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Epigenome Wide Association Studies in Asthma: A Systematic Review

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Abstract

Objective:

Asthma is a common, chronic respiratory airway disease influenced by environmental factors and possibly their interaction with the human genome causing epigenetic changes. Epigenome wide association studies (EWAS) have mainly investigated DNA methylation and its association with

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disease or traits, exposure factors or gene expression. This systematic review aimed to identify all EWAS assessing differentially methylated sites associated with asthma in humans.

Design:

Structured systematic literature search following PRISMA guidelines, Newcastle-Ottawa Scale (NOS) for cohort studies was used for bias assessment.

Data Sources:

We searched PubMed and Embase databases from 2005 through 2019.

Eligibility Criteria:

Epigenome wide association studies testing association between differential methylation and asthma in humans.

Results:

Overall, we identified sixteen EWAS studies complying with our search criteria. Twelve studies were conducted on children and ten were conducted on sample sizes less than 150 subjects. Four hundred and nineteen CpGs were reported in children studies after correction for multiple testing. In the adult studies, thousands of differentially methylated sites were identified. Differential methylation in inflammatory related genes correlated with higher levels of gene expressions of inflammatory modulators in asthma. Differentially methylated genes associated with asthma included *SMAD3*, *SERPINC1*, *PROK1*, *IL13*, *RUNX3* and *TIGIT*. Forty-one CpGs were replicated at least once in blood samples, and 28 CpGs were replicated in nasal samples.

Conclusion:

Although many differentially methylated CpGs in genes known to be involved in asthma have been identified in EWAS to date, we conclude that further studies of larger sample sizes and analyses of differential methylation between different phenotypes are needed in order to comprehensively evaluate the role of epigenetic factors in the pathophysiology and heterogeneity of asthma, and the potential clinical utility to predict or classify asthmatic patients.

1- Introduction

Asthma is a common, chronic respiratory airway disease, affecting respiratory health throughout life.

According to GINA (Global Initiative for Asthma), asthma prevalence is increasing in many countries, especially in childhood.[1] It is estimated that around 300 million people worldwide are affected by the disease (around 4.5% of adults and 11% of children worldwide).[2] The prevalence of asthma has more than doubled in the last decades in Western Europe, while developing countries have also suffered an increase in the disease prevalence.[3]

Family studies have shown that asthma is a heritable trait with heritability estimates around 50-60%.[4] Identified genetic variants associated with childhood asthma in large-scale GWA studies only account for a low fraction of variance in asthma severity, endotypes, heritability and clinical response to therapy.[5-8] Environmental exposures, such as tobacco smoke exposure during pregnancy, lack of breastfeeding and air pollutants that affect lung development and risk of asthma may alter gene activity and expression without changing underlying DNA sequence, referred to by the term epigenetics.[8-10] The genetic makeup of an individual and its interaction with the environment can both affect epigenetic mechanisms.[11, 12] Multiple epigenetic mechanisms, including DNA-methylation, histone modification and non-coding RNA-associated gene silencing are considered to initiate and sustain epigenetic changes.[13]

DNA methylation occurs by adding a methyl group to cytosine to form 5 methyl cytosine (5mc), and this happens primarily at DNA sites where a cytosine nucleotide is followed by a guanine nucleotide, called CpG.[14] Sixty to eighty percent of CpGs in the human DNA are highly methylated.[15] With gene transcription initiation mostly occurring at promoter sites rich in CG content (called CpG islands), their methylation has a large effect on gene expression of human DNA.[16] Another example of differentially methylated sites in the human DNA is the differentially methylated region (DMR), defined as a region of the genome where multiple CpG sites show different methylation patterns across different tissues or cells.[14] DMRs can therefore be tissue and ageing specific.[17]

Because DMRs are expected to affect gene transcriptional regulation, these are thought to alter gene expression profiles in humans as well.[14]

DNA methylation can be either assessed over the entire genome (whole genome methylation profiling) as explained in an overview by Rakyan et al.,[17] or by candidate studies designed to search specifically for DMRs or CpGs in specific genes or regions in the DNA.[18] Three techniques are currently available for detection of methylation status at DMRs or CpGs: bisulfite sequencing, microarrays or bead chips and methyl-sensitive cut counting (MSCC).[19]

Bisulfite sequencing exploits the current inability of DNA sequencing techniques to differentiate between methylcytosine and cytosine due to similar base pairing characteristics between both molecules.[20] DNA is treated with bisulfite, deaminating unmethylated cytosine to uracil. As a result unmethylated cytosine will be read as thymidine upon sequencing, enabling the detection of methylated regions in the DNA.[19] Microarrays detect differential methylation through hybridization of methylated DNA fractions.[19] Currently available array technologies include chips by Agilent and Affymetrix, some assays also focus on specific regions where DNA methylation is believed to have the largest effects (gene promoter regions, enhancer regulatory elements and 3' untranslated regions (3'UTRs) like the Infinium HumanMethylation450K Bead Chip array by Illumina, considered the most popular technique used to study methylation.[17, 19, 21] The recently released Illumina MethylationEPIC BeadChip has a higher coverage (850,000 CpG sites) than the HumanMethylation450K and high reproducibility, adding a potentially efficient platform to the currently available techniques.[22] The chip has been used and evaluated in a number of recent studies.[23, 24]

Epigenome wide association studies (EWAS) assess methylation of the DNA and its associations with gene expression, phenotypes or exposures. Although epigenetics as a general term also include histone modifications and other mechanisms that may alter gene expression, EWAS have been most widely used to study DNA methylation.

