometry analysis to determine DNA-methylation levels. DNA-methylation standards (0%, 20%, 40%, 60%, 80%, 100% methylated genomic DNA) were used to control for potential PCR bias (Supplementary Fig. 2).

RNA isolation, cDNA synthesis, and qPCR

For transcriptional analysis of *EPX*, *IL4* and *IL5RA*, total RNA of the blood was extracted by PAXgene Blood RNA kit (Qiagen, Hilden, Germany) and reverse transcribed using the ImProm-II™ Reverse Transcription System (Promega, Mannheim, Germany) according to the manufacturer's instructions. Gene expression was measured on the Biomark HD system using Universal ProbeLibrary (UPL) hydrolysis probes (Roche Life Sciences, Germany) and 96.96 Dynamic Arrays (Fluidigm, San Francisco, CA, USA) as previously described (6) (see Table S5 for qPCR primer sequences). All reactions were performed in triplicates. Gene expression values were determined by the $2^{-\Delta\Delta CT}$ method with *GAPD*, *PGK1*, and *GUSB*, as reference genes and normalized to the lowest measured value.

Cytokine measurement

Heparinized whole blood of six year-old participants of the LINA study were used to measure IL-4 concentration in pg/ml as previously described[15]. Briefly, whole blood (500µl) was stimulated 4 h at 37°C with the mitogen phytohemagglutinin (PHA, 50 µg/ml; Sigma Aldrich, Hamburg, Germany) and then diluted 1:1 with RPMI1640 medium without supplements. Samples were centrifuged and the supernatant was measured by flow cytometry using cytometric bead array (BD CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) according to manufactures protocol. The detection limit was 3 pg/ml. Values below the limit of detection were set as half of detection limit.

Natural Language Processing of network genes

First, target gene names included in the network analysis were mapped to human NCBI Gene identifiers by symbol, name, or synonym. In case more than one NCBI Gene ID was identified, target gene names were matched to GeneRIF data[16], which are brief descriptions of gene

functions frequently containing the respective gene name. Matches with GeneRIF were subsequently scored with Lucene's[17] BM25[18] implementation. The match with the highest score was used as the candidate target gene.

The BANNER[19] gene tagger was employed to identify any gene name mentioned in any publication listed in the PubMed and PubMed Central open access literature databases. The resulting document set was filtered by the terms “asthma”, “asthmatic”, “asthmatics”, “wheeze”, “bronchial hyperreactivity”, “airway hyperreactivity”, “bronchial hyperresponsiveness”, or “hyperreactive airway disease” co-occurring in the same sentence or the same paragraph with the already identified gene name (query date: November, 2022). Lastly, the mapped NCBI Gene IDs of the target genes were matched to the IDs extracted from the literature in the same manner as described above to filter the literature for sought genes.

Literature search of EWAS related to asthma or prenatal influencing factors

To identify previously published EWAS studies that investigated the effect of prenatal influencing factors ~~affecting DNA-methylation in cord blood that might overlap with our~~or EWAS investigated asthma-related ~~cord blood DMRs~~outcomes, we performed a literature search in PubMed ~~(query date: 25.04.2022) for previously published EWAS studies. Only~~. Previous asthma-outcome associated EWAS studies ~~that used array-based (Illumina’s HumanMethylation 27/450/850k BeadChips) or sequencing approaches were considered. A total of n=376 studies~~ in the PubMed database were identified by ~~using~~the search term: ("asthma" OR "wheeze") AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip") AND "blood" (query data 27.10.2022). This search retrieved n=68 publications, from which two reviews, one RCT and one systematic review were excluded. After manual curation n=22 EWAS studies (including meta-analyses) remained that reported DNA-methylation changes in blood related to asthma or lung function (Figure S1A).

Previous EWAS studies investigating prenatal influencing factors affecting DNA-methylation in cord blood in the PubMed database were identified by the search term: „DNA methylation" AND ("WGBS" OR "EWAS" OR "450k" OR "850k" OR "27k" OR "epigenome-wide" OR "HumanMethylation450K BeadChip")) AND ("prenatal" OR "utero" OR "maternal" OR "cord blood" OR "birth~~").~~") (query date: 25.04.2022). Only EWAS studies that used array-based (Illumina's HumanMethylation 27/450/850k BeadChips) or sequencing approaches were considered. A total of n=376 studies were identified. Subsequently, these n=376 papers were independently screened by two researchers to select only original studies conducted with cord blood samples. Additionally, studies examining only prospective outcomes, or those that described solely sex-related DNA-methylation differences were excluded. This resulted in n=89 EWAS of which five studies were excluded, since no significant DNA-methylation changes were described, leaving n=84 EWAS for overlap analysis with cord-blood asthma-related DMRs (~~Figure S1).~~Supplementary Fig. 1B).

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Table S1: Sample overview. Indicated are the number of samples of the three cohorts available for WG

	LINA n (asthma/control)	LISA n (asthma/control)	PASTURE n (asthma/control)
WGBS			
at birth (cord blood)	9/11	-/-	14/14
at phenotype	12/13	14/15	14/14
targeted analysis in phenotype			
DNA-methylation	19/108	25/115	-/-
RNA expression	19/107	25/115	-/-
protein concentration	19/96	-/-	-/-

GBS analysis and for analysis by targeted approaches.

Table S2: Quality of the whole genome bisulfite sequencing samples.

SAMPLE	COHORT	OUTCOME	AGE [YEARS]	CORD BLOOD AVAILABLE
Sample 1	LINA	asthma	6	Yes
Sample 2	LINA	asthma	8	Yes
Sample 3	LINA	asthma	6	Yes
Sample 4	LINA	asthma	6	Yes
Sample 5	LINA	asthma	6	Yes
Sample 6	LINA	asthma	6	-
Sample 7	LINA	asthma	5	-
Sample 8	LINA	asthma	5	-
Sample 9	LINA	asthma	5	Yes
Sample 10	LINA	asthma	6	Yes
Sample 11	LINA	asthma	5	Yes
Sample 12	LINA	asthma	6	Yes
Sample 13	LINA	control	8	-
Sample 14	LINA	control	8	Yes
Sample 15	LINA	control	6	Yes
Sample 16	LINA	control	5	Yes
Sample 17	LINA	control	6	Yes
Sample 18	LINA	control	6	-
Sample 19	LINA	control	5	Yes
Sample 20	LINA	control	6	Yes
Sample 21	LINA	control	6	Yes
Sample 22	LINA	control	5	Yes
Sample 23	LINA	control	6	Yes
Sample 24	LINA	control	6	Yes
Sample 25	LINA	control	6	Yes
Sample 26	LISA	asthma	15	-
Sample 27	LISA	asthma	15	-
Sample 28	LISA	asthma	15	-
Sample 29	LISA	asthma	15	-
Sample 30	LISA	asthma	15	-
Sample 31	LISA	asthma	15	-
Sample 32	LISA	asthma	15	-
Sample 33	LISA	asthma	15	-
Sample 34	LISA	asthma	15	-
Sample 35	LISA	asthma	15	-
Sample 36	LISA	asthma	15	-
Sample 37	LISA	asthma	15	-
Sample 38	LISA	asthma	15	-
Sample 39	LISA	asthma	15	-
Sample 40	LISA	control	15	-
Sample 41	LISA	control	15	-
Sample 42	LISA	control	15	-
Sample 43	LISA	control	15	-
Sample 44	LISA	control	15	-
Sample 45	LISA	control	15	-
Sample 46	LISA	control	15	-
Sample 47	LISA	control	15	-

Sample 48	LISA	control	15	-
Sample 49	LISA	control	15	-
Sample 50	LISA	control	15	-
Sample 51	LISA	control	15	-
Sample 52	LISA	control	15	-
Sample 53	LISA	control	15	-
Sample 54	LISA	control	15	-
Sample 55	PASTURE	asthma	6	Yes
Sample 56	PASTURE	asthma	6	Yes
Sample 57	PASTURE	asthma	6	Yes
Sample 58	PASTURE	asthma	6	Yes
Sample 59	PASTURE	asthma	6	Yes
Sample 60	PASTURE	asthma	6	Yes
Sample 61	PASTURE	asthma	6	Yes
Sample 62	PASTURE	asthma	6	Yes
Sample 63	PASTURE	asthma	6	Yes
Sample 64	PASTURE	asthma	6	Yes
Sample 65	PASTURE	asthma	6	Yes
Sample 66	PASTURE	asthma	6	Yes
Sample 67	PASTURE	asthma	6	Yes
Sample 68	PASTURE	asthma	6	Yes
Sample 69	PASTURE	control	6	Yes
Sample 70	PASTURE	control	6	Yes
Sample 71	PASTURE	control	6	Yes
Sample 72	PASTURE	control	6	Yes
Sample 73	PASTURE	control	6	Yes
Sample 74	PASTURE	control	6	Yes
Sample 75	PASTURE	control	6	Yes
Sample 76	PASTURE	control	6	Yes
Sample 77	PASTURE	control	6	Yes
Sample 78	PASTURE	control	6	Yes
Sample 79	PASTURE	control	6	Yes
Sample 80	PASTURE	control	6	Yes
Sample 81	PASTURE	control	6	Yes
Sample 82	PASTURE	control	6	Yes

cord blood samples

SAMPLE	COHORT	OUTCOME	AGE [YEARS]	CORD BLOOD AVAILABLE
Sample 1_CB	LINA	asthma	birth	NA
Sample 2_CB	LINA	asthma	birth	NA
Sample 3_CB	LINA	asthma	birth	NA
Sample 4_CB	LINA	asthma	birth	NA
Sample 5_CB	LINA	asthma	birth	NA
Sample 9_CB	LINA	asthma	birth	NA
Sample 10_CB	LINA	asthma	birth	NA
Sample 11_CB	LINA	asthma	birth	NA
Sample 12_CB	LINA	asthma	birth	NA
Sample 14_CB	LINA	control	birth	NA
Sample 15_CB	LINA	control	birth	NA
Sample 16_CB	LINA	control	birth	NA
Sample 17_CB	LINA	control	birth	NA

Sample 19_CB	LINA	control	birth	NA
Sample 20_CB	LINA	control	birth	NA
Sample 21_CB	LINA	control	birth	NA
Sample 22_CB	LINA	control	birth	NA
Sample 23_CB	LINA	control	birth	NA
Sample 24_CB	LINA	control	birth	NA
Sample 25_CB	LINA	control	birth	NA
Sample 55_CB	PASTURE	asthma	birth	NA
Sample 56_CB	PASTURE	asthma	birth	NA
Sample 57_CB	PASTURE	asthma	birth	NA
Sample 58_CB	PASTURE	asthma	birth	NA
Sample 59_CB	PASTURE	asthma	birth	NA
Sample 60_CB	PASTURE	asthma	birth	NA
Sample 61_CB	PASTURE	asthma	birth	NA
Sample 62_CB	PASTURE	asthma	birth	NA
Sample 63_CB	PASTURE	asthma	birth	NA
Sample 64_CB	PASTURE	asthma	birth	NA
Sample 65_CB	PASTURE	asthma	birth	NA
Sample 66_CB	PASTURE	asthma	birth	NA
Sample 67_CB	PASTURE	asthma	birth	NA
Sample 68_CB	PASTURE	asthma	birth	NA
Sample 69_CB	PASTURE	control	birth	NA
Sample 70_CB	PASTURE	control	birth	NA
Sample 71_CB	PASTURE	control	birth	NA
Sample 72_CB	PASTURE	control	birth	NA
Sample 73_CB	PASTURE	control	birth	NA
Sample 74_CB	PASTURE	control	birth	NA
Sample 75_CB	PASTURE	control	birth	NA
Sample 76_CB	PASTURE	control	birth	NA
Sample 77_CB	PASTURE	control	birth	NA
Sample 78_CB	PASTURE	control	birth	NA
Sample 79_CB	PASTURE	control	birth	NA
Sample 80_CB	PASTURE	control	birth	NA
Sample 81_CB	PASTURE	control	birth	NA
Sample 82_CB	PASTURE	control	birth	NA

GENOME.COVERAGE	N.READS.TOTAL	%READS.MAPPED	%READS.DUPLICATE
19.67x	693991264	99.9289	4.83477
70.97x	1906245228	99.814	13.6217
30.09x	1052026482	99.9593	2.96205
25.77x	1139206858	99.8375	20.3487
71.77x	1857564044	99.9433	10.3383
69.76x	1852178002	99.7495	11.8792
40.88x	1090744036	99.8996	14.0437
70.00x	1784776866	99.9886	8.77584
33.75x	1178216806	99.9599	4.07062
72.10x	1915004800	99.8456	12.1461
34.14x	1366319148	99.9469	11.2224
60.50x	1683132756	99.9964	10.6715
71.17x	1896576606	99.9662	13.2505
70.99x	1850929082	99.9663	11.8254
72.86x	1971011978	99.9784	14.49
70.05x	1794197820	99.882	9.76356
73.33x	1934162348	99.9251	11.7998
72.14x	1872337160	99.9326	10.4251
67.55x	1885776600	99.9009	16.1181
68.21x	1754252712	99.681	10.3399
68.84x	1861426578	99.5413	13.7267
68.16x	1834499120	99.4386	12.9616
63.96x	1608950928	99.8834	7.79976
67.59x	1786260654	99.808	11.7711
67.77x	1747698850	99.9937	10.1696
51.65x	1512092864	99.9964	17.987
55.47x	1609689028	99.9944	15.3155
66.32x	1837559770	99.9947	12.7407
55.37x	1631630110	99.9929	15.5801
53.83x	1597079574	99.9947	16.7433
58.80x	1755547360	99.9962	17.6279
59.98x	1679195204	99.9933	12.4333
57.13x	1618880414	99.9945	14.6753
38.07x	1127765154	99.9844	13.2486
41.90x	1230201776	99.991	13.8798
42.10x	1276053918	99.9832	15.4243
59.83x	1810243898	99.9935	19.066
58.12x	1676433958	99.9926	14.7855
57.97x	1687189844	99.9902	15.2089
44.13x	1281029040	99.9907	13.2701
53.89x	1592609790	99.9952	18.7372
61.92x	1785208038	99.9945	15.6971
57.88x	1733137894	99.9946	18.0195
47.95x	1418816250	99.9936	13.9537
58.56x	1710174000	99.9949	16.013
59.07x	1745663012	99.9943	18.6534
51.30x	1506590004	99.9964	18.1338

51.32x	1597645588	99.9834	19.4585
33.24x	958985622	99.9817	11.1782
50.80x	1504691006	99.9947	14.4048
61.33x	1805326342	99.9942	18.8988
55.62x	1566357966	99.9952	13.3532
55.15x	1613027942	99.9947	15.1037
44.83x	1327208096	99.9857	15.11
58.80x	1743773480	99.9902	13.8435
50.67x	1589584518	99.9953	20.0828
46.52x	1389207274	99.9933	15.8088
52.56x	1615200838	99.9949	17.7523
56.79x	1715524460	99.9939	18.1821
57.31x	1761683456	99.9914	20.2134
49.98x	1448160356	99.993	12.36
58.75x	1722466996	99.9944	16.2496
52.53x	1568529354	99.993	14.6647
63.16x	1742150544	99.9914	11.4903
57.88x	1619617724	99.9923	13.4572
59.90x	1724901362	99.9918	15.1492
60.66x	1758560262	99.9927	15.8641
55.84x	1599616226	99.9937	14.3395
51.89x	1481045980	99.9967	12.4388
54.86x	1575490514	99.9965	12.2724
49.01x	1426578848	99.9925	12.4112
58.45x	1731167634	99.9928	16.4242
53.73x	1526813250	99.9898	14.162
52.76x	1586733290	99.9938	15.2425
47.68x	1423679156	99.9949	16.7702
63.61x	1737126350	99.9907	11.5972
61.08x	1693428816	99.9898	13.2792
62.42x	1801565116	99.9935	16.8855
56.57x	1640722328	99.9925	15.9979
61.81x	1766375754	99.9914	14.2143
60.45x	1753325778	99.9907	15.7738
56.55x	1624341872	99.9923	15.8582

GENOME.COVERAGE	N.READS.TOTAL	%READS.MAPPED	%READS.DUPLICATE
20.79x	751617594	99.9546	7.19826
71.03x	1942327598	99.843	14.2724
72.01x	1898341440	99.9565	12.1736
29.52x	1018128990	99.9554	2.53578
23.98x	949678076	99.9115	13.2389
60.43x	2270218736	99.9596	8.4623
72.77x	1886872642	99.8963	10.1474
67.16x	1757016588	99.6791	10.7529
31.36x	1112479070	99.8437	4.07377
33.78x	1179778864	99.9866	4.04769
29.66x	1083408692	99.9424	8.23012
71.38x	1916729008	99.5566	13.5465
48.96x	1332085162	99.4344	14.0181

57.61x	2147226514	99.8503	8.1715
66.89x	1757963676	99.687	10.436
70.15x	1823912426	99.7549	11.3819
31.90x	1119565604	99.8336	4.02557
68.97x	1804699710	99.9848	10.4064
26.27x	914882810	99.437	3.23779
61.16x	1701576212	99.9954	10.1577
47.40x	1681248416	99.9634	21.6373
41.06x	1348251060	99.9522	17.8168
41.54x	1425212002	99.9392	18.3566
28.84x	1021633160	99.9611	16.9277
53.80x	1674572866	99.989	20.1724
45.78x	1411996092	99.9889	17.5372
37.13x	1250703984	99.9748	19.0577
44.40x	1376573536	99.9828	17.8292
39.52x	1400104320	99.9554	20.2197
54.40x	1713540620	99.9937	21.4606
30.28x	949641336	99.9834	15.9515
43.12x	1816647178	99.9688	35.5341
56.48x	1824221450	99.9941	23.9123
44.58x	1399540416	99.9922	19.6512
49.69x	1443849888	99.994	14.5689
53.94x	1711684586	99.9965	22.2758
57.14x	1643783012	99.9952	14.2099
57.76x	1724131284	99.9949	16.6513
56.64x	1676514264	99.9942	16.2174
57.47x	1692783842	99.9949	14.2283
40.38x	1195803488	99.9921	18.1724
59.73x	1769279528	99.9933	17.94
52.60x	1516148252	99.9919	15.224
61.97x	1799810708	99.9905	16.0565
60.12x	1826315026	99.9941	20.7314
64.02x	1856884426	99.993	16.6883
54.95x	1634705042	99.9937	18.3884
60.45x	1753325778	99.9907	15.7738

%READS.PROPERLY.PAIRED	MEDIAN.INSERT.SIZE	MEAN.METH.M
95.9698	166	0.001
95.5224	174	0.002162
94.76	169	0.001369
85.7961	168	0.000922
95.7028	172	0.005523
95.9109	165	0.001163
96.1313	176	0.001174
95.7048	168	0.001707
94.6928	170	0.000904
95.2094	171	0.001743
85.9563	161	0.001336
97.5981	171	0.001326
96.176	174	0.001514
96.2698	179	0.003596
95.5918	177	0.008509
96.4035	174	0.001837
95.7775	171	0.007843
95.75	171	0.001309
95.5586	168	0.001928
96.2993	178	0.001651
94.7389	174	0.00145
94.5134	178	0.001643
95.8533	170	0.00753
94.4895	172	0.002675
95.9918	170	0.00156
98.3565	187	0.001319
98.5583	185	0.001132
98.4524	182	0.001305
98.4763	186	0.002441
98.0562	179	0.002795
98.3746	179	0.001371
98.1293	170	0.001617
98.7154	184	0.001664
95.347	177	0.002434
97.7969	172	0.002663
94.2568	170	0.001921
95.8792	177	0.003665
97.6057	175	0.005991
95.607	177	0.001245
91.9185	174	0.001585
98.3787	196	0.001245
98.2153	176	0.000999
98.3738	179	0.001376
98.2341	184	0.001714
97.984	168	0.001473
98.6886	187	0.00118
98.3298	181	0.001131

81.1698	193	0.001755
98.976	166	0.001191
97.0319	172	0.00122
98.7567	185	0.00134
98.57	175	0.002218
97.2534	181	0.001579
98.1136	167	0.000922
97.5618	164	0.001394
96.6029	172	0.001269
98.0802	171	0.001624
96.2303	173	0.001417
98.2347	175	0.002416
89.2136	184	0.001818
96.9691	172	0.00252
98.3177	183	0.0015
96.7558	166	0.001679
98.2928	174	0.0016
98.827	183	0.001244
98.2923	174	0.001246
98.3698	192	0.001782
98.5455	181	0.002105
98.0746	176	0.001544
97.4816	167	0.001996
97.5002	165	0.001054
98.3761	174	0.00165
97.9548	169	0.001681
96.7135	170	0.001314
97.7413	167	0.001343
98.6355	181	0.001326
98.6772	181	0.003454
98.5722	183	0.001546
98.515	175	0.001242
98.5602	177	0.001092
98.5338	182	0.001448
98.6304	183	0.001385

%READS.PROPERLY.PAIRED	MEDIAN.INSERT.SIZE	MEAN.METH.M
95.574	167	0.000753
94.7167	170	0.001597
95.6004	171	0.00474
95.8668	168	0.00104
90.7398	172	0.000945
92.5755	158	0.000929
95.9102	169	0.001677
95.2517	172	0.001494
94.3848	167	0.000632
95.0006	167	0.000932
94.6879	170	0.000999
95.2659	174	0.001339
94.8751	176	0.001295

92.5511	155	0.000913
94.3015	171	0.003224
95.5631	177	0.002377
94.3821	166	0.000738
95.661	167	0.008504
94.4936	174	0.002666
97.451	167	0.002069
51.9115	151	0.002898
52.2734	163	0.002494
40.7474	156	0.003128
56.3601	149	0.002488
87.3635	181	0.001413
93.1921	174	0.0015
74.1209	161	0.002216
85.529	164	0.001616
64.134	152	0.002974
97.2115	164	0.001404
87.6861	168	0.003392
54.2747	156	0.002958
95.2772	173	0.002089
91.8211	170	0.002344
96.9519	169	0.001819
97.7126	171	0.002109
97.8123	170	0.002285
96.7482	169	0.002113
96.7956	167	0.001464
97.9939	171	0.001452
97.8913	183	0.001539
97.2994	182	0.001336
97.0914	178	0.001418
98.3292	192	0.001505
97.8585	173	0.007565
97.5942	173	0.015694
97.8842	179	0.001452
98.5338	182	0.001448

SEQUENCING PLATFORM
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Table S2B: Read coverage of n=158 asthma-related DMRs across all 82 samples.

chr	start	end	width	nCpGs	DNA-methylation difference	DNA-methylation direction
1	870565	871771	1206	68	0.06	hyper
1	6341136	6341683	547	28	-0.06	hypo
1	10436586	10436851	265	11	-0.05	hypo
1	22097059	22097227	168	8	-0.05	hypo
1	62364300	62364445	145	4	-0.04	hypo
1	67600417	67600715	298	21	-0.03	hypo
1	84744687	84744808	121	5	-0.04	hypo
1	93970706	93970977	271	9	-0.06	hypo
1	149162004	149162428	424	21	-0.09	hypo
1	202121664	202121815	151	9	-0.06	hypo
1	204479935	204480156	221	9	-0.04	hypo
2	24233600	24234117	517	24	-0.06	hypo
2	31154795	31155157	362	17	-0.07	hypo
2	70734255	70734341	86	5	-0.04	hypo
2	74213621	74213841	220	13	0.07	hyper
2	75089515	75089819	304	8	-0.05	hypo
2	97401278	97401372	94	6	-0.05	hypo
2	107082602	107082889	287	32	-0.06	hypo
2	113426404	113426419	15	3	-0.08	hypo
2	113956545	113956673	128	18	-0.07	hypo
2	118617427	118618163	736	73	-0.06	hypo
2	121816094	121816885	791	23	-0.05	hypo
2	130986715	130986828	113	20	-0.07	hypo
2	132404284	132404979	695	54	-0.05	hypo
2	241459177	241460047	870	54	-0.05	hypo
3	3150228	3150425	197	9	-0.05	hypo
3	39395430	39395805	375	12	-0.05	hypo
3	70560282	70560339	57	5	-0.05	hypo
3	75445094	75445699	605	53	-0.10	hypo
3	98476467	98476657	190	5	-0.05	hypo
3	128134844	128135029	185	8	-0.05	hypo
3	128317561	128317755	194	8	-0.05	hypo
3	128317793	128318091	298	12	-0.06	hypo
3	172243109	172243331	222	13	-0.06	hypo
3	184243755	184244149	394	22	-0.05	hypo
3	195964960	195965370	410	11	-0.04	hypo
4	1908638	1908946	308	10	-0.06	hypo
4	2366183	2366745	562	49	-0.03	hypo
4	144833125	144833346	221	30	-0.06	hypo
4	148634323	148634374	51	5	-0.06	hypo
5	8457869	8457980	111	13	-0.07	hypo
5	42923963	42924355	392	23	-0.08	hypo
5	42943969	42944684	715	41	-0.08	hypo
5	68700315	68700724	409	14	-0.04	hypo
5	77142381	77142899	518	26	-0.08	hypo
5	77146478	77147361	883	53	-0.07	hypo
5	132002374	132002507	133	5	-0.05	hypo

5	149145166	149145197	31	4	-0.06	hypo	
5	154224429	154224647	218	4	-0.05	hypo	
5	157117442	157117959	517	42	-0.07	hypo	
6	32063911	32064192	281	33	-0.04	hypo	
6	166674955	166675083	128	5	-0.05	hypo	
6	168435945	168436646	701	41	-0.06	hypo	
7	1914009	1914393	384	18	-0.04	hypo	
7	5382633	5382783	150	8	-0.05	hypo	
7	32357921	32358755	834	44	-0.08	hypo	
7	36007074	36007282	208	19	-0.09	hypo	
7	48887537	48887891	354	42	-0.08	hypo	
7	54900863	54901103	240	21	-0.04	hypo	
7	55412705	55412996	291	33	-0.09	hypo	
7	90895326	90896702	1376	91	-0.06	hypo	
7	102003600	102003767	167	9	-0.05	hypo	
7	127910860	127911680	820	49	-0.06	hypo	
7	150647915	150648063	148	8	-0.04	hypo	
8	599524	600398	874	60	-0.09	hypo	
8	58192499	58193338	839	50	-0.08	hypo	
8	128828626	128828794	168	5	-0.05	hypo	
8	131047175	131047345	170	5	-0.05	hypo	
9	5819260	5819334	74	4	-0.06	hypo	
9	32430999	32431303	304	9	-0.03	hypo	
9	38487906	38488165	259	25	-0.07	hypo	
9	38687606	38687992	386	18	-0.07	hypo	
9	69500968	69501070	102	14	-0.08	hypo	
9	123744449	123744762	313	10	-0.06	hypo	
9	125879001	125879080	79	7	-0.05	hypo	
9	128994302	128994390	88	4	-0.06	hypo	
9	135114516	135114649	133	9	-0.08	hypo	
9	140113368	140113559	191	9	-0.07	hypo	
10	1404948	1405307	359	31	-0.09	hypo	
10	1405351	1406102	751	99	-0.08	hypo	
10	46055866	46055919	53	4	-0.05	hypo	
10	134139414	134139779	365	11	-0.07	hypo	
11	1828650	1828783	133	5	-0.06	hypo	
11	12136161	12136468	307	10	-0.04	hypo	
11	59560470	59560549	79	4	-0.07	hypo	
11	65477123	65477452	329	9	-0.06	hypo	
11	128694096	128694425	329	32	-0.05	hypo	
11	132951692	132952492	800	45	-0.12	hypo	
12	16161553	16161815	262	7	-0.05	hypo	
12	57792999	57793110	111	6	-0.04	hypo	
12	102092915	102093110	195	11	-0.06	hypo	
12	107273279	107273681	402	7	-0.04	hypo	
12	111137400	111137596	196	11	-0.06	hypo	
12	117443273	117443444	171	7	-0.06	hypo	
12	119591663	119592119	456	29	-0.04	hypo	
12	124905467	124905759	292	15	-0.04	hypo	
12	125482583	125482829	246	18	-0.04	hypo	

13	20968573	20969085	512	37	-0.08	hypo	
13	20988857	20989415	558	41	-0.08	hypo	
13	24914323	24914905	582	37	-0.08	hypo	
13	25670023	25670239	216	21	-0.04	hypo	
13	111948367	111948559	192	9	-0.04	hypo	
14	75153156	75153308	152	3	-0.05	hypo	
14	93212312	93212487	175	11	-0.05	hypo	
14	100610169	100610668	499	22	-0.05	hypo	
14	103200841	103201128	287	12	-0.05	hypo	
15	30336647	30336863	216	29	-0.08	hypo	
15	31134409	31134668	259	9	-0.05	hypo	
15	40093789	40094023	234	7	-0.06	hypo	
15	52707259	52707363	104	5	-0.05	hypo	
15	52872030	52872160	130	6	-0.04	hypo	
15	57511786	57512216	430	18	-0.04	hypo	
15	74832028	74832090	62	5	-0.06	hypo	
16	30552372	30552613	241	9	-0.05	hypo	
16	57831974	57832180	206	18	-0.08	hypo	
16	69489543	69489665	122	5	-0.06	hypo	
16	85654156	85654324	168	13	-0.08	hypo	
16	88540019	88540526	507	39	-0.06	hypo	
16	88558082	88558379	297	17	-0.08	hypo	
16	88579452	88580072	620	21	-0.05	hypo	
17	8702637	8702756	119	16	-0.12	hypo	
17	8769570	8769884	314	14	-0.03	hypo	
17	17946397	17946585	188	7	-0.06	hypo	
17	19627951	19628166	215	27	-0.05	hypo	
17	21119605	21119845	240	9	-0.05	hypo	
17	28580392	28580614	222	5	-0.05	hypo	
17	36572579	36572897	318	11	-0.05	hypo	
17	49057182	49057239	57	5	-0.05	hypo	
17	56272299	56272502	203	10	-0.05	hypo	
17	56274149	56274598	449	23	-0.04	hypo	
17	56283478	56283523	45	7	-0.08	hypo	
17	56283687	56284009	322	10	-0.04	hypo	
17	78569835	78569888	53	4	-0.06	hypo	
17	79466178	79466419	241	34	-0.09	hypo	
18	8755023	8755343	320	13	-0.05	hypo	
18	12076398	12076622	224	30	-0.07	hypo	
18	14458381	14458937	556	38	-0.03	hypo	
18	22016574	22016800	226	6	-0.06	hypo	
18	71910027	71910089	62	6	-0.07	hypo	
18	77703283	77703521	238	15	-0.05	hypo	
19	1854531	1854766	235	24	-0.05	hypo	
19	3520495	3521154	659	32	-0.05	hypo	
19	4382715	4382768	53	4	-0.06	hypo	
19	10404092	10405285	1193	81	-0.04	hypo	
19	34859991	34860410	419	13	-0.06	hypo	
19	51373740	51374029	289	20	-0.07	hypo	
20	29515851	29515954	103	8	-0.14	hypo	

20	29525180	29525475	295	20	-0.10	hypo	
20	29550781	29551739	958	60	-0.05	hypo	
20	32232346	32232458	112	10	-0.05	hypo	
20	33416638	33416742	104	9	-0.08	hypo	
21	19184847	19184909	62	7	-0.07	hypo	
21	30298129	30298294	165	5	-0.05	hypo	
21	38750599	38750877	278	11	-0.04	hypo	
21	45705600	45705881	281	34	-0.08	hypo	
22	46762433	46763144	711	38	-0.11	hypo	
22	50616227	50617057	830	76	-0.09	hypo	
22	50985261	50985925	664	70	-0.10	hypo	

mean coverage
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28.1
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30.7
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38.9
17.0
41.0
36.8
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29.1
34.3
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21.6
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25.3
29.9
28.1
25.5
26.9
42.7
36.7
20.3
24.9
21.0
18.1

Table S3: Datasets used for DMR enhancer annotation.

Data set name
Enhancers_ENCODE
ROADMAP_6_EnhG/7_Enh.Blood&T-cell***
ROADMAP_6_EnhG/7_Enh.HSC&B-cell***
active_marks_LINA_children
GeneHancer

***Enhancer_ROADMAP_blood_Tcell**

E062 Primary.mononuclear.cells.from.peripheral.blood Blooc
E034 Primary.T.cells.from.peripheral.blood Blood.and.T-cell
E045 Primary.T.cells.effector.memory.enriched.from.peripher
E033 Primary.T.cells.from.cord.blood Blood.and.T-cell
E044 Primary.T.regulatory.cells.from.peripheral.blood Blood
E043 Primary.T.helper.cells.from.peripheral.blood Blood.and
E039 Primary.T.helper.naive.cells.from.peripheral.blood Bloc
E041 Primary.T.helper.cells.PMA-I.stimulated Blood.and.T-ce
E042 Primary.T.helper.17.cells.PMA-I.stimulated Blood.and.T
E040 Primary.T.helper.memory.cells.from.peripheral.blood.1
E037 Primary.T.helper.memory.cells.from.peripheral.blood.2
E048 Primary.T.CD8+.memory.cells.from.peripheral.blood Bl
E038 Primary.T.helper.naive.cells.from.peripheral.blood Bloc
E047 Primary.T.CD8+.naive.cells.from.peripheral.blood Blooc

****Enhancer_ROADMAP_HSC_Bcell**

E029 Primary.monocytes.from.peripheral.blood HSC&B-cell
E031 Primary.B.cells.from.cord.blood HSC&B-cell
E035 Primary.hematopoietic.stem.cells HSC&B-cell
E051 Primary.hematopoietic.stem.cells.G-CSF-mobilized.Male
E050 Primary.hematopoietic.stem.cells.G-CSF-mobilized.Fema
E036 Primary.hematopoietic.stem.cells.short.term.culture HS
E032 Primary.B.cells.from.peripheral.blood HSC&B-cell
E046 Primary.Natural.Killer.cells.from.peripheral.blood HSC8
E030 Primary.neutrophils.from.peripheral.blood HSC&B-cell

*****based on chromatin core 15-state model (5 marks, 127 epig**
STATE NO/ MNEMONIC/ Description
6/ EnhG/ Genic enhancers
7/ Enh/ Enhancers

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Definition for intersection of DMR with enhancer
intersection (at least 1bp) between known ENCODE enhancer and DMRs
intersection (at least 1bp) between known ROADMAP enhancer of blood or T-cell sets (n=14*) and DMRs
intersection (at least 1bp) between known ROADMAP enhancer of HSC or B-cell sets (n=9**) and DMRs
intersection (at least 1bp) between active marks of LINA children (according to Bauer et al.) and DMRs
intersection (at least 1bp) between known GeneHancer enhancer and DMRs

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d.and.T-cell

HSC&B-cell
le HSC&B-cell
SC&B-cell

&B-cell

genomes)

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(1);(2)
(3)
(3)
(4)
(5)

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Table S4: MassARRAY primer for targeted DNA-methylation analysis.

Gene	Genomic region covered	Forward primer
IL4	chr5:132002082-132002531	5'-x-GATGTTATATTAGTGAAAGGAG-3'
IL5RA	chr3:3150172-3150448	5'-x-TATTTTTTGTTAATTGTATATGGTG-3'
EPX	chr17:56272276-56272639	5'-x-GTGGGGTTAGGGAGTTTATG-3'

10Dimer forward tag X=aggaagagag; T7-reverse tag y-cagtaatacgactcactatagggagaaggct

Reverse primer	Temperature
5'-y-CAAAAAATAAACCAAAACACTC-3'	56°C
5'-y-ACTCATTCCTCTATTAAATTTC-3'	52°C
5'-y-CAAACAACTCCTTAAAAATAATAC-3'	52°C

Table S5: qPCR primer for targeted gene expression analysis.