Population based and case controlled EWAS studies investigating differential methylation in asthma have focused on two age categories: childhood (incorporating the links between maternal and perinatal exposures and the child epigenome and subsequent risk of developing asthma), and adulthood (incorporating the differential methylation in adults that lead to higher risk of asthma). Interest in the field of asthma epigenetics is growing, and several EWAS have been conducted.

2- Objective:

This systematic review aimed to identify all EWAS assessing the association between DNA methylation and asthma in humans. We assessed the quality of the included studies, including the validity and consistency of DNA methylation associated with asthma across different studies.

3- Methods:

The review was conducted and reported according to a predefined protocol compliant with the PRISMA guidelines for systematic reviews (Supplementary File 3) [25]. A structured systematic literature search was developed to identify all EWAS assessing epigenetic modifications associated with asthma in humans. Databases of PubMed and Embase were searched for articles published up to February 1st 2019. The search terms used in Embase were "Epigenome wide association" and "Asthma", PubMed was searched using Mesh terms: (((("Asthma" [MeSH Terms] OR "asthma" [Title]) AND ("Epigenetics" [MeSH Terms] OR "methylation" [All Fields]))). Conference or poster abstracts, studies not conducted on humans, studies not in English, studies conducted before 2005, studies not designed as epigenome wide association studies and studies where the association with asthma incidence was not the primary outcome, were excluded. The selection process was performed by two investigators (AE and LL), and in case of non-agreement, final decision was made during a consensus meeting. A flow diagram of the selection procedure is included in Figure (1).

Data Extraction

Data extraction was performed using a predesigned table outlining the information on first author, publishing date, size and age of samples (including replication and/or validation phases), number of differentially methylated sites detected, top genes involved, statistical threshold used and methylation detection technique, number of DMRs or CpG sites detected, types of cells, and P-value of the association.

The overall quality of studies was assessed based on an adapted set of pre-defined criteria (Supplementary file 1) [26, 28] and the risk of bias was assessed using the Newcastle-Ottawa Scale (NOS) for non-randomized studies (Supplementary file 2).[29]

4-Results:

Our search strategy identified 245 published studies, 227 from PubMed and additionally 18 from Embase. After exclusion of six duplicates, titles and abstracts were reviewed for 239 articles. After exclusion of 203 articles, full text was reviewed for 36 studies. Excluded studies were either conducted before 2005 (n=17), no observational epigenetic studies (n=77), candidate studies (n=58) not conducted on humans (n=23), not conducted on asthmatic patients or not investigating the association between different methylation profiles and asthma (n=23). One paper not in English was excluded as well.

After full text review, nineteen articles were additionally excluded and sixteen papers were included in the final review. Causes of exclusion after full text review were: article only available in abstract form (n=3), no comparison of asthmatics versus a control group (n=6), animal study (n= 2), not an observational epigenetic study (n=1), candidate study(n=7). All included studies were published between 2013 and 2018. Characteristics of the sixteen included studies are summarized in Table 1.

Quality Assessment:

Four studies (25%) reported a power analysis, all retrospectively. Ten out of 16 studies were conducted on a relatively small number of patients (between 10 and 150). Two studies did not describe sufficient baseline characteristics.[30, 31] Six studies included a validation and replication cohort [32-36] and one study replicated the reactions and reported a concordance rate.[32] Studies conducted on blood samples employed different approaches in adjusting for differential distribution of cell types: Xu et al. used estimated cell type proportions as co-variates in the sensitivity analyses[32], De Vries et al. used an algorithm to predict cell proportions from Illumina DNA methylation data and a cord blood reference panel [37], while Arathimos et al. and Reese et al. used the Houseman [38] method to estimate proportions of cells from DNA methylation data (Arathimos et al. used minify R package, built on the method) [34, 39]

Risk of bias was moderate; the median NOS score was 6. Six studies had a high risk of bias, and three studies had a low risk of bias as assessed by the scale. High risk of bias was generally due to limited representativeness of the sample (e.g. small unrepresentative sample sizes [31, 35, 37, 40]), subjective assessment of the outcome (e.g. the use of questionnaires filled by the subjects' mother to assess asthma by Arathimos et al., a method prone to recall bias) or the use of a calendar of history of events in Murphy et al.).[30, 39]

Study characteristics and results of EWAS

Overall, there were sixteen published EWAS assessing the association between differential methylation and asthma. Several laboratory techniques were used to identify DNA methylation: the Infinium human methylation 450k beadchip was used in eleven studies[30, 32-35, 39, 41, 42], the Methylated CpG Island Recovery Assay (MIRA)-chip and Illumina GoldenGate Methylation Cancer Panel I were used in one study each.[37, 43] Xu et al. also used Iplex genotyping method in the validation and replication phases.[32] Most studies focused on children and adolescents up to 17 years, only Xu et al. used a replication cohort involving adults.[32] Ten studies used blood samples,

three used nasal epithelial cells and three studies used airway epithelial cells. One study used both airway cells and blood samples. Blood samples were either peripheral, cord blood or whole blood.

Studies conducted on blood samples:

Rastogi et al. in 2013 studied differential methylation in peripheral blood samples on a genome-wide level [40] in children sampled from a study investigating inflammatory pathways in obesity-associated asthma.[44] The authors divided 32 children into four subgroups: obese asthmatics, normal-weight asthmatics, obese non-asthmatics and healthy controls. Massively-parallel sequencing (MPS)-based HELP-tagging assay was used to test DNA methylation genome wide. When compared to obese asthmatics, 7,119 loci were differentially methylated in normal-weight asthmatics, 12,875 loci in obese non-asthmatics and 6,773 loci were differentially methylated in healthy controls.[40] Of these, 215 loci were consistent differentially methylated in all comparisons. IPA Analysis of the differentially methylated loci revealed hypomethylation of promotor loci associated with Th Cell differentiation (e.g.: *IL2RA*, *TBX21*). Promoters of *FCER2* gene, encoding a low-affinity receptor for IgE (CD23)[45] had higher levels of methylation.[40] The study is restricted by limited power due to very small sample size. Also the authors did not use a false discovery rate or adjust for multiple testing in their statistical analysis and reported the top 1% most consistently different loci between the four groups.