Gene	Forward primer	Reverse primer	UPL#
PGK1	5'-tgcaaaggccttggagag-3'	5'-tggatcttgtctgcaactttagc-3'	72
GAPD	5'-gctctctgctcctcctgttc-3'	5'-acgaccaaatccgttgactc-3'	60
GUSB	5'-cgccctgcctatctgtattc-3'	5'-tccccacagggagtgtgtag-3'	57
IL5RA	5'-cagcaccaaaaagtaatatcaaagat-3'	5'-ccaaagtgacagtcaaaacacag-3'	65
EPX	5'-ccctgtctcctcaccaacc-3'	5'-gtttccgttgatcgggtgt-3'	38
IL4	5'-agctgatccgattcctgaaa-3'	5'-agctgcttgtgcctgtggaactg-3'	57

Table S6A: Overview of n=158 asthma-related DMRs (comparing n=40 asthmatics vs. n=42 co

* enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annotation was ava

DMR coordinates					DNA-methylation			
chr	start	end	width	nCpGs	mean asthma	mean controls	mean difference	direction
1	870565	871771	1206	68	0.35	0.29	0.06	hyper
1	6341136	6341683	547	28	0.79	0.84	-0.06	hypo
1	10436586	10436851	265	11	0.86	0.91	-0.05	hypo
1	22097059	22097227	168	8	0.88	0.93	-0.05	hypo
1	62364300	62364445	145	4	0.89	0.93	-0.04	hypo
1	67600417	67600715	298	21	0.04	0.07	-0.03	hypo
1	84744687	84744808	121	5	0.92	0.95	-0.04	hypo
1	93970706	93970977	271	9	0.83	0.89	-0.06	hypo
1	149162004	149162428	424	21	0.47	0.57	-0.09	hypo
1	202121664	202121815	151	9	0.86	0.91	-0.06	hypo
1	204479935	204480156	221	9	0.92	0.96	-0.04	hypo
2	24233600	24234117	517	24	0.54	0.60	-0.06	hypo
2	31154795	31155157	362	17	0.87	0.94	-0.07	hypo
2	70734255	70734341	86	5	0.91	0.95	-0.04	hypo
2	74213621	74213841	220	13	0.70	0.63	0.07	hyper
2	75089515	75089819	304	8	0.85	0.90	-0.05	hypo
2	97401278	97401372	94	6	0.88	0.93	-0.05	hypo
2	107082602	107082889	287	32	0.89	0.95	-0.06	hypo
2	113426404	113426419	15	3	0.85	0.93	-0.08	hypo
2	113956545	113956673	128	18	0.22	0.29	-0.07	hypo
2	118617427	118618163	736	73	0.44	0.50	-0.06	hypo
2	121816094	121816885	791	23	0.82	0.87	-0.05	hypo
2	130986715	130986828	113	20	0.49	0.56	-0.07	hypo
2	132404284	132404979	695	54	0.25	0.31	-0.05	hypo
2	241459177	241460047	870	54	0.35	0.40	-0.05	hypo
3	3150228	3150425	197	9	0.88	0.93	-0.05	hypo
3	39395430	39395805	375	12	0.85	0.91	-0.05	hypo
3	70560282	70560339	57	5	0.88	0.93	-0.05	hypo
3	75445094	75445699	605	53	0.40	0.50	-0.10	hypo
3	98476467	98476657	190	5	0.89	0.93	-0.05	hypo
3	128134844	128135029	185	8	0.86	0.91	-0.05	hypo
3	128317561	128317755	194	8	0.88	0.93	-0.05	hypo
3	128317793	128318091	298	12	0.67	0.73	-0.06	hypo
3	172243109	172243331	222	13	0.84	0.89	-0.06	hypo
3	184243755	184244149	394	22	0.18	0.23	-0.05	hypo
3	195964960	195965370	410	11	0.89	0.93	-0.04	hypo
4	1908638	1908946	308	10	0.86	0.91	-0.06	hypo
4	2366183	2366745	562	49	0.20	0.23	-0.03	hypo
4	144833125	144833346	221	30	0.15	0.20	-0.06	hypo

4	148634323	148634374	51	5	0.88	0.94	-0.06	hypo
5	8457869	8457980	111	13	0.16	0.23	-0.07	hypo
5	42923963	42924355	392	23	0.64	0.72	-0.08	hypo
5	42943969	42944684	715	41	0.34	0.42	-0.08	hypo
5	68700315	68700724	409	14	0.92	0.95	-0.04	hypo
5	77142381	77142899	518	26	0.53	0.61	-0.08	hypo
5	77146478	77147361	883	53	0.58	0.65	-0.07	hypo
5	132002374	132002507	133	5	0.86	0.91	-0.05	hypo
5	149145166	149145197	31	4	0.88	0.94	-0.06	hypo
5	154224429	154224647	218	4	0.88	0.93	-0.05	hypo
5	157117442	157117959	517	42	0.43	0.50	-0.07	hypo
6	32063911	32064192	281	33	0.21	0.26	-0.04	hypo
6	166674955	166675083	128	5	0.88	0.93	-0.05	hypo
6	168435945	168436646	701	41	0.27	0.33	-0.06	hypo
7	1914009	1914393	384	18	0.87	0.91	-0.04	hypo
7	5382633	5382783	150	8	0.90	0.95	-0.05	hypo
7	32357921	32358755	834	44	0.20	0.27	-0.08	hypo
7	36007074	36007282	208	19	0.35	0.44	-0.09	hypo
7	48887537	48887891	354	42	0.28	0.36	-0.08	hypo
7	54900863	54901103	240	21	0.84	0.89	-0.04	hypo
7	55412705	55412996	291	33	0.38	0.47	-0.09	hypo
7	90895326	90896702	1376	91	0.67	0.73	-0.06	hypo
7	102003600	102003767	167	9	0.84	0.89	-0.05	hypo
7	127910860	127911680	820	49	0.44	0.50	-0.06	hypo
7	150647915	150648063	148	8	0.89	0.94	-0.04	hypo
8	599524	600398	874	60	0.84	0.92	-0.09	hypo
8	58192499	58193338	839	50	0.50	0.58	-0.08	hypo
8	128828626	128828794	168	5	0.89	0.95	-0.05	hypo
8	131047175	131047345	170	5	0.90	0.94	-0.05	hypo
9	5819260	5819334	74	4	0.89	0.95	-0.06	hypo
9	32430999	32431303	304	9	0.91	0.95	-0.03	hypo
9	38487906	38488165	259	25	0.48	0.56	-0.07	hypo
9	38687606	38687992	386	18	0.35	0.42	-0.07	hypo
9	69500968	69501070	102	14	0.72	0.80	-0.08	hypo
9	123744449	123744762	313	10	0.83	0.89	-0.06	hypo
9	125879001	125879080	79	7	0.88	0.93	-0.05	hypo
9	128994302	128994390	88	4	0.88	0.93	-0.06	hypo
9	135114516	135114649	133	9	0.32	0.39	-0.08	hypo
9	140113368	140113559	191	9	0.80	0.87	-0.07	hypo
10	1404948	1405307	359	31	0.60	0.68	-0.09	hypo
10	1405351	1406102	751	99	0.34	0.42	-0.08	hypo
10	46055866	46055919	53	4	0.88	0.94	-0.05	hypo
10	134139414	134139779	365	11	0.87	0.93	-0.07	hypo
11	1828650	1828783	133	5	0.79	0.86	-0.06	hypo
11	12136161	12136468	307	10	0.86	0.90	-0.04	hypo
11	59560470	59560549	79	4	0.85	0.92	-0.07	hypo
11	65477123	65477452	329	9	0.81	0.87	-0.06	hypo
11	128694096	128694425	329	32	0.20	0.25	-0.05	hypo
11	132951692	132952492	800	45	0.47	0.59	-0.12	hypo
12	16161553	16161815	262	7	0.87	0.91	-0.05	hypo

12	57792999	57793110	111	6	0.91	0.95	-0.04	hypo
12	102092915	102093110	195	11	0.86	0.92	-0.06	hypo
12	107273279	107273681	402	7	0.90	0.94	-0.04	hypo
12	111137400	111137596	196	11	0.87	0.93	-0.06	hypo
12	117443273	117443444	171	7	0.86	0.92	-0.06	hypo
12	119591663	119592119	456	29	0.17	0.21	-0.04	hypo
12	124905467	124905759	292	15	0.88	0.92	-0.04	hypo
12	125482583	125482829	246	18	0.86	0.90	-0.04	hypo
13	20968573	20969085	512	37	0.50	0.58	-0.08	hypo
13	20988857	20989415	558	41	0.53	0.61	-0.08	hypo
13	24914323	24914905	582	37	0.67	0.75	-0.08	hypo
13	25670023	25670239	216	21	0.11	0.15	-0.04	hypo
13	111948367	111948559	192	9	0.86	0.91	-0.04	hypo
14	75153156	75153308	152	3	0.87	0.92	-0.05	hypo
14	93212312	93212487	175	11	0.89	0.94	-0.05	hypo
14	100610169	100610668	499	22	0.84	0.89	-0.05	hypo
14	103200841	103201128	287	12	0.88	0.93	-0.05	hypo
15	30336647	30336863	216	29	0.62	0.70	-0.08	hypo
15	31134409	31134668	259	9	0.87	0.92	-0.05	hypo
15	40093789	40094023	234	7	0.88	0.94	-0.06	hypo
15	52707259	52707363	104	5	0.90	0.94	-0.05	hypo
15	52872030	52872160	130	6	0.91	0.95	-0.04	hypo
15	57511786	57512216	430	18	0.90	0.94	-0.04	hypo
15	74832028	74832090	62	5	0.87	0.93	-0.06	hypo
16	30552372	30552613	241	9	0.88	0.92	-0.05	hypo
16	57831974	57832180	206	18	0.53	0.61	-0.08	hypo
16	69489543	69489665	122	5	0.88	0.94	-0.06	hypo
16	85654156	85654324	168	13	0.83	0.90	-0.08	hypo
16	88540019	88540526	507	39	0.84	0.90	-0.06	hypo
16	88558082	88558379	297	17	0.80	0.87	-0.08	hypo
16	88579452	88580072	620	21	0.83	0.88	-0.05	hypo
17	8702637	8702756	119	16	0.26	0.38	-0.12	hypo
17	8769570	8769884	314	14	0.90	0.93	-0.03	hypo
17	17946397	17946585	188	7	0.87	0.93	-0.06	hypo
17	19627951	19628166	215	27	0.10	0.15	-0.05	hypo
17	21119605	21119845	240	9	0.84	0.89	-0.05	hypo
17	28580392	28580614	222	5	0.88	0.93	-0.05	hypo
17	36572579	36572897	318	11	0.85	0.90	-0.05	hypo
17	49057182	49057239	57	5	0.88	0.94	-0.05	hypo
17	56272299	56272502	203	10	0.85	0.90	-0.05	hypo
17	56274149	56274598	449	23	0.87	0.91	-0.04	hypo
17	56283478	56283523	45	7	0.87	0.95	-0.08	hypo
17	56283687	56284009	322	10	0.90	0.94	-0.04	hypo
17	78569835	78569888	53	4	0.85	0.91	-0.06	hypo
17	79466178	79466419	241	34	0.58	0.67	-0.09	hypo
18	8755023	8755343	320	13	0.83	0.87	-0.05	hypo
18	12076398	12076622	224	30	0.24	0.31	-0.07	hypo
18	14458381	14458937	556	38	0.04	0.07	-0.03	hypo
18	22016574	22016800	226	6	0.87	0.93	-0.06	hypo
18	71910027	71910089	62	6	0.83	0.90	-0.07	hypo

18	77703283	77703521	238	15	0.89	0.94	-0.05	hypo
19	1854531	1854766	235	24	0.80	0.85	-0.05	hypo
19	3520495	3521154	659	32	0.81	0.86	-0.05	hypo
19	4382715	4382768	53	4	0.85	0.91	-0.06	hypo
19	10404092	10405285	1193	81	0.18	0.22	-0.04	hypo
19	34859991	34860410	419	13	0.81	0.87	-0.06	hypo
19	51373740	51374029	289	20	0.56	0.63	-0.07	hypo
20	29515851	29515954	103	8	0.47	0.62	-0.14	hypo
20	29525180	29525475	295	20	0.19	0.29	-0.10	hypo
20	29550781	29551739	958	60	0.16	0.21	-0.05	hypo
20	32232346	32232458	112	10	0.86	0.90	-0.05	hypo
20	33416638	33416742	104	9	0.84	0.92	-0.08	hypo
21	19184847	19184909	62	7	0.82	0.89	-0.07	hypo
21	30298129	30298294	165	5	0.89	0.94	-0.05	hypo
21	38750599	38750877	278	11	0.85	0.89	-0.04	hypo
21	45705600	45705881	281	34	0.33	0.41	-0.08	hypo
22	46762433	46763144	711	38	0.61	0.72	-0.11	hypo
22	50616227	50617057	830	76	0.41	0.49	-0.09	hypo
22	50985261	50985925	664	70	0.56	0.65	-0.10	hypo

ɔntrols).

ilable the closest TSS gene is given

DMR information

statistics			
q-value (metilene)	raw *p*-value (ANOVA)		
	LINA	LISA	PASTURE
3.90E-03	2.84E-08	2.06E-29	1.76E-23
1.20E-14	2.96E-17	1.83E-17	1.40E-09
1.60E-05	2.33E-07	4.70E-15	7.23E-08
2.10E-04	6.41E-05	2.44E-11	2.26E-08
3.80E-03	7.70E-05	1.99E-07	1.81E-05
3.70E-05	1.61E-12	2.78E-06	1.95E-10
4.20E-05	2.00E-05	2.31E-07	1.28E-07
3.70E-04	1.21E-09	1.94E-10	5.57E-10
1.70E-05	4.23E-10	6.52E-19	4.90E-06
9.50E-04	2.55E-09	3.44E-15	5.17E-05
2.70E-05	5.63E-08	5.81E-16	4.35E-06
3.60E-04	3.09E-09	2.92E-10	1.92E-06
8.50E-26	1.69E-16	9.00E-40	3.01E-15
6.00E-03	3.18E-05	2.78E-07	1.89E-05
2.20E-02	4.32E-07	7.47E-12	5.27E-06
4.50E-04	3.38E-05	2.35E-15	1.54E-07
6.20E-04	4.06E-05	2.02E-11	2.90E-05
5.80E-19	1.17E-06	7.56E-14	6.83E-08
3.40E-04	4.98E-05	9.92E-10	1.29E-06
2.00E-04	3.94E-12	6.76E-06	2.93E-10
1.30E-03	3.82E-29	2.80E-07	7.77E-08
4.40E-10	8.71E-18	2.67E-17	1.06E-08
4.50E-05	5.24E-07	1.54E-09	3.56E-06
1.70E-09	1.56E-11	4.12E-31	6.11E-20
1.50E-03	2.43E-24	3.32E-11	8.95E-06
4.70E-13	3.21E-11	4.29E-19	5.41E-08
3.30E-05	6.31E-07	2.24E-21	2.06E-06
5.40E-03	6.31E-05	5.35E-09	3.24E-06
1.70E-25	3.10E-39	3.07E-05	1.05E-14
4.20E-04	5.87E-05	5.66E-10	1.33E-05
2.20E-05	2.41E-06	1.33E-07	1.41E-12
2.90E-06	1.96E-07	6.42E-12	2.16E-05
1.20E-04	1.39E-11	7.67E-09	3.49E-09
2.20E-04	4.01E-08	2.36E-23	5.93E-06
4.90E-03	6.11E-19	1.37E-06	2.53E-06
3.30E-02	6.45E-08	1.43E-10	1.78E-07
1.80E-10	3.18E-09	5.71E-21	1.24E-11
5.80E-06	4.44E-22	2.29E-11	2.13E-10
3.10E-15	2.53E-18	1.87E-19	9.76E-08

6.20E-08	3.31E-09	8.07E-10	2.89E-07
5.40E-11	4.10E-20	1.04E-05	2.02E-05
4.70E-08	1.29E-08	1.08E-14	1.22E-04
1.40E-09	5.13E-18	1.86E-39	7.51E-11
3.10E-08	3.23E-08	2.74E-24	1.79E-07
9.20E-05	6.22E-09	1.10E-06	7.66E-09
2.70E-07	2.46E-12	4.25E-05	5.93E-19
3.10E-03	1.10E-05	7.67E-09	8.19E-06
1.60E-03	2.22E-07	3.93E-07	4.13E-07
4.60E-03	5.58E-05	3.08E-08	1.64E-05
5.90E-09	9.14E-19	6.14E-07	7.73E-05
1.70E-03	4.49E-08	9.47E-13	2.34E-07
9.00E-06	5.55E-10	1.16E-07	3.11E-06
4.70E-03	4.69E-09	2.06E-09	1.10E-15
1.10E-05	2.87E-05	2.09E-07	3.94E-08
1.90E-09	2.95E-09	6.65E-17	1.36E-05
2.30E-23	1.67E-16	2.33E-27	2.46E-35
2.00E-06	1.21E-11	8.81E-05	8.07E-13
8.80E-18	1.34E-25	2.25E-16	2.11E-05
3.70E-03	7.41E-07	3.21E-07	3.16E-13
1.00E-09	4.17E-23	2.52E-07	6.02E-07
8.60E-09	3.26E-09	6.87E-12	1.96E-37
4.10E-04	2.01E-07	2.42E-06	7.01E-07
1.70E-04	2.70E-09	4.29E-07	4.00E-20
3.00E-06	3.37E-06	3.82E-06	2.81E-07
6.10E-35	2.44E-09	6.57E-43	1.36E-29
8.10E-07	7.37E-13	3.19E-11	6.36E-22
2.00E-04	1.07E-07	4.57E-08	1.24E-06
6.30E-05	8.12E-07	9.04E-12	1.34E-05
3.70E-05	2.30E-05	1.92E-10	1.94E-05
1.20E-03	1.89E-06	1.68E-07	3.03E-09
7.50E-06	1.00E-04	6.74E-08	9.78E-17
3.70E-04	5.54E-12	2.94E-06	5.12E-09
2.10E-07	1.58E-07	2.75E-09	1.12E-05
8.80E-07	6.86E-11	6.28E-12	3.13E-06
2.60E-04	7.81E-06	1.85E-17	1.36E-05
2.90E-03	2.67E-05	2.18E-10	1.30E-04
6.10E-04	1.77E-05	1.76E-14	3.73E-06
8.90E-06	4.59E-16	3.60E-14	1.51E-06
1.60E-08	3.27E-09	2.68E-06	1.04E-17
2.20E-26	1.80E-48	1.61E-06	1.71E-45
6.20E-03	1.10E-05	1.31E-08	1.16E-04
9.10E-18	1.75E-18	3.33E-23	7.38E-08
1.50E-02	1.11E-05	7.94E-11	1.95E-06
1.80E-04	9.25E-07	7.12E-07	1.81E-07
7.90E-05	2.29E-06	4.09E-07	2.23E-06
7.80E-04	6.11E-08	1.41E-12	3.72E-05
1.00E-09	2.96E-08	1.30E-10	1.22E-11
2.50E-26	7.73E-24	4.78E-12	1.06E-19
4.40E-04	7.97E-07	4.66E-14	1.72E-05

1.20E-04	3.08E-05	5.73E-09	1.10E-04
4.20E-10	2.27E-13	6.48E-14	6.57E-10
4.70E-06	1.26E-11	2.14E-10	2.35E-06
6.80E-12	5.48E-20	4.67E-19	6.37E-05
1.30E-07	3.70E-09	2.63E-12	2.29E-05
1.20E-02	4.28E-18	1.91E-07	2.71E-05
2.90E-06	6.54E-12	1.66E-10	3.27E-11
4.50E-06	3.30E-06	8.12E-09	1.82E-15
3.30E-09	6.92E-07	3.26E-16	1.62E-35
1.70E-10	3.92E-29	2.54E-05	1.66E-12
2.20E-14	2.18E-08	4.16E-18	1.70E-08
5.50E-06	3.31E-12	3.49E-09	1.59E-06
9.80E-05	7.41E-07	7.83E-07	3.82E-07
8.50E-03	1.97E-05	1.22E-06	1.19E-05
5.00E-06	4.60E-08	1.91E-11	1.87E-11
8.40E-12	2.00E-09	1.40E-22	3.82E-13
5.90E-07	1.99E-09	4.86E-17	5.24E-10
1.10E-04	4.69E-05	3.62E-07	2.75E-08
8.20E-07	1.62E-10	9.73E-14	1.78E-05
1.40E-12	2.50E-09	5.21E-25	6.90E-05
2.80E-04	1.91E-07	1.09E-07	6.88E-06
4.00E-04	9.30E-05	1.32E-13	2.76E-05
1.80E-12	4.14E-14	2.80E-21	4.86E-05
5.30E-03	6.10E-10	1.16E-04	3.86E-05
8.40E-06	4.48E-10	1.31E-16	3.31E-05
2.00E-05	2.98E-05	2.52E-10	1.41E-11
2.80E-06	3.18E-07	3.11E-12	3.19E-06
7.20E-16	5.26E-15	8.44E-12	2.34E-20
1.70E-23	3.17E-09	9.73E-12	2.90E-16
5.00E-17	3.19E-06	5.15E-31	1.33E-10
5.20E-06	2.17E-16	2.84E-11	2.20E-06
6.30E-06	5.11E-10	2.66E-12	3.06E-08
9.10E-06	3.53E-06	9.76E-12	1.30E-05
1.60E-06	1.91E-09	8.33E-19	4.76E-06
1.70E-18	2.43E-07	1.43E-10	4.13E-37
3.40E-05	8.77E-07	8.76E-09	1.88E-06
1.30E-02	3.05E-05	5.00E-08	1.74E-05
6.50E-04	8.81E-10	4.10E-20	1.97E-05
6.20E-04	2.66E-05	3.92E-08	9.04E-06
3.60E-05	9.90E-06	7.82E-18	4.92E-07
2.80E-07	1.16E-08	4.04E-18	1.03E-06
6.30E-06	1.54E-07	1.27E-15	9.68E-06
6.90E-05	6.51E-09	2.64E-13	1.17E-04
1.30E-02	4.64E-05	1.12E-12	5.02E-05
3.40E-07	2.49E-08	4.20E-09	2.00E-12
1.60E-04	3.72E-08	2.72E-13	5.24E-06
1.30E-15	1.12E-05	3.41E-11	4.74E-12
1.10E-08	4.30E-12	3.31E-14	5.24E-07
3.70E-04	6.01E-05	1.87E-14	8.62E-06
9.90E-09	5.12E-07	1.20E-13	3.90E-11

2.20E-11	3.10E-10	2.88E-22	1.25E-07
2.10E-04	3.89E-11	8.28E-18	9.38E-05
4.30E-11	6.06E-08	8.17E-13	2.76E-06
1.60E-05	2.08E-06	1.21E-07	1.94E-06
4.20E-07	1.94E-44	1.88E-08	1.28E-12
1.40E-06	7.49E-08	9.95E-14	3.05E-09
1.00E-04	4.39E-05	4.66E-13	7.89E-07
1.50E-07	4.39E-05	2.03E-14	3.46E-05
1.10E-12	8.82E-13	1.30E-06	9.48E-08
1.70E-11	3.67E-11	6.07E-18	1.13E-14
2.10E-03	3.20E-06	5.11E-08	1.49E-07
4.40E-12	2.32E-10	2.32E-15	1.40E-09
1.10E-05	2.22E-09	1.83E-10	1.35E-05
4.40E-05	9.00E-05	2.33E-12	7.23E-06
1.60E-02	4.33E-09	2.20E-09	3.23E-05
1.20E-09	2.81E-08	6.04E-18	5.78E-12
6.70E-16	1.72E-16	3.82E-22	5.49E-16
9.50E-08	1.03E-21	6.46E-24	2.54E-06
4.60E-19	3.66E-29	2.31E-15	1.04E-38

associated genes*	ngDMR/ gDMR	eosinophils
AL645608.1	ngDMR	-
RP1-202O8.3;ACOT7;GPR153;RP1-202O8.3;ACOT7	ngDMR	-
PGD;TARDBP;RP4-635E18.8;EXOSC10;MTOR;KIF1B	ngDMR	YES
USP48	ngDMR	-
INADL	ngDMR	-
C1orf141;IL23R	gDMR	-
PRKACB;SNORA2;RP11-376N17.4;SAMD13	ngDMR	YES
FNBP1L	ngDMR	-
HIST2H3PS2	ngDMR	-
PTPN7;PTPRVP;ARL8A	ngDMR	YES
MDM4	ngDMR	YES
RP11-443B20.1;CENPO;ATAD2B;MFSD2B;UBXN2A	ngDMR	-
CAPN13	ngDMR	YES
TGFA	ngDMR	-
AC073046.25;TET3;MGC10955;ALMS1;CCT7;MOB1A;SLC4A5;HTRA2;DGUOK-A	ngDMR	-
HK2;AC104135.2	ngDMR	YES
LMAN2L	ngDMR	-
RGPD3	gDMR	-
SLC20A1	ngDMR	YES
AC016683.5;PSD4	ngDMR	-
DDX18	gDMR	-
TFCP2L1	ngDMR	YES
TUBA3E	ngDMR	-
C2orf27A	ngDMR	-
GPC1;MIR149;ANKMY1	gDMR	-
IL5RA	ngDMR	YES
EEF1A1P24;RP11-241K18.2;RPSA;CCR8;CX3CR1	gDMR	-
MITF	ngDMR	-
LINC00960;RP11-803B1.3;LSP1P2	gDMR	-
CPOX;ST3GAL6;PDLIM1P4	ngDMR	-
DNAJB8;GATA2;DNAJB8-AS1;EFCC1;TPRA1;EEFSEC;RPL32P3;RP11-529F4.1;H1	ngDMR	-
C3orf27	ngDMR	YES
C3orf27	gDMR	-
TNFSF10	ngDMR	YES
CHRD;EIF4G1;POLR2H;THPO;YEATS2;CLCN2;EPHB3;MAGEF1;FAM131A	gDMR	-
SLC51A	ngDMR	YES
WHSC1	ngDMR	-
RP11-478C1.7;ZFYVE28;RNF4;NOP14-AS1;NELFA;POLN;HAUS3;RP11-317B7.3;	ngDMR	-
FREM3;GYPE	ngDMR	-

ARHGAP10	ngDMR	YES
MIR4458HG;RP11-480D4.2;MTRR	ngDMR	-
SEPP1	ngDMR	-
CTD-2201E18.5;ZNF131	gDMR	-
MARVELD2	ngDMR	YES
TBCA	gDMR	-
TBCA	gDMR	-
RP11-485M7.1;RAD50;IL4;AFF4;AC063976.7;ZCCHC10	ngDMR	YES
PPARGC1B	ngDMR	YES
MIR378H;CNOT8;FAXDC2	ngDMR	YES
C5orf52	gDMR	-
TNXB	gDMR	-
PRR18	ngDMR	YES
KIF25;KIF25-AS1;FRMD1	ngDMR	-
MAD1L1;MIR4655	gDMR	-
SLC29A4	ngDMR	YES
PDE1C	gDMR	-
SEPT7P3	ngDMR	-
AC004899.1	ngDMR	-
SEC61G	ngDMR	-
LANCL2	gDMR	-
AKAP9;AC000120.7;FZD1;KRIT1;GTPBP10;AC002064.4	ngDMR	-
ORAI2;RP11-163E9.1;RP11-163E9.2;PRKRIP1;ALKBH4	ngDMR	YES
RP11-62J1.4;RBM28;LEP	ngDMR	-
KCNH2	ngDMR	YES
ERICH1	gDMR	-
IMPAD1	gDMR	-
PVT1;MYC	ngDMR	YES
FAM49B	ngDMR	YES
ERMP1	ngDMR	-
ACO1;DDX58;TMEM215	ngDMR	YES
IGFBPL1	ngDMR	-
ANKRD18A	gDMR	-
ANKRD20A4	ngDMR	-
TRAF1	gDMR	YES
GPR21	ngDMR	-
MVB12B	ngDMR	YES
NTNG2	ngDMR	-
RNF208;NDOR1;NPDC1	ngDMR	YES
WDR37	ngDMR	-
WDR37	ngDMR	-
MARCH8	ngDMR	YES
ZNF511;TUBGCP2;RP11-122K13.12;LRRC27;STK32C	ngDMR	YES
SYT8	ngDMR	YES
MICAL2;MICALCL	gDMR	-
MRPL16	ngDMR	-
RBM4;RBM14;ZFPL1;CCS;CTSF;MRPL11;RP11-755F10.3;RP11-658F2.8;SSSCA1	ngDMR	YES
KCNJ1	gDMR	-
OPCML	ngDMR	-
DERA	ngDMR	-

AC126614.1	ngDMR	YES
CHPT1;ARL1;GNPTAB	ngDMR	YES
C12orf23	ngDMR	YES
RN7SL387P;PCNPP1;HVCN1;FAM216A	ngDMR	YES
FBXW8	ngDMR	-
SRRM4	gDMR	-
RP11-408I18.9;NCOR2	ngDMR	-
BRI3BP	gDMR	-
GJB2;MIR4499	ngDMR	-
GJB2;MIR4499;CRYL1	ngDMR	-
AL359736.1	ngDMR	-
PABPC3	ngDMR	-
TEX29	ngDMR	-
AREL1;SYNDIG1L;FCF1;SNORA7;LTBP2	ngDMR	YES
LGMN	ngDMR	YES
DEGS2;SLC25A29;MIR770;EVL	ngDMR	YES
TRAF3	ngDMR	YES
GOLGA8J	ngDMR	-
HERC2P10;CHRFAM7A;RP11-540B6.3;FAN1	gDMR	YES
FSIP1	ngDMR	-
MYO5A	ngDMR	-
ARPP19	ngDMR	-
LINC00926;TCF12	gDMR	YES
ARID3B	ngDMR	-
ITGAL;AC002310.7;ZNF764;AC002310.13;TAOK2;INO80E;SLX1A;DCTPP1;CD2B	ngDMR	-
CNOT1;SETD6;RSPRY1;KIFC3	ngDMR	-
CYB5B	ngDMR	YES
CTD-2542L18.1;GSE1	ngDMR	YES
ZNF469;ZFPM1;AC137932.1;RP11-46C24.7;LINC00304;APRT;PIEZO1;CTU2;RN	ngDMR	YES
ACSF3;CBFA2T3;ZC3H18;GALNS;AC137932.1;PIEZO1;RP5-1142A6.9;ANKRD11;	ngDMR	-
MIR5189;ZC3H18;ANKRD11;ZFPM1;BANP	ngDMR	YES
MFSD6L	gDMR	-
CNTROB;PIK3R6;PFAS;MFSD6L;RP11-849F2.5;RPL26	ngDMR	YES
GID4	gDMR	-
CTC-457L16.2;SNORA31;SLC47A2;MAPK7;CCDC144CP;RP11-311F12.2;RP11-31	gDMR	-
FAM106B;DHRS7B;AC087294.2;USP22;TMEM11	ngDMR	YES
NSRP1;TEFM;ATAD5;SH3GL1P2;LRRC37BP1;GOSR1;NUFIP2;BLMH	ngDMR	-
GPR179;FBXL20;RPL19	ngDMR	-
TOB1	ngDMR	YES
EPX	ngDMR	YES
EPX	ngDMR	YES
LPO	ngDMR	YES
LPO	ngDMR	YES
RPTOR	ngDMR	YES
ACTG1	gDMR	-
RP11-674N23.4	ngDMR	-
ANKRD62	ngDMR	-
LONRF2P1;CXADRP3;GRAMD4P7	gDMR	-
IMPACT	ngDMR	YES
CYB5A;RP11-669I1.1	ngDMR	YES

PQLC1;RBFADN;PQLC1	ngDMR	YES
PLEKHJ1;CTB-31O20.6;AC005306.3;CSNK1G2;ADAT3;SCAMP4;KLF16;TCF3;TM	ngDMR	-
MFSD12;C19orf71;SNORD38;FZR1	gDMR	YES
SH3GL1;UBXN6;AC007292.6	ngDMR	-
ZNF426;ZNF846;ICAM1;ZNF121;DOCK6;ILF3;KRI1;CTC-325H20.4;DNMT1;ZNF5	gDMR	-
GPI	ngDMR	-
C19orf48;SNORD88C;KLK3;KLK2;KLKP1	gDMR	-
FRG1B	ngDMR	-
FRG1B	ngDMR	-
FRG1B	ngDMR	-
C20orf144	gDMR	-
NCOA6	ngDMR	YES
C21orf91	ngDMR	-
N6AMT1	ngDMR	-
DYRK1A	ngDMR	-
AIRE	ngDMR	-
TRMU	gDMR	-
PANX2	gDMR	-
TYMP;CTA-384D8.31;TRABD;KLHDC7B;PANX2;SYCE3;CPT1B;HDAC10;CHKB;AR	gDMR	-

cell type dependency						cord blood DMR
B cells	NK cells	$CD4^+$ T cells	$CD8^+$ T cells	monocytes	neutrophils	
-	-	-	-	-	-	YES
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enhancer annotation			
	database-specific enhancer annotation		
enhancer total	GeneHancer	ENCODE	histone marks (LINA)
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ROADMAP	Peripheral_Blood Mononuclear Primary_Cells	CD3_Primary_Cells Peripheral_UW	CD4+_CD25int_CD127+ Tmem_Primary_Cells	CD3 Primary_Cells Cord_Biood
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Enhancer (Blood & T cell ROADMAP track)				
CD4+_CD25+_CD127-Treg_Primary_Cells	CD4+_CD25-_Th Primary_Cells	CD4+_CD25-_CD45RA+_Naive Primary_Cells	CD4+_CD25-_IL17- PMA-Ionomycin_stimulated MACS_purified Th_Primary_Cells	CD4+_CD25-_IL17+ PMA-Ionomcyin_stimulated Th17_Primary_Cells
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YES	YES	-	YES	YES
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YES	YES	YES	YES	YES
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-	-	-	-	YES
-	YES	-	-	-
-	YES	-	-	-

cell type specific enhancer annotation (ROADMAP Epigenomic Project)				
CD4+_CD25-_CD45RO+ Memory Primary_Cells	CD4_Memory Primary_Cells	CD8_Memory Primary_Cells	CD4 Naive_Primary_Cells	CD8 Naive_Primary_Cells
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	YES	-	-	-
-	-	-	-	-
-	YES	-	-	-
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-	-	YES	YES	-
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YES	YES	YES	-	-
YES	YES	-	YES	-
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YES	YES	-	YES	YES
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YES	YES	-	YES	-
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YES	YES	YES	YES	YES
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Enhancer (HSC&B-cell ROADMAP				
CD14 Primary_Cells	CD19 Primary_Cells_Cord_Blood	CD34_Primary_Cells	Mobilized_CD34 Primary_Cells_Male	Mobilized_CD34 Primary_Cells_Female
-	-	-	-	-
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YES	YES	YES	YES	YES
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YES	-	-	YES	YES
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YES	-	-	-	YES
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track)

CD34 Cultured_Cells	CD19 Primary Cells Peripheral_UW	CD56 Primary_Cells	CD15 Primary_Cells
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YES	-	-	YES
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YES	-	-	-
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YES	YES	YES	-
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YES	YES	YES	YES
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YES	-	-	-
YES	YES	-	-

Table S6B: List of n=38 asthma-related gDMRs with their corresponding meQTLs and associated GWAS trait

* enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annotation was avai

SNPs and the respective reported GWAS trait with loose association to asthma are indicated in bold

chr	start	end	associated genes*
5	77146478	77147361	*TBCA*
8	58192499	58193338	*IMPAD1*
8	599524	600398	*ERICH1*
22	50616227	50617057	*PANX2*
5	77142381	77142899	*TBCA*

17	79466178	79466419	*ACTG1*
22	46762433	46763144	*TRMU*
7	1914009	1914393	*MAD1L1;MIR4655*
19	51373740	51374029	*C19orf48;SNORD88C;KLK3;KLK2;KLKP1*
19	10404092	10405285	*ZNF426;ZNF846;ICAM1;ZNF121;DOCK6;ILF3;KRI1;CTC-325H20.4;DNMT1;ZNF561;ZNF699;FBXL12;ZNF559;CDC37;ZNF266;S1PR2;CTD-2369P2.8;ICAM4;ICAM5;EIF3G;RAVER1;PPAN;ZNF562*
1	67600417	67600715	*C1orf141;IL23R*
11	128694096	128694425	*KCNJ1*
3	128317793	128318091	*C3orf27*
2	107082602	107082889	*RGPD3*
2	118617427	118618163	*DDX18*
2	241459177	241460047	*GPC1;MIR149;ANKMY1*
3	39395430	39395805	*EEF1A1P24;RP11-241K18.2;RPSA;CCR8;CX3CR1*
3	75445094	75445699	*LINC00960;RP11-803B1.3;LSP1P2*
3	184243755	184244149	*CHRD;EIF4G1;POLR2H;THPO;YEATS2;CLCN2;EPHB3;MAGEF1;FAM131A*
5	42943969	42944684	*CTD-2201E18.5;ZNF131*

5	157117442	157117959	*C5orf52*
6	32063911	32064192	*TNXB*
7	32357921	32358755	*PDE1C*
7	55412705	55412996	*LANCL2*
9	38687606	38687992	*ANKRD18A*
9	123744449	123744762	*TRAF1*
11	12136161	12136468	*MICAL2;MICALCL*
12	119591663	119592119	*SRRM4*
12	125482583	125482829	*BRI3BP*
15	31134409	31134668	*HERC2P10;CHRFAM7A;RP11-540B6.3;FAN1*
15	57511786	57512216	*LINC00926;TCF12*
17	8702637	8702756	*MFSD6L*
17	17946397	17946585	*GID4*
17	19627951	19628166	*CTC-457L16.2;SNORA31;SLC47A2;MAPK7;CCDC144CP;RP11-311F12.2;RP11-311F12.1*
18	14458381	14458937	*LONRF2P1;CXADRP3;GRAMD4P7*
19	3520495	3521154	*MFSD12;C19orf71;SNORD38;FZR1*
20	32232346	32232458	*C20orf144*
22	50985261	50985925	*TYMP;CTA-384D8.31 ;TRABD ;KLHDC7B;PANX2;SYCE3;CPT1B; HDAC10; CHKB;ARSA;TUBGCP6;ODF3B;CTA-384D8.36;SCO2*

ts.

ilable the closest TSS gene is given

enhancer total	cord blood DMR
YES	YES
-	YES
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YES	YES
YES	YES

YES	YES
YES	YES
YES	YES
YES	YES
YES	YES
YES	-
YES	-
YES	YES
YES	-
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YES	YES
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YES	YES
YES	YES
YES	YES

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YES	-
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YES	YES

SNP_ID[#]
rs10079675,rs10942834,rs12658675,rs12697875,rs13159821,rs13162500,rs13165722,rs13172056,rs13180688,rs13187880,rs2361312,rs2361313,rs386488634,rs4262083,rs6877994,rs6880703,rs6881736,rs6886200,rs6889917,**rs7700998**,rs7704525,rs7708151,rs7717920,rs7728693,rs7732015
rs111915672,rs112284078,rs112793785,rs113745779,rs113857287,rs1390411,rs2270607,rs2270608,rs2270609,rs2270610,rs3814486,rs58371676,rs58608483,rs58720297,rs58947041,rs58957714,rs58982816,rs59265461,rs59275611,rs59341413,rs59383954,rs59843034,rs60646469,rs60786705,rs61638902,rs61733801,rs61998258,rs61998259,rs66477954,rs66479724,rs66731170,rs66840104,rs66886949,rs67042991,rs67105789,rs67344620,rs67389108,rs67966192,rs68076606,rs68112919,rs72652905,rs72652906,rs72652908,rs73591706,rs73609747,rs73609760,rs73609762,rs73609764,rs75585481,rs76023408,rs76270388,rs76944716,rs78138632,rs79311869,rs9650139,rs10504229,rs10504230,rs55912204,rs55977795,rs56130194,rs57314710,rs59708460,rs68114352,rs6981914,rs6999878
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0.05
0.0239902693432153,0.0239902693432153
0.00259559688210291,0.00770774419181276,0.00118571116321647,0.00118571116321647,0.00016352423
0.0309967902426561,0.0309967902426561
0.06