In 2014, Gunawardhana et al. investigated the differential methylation between different asthma phenotypes in adult patients. Nine neutrophilic asthma patients, 21 eosinophilic asthma patients and 22 paucigranulocytic asthma (PGA) patients were compared to 10 healthy subjects: 413 CpGs were differentially methylated in eosinophilic asthma, 49 in neutrophilic asthma and 374 in PGA compared to healthy controls. Nine CpGs annotated to 9 genes were differentially methylated in all three phenotypes compared to controls. The study is limited by its sample size, and the lack of adjustment for multiple testing.

Yang et al.[41] assessed differential methylation in a cohort of African Americans and Hispanic children with atopic asthma compared to controls. The study was validated in a cohort of 101 African Americans from the Inner-City Asthma Consortium. The researchers initially identified 81 DMRs associated with childhood asthma. Of these, 55 DMRs remained statistically significant after adjustment for cell types. The most important genes (passing statistical significance level) detected in a pathway analysis of traditional asthma pathways were *IL13*, *RUNX3*, and specific genes relevant to T lymphocytes (*TIGIT*). The study was conducted on a relatively small sample size of children reporting to be of either African American or Hispanic ancestry, as the researchers primarily targeted to study asthma in families living in poverty.

Devries et al.[37] more recently conducted an EWAS in children to detect if DMRs at birth would be associated with later asthma in childhood. The study used data from the Infant Immune Study (IIS) cohort and results were replicated in the Manchester Asthma and Allergy Study and the Childhood Origins of Asthma Study. Overall, the researchers identified 589 DMRs, of which the researchers focused primarily on the *SMAD3* gene as it was most connected to other genes within a gene network of molecular interactions between DMR-associated genes. The researchers further investigated the methylation sites related to this gene in 60 neonates from the IIS cohort, but the difference in the methylation site at the gene promoter did not reach statistical significance between asthmatics and non—asthmatics in this replication dataset. However, in the sub-population of children of asthmatic mothers, DNA methylation significantly increased in asthmatic children compared to non-asthmatics. Asthmatic children of non-asthmatic mothers did not differ in their *SMAD3* methylation levels compared to non-asthmatic children. The study had a limited sample size and did not control for the child sex.

Arathimos et al.[39] in 2017, applied Mendelian randomization on data obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC), which followed children from birth until the age of 7.5 and 16.5 years where asthma status was identified using questionnaires. In total, 302

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differentially methylated CpGs were associated with asthma in the 7.5 years age group (149 cases vs 632 controls) after adjustment for cell counts. Cg04983687, a CpG site mapped to *ZFPM1* had the strongest association amongst the CpGs. At 16.5 years (194 cases vs 554 controls), only 2 CpG sites were associated with asthma after adjustment for cell counts: cg17676835 annotated to *AP2A2* and cg10159529 annotated to *IL5RA*. The researchers also evaluated two longitudinal models of EWAS to establish the direction of association between DNA methylation and asthma status in childhood and adolescence. In this analysis, 4 CpGs were associated with asthma, however, the associations did not remain significant after adjustment for cell counts and multiple testing.[39] One limitation in the study was the use of questionnaires to assess asthma, subjecting the outcome to potential misreporting.

A large EWAS by Xu et al. in 2018[32], was initially conducted on 392 asthmatic children vs 1156 controls from four European cohorts, within the Mechanisms of the Development of ALLergy (MeDALL) project. Results were replicated in children (0-16 years) selected from seven other MeDALL cohorts and a Canadian cohort comprising both adults and children (1-79 years).[46] Fourteen methylation sites in total were replicated and found to be associated (after Bonferroni correction for multiple testing) with asthma in the different age groups. However, no associations were found for methylation levels at these 14 CpGs at birth (cord blood) and later childhood asthma, and the authors noted that their results are indicative on postnatal environmental effects at these CpGs. The strongest associated CpG, annotated to *DICER1* gene, is involved in the generation of microRNAs, potentially a very important link to the post-translational level of gene expression. *DICER1* deficient mice have shown impaired T-cell development and an increase in interferon- γ expression, an important cytokine of Th1 pathway.[47] The study also highlighted that eosinophils seem to be the main driver of differential DNA methylation (on average 18% difference in methylation levels between asthmatics and non-asthmatics in eosinophils) seen in whole blood samples, which is likely to have a significant effect on asthma phenotype. As the study showed a major difference in eosinophil methylation between asthmatics and controls, the authors also

suggested that eosinophil methylation could be used in the future as a rapid diagnostic test for asthma.

Reese et al. published the latest meta-analysis of DNA methylation and asthma using data from the Pregnancy And Childhood Epigenetics (PACE) consortium.[34] PACE is an international consortium of cohorts with methylation data at birth or in childhood.[48] The authors performed two independent analyses: a prospective analysis in newborns and a cross sectional analysis in school-age children. In newborns (668 cases vs 2904 controls), 9 CpGs and 35 regions were differentially methylated with an FDR <0.05. The annotated genes were different from the earlier work by De Vries et al. on newborns.[49] In school-age children (631 cases vs 2231 controls), 179 CpGs were identified to be differentially methylated, 173 of which showed decreased methylation. Thirty six DMRs were identified. The authors reported that of the FDR significant CpGs, 31(17%) fell within a significant DMR, and 21 (58%) DMRs contained at least one significant CpG.[34] Upon comparison, no CpGs significant for newborns were significant in childhood. Six CpGs significant for asthma in childhood were at least nominally significant for newborns, two of which had the same direction of effect. The authors sought to replicate the childhood cohort findings in both nasal epithelium and purified eosinophils. Differential methylation in the 179 significant CpGs was compared to findings from two studies in Dutch and African-American children cohorts, testing methylation using nasal epithelium.[50, 51] Twenty and 128 (out of 179) had nominal significance and the same direction of effect in the two cohorts, respectively. In purified eosinophils, 148 CpGs were nominally significant.[37] When compared to the meta-analysis of Xu et al., 7 CpGs reached genome-wide significance in both studies, and 13 (out of 14 reported in Xu et al.) were nominally significant and had the same direction of association. Interestingly, the study reported two significant CpGs (cg01310029 and cg10159529) in the *IL5RA* gene, the target for benralizumab, an anti-interleukin-5 receptor α monoclonal antibody. This study is unique in its comparison between methylation in newborns versus young children, allowing a deeper understanding of possible environmental effects on asthma development. A limitation of the study is the possible heterogeneity due to diversity in

ancestry and age ranges (two cohorts of older adolescents were included in the childhood analysis as well).

A study by Lund et al. in 2018 also examined epigenomic changes related to rhinovirus induced wheezing and their association with asthma in 48 children.[52] Rhinovirus positive children aged 3-23 months were recruited and followed until the age of four. The strongest association was annotated to *SMAD3* gene for atopic asthma and to prostaglandin-H2 D-isomerase (*PTGDS*) gene for non-atopic asthma.[52] These changes were still present in an additional follow up at 8 years of age. However, the authors detected no changes in gene expression due to these differences.

Studies conducted on nasal epithelial cells:

Three studies investigating the genome-wide methylation of nasal epithelial cells in asthmatics have been published: the first by Yang et al. in 2017[53] and two studies in 2018 by Forno et al. and Zhang et al., respectively [33, 54]

Yang et al. compared DNA methylation and gene expression in nasal epithelial samples from 36 African American children vs 36 healthy controls. Two independent cohorts of 30 asthmatic African American children and 12 asthmatic adults were used for validation and replication. The study identified 119 DMRs annotated to 118 genes, and 109 CpGs associated with 107 genes.[53] The associated genes were mainly related to immunity, components of the extra-cellular matrix, airway obstruction and obesity. Histamine N-methyltransferase (*HNMT*) and arachidonate 15-lipoxygenase (*ALOX15*), two genes previously linked to asthma [55, 56], were also among the significant genes associated with differential methylation. The authors also compared their results with their earlier work on differential methylation in PBMC. [41] Three DMRs (associated with eQTLs) overlapped between the two studies. In their validation cohort of 12 Caucasian adults, 36% of the significant genes in African American children had at least one genome wide significant region in the Caucasian adult analysis. Ten loci selected for validation with pyrosequencing in the validation cohort were significant compared to controls.

Zhang et al. studied differential methylation in 29 African American sibling pairs discordant for asthma.[54] Six CpGs were identified as differentially methylated, but only achieved nominal significance.[54] Only one CpG, cg14830002, was externally replicated in two other asthma cohorts, and the authors validated three of the CpGs in a larger cohort of 54 sibling pairs using pyrosequencing.[54]

Forno et al. studied differential methylation in a cohort of 483 Puerto Rican children, using data from the (EVA-PR) study.[33] The study initially focused on the differential methylation in atopic versus non-atopic patients (8664 differentially methylated CpGs by atopy) with a subgroup analysis of subjects with atopic asthma compared to subjects without atopy or asthma (7104 differentially methylated CpGs, indicating a large overlap between atopy and asthma effects on the methylome). In those patients, the authors reported that there was substantial overlap between the genes detected in the atopy vs non-atopy analysis and the asthma analysis. The study reported 30 CpGs at the top loci in their asthma analysis, including genes previously linked to asthma like *CDHR3*. [57] The authors compared their findings to the previous mentioned study by Yang et al., 29 out of their 30 top CpGs were genome-wide significant in both studies.[53] The authors additionally put part of their results (i.e. 500 CpGs) into a machine learning model to retrieve a panel of 30 CpGs to classify asthmatic patients vs non-asthmatics. [33] With a 30 CpGs panel, they achieved an accuracy of 88% in classifying asthmatic patients.[33]

A unique genome wide assessment of genetic epigenetic interactions using whole blood samples was published by Kogan et al. in early 2019.[58] The authors applied a novel statistical gene centric approach (canonical analysis of set interactions) to test genetic and epigenetics interactions between SNPs and CpGs in the same genomic region in cases versus controls. Methylation and genetic data from 576 patients available in the BRIDGE consortium was used.[59] Twelve genes were considered statistically significant and additional analysis revealed 19 significant pairs related to 4 of the 12 genomic regions. The model revealed complex associations between methylation, genetic variation and asthma incidence. For instance, methylation level of a cg16688533 was higher in

asthmatics with an AA allele of rs9969426 –a SNP in stanniocalcin-1 (*STC1*) gene- compared to asthmatics with the GG allele who had lower methylation levels.[58] Despite the innovative approach, the study was limited by their definition of genomic regions where interaction was tested for (only 2 kb up and downstream of each gene) therefore missing possible important interactions.