0.0537817878318614,0.0633602627964685,4.41497036587729e-07,4.41497036587729e-
07,4.85088512121949e-06,4.20931295085696e-06,0.000112948559303754,1.16060502734135e-
05,6.46480649774955e-06,4.41497036587729e-07,0.0287981898659266,5.57229712724854e-
08,4.20931295085696e-06,4.41497036587729e-07,4.20931295085696e-06,1.45472990605012e-
05,8.18873998554563e-06,1.34383733419466e-06,8.09623522352935e-07,4.20931295085696e-
06,2.85723379169662e-06,4.41497036587729e-07,4.41497036587729e-
07,0.000291711900536549,4.58512373615944e-06,4.41497036587729e-07

0.0930043252481612,0.0930043252481612,0.0605631940081978,0.0605631940081978,0.09300432524816
12,0.0623306426087032,0.0930043252481612

0.04

5.85875120780481e-07,1.08125931953341e-09,0.0032080374185556 4,0.000190689502204085,
0.0432464052640247, 7.35311206666787e-10,6.51870717691192e-14,2.4410680274637e-
08,0.00260014656838954, 0.000745525403369278,1.57385094445255e-07,1.36001537607332e-
11,6.2395831870858e-08,9.06246669416187e-10,0.000248934235204621
0.0262203974771051,0.0480742029621163
0.02

0.0676542926704181,0.0694406275209425,0.0694406275209425

GWAS trait[#]
Appendicular lean mass, Height, **Lung function (FVC)**
Developmental language disorder (linguistic errors)
Educational attainment
educational attainment, Brain region volumes
Electrocardiogram morphology (amplitude at temporal datapoints), **Lung function (FVC)**, Appendicular lean mass, Height

Hand grip strength

Left–right brain asymmetry, Brain shape (segment 3), Cortical thickness, Vertex-wise cortical thickness, Cortical surface area, brain measurement

Number of sexual partners, Longevity, Refractive error, **Idiopathic pulmonary fibrosis**

PCA3 expression level, Celiac disease, Prostate cancer

Protein quantitative trait loci, Inflammatory skin disease, Platelet-to-lymphocyte ratio, **Lymphocyte counts**, Serum levels of protein ICAM5, Sex hormone-binding globulin levels adjusted for BMI, Blood protein levels

Psoriatic arthritis, Crohn's disease, Leprosy, L1-L4 bone mineral density x serum urate levels interaction, Sarcoidosis, QT interval in Tripanosoma cruzi seropositivity

Spherical equivalent, **Lung Function (FVC)**, Estimated glomerular filtration rate (creatinine), Hip circumference adjusted for BMI

Basophil count, White blood cell count (basophil), White blood cell count (eosinophil), White blood cell count, leukocyte count, Neutrophil percentage of white cells, eosinophil percentage of leukocytes, eosinophil count

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Table S6C: List of n=56 asthma-related DMRs present in cord blood.

* enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annota

[#] GWAS trait with loose association to asthma are indicated in bold

** Reference of EWAS, which investigated the prenatal influencing factors:

1 *Joubert BR, Felix JF, Yousefi P et al.:* ***DNA Methylation in Newborns and Maternal Sm***

2 *Gruzieva O, Xu CJ, Yousefi P, et al.:* ***Prenatal Particulate Air Pollution and DNA Methy***

3 *Canouil M, Khamis A, Keikkala E et al.:* ***Epigenome-Wide Association Study Reveals M***

4 *Solomon O, Yousefi P, Huen K, et al.:* ***Prenatal phthalate exposure and altered patter***

5 *Miura R, Araki A, Miyashita C, et al.:* ***An epigenome-wide study of cord blood DNA m***

6 *Fuemmeler BF, Dozmorov MG, Do EK, et al.:* ***DNA Methylation in Babies Born to Non***

7 *Herzog EM, Eggink AJ, Willemsen SP, et al.:* ***Early- and late-onset preeclampsia and t***

8 *Weng X, Liu F, Zhang H, et al.:* ***Genome-wide DNA methylation profiling in infants bo***

9 *Joubert BR, den Dekker HT, Felix JF,et al.:* ***Maternal plasma folate impacts differentia***

10 *McCabe CF, LaBarre JL, Domino SE, et al.:* ***Maternal and neonatal one-carbon metab***

11 *Wu S, Hivert MF, Cardenas A, et al.* ***Exposure to Low Levels of Lead in Utero and Umb***

12 *Leung YK, Ouyang B, Niu L,at al.:* ***Identification of sex-specific DNA methylation chan***

13 *Bauer T, Trump S, Ishaque N, et al.:* ***Environment-induced epigenetic reprogramming***

Abbreviation

PFOS perfluorooctane sulfonic acid

chr	start	end	DMR width	# of CGs in DMR	mean difference of DNA-methylation	direction of DNA-methylation
19	10404092	10405285	1193	81	-0.010	hypo
22	50616227	50617057	830	76	-0.040	hypo
5	42943969	42944684	715	41	-0.021	hypo
22	46762433	46763144	711	38	-0.056	hypo
8	58192499	58193338	839	50	-0.098	hypo
2	241459177	241460047	870	54	-0.016	hypo
3	75445094	75445699	605	53	-0.066	hypo
7	32357921	32358755	834	44	-0.041	hypo
2	118617427	118618163	736	73	-0.052	hypo
3	128317793	128318091	298	12	-0.042	hypo
3	184243755	184244149	394	22	-0.031	hypo
5	77142381	77142899	518	26	-0.076	hypo
5	77146478	77147361	883	53	-0.070	hypo
7	1914009	1914393	384	18	-0.030	hypo
8	599524	600398	874	60	-0.068	hypo
17	19627951	19628166	215	27	-0.043	hypo
17	79466178	79466419	241	34	-0.060	hypo

19	51373740	51374029	289	20	-0.054	hypo
22	50985261	50985925	664	70	-0.062	hypo
11	132951692	132952492	800	45	-0.100	hypo
2	24233600	24234117	517	24	-0.041	hypo
13	20988857	20989415	558	41	-0.049	hypo
4	2366183	2366745	562	49	-0.022	hypo
1	870565	871771	1206	68	0.061	hyper
2	74213621	74213841	220	13	0.041	hyper
6	168435945	168436646	701	41	-0.048	hypo
13	24914323	24914905	582	37	-0.062	hypo
13	20968573	20969085	512	37	-0.051	hypo
2	121816094	121816885	791	23	-0.028	hypo
5	8457869	8457980	111	13	-0.068	hypo
10	1405351	1406102	751	99	-0.045	hypo
16	88558082	88558379	297	17	-0.045	hypo
16	85654156	85654324	168	13	-0.054	hypo
7	127910860	127911680	820	49	-0.063	hypo
1	6341136	6341683	547	28	-0.021	hypo
1	149162004	149162428	424	21	-0.052	hypo
1	202121664	202121815	151	9	-0.029	hypo
2	31154795	31155157	362	17	-0.038	hypo
2	132404284	132404979	695	54	-0.032	hypo
4	144833125	144833346	221	30	-0.020	hypo
5	42923963	42924355	392	23	-0.063	hypo
7	5382633	5382783	150	8	-0.023	hypo
7	36007074	36007282	208	19	-0.068	hypo
7	90895326	90896702	1376	91	-0.048	hypo
9	38487906	38488165	259	25	-0.046	hypo
9	69500968	69501070	102	14	-0.105	hypo
10	1404948	1405307	359	31	-0.084	hypo
10	134139414	134139779	365	11	-0.030	hypo
13	111948367	111948559	192	9	-0.035	hypo
16	30552372	30552613	241	9	-0.044	hypo
16	57831974	57832180	206	18	-0.043	hypo
16	88579452	88580072	620	21	-0.039	hypo
18	8755023	8755343	320	13	-0.023	hypo
20	29525180	29525475	295	20	-0.068	hypo
20	29550781	29551739	958	60	-0.038	hypo
21	38750599	38750877	278	11	-0.024	hypo

ıtion was available the closest TSS gene is given

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ısmoking Mothers Exposed to Secondhand Smoke during Pregnancy: An Epigenome-Wide A
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olites and the epigenome-wide infant response. J Nutr Biochem. 2022 Mar;101:108938.
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nges driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics. 2018;1
g in genomic regulatory elements in smoking mothers and their children. Mol Syst Biol. 201(

DMR annotation			
***raw p*-value (ANOVA)**	**associated genes***	**enhancer total**	**ngDMR/ gDMR**
6.57E-07	*ZNF426;ZNF846;ICAM1;ZNF121;DOCK6;ILF3;KRI1;CTC-*	YES	gDMR
1.63E-20	*PANX2*	YES	gDMR
1.16E-06	*CTD-2201E18.5;ZNF131*	YES	gDMR
1.41E-10	*TRMU*	YES	gDMR
4.98E-37	*IMPAD1*	-	gDMR
8.95E-05	*GPC1;MIR149;ANKMY1*	YES	gDMR
6.85E-19	*LINC00960;RP11-803B1.3;LSP1P2*	YES	gDMR
7.67E-10	*PDE1C*	-	gDMR
9.38E-27	*DDX18*	-	gDMR
1.47E-07	*C3orf27*	YES	gDMR
8.49E-10	*CHRD;EIF4G1;POLR2H;THPO;YEATS2;CLCN2;EPHB3;MA*	YES	gDMR
3.14E-11	*TBCA*	YES	gDMR
2.25E-14	*TBCA*	YES	gDMR
4.45E-06	*MAD1L1;MIR4655*	YES	gDMR
2.11E-35	*ERICH1*	-	gDMR
5.97E-17	*CTC-457L16.2;SNORA31;SLC47A2;MAPK7;CCDC144CP;*	YES	gDMR
6.09E-13	*ACTG1*	YES	gDMR

1.20E-11	*C19orf48;SNORD88C;KLK3;KLK2;KLKP1*	YES	gDMR
3.01E-20	*TYMP;CTA-384D8.31;TRABD;KLHDC7B;PANX2;SYCE3;C*	YES	gDMR
3.49E-19	*OPCML*	YES	ngDMR
7.75E-10	*RP11-443B20.1;CENPO;ATAD2B;MFSD2B;UBXN2A*	YES	ngDMR
1.10E-08	*GJB2;MIR4499;CRYL1*	YES	ngDMR
1.44E-12	*RP11-478C1.7;ZFYVE28;RNF4;NOP14-AS1;NELFA;POLN*	YES	ngDMR
1.18E-10	*AL645608.1*	-	ngDMR
2.32E-07	*AC073046.25;TET3;MGC10955;ALMS1;CCT7;MOB1A;S*	YES	ngDMR
2.65E-08	*KIF25;KIF25-AS1;FRMD1*	YES	ngDMR
1.05E-12	*AL359736.1*	YES	ngDMR
3.76E-17	*GJB2;MIR4499*	YES	ngDMR
1.14E-09	*TFCP2L1*	-	ngDMR
2.08E-06	*MIR4458HG;RP11-480D4.2;MTRR*	YES	ngDMR
5.95E-33	*WDR37*	-	ngDMR
3.26E-11	*ACSF3;CBFA2T3;ZC3H18;GALNS;AC137932.1;PIEZO1;RI*	YES	ngDMR
1.41E-12	*CTD-2542L18.1;GSE1*	YES	ngDMR
4.93E-10	*RP11-62J1.4;RBM28;LEP*	YES	ngDMR
3.03E-07	*RP1-202O8.3;ACOT7;GPR153;RP1-202O8.3;ACOT7*	YES	ngDMR
1.50E-05	*HIST2H3PS2*	-	ngDMR
1.34E-05	*PTPN7;PTPRVP;ARL8A*	YES	ngDMR
1.64E-10	*CAPN13*	YES	ngDMR
2.58E-10	*C2orf27A*	YES	ngDMR
3.73E-05	*FREM3;GYPE*	YES	ngDMR
2.57E-12	*SEPP1*	YES	ngDMR
1.65E-05	*SLC29A4*	-	ngDMR
5.51E-16	*SEPT7P3*	YES	ngDMR
3.11E-23	*AKAP9;AC000120.7;FZD1;KRIT1;GTPBP10;AC002064.4*	YES	ngDMR
2.02E-07	*IGFBPL1*	-	ngDMR
1.75E-09	*ANKRD20A4*	-	ngDMR
8.94E-18	*WDR37*	-	ngDMR
5.31E-07	*ZNF511;TUBGCP2;RP11-122K13.12;LRRC27;STK32C*	YES	ngDMR
1.35E-05	*TEX29*	YES	ngDMR
5.31E-08	*ITGAL;AC002310.7;ZNF764;AC002310.13;TAOK2;INO8(*	YES	ngDMR
1.27E-08	*CNOT1;SETD6;RSPRY1;KIFC3*	YES	ngDMR
6.07E-13	*MIR5189;ZC3H18;ANKRD11;ZFPM1;BANP*	YES	ngDMR
7.88E-05	*RP11-674N23.4*	YES	ngDMR
5.12E-07	*FRG1B*	YES	ngDMR
7.47E-13	*FRG1B*	-	ngDMR
3.56E-05	*DYRK1A*	YES	ngDMR

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nternal Methylome. *Diabetes Care. 2021 Sep;44(9):1992-1999.*
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. *Environmental health perspectives. 2017;125(8):087019.*
13(3):290-300.
6;12(3):861.

GWAS trait[#]
Protein quantitative trait loci, Inflammatory skin disease, Platelet-to-lymphocyte ratio, **Lymphocyte counts**, **Serum levels of protein ICAM5**, Sex hormone-binding globulin levels adjusted for BMI, Blood protein levels
educational attainment, Brain region volumes
Left–right brain asymmetry, Brain shape (segment 3), Cortical thickness, Vertex-wise cortical
Developmental language disorder (linguistic errors)
Basophil count, **White blood cell count (basophil), White blood cell count (eosinophil)**, White blood cell count, leukocyte count, Neutrophil percentage of white cells, eosinophil percentage of leukocytes, eosinophil count
Electrocardiogram morphology (amplitude at temporal datapoints), **Lung function (FVC)**, Appendicular lean mass, Height
Appendicular lean mass, Height, **Lung function (FVC)**
Number of sexual partners, Longevity, Refractive error, **Idiopathic pulmonary fibrosis**
Educational attainment
Hand grip strength

PCA3 expression level, Celiac disease, Prostate cancer

prenatal influencing factor[Reference**]
lead[11]
gestational diabetis[3], lead[11]
nPFOS[12]
phthalate[4], lead[11]
S-adenosyl-homocysteine[10]
tobacco smoke[6]
tobacco smoke[13]
tobacco smoke[6,13], gestational diabetis[8]

folate[9], tobacco smoke[13]
gestational diabetis[3]

air pollution[2]
lead[11]
lead[11]
lead[11]
lead[11]
lead[11], tobacco smoke[13]
gestational diabetis[3]
gestational diabetis[3]
gestational diabetis[3]
nPFOS[5]
preeclampsia[7], lead[11]
tobacco smoke[1]

Table S7: Overlap between DMRs and previous asthma EWASs using Illumina's 450k array appr

chr	start	end	direction of DNA-methylation	gene closest TSS
1	6,341,136	6,341,683	hypo	*GPR153*
1	84,744,687	84,744,808	hypo	*SAMD13*
11	1,828,650	1,828,783	hypo	*SYT8*
11	12,136,161	12,136,468	hypo	*MICAL2*
14	100,610,169	100,610,668	hypo	*DEGS2*
14	75,153,156	75,153,308	hypo	*AREL1*
15	40,093,789	40,094,023	hypo	*FSIP1*
16	88,540,019	88,540,526	hypo	*ZFPM1*
16	85,654,156	85,654,324	hypo	*GSE1*
16	88,558,082	88,558,379	hypo	*ZFPM1*
17	17,946,397	17,946,585	hypo	*GID4*
17	56,274,149	56,274,598	hypo	*EPX*
17	56,283,687	56,284,009	hypo	*LPO*
19	34,859,991	34,860,410	hypo	*GPI*
19	1,854,531	1,854,766	hypo	*REXO1*
2	75,089,515	75,089,819	hypo	*HK2*
2	24,233,600	24,234,117	hypo	*MFSD2B*
2	121,816,094	121,816,885	hypo	*TFCP2L1*
3	128,134,844	128,135,029	hypo	*DNAJB8*
3	195,964,960	195,965,370	hypo	*SLC51A*
5	8,457,869	8,457,980	hypo	*FASTKD3*
6	32,063,911	32,064,192	hypo	*TNXB*
7	1,914,009	1,914,393	hypo	*AC110781.3*
7	150,647,915	150,648,063	hypo	*KCNH2*
8	599,524	600,398	hypo	*ERICH1*

*** enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annot**

****Reference of EWAS, which investigated asthma or lung function:**

1 Reese SE, Xu CJ, den Dekker HT et al.: **Epigenome-wide meta-analysis of DNA methy**

2 Everson TM, Zhang H, Lockett GA et al.: **Epigenome-wide association study of asthm**

3 Arathimos R, Suderman M, Sharp GC et al.: **Epigenome-wide association study of ast**

4 Hoang TT, Sikdar S, Xu CJ et al.: **Epigenome-wide association study of DNA methylati**

5 Peng C, Van Meel ER, Cardenas A et al.: **Epigenome-wide association study reveals r**

6 Lin PI, Shu H, Mersha TB.: **Comparing DNA methylation profiles across different tissu**

7 Xu CJ, Gruzieva O, Qi C, et al.: **Shared DNA methylation signatures in childhood aller**

8 Herrera-Luis E, Li A, Mak ACY et al.: **Epigenome-wide association study of lung functi**

9 Xu CJ, Söderhäll C, Bustamante M et al.: **DNA methylation in childhood asthma: an e**

roach.

associated genes*
RP1-202O8.3;ACOT7;GPR153
PRKACB;SNORA2;RP11-376N17.4;SAMD13
SYT8
MICAL2;MICALCL
DEGS2;SLC25A29;MIR770;EVL
AREL1;SYNDIG1L;FCF1;SNORA7;LTBP2
FSIP1
ZNF469;ZFPM1;AC137932.1;RP11-46C24.7;LINC00304;APRT;PIEZO1;CTU2;RNF166;SNAI3;MIR5189;ZC3H18;BA
CTD-2542L18.1;GSE1
ACSF3;CBFA2T3;ZC3H18;GALNS;AC137932.1;PIEZO1;RP5-1142A6.9;ANKRD11;BANP;ZFPM1;MIR5189
GID4
-
LPO
GPI
PLEKHJ1;CTB-31O20.6;AC005306.3;CSNK1G2;ADAT3;SCAMP4;KLF16;TCF3;TMEM259;GRIN3B;CTB-31O20.8;DAZAP1;BTBD2;MOB3A;SF3A2;NDUFS7;SPPL2B;LSM7;ZNF555;REXO1;MED16;CIRBP;ATP8B3;C19orf HK2;AC104135.2
RP11-443B20.1;CENPO;ATAD2B;MFSD2B;UBXN2A
TFCP2L1
DNAJB8;GATA2;DNAJB8-AS1;EFCC1;TPRA1;EEFSEC;RPL32P3;RP11-529F4.1;H1FX-AS1
SLC51A
MIR4458HG;RP11-480D4.2;MTRR
TNXB
MAD1L1;MIR4655
KCNH2
ERICH1