Studies conducted on airway epithelial cells:

Airway epithelial cells were used in the single study investigating histone modifications in asthma.[31] The study had a very limited sample size (n=3 asthmatics vs n=3 controls) and used chromatin immunoprecipitation (ChIP) to test for histone modifications. An increase in the acetylation of lysine 18 on histone 3 and trimethylation of lysine 9 on the same histone were reported in asthmatics as compared to non-asthmatics. However, the small sample size and overall high risk of bias limit the impact of these findings.

The only published study testing for differential methylation in adults using airway epithelial cells was by Nicodemus-Johnson et al. in 2016.[42] This study included both genetics and epigenetics, and explored the differentially methylated sites in an adult cohort of asthmatics and non-asthmatics and related the different sites to gene expression. The researchers then used Mendelian randomization to explore the relation between the different alleles, methylation sites and gene expression. The study was only conducted on 115 subjects (74 asthmatics and 41 controls). At a false discovery rate of 5%, there were 40,892 differentially methylated sites, including 22,216 hyper methylated and 18,676 hypomethylated sites in asthmatics compared to controls. When the methylated regions were co-related with genetic variants from two large asthma GWAS, several eQTL SNPs were found to be co-related with both methylation and gene expression for their genes (i.e., both eQTL and meQTL). Most importantly for future understanding of asthma, this finding was true for SNPs in the 17q12-21 locus. For example, an eQTL SNP for *ORMDL3*, rs2517955, was also a significant meQTLs for a nearby CpG site (cg05616858). Methylation at this site was co-related only to *ORMDL3*.

Studies conducted on buccal cells:

Only one included study was conducted using buccal cell samples.[30] The study was conducted on discordant monozygotic twins (MZ), a design believed to be important for epigenetic studies.[60] The study was conducted longitudinally on a cohort of MZ, recruited from the E-Risk study. Asthma outcomes were assessed using an event history calendar at ages 5 and 10, and an interview based on the European Community Respiratory Health Survey (ECRHS) II screening questionnaire.[61] The study tested for differential methylation in buccal cells in ages 10 and 18, aiming to identify the methylation sites related to the development of asthma and its persistence. However, no differentially methylated sites were statistically significant after adjustment for multiple testing, despite several nominally significant CpGs. Enrichment analysis of nominally significant CpGs identified 142 enriched genes, including genes linked to IL2 and interferon production.

Network Interaction Analysis:

To visualize differences between findings of studies conducted on different cell types, we performed gene interaction network and pathway analysis using GeneMania [62] entering all top-hits mentioned in the reported studies using nasal epithelial cells (57 genes)(Figure 2), and again for all studies using blood cells (43 genes).(Figure 3)

5- Discussion

We identified sixteen EWAS studies, twelve studies conducted on children and four on adults. The researchers identified 419 CpGs associated with asthma in children, while in the adult methylation study a surprisingly high number of 40,892 differentially methylated loci were identified.

To our best knowledge this is the first systematic review of EWAS in asthma. Differential methylation studies appear to be published more frequently in childhood onset asthma, as can be observed by the number of reports found in our search. Lower methylation levels in specific inflammatory gene clusters was generally shown to correlate in genome-wide studies with increased expression of

inflammatory mediators in asthma. For example, Xu et al.[32], identified three clusters with genes related to eosinophils, CD4 and CD8 T-cells. The authors noted that the lower methylation levels at those sites were associated with an expression profile suggesting reduced activity of naive T cells and increased activity of effector/memory CD8 T cells. Arathimos et al. compared their results to the EWAS by Yang et al., highlighting that both studies found associations with CpG sites of the *ACOT7* and *IL13* genes. Differences in study power and discovered methylation sites were also noted between the two studies, possibly due to differences in samples (peripheral blood mononuclear cells vs whole blood).[39] Reese et al. also reported differential methylation in genes related to immune functions. Associations annotated to the *ACOT7* gene were also reported, highlighting the potential importance of this gene in the inflammatory pathway. Thirteen CpGs detected in Xu et al. were replicated in the study by Reese et al. (7 genome-wide significant, 6 nominally significant).[34] CpGs annotated to the *IL5RA* gene, a gene where differential methylation was first associated with asthma by Arathimos et al., were also replicated by Reese et al.[34, 39]

We compared the available data from CpGs in three blood studies (Arathimos et al., Reese et al., Xu et al.)[32, 34, 39] and two nasal epithelium studies (Yang et al. and Forno et al.).[33, 35] In blood studies, 41 CpGs were replicated in at least two studies, cg01901579(*DICER1*) and cg03131767(*ABCB9*) were replicated in three studies. In the two nasal epithelium studies, 28 out of the top 30 CpGs from Forno et al. were replicated in Yang et al. [33, 35]

Our results outline several epigenetic mechanisms involved in asthma development. T-cells are very important in the inflammatory process in asthma. Two asthma endotypes which differ in several aspects of the disease, have been described based on involvement of T-cells: Th2 high and Th2 low.[63] Th2 high is characterized by elevated eosinophils due to activity of interleukins. The gene clusters detected as discussed above are responsible for expression of related genes and may help explain part of the phenotypic variability seen between the two endotypes. Future work on those methylation sites may help develop methods to predict phenotypes and adjust therapy based on differential methylation. As an example, *FCER2* differential methylation as outlined by Rastogi et al.,

may be of particular importance as genetic polymorphisms in this gene have previously been linked to both incidence of asthma and increased risk of exacerbations in children, despite ICS use.[64-67] Such results, if consistent in specific asthma phenotypes or ages, may be used in future precision medicine studies where information about methylation levels and gene variants may guide therapeutic decisions in different asthmatic populations. This hypothesis is also backed up by the EWAS by Rastogi et al.[40] who conducted an EWAS on a small sample size and aimed to investigate a specific asthma phenotype (obese asthmatic children). Although this limits the generalizability of their findings to a general asthmatic population, still some of their findings were consistent with other studies as regions with lower methylation levels in asthma were in shared immune pathways (T-cell signaling and macrophage activation).

There is a big challenge in validating epigenetic findings and establishing their clinical utility. An interesting approach was employed by Forno et al. by using machine learning to select CpGs predicting atopy or atopic asthma. The performance was better compared to two negative controls (30 randomly selected CpGs and a model using only demographic or questionnaire variables). This interdisciplinary approach may provide an important method for future research in the clinical utility of epigenetic markers. Similar studies may also help evaluate differential methylation between different asthma phenotypes and determine the potential benefits of epigenetic variations in phenotyping asthmatic patients for a more precise approach in treatment.

The current epigenetic studies do not fully provide information about the direction of genetic variation effects on DNA methylation, and their combined effect on the level of protein. For example, it must be acknowledged that although differential methylation may exist, the resulting phenotype observed may also have been caused by genetic variation, with methylation being a result of this variation rather than the cause of the phenotypical differences. An example of such a methylation site is the cg05616858 linked to an eQTL SNP, rs2517955, and annotated to the ORM DL3 gene. Kogan et al. also analyzed interactions between SNPs and CpGs and found 12 genes

in interaction with the methylome, possibly showing a joint effect of genetics and epigenetics in asthma pathogenesis.[58]

Current studies have not fully revealed the complex relationship between environmental factors, the epigenome and the incidence of asthma. Exposure to allergens or environmental factors with established effects on asthma incidence, such as smoking, has already been shown to alter the epigenome. Smoking, for example has known effects on asthma[69], maternal smoking has been linked to asthma incidence in childhood[70], even on a transgenerational level.[71] Maternal smoking has also been linked to reduced inhaled corticosteroid efficacy in the child.[72] Epigenetically, cigarette smoking was linked to differential methylation in 15 sites across the adult genome.[73] Hundreds of CpGs differentially methylated in newborns, have been linked to maternal smoking.[74-76] CpGs in ESR1 and IL32 genes, previously linked to asthma, were differentially methylated in an EWAS study by Joubert et al. investigating differential methylation in newborns due to maternal smoking.[76] These studies suggest that smoking effects on asthma may be mediated through epigenetic mechanisms. Although the link between environmental exposure and epigenetic mechanisms associated with asthma appears to be strong, no studies to date have actually shown evidence that environmental factors such as smoking increase the risk of asthma through epigenetic changes. For atopic dermatitis, Wang et al. have investigated the effects of prenatal smoke exposure on the methylation status as well as the methylation status associated with disease and found overlapping CpGs within the same study.[77] The current studies also did not take into account the possible confounding effects of drugs on the observed differential methylation in asthmatics, e.g.: inhaled corticosteroids.[78]

The only methylation study comparing asthmatics to healthy controls conducted in adults[42] used airway epithelial cells and reported a very large number of DMRs. This could either be due to technical limitations (more false positive findings due to a small number of subjects and no replication) or due to a difference in methylome associated with asthma between adults and

children, as all other studies were conducted on children. The use of epithelial airway cells may have also contributed to the difference. The use of different type of cells indeed impacted methylation results. Yang et al. showed only a small degree of overlap between their nasal epithelial results and their previous results in PBMC.[35, 36] Conversely, their results were consistent with more recent findings from Forno et al.[33] In comparison, 13 CpGs of 14 detected in Xu et al. were replicated in Reese et al.'s results, both studies were conducted on blood samples.[34, 79] A comparison between DMRs associated with asthma in PBMCs vs in epithelial cells also highlights this difference (36 DMRs in PBMCs in Reese et al. vs 7104 DMRs in nasal epithelial cells in Forno et al.).[33, 34] Age effects are also a possible cause of this difference. However, interpretation of the different numbers of retrieved methylation sites is further complicated by difficulties in replication. For example, Arathimos et al. found only 2 CpG sites associated with asthma in their analysis of patients at 16 years as compared to 302 CpG sites in asthma diagnosed at 7.5 years.[39] In contrast, the initial smaller number of 14 CpG sites associated with childhood asthma by Xu et al. were all replicated in a cohort comprising adults.[32] The best age category to investigate differential methylation thus remains unclear.

Forno et al. compared their findings of DNA methylation in nasal epithelium to Xu et al. and only 3 CpGs significant in whole blood were genome-wide significant in both analyses. Using whole blood samples in epigenetic studies with blood cells used as a surrogate to understand methylation in lung airway cells is a double-edged blade. Blood may be useful in detecting differential methylation in important white blood cell components involved in asthmatic immune pathways like eosinophils (especially Th2 T-cells) and neutrophils, and their relation to the development of varying asthma phenotypes. On the other hand, it may miss important features of the target airway cells and contribution of their methylome and gene expression to varying asthma phenotypes as well. As pointed out by Yang et al.[47], nasal epithelial cells are directly exposed to the environment, and therefore may affect the disease pathophysiology differently. They also represent a pure cell

population and changes may therefore be less diluted by the mixture of cell types like in whole blood.

We performed a network interaction analysis to illustrate pathway interaction between several genes detected in the studies and to compare results from studies conducted on different types of cells. The networks show the different pattern between the differentially methylated genes in the two types of cells. The nasal epithelial network reveals a co-expression network between several genes detected (most importantly between the POSTN gene and COL5A1, COL5A2, COL1A2 and COL4A1). The blood results' network revealed pathway interactions among two new genes, AGO2 and CBFP, linked to DICER1, SMAD3 and RUNX3 genes. The detected pathways and interactions were mostly linked to miRNA production and processing, further highlighting the role of gene expression in asthma inflammatory pathways and suggesting possible roles for noncoding RNA in the process.