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ion and adult asthma in the Agricultural Lung Health Study. Eur Respir J. 2020 Sep 3;56(3):2000217.
methylation pathways associated with childhood allergic sensitization. Epigenetics. 2019 May;14(5):445-466.
ues associated with the diagnosis of pediatric asthma. Sci Rep. 2020 Jan 13;10(1):151
gy: The MeDALL study. J Allergy Clin Immunol. 2021 Mar;147(3):1031-1040
ion in Latino children and youth with asthma. Clin Epigenetics. 2022 Jan 15;14(1):9.
pigenome-wide meta-analysis. Lancet Respir Med. 2018 May;6(5):379-388.

enhancer total	ngDMR/ gDMR
YES	ngDMR
YES	ngDMR
	gDMR
	gDMR
YES	ngDMR
YES	ngDMR
	gDMR
YES	ngDMR
YES	ngDMR
YES	gDMR
YES	ngDMR
	ngDMR
	gDMR
	ngDMR
YES	gDMR
YES	ngDMR
YES	ngDMR
	gDMR
YES	ngDMR
	ngDMR
YES	gDMR
	gDMR
YES	gDMR
	gDMR
	gDMR

450K overlap (cg number)**
cg13066938 [1,2];cg21220721 [1,3,4,5,6];cg09249800 [1,3,4];cg11699125 [1,3,4,7]
cg17772438 [4]
cg21992676 [4]
cg23044178 [2,3,4], cg01450133 [4]
cg16409452 [1,2];cg14084609 [1,2,4];cg18550847 [1,2,4];cg06756385 [3,7,9];cg01000631 [1,2]
cg26103369 [4]
cg18852698 [4]
cg08940169 [1,2,3];cg16627358 [2];cg00986350 [3]
cg04847043 [2,3,4];cg07098502 [4]
cg04983687 [1,3,7,9], cg05958985 [2,3,4]
cg14611258 [4]
cg03519593 [4]; cg08105265 [4]
cg11112605 [4]
cg02359181 [1,2,3,4]
cg01373896 [3]
cg12077754 [1,2,3,4,9]
cg19273694 [3]
cg22982094 [4]
cg03278639 [4]
cg22221575 [4]
cg18650626 [2,4]

previously reported DMRs**
chr1: 6341140-6341328 [7]; chr1: 6341139-6341327 [8]
chr14: 100610071-100610668 [1]; chr14: 100610186-100610668 [7]
chr16: 88539861-88540397 [1,7]
chr16: 85654157-85656261 [7]
chr16: 88558065-88558238 [7]
chr17: 56274480-56274598 [7]
chr19: 1854549-1854820 [7]
chr2: 24233923-24234018 [7]
chr5: 8457720-8458089 [8]
chr6: 32063402-32064837 [8]
chr7: 1913505-1914524 [7]
chr7: 150647757-150648498 [7]
chr8: 599525-600556 [7]

Table S8: Cell type composition of WGBS samples estimated by deconvolution. Indicated is Man

Cell type	control			
	mean [%]	-/+ 95% CI for mean	median [%]	lower quartile [%]
B cell	4.4	3.4 / 5.4	4.0	2.0
NK	2.9	1.6 / 4.1	1.2	0.0
T cell	25.8	23.2 / 28.4	27.0	20.0
monocytes	8.8	8 / 9.6	8.2	6.9
neutrophils	58.1	54.6 / 61.6	56.1	49.7
eosinophils	0.1	0 / 0.2	0.0	0.0

ın Whitney U-test statistics comparing controls (n=42) vs. asthma (n=40) samples.

	asthma			
upper quartile [%]	mean [%]	-/+ 95% CI for mean	median [%]	lower quartile [%]
5.9	4.8	3.7 / 5.8	4.3	2.2
4.7	3.3	2.2 / 4.3	3.6	0.0
32.0	23.5	20.6 / 26.4	25.0	16.0
10.5	8.7	7.5 / 9.8	8.6	6.8
64.7	58.5	54.4 / 62.7	58.1	48.5
0.0	1.3	0.5 / 2.1	0.0	0.0

	control vs. asthma		
upper quartile [%]	***Z***	***p*-value**	**r_{pb} correlation coefficient**
7.4	-0.37	0.715	0.057
5.3	-1.03	0.316	0.056
30.7	1.10	0.273	-0.133
10.8	-0.09	0.93	-0.012
66.5	-0.29	0.771	0.018
0.9	-3.43	0.017	0.317

Table S9: Adjusted multiple regression analysis of n=60 cell type dependent asthma-realated DMRs. Sl

* enhancer target genes were derived from GeneHancer, in cases where no GeneHancer annotation was

Note: Multiple regression analysis adjusted for asthma outcome, child's sex, cohort, prenatal tobacco

Shown are standardized Beta (ß), ±95% CI , and *p*-value of the independent variables asthma o

DMR				enhancer total
chr	start	end	associated genes*	
1	10436586	10436851	*PGD;TARDBP;RP4-635E18.8;EXOSC10;MTOR;KIF1B*	YES
1	84744687	84744808	*PRKACB;SNORA2;RP11-376N17.4;SAMD13*	YES
1	202121664	202121815	*PTPN7;PTPRVP;ARL8A*	YES
1	204479935	204480156	*MDM4*	YES
2	31154795	31155157	*CAPN13*	YES
2	75089515	75089819	*HK2;AC104135.2*	YES
2	113426404	113426419	*SLC20A1*	-
2	121816094	121816885	*TFCP2L1*	-
3	3150228	3150425	*IL5RA*	YES
3	128317561	128317755	*C3orf27*	YES
3	172243109	172243331	*TNFSF10*	YES
3	195964960	195965370	*SLC51A*	-
4	148634323	148634374	*ARHGAP10*	-
5	68700315	68700724	*MARVELD2*	YES
5	132002374	132002507	*RP11-485M7.1;RAD50;IL4;AFF4;AC063976.7;ZCCHC*	YES
5	149145166	149145197	*PPARGC1B*	YES
5	154224429	154224647	*MIR378H;CNOT8;FAXDC2*	YES
6	166674955	166675083	*PRR18*	YES
7	5382633	5382783	*SLC29A4*	-
7	102003600	102003767	*ORAI2;RP11-163E9.1;RP11-163E9.2;PRKRIP1;ALKBH*	YES
7	150647915	150648063	*KCNH2*	-
8	128828626	128828794	*PVT1;MYC*	YES
8	131047175	131047345	*FAM49B*	YES
9	32430999	32431303	*ACO1;DDX58;TMEM215*	YES
9	123744449	123744762	*TRAF1*	-
9	128994302	128994390	*MVB12B*	-
9	140113368	140113559	*RNF208;NDOR1;NPDC1*	YES
10	46055866	46055919	*44628*	YES
10	134139414	134139779	*ZNF511;TUBGCP2;RP11-122K13.12;LRRC27;STK32C*	YES
11	1828650	1828783	*SYT8*	YES
11	65477123	65477452	*RBM4;RBM14;ZFPL1;CCS;CTSF;MRPL11;RP11-755F1*	YES
12	57792999	57793110	*AC126614.1*	-
12	102092915	102093110	*CHPT1;ARL1;GNPTAB*	YES
12	107273279	107273681	*C12orf23*	-
12	111137400	111137596	*RN7SL387P;PCNPP1;HVCN1;FAM216A*	YES
14	75153156	75153308	*AREL1;SYNDIG1L;FCF1;SNORA7;LTBP2*	YES
14	93212312	93212487	*LGMN*	YES
14	100610169	100610668	*DEGS2;SLC25A29;MIR770;EVL*	YES
14	103200841	103201128	*TRAF3*	-
15	31134409	31134668	*HERC2P10;CHRFAM7A;RP11-540B6.3;FAN1*	YES

15	57511786	57512216	*LINC00926;TCF12*	YES
16	69489543	69489665	*CYB5B*	YES
16	85654156	85654324	*CTD-2542L18.1;GSE1*	YES
16	88540019	88540526	*ZNF469;ZFPM1;AC137932.1;RP11-46C24.7;LINC003*	YES
16	88579452	88580072	*MIR5189;ZC3H18;ANKRD11;ZFPM1;BANP*	YES
17	8769570	8769884	*CNTROB;PIK3R6;PFAS;MFSD6L;RP11-849F2.5;RPL26*	YES
17	21119605	21119845	*FAM106B;DHRS7B;AC087294.2;USP22;TMEM11*	YES
17	49057182	49057239	*TOB1*	YES
17	56272299	56272502	*EPX*	-
17	56274149	56274598	*EPX*	-
17	56283478	56283523	*LPO*	-
17	56283687	56284009	*LPO*	-
17	78569835	78569888	*RPTOR*	YES
18	22016574	22016800	*IMPACT*	-
18	71910027	71910089	*CYB5A;RP11-669I1.1*	YES
18	77703283	77703521	*PQLC1;RBFADN;PQLC1*	YES
19	3520495	3521154	*MFSD12;C19orf71;SNORD38;FZR1*	YES
20	33416638	33416742	*NCOA6*	YES
9	135114516	135114649	*NTNG2*	YES
2	74213621	74213841	*AC073046.25;TET3;MGC10955;ALMS1;CCT7;MOB1A*	YES
2	113956545	113956673	*AC016683.5;PSD4*	YES

hown are regression results for asthma outcome.

s available the closest TSS gene is given

ɔ smoke exposure, family history of atopy, parental school education, maternal age at birth, growing up

ıutcome, and specific cell type# frequency.

	DMR classification					
ngDMR/ gDMR	**cell type dependency**					
	eosinophils	B cells	NK cells	CD4$^+$ T cells	CD8$^+$ T cells	monocytes
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
gDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
gDMR	YES	-	-	-	-	-

gDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
gDMR	YES	-	-	-	-	-
ngDMR	YES	-	-	-	-	-
ngDMR	-	YES	-	YES	YES	-
ngDMR	-	-	-	YES	-	-
ngDMR	-	-	-	-	-	-

on a farm and #specific cell type frequency (=independent variables). Dependent variable w

		adjusted multip			
	cord blood DMR	independent variable: asthma outcome			
neutrophils		*p*-value	Beta(ß)	CI-95%	CI+95%
-	-	2.5E-05	-0.04	-0.06	-0.02
-	-	3.3E-06	-0.03	-0.04	-0.02
-	YES	5.5E-07	-0.05	-0.07	-0.03
-	-	2.0E-05	-0.03	-0.04	-0.02
-	YES	1.0E-06	-0.06	-0.08	-0.04
-	-	8.8E-05	-0.04	-0.07	-0.02
-	-	1.7E-05	-0.07	-0.10	-0.04
-	YES	9.1E-09	-0.05	-0.06	-0.03
-	-	2.1E-05	-0.04	-0.06	-0.02
-	-	7.3E-05	-0.04	-0.06	-0.02
-	-	3.1E-04	-0.05	-0.07	-0.02
-	-	6.0E-05	-0.03	-0.04	-0.02
-	-	5.0E-06	-0.06	-0.08	-0.03
-	-	5.0E-06	-0.03	-0.04	-0.02
-	-	1.8E-06	-0.04	-0.06	-0.03
-	-	8.5E-06	-0.05	-0.07	-0.03
-	-	3.1E-05	-0.04	-0.05	-0.02
-	-	3.3E-05	-0.04	-0.06	-0.02
-	YES	3.3E-04	-0.04	-0.06	-0.02
-	-	1.4E-04	-0.04	-0.06	-0.02
-	-	1.6E-03	-0.03	-0.05	-0.01
-	-	1.8E-05	-0.04	-0.06	-0.02
-	-	1.9E-06	-0.04	-0.05	-0.02
-	-	3.9E-05	-0.03	-0.04	-0.02
-	-	4.7E-07	-0.05	-0.07	-0.03
-	-	4.8E-04	-0.04	-0.06	-0.02
-	-	6.8E-08	-0.06	-0.09	-0.04
-	-	3.8E-04	-0.04	-0.07	-0.02
-	YES	7.8E-07	-0.06	-0.09	-0.04
-	-	8.3E-08	-0.06	-0.08	-0.04
-	-	3.7E-06	-0.05	-0.07	-0.03
-	-	5.8E-05	-0.03	-0.04	-0.02
-	-	8.2E-05	-0.05	-0.07	-0.02
-	-	5.5E-07	-0.03	-0.05	-0.02
-	-	8.9E-07	-0.05	-0.07	-0.03
-	-	4.5E-05	-0.04	-0.06	-0.02
-	-	1.1E-03	-0.03	-0.05	-0.01
-	-	8.4E-05	-0.04	-0.05	-0.02
-	-	1.1E-03	-0.03	-0.05	-0.01
-	-	5.9E-04	-0.03	-0.05	-0.01

-	-	6.9E-05	-0.03	-0.05	-0.02
-	-	3.7E-06	-0.05	-0.07	-0.03
-	YES	7.3E-05	-0.06	-0.09	-0.03
-	-	3.5E-05	-0.06	-0.08	-0.03
-	YES	7.4E-05	-0.04	-0.06	-0.02
-	-	1.8E-03	-0.03	-0.04	-0.01
-	-	2.4E-06	-0.04	-0.06	-0.03
-	-	3.7E-04	-0.05	-0.07	-0.02
-	-	6.2E-05	-0.04	-0.06	-0.02
-	-	6.4E-05	-0.03	-0.05	-0.02
-	-	1.9E-03	-0.06	-0.09	-0.02
-	-	2.3E-04	-0.03	-0.05	-0.01
-	-	6.0E-07	-0.05	-0.07	-0.03
-	-	9.7E-05	-0.04	-0.06	-0.02
-	-	1.1E-07	-0.07	-0.10	-0.05
-	-	1.1E-03	-0.04	-0.06	-0.02
-	-	2.3E-05	-0.05	-0.07	-0.03
-	-	3.9E-05	-0.06	-0.08	-0.03
YES	-	4.5E-06	-0.08	-0.11	-0.05
YES	YES	3.3E-03	0.06	0.02	0.10
YES	-	1.5E-02	-0.06	-0.11	-0.01

ʼas the mean DNA-methylation of the DMR.

ɔle regression

#specific cell type adjusted for in the model
eosinophils
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eosinophils
B cells, CD4+ T cells, CD8+ T cells, neutrophils
CD4+ T cells, neutrophils
neutrophils

Table S10: List of DMRs included in enriched pathways from GREAT analysis.

chr	start	end	width	nCpGs	direction
Asthma pathway					
17	56272299	56272502	203	10	hypo
17	56274149	56274598	449	23	hypo
17	56283478	56283523	45	7	hypo
17	56283687	56284009	322	10	hypo
5	132002374	132002507	133	5	hypo
Signaling events mediated by HDAC Class I					
3	128317561	128317755	194	8	hypo
3	128317793	128318091	298	12	hypo
12	124905467	124905759	292	15	hypo
16	88558082	88558379	297	17	hypo
16	88579452	88580072	620	21	hypo
16	88540019	88540526	507	39	hypo
Bladder Cancer					
7	55412705	55412996	291	33	hypo
7	54900863	54901103	240	21	hypo
8	128828626	128828794	168	5	hypo
15	40093789	40094023	234	7	hypo
22	50985261	50985925	664	70	hypo
Genes involved in factors involved in megakaryocyte development and platelet production					
3	128317561	128317755	194	8	hypo
3	128317793	128318091	298	12	hypo
1	84744687	84744808	121	5	hypo
14	103200841	103201128	287	12	hypo
16	88558082	88558379	297	17	hypo
16	88579452	88580072	620	21	hypo
16	88540019	88540526	507	39	hypo
Genes involved in beta defensins					
20	29525180	29525475	295	20	hypo
20	29515851	29515954	103	8	hypo
20	29550781	29551739	958	60	hypo
CD40/CD40L signaling					
5	132002374	132002507	133	5	hypo
8	128828626	128828794	168	5	hypo
9	123744449	123744762	313	10	hypo
14	103200841	103201128	287	12	hypo
Genes involved in membrane trafficking					
5	77142381	77142899	518	26	hypo
5	77146478	77147361	883	53	hypo
17	78569835	78569888	53	4	hypo
13	20968573	20969085	512	37	hypo
13	20988857	20989415	558	41	hypo
15	30336647	30336863	216	29	hypo
Genes Involved in defensins					
20	29525180	29525475	295	20	hypo
20	29515851	29515954	103	8	hypo
20	29550781	29551739	958	60	hypo

Map kinase Inactivation of SMRT corepressor					
7	55412705	55412996	291	33	hypo
7	54900863	54901103	240	21	hypo
12	124905467	124905759	292	15	hypo
C-MYC pathway					
11	65477123	65477452	329	9	hypo
8	128828626	128828794	168	5	hypo
5	68700315	68700724	409	14	hypo
IL5 Signaling Pathway					
5	132002374	132002507	133	5	hypo
3	3150228	3150425	197	9	hypo
Lysosome					
5	77142381	77142899	518	26	hypo
5	77146478	77147361	883	53	hypo
11	1828650	1828783	133	5	hypo
1	84744687	84744808	121	5	hypo
14	93212312	93212487	175	11	hypo
Telomeres, telomerase, cellular aging, and immortality					
7	55412705	55412996	291	33	hypo
7	54900863	54901103	240	21	hypo
8	128828626	128828794	168	5	hypo
Sonic hedgehog (Shh) pathway					
21	38750599	38750877	278	11	hypo
2	121816094	121816885	791	23	hypo
1	84744687	84744808	121	5	hypo
Genes involved in TRAF3-dependent IRF activation pathway					
9	32430999	32431303	304	9	hypo
14	103200841	103201128	287	12	hypo

mean DNA-methylation difference
-0.050
-0.040
-0.078
-0.043
-0.051
-0.047
-0.061
-0.044
-0.075
-0.049
-0.064
-0.090
-0.043
-0.054
-0.059
-0.098
-0.047
-0.061
-0.035
-0.045
-0.075
-0.049
-0.064
-0.098
-0.141
-0.049
-0.051
-0.054
-0.055
-0.045
-0.080
-0.073
-0.057
-0.076
-0.079
-0.080
-0.098
-0.141
-0.049

-0.090
-0.043
-0.044
-0.057
-0.054
-0.037
-0.051
-0.054
-0.080
-0.073
-0.062
-0.035
-0.045
-0.090
-0.043
-0.054
-0.040
-0.048
-0.035
-0.033
-0.045

associated genes*
EPX
EPX
LPO
LPO
RP11-485M7.1;RAD50;IL4;AFF4;AC063976.7;ZCCHC10
C3orf27
C3orf27
RP11-408I18.9;NCOR2
ACSF3;CBFA2T3;ZC3H18;GALNS;AC137932.1;PIEZO1;RP5-1142A6.9;ANKRD11;BANP;ZFPM1;MIR5189
MIR5189;ZC3H18;ANKRD11;ZFPM1;BANP
ZNF469;ZFPM1;AC137932.1;RP11-46C24.7;LINC00304;APRT;PIEZO1;CTU2;RNF166;SNAI3;MIR5189;ZC3H
LANCL2
SEC61G
PVT1;MYC
FSIP1
TYMP;CTA-384D8.31;TRABD;KLHDC7B;PANX2;SYCE3;CPT1B;HDAC10;CHKB;ARSA;TUBGCP6;ODF3B;CT
C3orf27
C3orf27
PRKACB;SNORA2;RP11-376N17.4;SAMD13
TRAF3
ACSF3;CBFA2T3;ZC3H18;GALNS;AC137932.1;PIEZO1;RP5-1142A6.9;ANKRD11;BANP;ZFPM1;MIR5189
MIR5189;ZC3H18;ANKRD11;ZFPM1;BANP
ZNF469;ZFPM1;AC137932.1;RP11-46C24.7;LINC00304;APRT;PIEZO1;CTU2;RNF166;SNAI3;MIR5189;ZC3H
FRG1B
FRG1B
FRG1B
RP11-485M7.1;RAD50;IL4;AFF4;AC063976.7;ZCCHC10
PVT1;MYC
TRAF1
TRAF3
TBCA
TBCA
RPTOR
GJB2;MIR4499
GJB2;MIR4499;CRYL1
GOLGA8J
FRG1B
FRG1B
FRG1B

LANCL2
SEC61G
RP11-408I18.9;NCOR2
RBM4;RBM14;ZFPL1;CCS;CTSF;MRPL11;RP11-755F10.3;RP11-658F2.8;SSSCA1;SIPA1;RP11-867G23.3;RN
PVT1;MYC
MARVELD2
RP11-485M7.1;RAD50;IL4;AFF4;AC063976.7;ZCCHC10
IL5RA
TBCA
TBCA
SYT8
PRKACB;SNORA2;RP11-376N17.4;SAMD13
LGMN
LANCL2
SEC61G
PVT1;MYC
DYRK1A
TFCP2L1
PRKACB;SNORA2;RP11-376N17.4;SAMD13
ACO1;DDX58;TMEM215
TRAF3

L1;RNASEH2C;SCYL1;RELA;POLA2;KAT5;KRT8P26;MUS81;SART1;FRMD8;EIF1AD;SF3B2;RP11-1

167A19.2;RP11-755F10.1

Table S11: List of n=103 DMRs with at least one enriched transcription factor binding motif 1 to 20

Note:

motif number	*transcription factor binding motif*	*transcription factor (HOCOMOCO ID)**
Motif_1		*ZN264*
Motif_2		*ZN770, SP2, PATZ1,* ***SP3****, TAF1, SP4,* ***WT1****,* ***EGR1****,* ***SP1****, GABPA*
Motif_3		*ZFP28, ZN586*
Motif_4		
Motif_5		
Motif_6		***THA****,* ***THB****, USF1,* ***MITF****,* ***RARA****,* ***NR1H3***
Motif_7		
Motif_8		*GATA4, TAL1,* ***GATA6****,* ***GATA3****, GATA1, GATA2, EVI1*
Motif_9		*ZN329*
Motif_10		
Motif_11		***NFAC1****, NF2L1, SOX17*
Motif_12		***CEBPB, CEBPA***
Motif_13		
Motif_14		*BC11A, ETV5,* ***RXRA***
Motif_15		
Motif_16		***FOXC1****,* ***FOXQ1****,* ***FOXA3****, FOXO4, FOXK1*
Motif_17		
Motif_18		*PRDM6, ZN586*
Motif_19		
Motif_20		*PRDM6,* ***IRF1****,* ***STAT2****, ZFP28,* ***LEF1****, ZIM3, ANDR,* ***NFAC1****, SYR*

**previously associated with asthma in bold*

chr	start	end	width	nCpGs	direction
1	10436586	10436851	265	11	hypo
1	149162004	149162428	424	21	hypo
1	204479935	204480156	221	9	hypo
1	22097059	22097227	168	8	hypo
1	62364300	62364445	145	4	hypo
1	6341136	6341683	547	28	hypo
1	67600417	67600715	298	21	hypo
1	84744687	84744808	121	5	hypo
1	870565	871771	1206	68	hypo
1	93970706	93970977	271	9	hypo
11	12136161	12136468	307	10	hypo
11	128694096	128694425	329	32	hypo
11	132951692	132952492	800	45	hypo
11	1828650	1828783	133	5	hypo
11	59560470	59560549	79	4	hypo
11	65477123	65477452	329	9	hypo
12	102092915	102093110	195	11	hypo
12	107273279	107273681	402	7	hypo
12	111137400	111137596	196	11	hypo
12	117443273	117443444	171	7	hypo
12	124905467	124905759	292	15	hypo

12	125482583	125482829	246	18	hypo
12	16161553	16161815	262	7	hypo
12	57792999	57793110	111	6	hypo
13	111948367	111948559	192	9	hypo
14	100610169	100610668	499	22	hypo
14	103200841	103201128	287	12	hypo
14	75153156	75153308	152	3	hypo
14	93212312	93212487	175	11	hypo
15	30336647	30336863	216	29	hypo
15	31134409	31134668	259	9	hypo
15	40093789	40094023	234	7	hypo
15	52872030	52872160	130	6	hypo
15	57511786	57512216	430	18	hypo
15	74832028	74832090	62	5	hypo
16	57831974	57832180	206	18	hypo
16	69489543	69489665	122	5	hypo
16	85654156	85654324	168	13	hypo
16	88579452	88580072	620	21	hypo
17	17946397	17946585	188	7	hypo
17	21119605	21119845	240	9	hypo
17	28580392	28580614	222	5	hypo
17	36572579	36572897	318	11	hypo
17	56272299	56272502	203	10	hypo
17	8702637	8702756	119	16	hypo
17	8769570	8769884	314	14	hypo
18	12076398	12076622	224	30	hypo
18	22016574	22016800	226	6	hypo
18	71910027	71910089	62	6	hypo
18	8755023	8755343	320	13	hypo
19	10404092	10405285	1193	81	hypo
19	34859991	34860410	419	13	hypo
19	4382715	4382768	53	4	hypo
2	113426404	113426419	15	3	hypo
2	118617427	118618163	736	73	hypo
2	121816094	121816885	791	23	hypo
2	130986715	130986828	113	20	hypo
2	70734255	70734341	86	5	hypo
2	74213621	74213841	220	13	hypo
2	75089515	75089819	304	8	hypo
2	97401278	97401372	94	6	hypo
20	29525180	29525475	295	20	hypo
20	29550762	2955175			hypo
20	33416638	33416742	104	9	hypo
21	19184847	19184909	62	7	hypo
21	30298129	30298294	165	5	hypo
21	38750599	38750877	278	11	hypo
22	46762433	46763144	711	38	hypo
22	50616227	50617057	830	76	hypo
3	128134844	128135029	185	8	hypo
3	128317793	128318091	298	12	hypo

3	172243109	172243331	222	13	hypo
3	195964960	195965370	410	11	hypo
3	39395430	39395805	375	12	hypo
3	70560282	70560339	57	5	hypo
3	98476467	98476657	190	5	hypo
4	144833125	144833346	221	30	hypo
4	148634323	148634374	51	5	hypo
4	1908638	1908946	308	10	hypo
4	2366183	2366745	562	49	hypo
5	132002374	132002507	133	5	hypo
5	149145166	149145197	31	4	hypo
5	154224429	154224647	218	4	hypo
5	157117442	157117959	517	42	hypo
5	68700315	68700724	409	14	hypo
5	77142381	77142899	518	26	hypo
6	166674955	166675083	128	5	hypo
7	102003600	102003767	167	9	hypo
7	127910860	127911680	820	49	hypo
7	1914009	1914393	384	18	hypo
7	32357921	32358755	834	44	hypo
7	48887537	48887891	354	42	hypo
7	5382633	5382783	150	8	hypo
7	54900863	54901103	240	21	hypo
7	90895326	90896702	1376	91	hypo
8	128828626	128828794	168	5	hypo
8	131047175	131047345	170	5	hypo
8	58192499	58193338	839	50	hypo
8	599524	600398	874	60	hypo
9	123744449	123744762	313	10	hypo
9	128994302	128994390	88	4	hypo
9	32430999	32431303	304	9	hypo
9	5819260	5819334	74	4	hypo
9	69500968	69501070	102	14	hypo

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	Transcription factor (HOCOMOCO ID)	reference (only one representative reference is give
	ANDR	*R. Satyanarayana Raju Kalidhindi et al. Androgen r*
A	*CEBPA*	*G. Caramori et al. Role of transcription factors in th*
	CEBPB	*G. Caramori et al. Role of transcription factors in th*
	ERG1	*K. Golebski. EGR-1 as a potential biomarker in asth*
	FOXA3	*G. Chen et al. Foxa3 induces goblet cell metaplasia*
	FOXC1	*S. Shamsadin Athari. Targeting cell signaling in alle*
	FOXQ1	*H. Ying. Transcriptomic Analysis Exploring the Mole*
	GATA3	*Shrine N, Portelli MA, John C, et al. Moderate-to-se*
	GATA6	*Fang P, Shi HY, Wu XM, et al. Targeted inhibition o*
	IRF1	*Landgraf-Rauf K, Boeck A, Siemens D, et al. IRF-1 S*
	LEF1	*H. Xiiao et al. Hippo Signaling Pathway and Macro*
	MITF	*E. Morii et al. MITF is necessary for generation of p*
	NFAC1	*Koch S, Graser A, Mirzakhani H, et al. Increased exp*
	NR1H3	*Duan QL, Du R, Lasky-Su J, et al. A polymorphism in*
	RARA	*J. K. Novak et al. Expression profiling of ileal mucos*
	RXRA	*J. Suurmond et al. Repeated FcεRI triggering revea*
	SP1	*B. Diao et al. Functional network analysis with the*
	SP3	*B. Diao et al. Functional network analysis with the*
	STAT2	*A. Bergauer et al. IFNα/IFN-λ responses to respirat*
R	*THA*	*Duan QL, Du R, Lasky-Su J, et al. A polymorphism in*
	THB	*Duan QL, Du R, Lasky-Su J, et al. A polymorphism in*
	WT1	*X. Wu, R. Li, Q. Xu, F. Liu, Y. Jiang , M. Zhang, M. To*

motif number	Transcription factor (HOCOMOCO ID)
3, 14	BC11A, ETV5, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
3, 10	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
1, 3, 4, 5, 7	ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
1, 3, 4, 5, 7	ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
8, 14, 19	BC11A, ETV5, GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
15	
10	
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
3	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
3, 5, 11	NFAC1, NF2L1, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
3	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
8, 14	BC11A, ETV5, GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
8, 13	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
1, 2, 3, 5	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
3, 8	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3, GATA4, TAL1, G
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
3	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
14	BC11A, ETV5

1, 2, 3, 4, 5	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
17	
1, 2, 3, 4, 5	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
4	
4	
8, 10	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
8, 10	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
1, 2, 5, 6, 7	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
10	
13	
2, 8, 17	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2, 9, 15	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 2, 4, 6, 7	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 4, 8, 18	PRDM6, ZN586, ZN264, GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
2, 3	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
1, 2, 6	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
3	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
1, 4	ZN264
14	BC11A, ETV5
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 2, 3, 4, 11	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2, 15	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1,3, 4, 5, 7	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3, ZN264
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
15	
15	
1, 4, 6, 7	THA, THB, USF1, MITF, RARA, NR1H3, ZN264
14	BC11A, ETV5
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
15	
18	PRDM6, ZN587
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2, 15	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
3, 15	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
1, 2, 4, 6	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
10	
10	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 2, 4, 6, 13	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
20	PRDM6, IRF1, STAT2, ZFP28, LEF1, ZIM3, ANDR, NFAC1, SRY
1, 2, 9	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
15	
14	BC11A, ETV5
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1

6, 8, 13	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1, THA, THB, USF1, MITF, RARA,
19	
1, 2, 3, 4, 9	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
14	BC11A, ETV5
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 2, 4, 6	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
2, 3, 6	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
8, 16	FOXC1, FOXQ1, GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2, 9, 15	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 4, 8, 10, 12, 16	CEBPB, CEBPA, ZN264, FOXC1, FOXQ2, GATA4, TAL1, GATA6, GATA3, GATA1, GAT
2, 3, 5, 6, 7	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
14	BC11A, ETV5
3	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
14, 17	BC11A, ETV5
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
1, 2, 3, 4, 5	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
20	PRDM6, IRF1, STAT2, ZFP28, LEF1, ZIM3, ANDR, NFAC1, SRY
2	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,
3, 8	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3, GATA4, TAL1, (
8	GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
17	
14	BC11A, ETV5
4, 8, 12, 17	CEBPB, CEBPA, GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
3, 10	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
3, 17	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
3	PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
2, 4	ZN770, SP2, PATZ1, SP3,TAF1, SP4, WT1, EGR1, SP1, GABPA, KLF15, MAZ, ZN467,

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ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2, ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3
GATA6, GATA3, GATA1, GATA2, EVI1

ZN281, ZN263, KLF3, EGR2, ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3

ZN281, ZN263, KLF3, EGR2, ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3

ZN281, ZN263, KLF3, EGR2, ZN264, THA, THB, USF1, MITF, RARA, NR1H3
ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2, GATA4, TAL1, GATA6, GATA3, GATA1, GATA2, EVI1
ZN281, ZN263, KLF3, EGR2, ZN329
ZN281, ZN263, KLF3, EGR2, ZN264, THA, THB, USF1, MITF, RARA, NR1H3
ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3

ZN281, ZN263, KLF3, EGR2, ZN264, THA, THB, USF1, MITF, RARA, NR1H3

ZN281, ZN263, KLF3, EGR2
ZN281, ZN263, KLF3, EGR2, ZN264, NFAC1, NF2L1, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6
ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2
ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2, ZN264, THA, THB, USF1, MITF, RARA, NR1H3
ZN281, ZN263, KLF3, EGR2

ZN281, ZN263, KLF3, EGR2, ZN330
ZN281, ZN263, KLF3, EGR2, ZN264, THA, THB, USF1, MITF, RARA, NR1H3

ZN281, ZN263, KLF3, EGR2, ZN264, ZN331

, NR1H3

. ZN281, ZN263, KLF3, EGR2, ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3,

. ZN281, ZN263, KLF3, EGR2
. ZN281, ZN263, KLF3, EGR2, ZN264, THA, THB, USF1, MITF, RARA, NR1H3

. ZN281, ZN263, KLF3, EGR2

. ZN281, ZN263, KLF3, EGR2, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3, THA, TH

. ZN281, ZN263, KLF3, EGR2
. ZN281, ZN263, KLF3, EGR2, ZN333
. ZN281, ZN263, KLF3, EGR2
TA2, EVI1
. ZN281, ZN263, KLF3, EGR2, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3, THA, TH

. ZN281, ZN263, KLF3, EGR2
. ZN281, ZN263, KLF3, EGR2, ZN264, PRDM6, FOXJ3, GATA3, GATA6, SOX5, ANDR, HNF6, NFAC1, LHX3

. ZN281, ZN263, KLF3, EGR2
GATA6, GATA3, GATA1, GATA2, EVI1

. ZN281, ZN263, KLF3, EGR2

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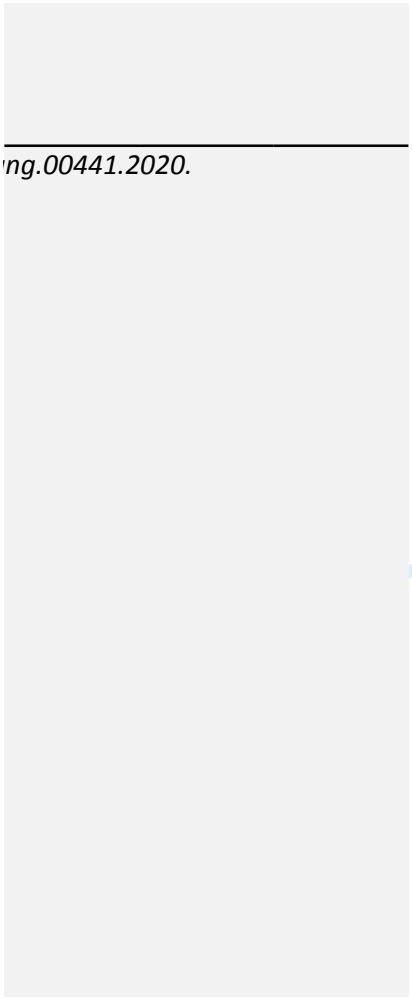
armacogenomics journal. 2013;13(2):130-136
armacogenomics journal. 2013;13(2):130-136
nformatics analysis. Sci Rep. 2022 Feb 15;12(1):2549.

6, NFAC1, LHX3

, ZN332

HB, USF1, MITF, RARA, NR1H3

HB, USF1, MITF, RARA, NR1H3



ng.00441.2020.