Analysis in the Reactome database revealed involvement of signaling pathways (e.g: IL13 signaling pathway, cytokine signaling and IL4 signaling) which were the most commonly detected in the studied gene set resulting from blood cell studies (13 genes out of 43).[80] In comparison, the nasal epithelium network shows that the detected genes were mostly involved in extracellular matrix organization, collagen degradation and cellular responses to external stimuli. This analysis clearly shows that the detected inflammatory pathways differ between blood cells and nasal epithelial cells.

Larger studies in purified cell populations may reveal other important genes or pathophysiological pathways involved in asthma. **These differences in tissue-specific methylation highlight both the strong contribution of tissue types to differential methylation and the complexity of EWAS results interpretation.** For example, as outlined by Guo et al., functional investigation of associated CpGs using animal and cell models in a complex and heterogeneous disease (obesity) proved difficult.[81] Integrative analysis has been consequently suggested as an approach to correlate differential methylation with matching, cell and tissue type specific gene expression data.[81-83] Future studies integrating cell and tissue specific gene expression analyses are recommended for an adequate interpretation of the epigenetic effects on the biological processes affecting the

disease.[20]

Although our search strategy was not limited to DNA methylation, all except one of the included studies focused solely on methylation as an epigenetic mechanism. The study investigating histone modifications had a limited sample size and a high risk of bias. DNA methylation is the most extensively studied epigenetic mechanism most likely due to technical reasons.[84] However, It should be noted that other epigenetic techniques (e.g.: histone modifications and miRNA) may be very important in asthma as well.[85-87]

All EWAS reviewed were limited by the current platforms and techniques used in respective study. The HumanMethylation450k BeadChip used in eleven of the sixteen studies, covers only 2% of the human DNA, which nevertheless accounts for 96% of all known CpG-islands. However, it only covers about 1.6% of all CpGs in the human genome, which means that many (unknown) CpGs and genes are not or only barely covered by the array.[88] Furthermore, the chip technology relies on hybridization of genomic fragments to probes on the chip. However, certain genomic factors may compromise the ability to measure methylation using the array such as SNPs, small insertions and deletions, repetitive DNA, and regions with reduced genomic complexity.[88] Annotation represents another important technical limitation since current annotation approaches use proximity to nearest gene for annotation (e.g: Reese et al.[34]). However, new references for the human genome e.g: (GRCh38) may cause differences in annotation compared to old references. Heterogeneity between studies could partly be explained by different parameters used to define and annotate CpGs. For example, Xu et al. used a tool (GREAT) that incorporates distal binding sites while most other studies annotate CpGs to the nearest gene within 10 Mb.[89] The annotation to certain genes may also differ according to the length of the CpG.[90] More harmonization among CpGs in terms of length, GC content and detection methods may help avoid this discrepancy.

Ten of the identified studies were conducted on a relatively small number of subjects and only four studies reported power calculations. This is a particular limitation in Rastogi et al. as the study was conducted on four groups each consisting of only 8 subjects. The statistical analysis had to be

adjusted and no false discovery rate was used as a result of the small sample size. The absence of a validation cohort and limited sample sizes may have also greatly affected the ability to replicate and validate the results. Although it may be difficult to estimate power and sample size for epigenetic studies, an example of power analysis has been published by Tsai and Bell, concluding that a sample size of 112 cases and 112 controls may be appropriate for epigenetic case-control studies.[91]

A final quality concern would be the adjustment based on differential numbers of white blood cells in blood studies which has proven to be an important quality measure in the studies assessed, as association levels in several studies differed significantly when adjusted by cell types. Three studies reported markedly lower number of CpGs/DMRs with adjustment for cell type.[32, 39, 41]

6- Conclusion

Our systematic review has shown that the current knowledge of differential methylation and its contribution to asthma is still limited. Sixteen epigenome wide studies were identified in the literature, and only six studies were conducted on a large sample size. Large numbers of differentially methylated regions have been identified in asthmatics through the different studies, and the methylated regions were generally related to genes involved in inflammatory pathways and immune functions. The first EWAS conducted on asthmatics was in 2012 with a growing interest in the field and a more rapid succession of papers very recently. The general findings in the different EWAS suggest that DNA methylation may be directing the expression of several important genes in the inflammatory pathway (e.g.: cytokine signaling pathways, collagen degradation pathways, and cellular responses to external stimuli). Future well-design studies will likely identify additional methylation sites associated with asthma in children and adults, and expand knowledge on the complex interplay between genetics and environmental factors in asthma pathogenesis.