Table S12: Module pathway enrichment

(B) BioCarta
(R) Reactome
(N) Pathway Interaction Database
(K) KEGG

module	
0	**Immune response**
1	**Carbon metab. in cancer**
2	**mRNA metabolism**

3	**Integrin signaling**
4	**Transcription**
5	**Translation**
6	**DNA damage control**
7	**RNA degradation**
8	**Lipid metabolism**
9	**Mitosis**
10	**Hedgehog signaling/ addiction**
11	**rRNA processing**
	Tight junction / Protein

12 folding

t for all enhancer target genes, their host genes and promoter genes (FDR <0.01)

pathway
JAK-STAT signaling pathway(K)
Pathways in cancer(K)
CD40/CD40L signaling(N)
NF-kappa B signaling pathway(K)
Th17 cell differentiation(K)
Cytokine-cytokine receptor interaction(K)
tnfr2 signaling pathway(B)
IL12-mediated signaling events(N)
Inflammatory bowel disease(K)
Adipocytokine signaling pathway(K)
RIG-I-like receptor signaling pathway(K)
Viral carcinogenesis(K)
DDX58/IFIH1-mediated induction of interferon-alpha/beta(R)
the 41bb-dependent immune response(B)
Longevity regulating pathway(K)
Small cell lung cancer(K)
IL-17 signaling pathway(K)
Hematopoietic cell lineage(K)
TNF signaling pathway(K)
AMPK signaling pathway(K)
hiv-1 nef: negative effector of fas and tnf(B)
Measles(K)
Autophagy - other(K)
HIV-1 Nef: Negative effector of Fas and TNF-alpha(N)
PI3K-Akt signaling pathway(K)
IL2 signaling events mediated by PI3K(N)
Hepatitis C(K)
IL23-mediated signaling events(N)
Hepatitis B(K)
Influenza A(K)
MTOR signalling(R)
LKB1 signaling events(N)
TNF receptor signaling pathway(N)
keratinocyte differentiation(B)
Central carbon metabolism in cancer(K)
Processing of Capped Intron-Containing Pre-mRNA(R)
Spliceosome(K)
Beta2 integrin cell surface interactions(N)
Integrin signalling pathway(P)
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell(R)
Viral myocarditis(K)
Extracellular matrix organization(R)

Leukocyte transendothelial migration(K)
SNARE interactions in vesicular transport(K)
Malaria(K)

RNA Polymerase II Transcription(R)
Signaling events mediated by HDAC Class I(N)
Factors involved in megakaryocyte development and platelet production(R)

SRP-dependent cotranslational protein targeting to membrane(R)
Response of EIF2AK4 (GCN2) to amino acid deficiency(R)
Selenoamino acid metabolism(R)
Eukaryotic Translation Termination(R)
Eukaryotic Translation Elongation(R)
Nonsense-Mediated Decay (NMD)(R)
Eukaryotic Translation Initiation(R)
Insertion of tail-anchored proteins into the endoplasmic reticulum membrane(R)
Ribosome(K)
rRNA processing(R)
Signaling by ROBO receptors(R)
Coronavirus disease - COVID-19(K)
Protein processing in endoplasmic reticulum(K)

HDR through Homologous Recombination (HRR) or Single Strand Annealing (SSA)(R)
Nonhomologous End-Joining (NHEJ)(R)
Fanconi Anemia Pathway(R)
DNA Double Strand Break Response(R)
Fanconi anemia pathway(K)
DNA Damage/Telomere Stress Induced Senescence(R)
ATM pathway(N)
Homologous recombination(K)
Fanconi anemia pathway(N)
Cell Cycle Checkpoints(R)

Deadenylation-dependent mRNA decay(R)
RNA degradation(K)
RNA transport(K)
mRNA surveillance pathway(K)
Eukaryotic Translation Initiation(R)

Regulation of lipid metabolism by PPARalpha(R)
Transcriptional regulation of white adipocyte differentiation(R)
RNA Polymerase II Transcription(R)
Signaling events mediated by HDAC Class II(N)
Signaling by NOTCH1(R)
Mitochondrial biogenesis(R)
Cytoprotection by HMOX1(R)

Mitotic G2-G2/M phases(R)
Mitotic Prometaphase(R)
Cilium Assembly(R)

Cocaine addiction(K)
Hedgehog signaling pathway(K)
Amphetamine addiction(K)

rRNA processing(R)
Ribosome biogenesis in eukaryotes(K)

Protein folding(R)

Tight junction(K)

ratio of proteins in gene set	genes observed/ genes in pathway	*p*-value	FDR
0.0136	162/ 6	2.91E-08	6.57E-06
0.0447	531/ 7	1.68E-06	1.89E-04
0.0024	29/ 3	6.49E-06	3.74E-04
0.0088	104/ 4	7.44E-06	3.74E-04
0.009	107/ 4	8.32E-06	3.74E-04
0.0249	295/ 5	2.31E-05	8.55E-04
0.0008	9/ 2	6.00E-05	1.73E-03
0.0052	62/ 3	6.18E-05	1.73E-03
0.0055	65/ 3	7.11E-05	1.77E-03
0.0058	69/ 3	8.48E-05	1.77E-03
0.0059	70/ 3	8.85E-05	1.77E-03
0.0172	204/ 4	1.02E-04	1.84E-03
0.0066	78/ 3	1.22E-04	2.07E-03
0.0012	14/ 2	1.45E-04	2.31E-03
0.0075	89/ 3	1.79E-04	2.69E-03
0.0077	92/ 3	1.98E-04	2.73E-03
0.0079	94/ 3	2.10E-04	2.73E-03
0.0083	99/ 3	2.45E-04	2.94E-03
0.0094	112/ 3	3.51E-04	3.86E-03
0.0101	120/ 3	4.29E-04	4.72E-03
0.0025	30/ 2	6.56E-04	6.56E-03
0.0117	139/ 3	6.57E-04	6.57E-03
0.0027	32/ 2	7.45E-04	6.71E-03
0.0028	33/ 2	7.92E-04	7.12E-03
0.0298	354/ 4	8.29E-04	7.12E-03
0.0029	35/ 2	8.90E-04	7.12E-03
0.0132	157/ 3	9.34E-04	7.16E-03
0.0031	37/ 2	9.93E-04	7.16E-03
0.0136	162/ 3	1.02E-03	7.16E-03
0.0144	171/ 3	1.19E-03	8.36E-03
0.0035	41/ 2	1.22E-03	8.51E-03
0.0036	43/ 2	1.34E-03	9.15E-03
0.0039	46/ 2	1.52E-03	9.15E-03
0.004	47/ 2	1.59E-03	9.54E-03
0.0059	70/ 3	5.61E-05	7.63E-03
0.0199	236/ 8	3.82E-12	9.92E-11
0.0127	151/ 5	1.44E-07	1.88E-06
0.0024	29/ 3	1.73E-06	1.35E-04
0.0133	158/ 4	6.18E-06	2.41E-04
0.0167	198/ 4	1.50E-05	2.87E-04
0.0051	60/ 3	1.51E-05	2.87E-04
0.0219	260/ 4	4.35E-05	6.52E-04

0.0096	114/ 3	1.01E-04	1.31E-03
0.0028	33/ 2	3.43E-04	3.77E-03
0.0042	50/ 2	7.81E-04	7.03E-03
0.0769	913/ 5	1.51E-05	3.32E-04
0.0047	56/ 2	3.30E-04	3.63E-03
0.009	107/ 2	1.19E-03	8.33E-03
0.0087	103/ 5	2.69E-09	6.20E-08
0.0079	94/ 4	2.68E-07	2.70E-06
0.0087	103/ 4	3.86E-07	2.70E-06
0.0072	85/ 3	2.00E-05	8.00E-05
0.0072	85/ 3	2.00E-05	8.00E-05
0.0091	108/ 3	4.08E-05	1.22E-04
0.0094	112/ 3	4.54E-05	1.36E-04
0.0019	22/ 2	9.55E-05	1.91E-04
0.0133	158/ 3	1.26E-04	2.51E-04
0.0161	191/ 3	2.20E-04	3.88E-04
0.0169	201/ 3	2.55E-04	3.88E-04
0.0195	232/ 3	3.88E-04	3.88E-04
0.0144	171/ 2	5.48E-03	5.48E-03
0.0072	85/ 6	9.38E-13	3.19E-11
0.003	36/ 3	9.67E-07	1.06E-05
0.003	36/ 3	9.67E-07	1.06E-05
0.0039	46/ 3	2.01E-06	1.61E-05
0.0045	54/ 3	3.25E-06	1.95E-05
0.0023	27/ 2	1.08E-04	5.39E-04
0.0029	34/ 2	1.71E-04	6.83E-04
0.0035	41/ 2	2.48E-04	8.94E-04
0.0038	45/ 2	2.98E-04	8.94E-04
0.0209	248/ 3	3.00E-04	8.99E-04
0.0046	55/ 3	3.43E-06	5.84E-05
0.0067	79/ 3	1.01E-05	8.09E-05
0.0157	186/ 3	1.28E-04	6.42E-04
0.0082	97/ 2	1.36E-03	5.43E-03
0.0094	112/ 2	1.81E-03	5.43E-03
0.0099	117/ 4	1.39E-07	4.18E-06
0.0071	84/ 3	6.97E-06	1.05E-04
0.0769	913/ 5	1.51E-05	1.51E-04
0.0032	38/ 2	1.52E-04	1.07E-03
0.0051	61/ 2	3.91E-04	2.34E-03
0.0076	90/ 2	8.45E-04	4.22E-03
0.0102	121/ 2	1.52E-03	6.07E-03
0.0149	177/ 5	4.37E-09	8.98E-09
0.015	178/ 5	4.49E-09	8.98E-09
0.0152	180/ 4	7.74E-07	7.74E-07
0.0041	49/ 2	1.02E-04	6.14E-03
0.0042	50/ 2	1.06E-04	6.14E-03
0.0058	69/ 2	2.01E-04	7.84E-03
0.0161	191/ 3	4.17E-06	1.25E-05
0.0093	110/ 2	2.56E-04	2.56E-04
0.0051	60/ 2	7.64E-05	9.93E-04

0.0142	169/ 2	6.02E-04	3.61E-03

node genes
IL23R,IL5RA,MTOR,IL4,THPO,LEP
IL23R,IL5RA,TRAF1,RELA,MTOR,IL4,TRAF3
TRAF1,RELA,TRAF3
DDX58,TRAF1,RELA,TRAF3
IL23R,RELA,MTOR,IL4
IL23R,IL5RA,IL4,THPO,LEP
TRAF1,TRAF3
RELA,MTOR,IL4
IL23R,RELA,IL4
RELA,MTOR,LEP
DDX58,RELA,TRAF3
TRAF1,RELA,TRAF3,MAD1L1
DDX58,RELA,TRAF3
RELA,IL4
RELA,MTOR,RPTOR
TRAF1,RELA,TRAF3
RELA,IL4,TRAF3
IL5RA,IL4,THPO
TRAF1,RELA,TRAF3
MTOR,RPTOR,LEP
TRAF1,RELA
DDX58,RELA,TRAF3
MTOR,RPTOR
TRAF1,RELA
RELA,MTOR,IL4,RPTOR
RELA,MTOR
DDX58,RELA,TRAF3
IL23R,RELA
DDX58,RELA,TRAF3
DDX58,RELA,TRAF3
MTOR,RPTOR
MTOR,RPTOR
TRAF1,RELA
TRAF1,RELA
HK2,SCO2,MYC
SART1,LSM7,SF3B2,SF3A2,FUS,CD2BP2,POLR2H,SF1
SART1,LSM7,SF3B2,SF3A2,FUS
ICAM4,ITGAL,ICAM1
ARHGAP10,ARL1,ITGAL,ACTG1
ICAM4,ICAM5,ITGAL,ICAM1
ITGAL,ACTG1,ICAM1
ICAM4,ICAM5,ITGAL,ICAM1

ITGAL,ACTG1,ICAM1
GOSR1,STX4
ITGAL,ICAM1

SETD1A,TCF12,TCF3,GATA2,ZFPM1
GATA2,ZFPM1
GATA2,ZFPM1

SEC61G,RPN1,RPSA,RPL26,RPL19
IMPACT,RPSA,RPL26,RPL19
EEFSEC,RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
CYB5A,SEC61G
RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
RPSA,RPL26,RPL19
SEC61G,RPN1

MUS81,RAD50,KAT5,NSD2,SLX1A,RNF4
RAD50,KAT5,NSD2
MUS81,SLX1A,FAN1
RAD50,KAT5,NSD2
MUS81,SLX1A,FAN1
RAD50,KAT5
RAD50,KAT5
MUS81,RAD50
RAD50,FAN1
RAD50,KAT5,NSD2

CNOT1,CNOT8,EIF4G1
CNOT1,PABPC3,CNOT8
EIF3G,PABPC3,EIF4G1
DAZAP1,PABPC3
EIF3G,EIF4G1

NCOR2,NCOA6,MED16,PPARGC1B
NCOR2,NCOA6,MED16
NCOR2,RBM14,HDAC10,MED16,PPARGC1B
NCOR2,HDAC10
NCOR2,HDAC10
NCOA6,PPARGC1B
NCOR2,NCOA6

TUBGCP2,AKAP9,ALMS1,TUBGCP6,HAUS3
TUBGCP2,AKAP9,ALMS1,TUBGCP6,HAUS3
AKAP9,ALMS1,MKS1,HAUS3

GRIN3B,PRKACB
PRKACB,CSNK1G2
GRIN3B,PRKACB

RBM28,EXOSC10,FCF1
RBM28,FCF1

TUBA3E,CCT7

TUBA3E,MARVELD2

Table S13A:

gene name
ACGT1
AFF4
CYB5A
DDX58
DNMT1
EEFSEC
EVL
FZD1
FZR1
GATA2
HDAC10
HK2
HTRA2
ICAM1
IL23R
IL4
IL5RA
ILF3
ITGAL
LEP
MAD1L1
MAPK7
MARVLD2
MTOR
MYC
NCOR2
PPARGC1B
RAD50
RELA
RPSA
RPTOR
STX4
TRAF1
ZFPM1

Natural language processing (NLP) reference list of genes included in the network and which were p

reference
Chen A, Diaz-Soto MP, Sanmamed MF et al. **Single-cell characterization of a model of poly I:C-stimulated peri**
Murphy TM, Wong CC, Arseneault L et al. **Methylomic markers of persistent childhood asthma: a longitudina**
Jeong H, Rhim T, Ahn MH, Yoon PO et al. **Proteomic analysis of differently expressed proteins in a mouse mo**
Landgraf-Rauf K, Anselm B, Schaub B. **The puzzle of immune phenotypes of childhood asthma**. Mol Cell Pedia
Li H, Ryu MH, Rider CF et al. **Predominant DNMT and TET mediate effects of allergen on the human bronchia**
Johansson Å, Rask-Andersen M, Karlsson T, Ek WE. **Genome-wide association analysis of 350 000 Caucasians f**
Cardenas A, Sordillo JE, Rifas-Shiman SL et al. **The nasal methylome as a biomarker of asthma and airway infl**
Altman MC, Gill MA, Whalen E et al. **Transcriptome networks identify mechanisms of viral and nonviral asthr**
Yang L, Zheng Y, Miao YM et al. **Bergenin, a PPARγ agonist, inhibits Th17 differentiation and subsequent neut**
Yang IV, Tomfohr J, Singh J et al. **The clinical and environmental determinants of airway transcriptional profil**
Zhang HP, Wang L, Fu JJ et al. **Association between histone hyperacetylation status in memory T lymphocyte**
Yu H, Cheng Y, Zhang G et al. **p62-dependent autophagy in airway smooth muscle cells regulates metabolic r**
Renke J, Wasilewska E, Kędzierska-Mieszkowska S et al. **Tumor Suppressors-HTRA Proteases and Interleukin-1**
Tang ML, Fiscus LC. **Important roles for L-selectin and ICAM-1 in the development of allergic airway inflamm**
Abdollahi E, Tavasolian F, Momtazi-Borojeni AA et al. **Protective role of R381Q (rs11209026) polymorphism in**
Cui A-H, Zhao J, Liu S-X, Hao Y-S. **Associations of IL-4, IL-6, and IL-12 levels in peripheral blood with lung funct**
Liang L, Willis-Owen SA, Laprise C et al. **An epigenome-wide association study of total serum immunoglobulir**
Gill AS, Pulsipher A, Sumsion JS et al. **Transcriptional Changes in Chronic Rhinosinusitis with Asthma Favor a T**
Gauvreau GM, Becker AB, Boulet LP et al. **The effects of an anti-CD11a mAb, efalizumab, on allergen-induced**
Kato H, Ueki S, Kamada R et al. **Leptin has a priming effect on eotaxin-induced human eosinophil chemotaxis**
McErlean P, Favoreto S, Jr., Costa FF et al. **Human rhinovirus infection causes different DNA methylation char**
Ghosh D, Ding L, Bernstein JA, Mersha TB. **The Utility of Resolving Asthma Molecular Signatures Using Tissue-**
Smyth T, Veazey J, Eliseeva S et al. **Diesel exhaust particle exposure reduces expression of the epithelial tight**
Ma B, Athari SS, Mehrabi Nasab E, Zhao L. **PI3K/AKT/mTOR and TLR4/MyD88/NF-κB Signaling Inhibitors Atte**
Ye L, Pan J, Liang M, Pasha MA et al. **A critical role for c-Myc in group 2 innate lymphoid cell activation**. Allerg
Portas L, Pereira M, Shaheen SO et al. **Lung Development Genes and Adult Lung Function**. Am J Respir Crit Car
Lee SH, Jang AS, Woo Park S et al. **Genetic effect of single-nucleotide polymorphisms in the PPARGC1B gene**
Li X, Hawkins GA, Moore WC et al. **Expression of asthma susceptibility genes in bronchial epithelial cells and l**
Gagliardo R, Chanez P, Mathieu M et al. **Persistent activation of nuclear factor-kappaB signaling pathway in s**
Christie PE, Jonas M, Tsai CH et al. **Increase in laminin expression in allergic airway remodelling and decrease**
Yick CY, Zwinderman AH, Kunst PW et al. **Gene expression profiling of laser microdissected airway smooth mu**
Fettrelet T, Gigon L, Karaulov A et al. **The Enigma of Eosinophil Degranulation**. Int J Mol Sci. 2021 Jun 30;22(13
Pedros C, Altman A, Kong K-F. **Role of TRAFs in Signaling Pathways Controlling T Follicular Helper Cell Differe**
Jahreis S, Trump S, Bauer M et al. **Maternal phthalate exposure promotes allergic airway inflammation over**

Table S13B: Na

gene name
TFCP2L1
PDE1C
TET3
HTRA2
ANKRD11
ZFPM1
BANP
LEP
S1PR2
PANX2
ICAM1
ILF3
DNMT1

ıtural language processing (NLP) reference list of genes previously related to asthma and to a asthm

reference
Mostafa MM, Rider CF, Shah S, et al. **Glucocorticoid-driven transcriptomes in human airway epithelial cells: c**
Ding L, Abebe T, Beyene J et al. **Rank-based genome-wide analysis reveals the association of ryanodine recep**
Li H, Ryu MH, Rider CF et al. **Predominant DNMT and TET mediate effects of allergen on the human bronchia**
Renke J, Wasilewska E, Kędzierska-Mieszkowska S et al. **Tumor Suppressors-HTRA Proteases and Interleukin-1**
Banerjee P, Balraj P, Ambhore NS, et al. **Network and co-expression analysis of airway smooth muscle cell tra**
Jahreis S, Trump S, Bauer M et al. **Maternal phthalate exposure promotes allergic airway inflammation over**
Chemmannur SV, Badhwar AJ, Mirlekar B et al. **Nuclear matrix binding protein SMAR1 regulates T-cell differe**
Kato H, Ueki S, Kamada R et al. **Leptin has a priming effect on eotaxin-induced human eosinophil chemotaxis**
Chiba Y, Suzuki K, Uechi M et al. **Downregulation of sphingosine-1-phosphate receptors in bronchial smooth**
Wang T, Wang W, Li W et al. **Genome-wide DNA methylation analysis of pulmonary function in middle and o**
Tang ML, Fiscus LC. **Important roles for L-selectin and ICAM-1 in the development of allergic airway inflamm**
Gill AS, Pulsipher A, Sumsion JS et al. **Transcriptional Changes in Chronic Rhinosinusitis with Asthma Favor a T**
Li H, Ryu MH, Rider CF et al. **Predominant DNMT and TET mediate effects of allergen on the human bronchia**