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References

1. Rosa MJ, Lee AG, Wright RJ. Evidence establishing a link between prenatal and early-life stress and asthma development. *Curr Opin Allergy Clin Immunol*. 2018;18(2):148-58.
2. Ferrante G, La Grutta S. The Burden of Pediatric Asthma. *Front Pediatr*. 2018;6:186.
3. WHO. Bronchial asthma Fact sheet N°206 [Available from: <http://www.who.int/mediacentre/factsheets/fs206/en/>].
4. Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res*. 2001;4(2):81-93.
5. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461(7265):747-53.
6. Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet*. 2011;43(11):1082-90.
7. Vicente CT, Revez JA, Ferreira MAR. Lessons from ten years of genome-wide association studies of asthma. *Clin Transl Immunology*. 2017;6(12):e165.
8. Yang IV, Lozupone CA, Schwartz DA. The environment, epigenome, and asthma. *J Allergy Clin Immunol*. 2017;140(1):14-23.
9. DeVries A, Vercelli D. Epigenetic Mechanisms in Asthma. *Ann Am Thorac Soc*. 2016;13 Suppl 1:S48-50.
10. Yang IV, Schwartz DA. Epigenetic mechanisms and the development of asthma. *J Allergy Clin Immunol*. 2012;130(6):1243-55.
11. Liu L, Li Y, Tollefsbol TO. Gene-environment interactions and epigenetic basis of human diseases. *Curr Issues Mol Biol*. 2008;10(1-2):25-36.
12. Gref A, Merid SK, Gruziova O, Ballereau S, Becker A, Bellander T, et al. Genome-Wide Interaction Analysis of Air Pollution Exposure and Childhood Asthma with Functional Follow-up. *Am J Respir Crit Care Med*. 2017;195(10):1373-83.
13. Flanagan JM. Epigenome-wide association studies (EWAS): past, present, and future. *Methods Mol Biol*. 2015;1238:51-63.
14. Neidhart M. Chapter 1 - DNA Methylation – Introduction. In: Neidhart M, editor. *DNA Methylation and Complex Human Disease*. Oxford: Academic Press; 2016. p. 1-8.
15. Ziller MJ, Gu H, Muller F, Donaghey J, Tsai LT, Kohlbacher O, et al. Charting a dynamic DNA methylation landscape of the human genome. *Nature*. 2013;500(7463):477-81.
16. Jang HS, Shin WJ, Lee JE, Do JT. CpG and Non-CpG Methylation in Epigenetic Gene Regulation and Brain Function. *Genes (Basel)*. 2017;8(6).
17. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. *Nat Rev Genet*. 2011;12(8):529-41.
18. Sugawara H, Murata Y, Ikegame T, Sawamura R, Shimanaga S, Takeoka Y, et al. DNA methylation analyses of the candidate genes identified by a methylome-wide association study revealed common epigenetic alterations in schizophrenia and bipolar disorder. *Psychiatry Clin Neurosci*. 2018;72(4):245-54.

19. Kurdyukov S, Bullock M. DNA Methylation Analysis: Choosing the Right Method. *Biology* (Basel). 2016;5(1).
20. Sant KE, Nahar MS, Dolinoy DC. DNA methylation screening and analysis. *Methods Mol Biol*. 2012;889:385-406.
21. Laird PW. Principles and challenges of genome-wide DNA methylation analysis. *Nature Reviews Genetics*. 2010;11:191.
22. Pidsley R, Zotenko E, Peters TJ, Lawrence MG, Risbridger GP, Molloy P, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biol*. 2016;17(1):208.
23. van Dongen J, Ehli EA, Jansen R, van Beijsterveldt CEM, Willemsen G, Hottenga JJ, et al. Genome-wide analysis of DNA methylation in buccal cells: a study of monozygotic twins and mQTLs. *Epigenetics Chromatin*. 2018;11(1):54.
24. Gujar H, Liang JW, Wong NC, Mozhui K. Profiling DNA methylation differences between inbred mouse strains on the Illumina Human Infinium MethylationEPIC microarray. *PLoS One*. 2018;13(3):e0193496.
25. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol*. 2009;62(10):1006-12.
26. van Vugt LJ, van den Reek J, Coenen MJH, de Jong E. A systematic review of pharmacogenetic studies on the response to biologics in patients with psoriasis. *Br J Dermatol*. 2018;178(1):86-94.
27. Gao X, Jia M, Zhang Y, Breitling LP, Brenner H. DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies. *Clin Epigenetics*. 2015;7:113.
28. MILL J. ERROR, BIAS, PROBLEMS AND PITFALLS IN EPIGENETIC EPIDEMIOLOGY. 2012.
29. GA Wells BS, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
30. Murphy TM, Wong CC, Arseneault L, Burrage J, Macdonald R, Hannon E, et al. Methyloic markers of persistent childhood asthma: a longitudinal study of asthma-discordant monozygotic twins. *Clin Epigenetics*. 2015;7:130.
31. Stefanowicz D, Lee JY, Lee K, Shaheen F, Koo HK, Booth S, et al. Elevated H3K18 acetylation in airway epithelial cells of asthmatic subjects. *Respir Res*. 2015;16:95.
32. Xu CJ, Soderhall C, Bustamante M, Baiz N, Gruziova O, Gehring U, et al. DNA methylation in childhood asthma: an epigenome-wide meta-analysis. *Lancet Respir Med*. 2018;6(5):379-88.
33. Forno E, Wang T, Qi C, Yan Q, Xu CJ, Boutaoui N, et al. DNA methylation in nasal epithelium, atopy, and atopic asthma in children: a genome-wide study. *Lancet Respir Med*. 2018.
34. Reese SE, Xu CJ, den Dekker HT, Lee MK, Sikdar S, Ruiz-Arenas C, et al. Epigenome-wide Meta-analysis of DNA Methylation and Childhood Asthma. *J Allergy Clin Immunol*. 2018.
35. Yang IV, Pedersen BS, Liu AH, O'Connor GT, Pillai D, Kattan M, et al. The nasal methylome and childhood atopic asthma. *J Allergy Clin Immunol*. 2017;139(5):1478-88.
36. Yang IV, Pedersen BS, Liu A, O'Connor GT, Teach SJ, Kattan M, et al. DNA methylation and childhood asthma in the inner city. *J Allergy Clin Immunol*. 2015;136(1):69-80.
37. DeVries A, Wlasiuk G, Miller SJ, Bosco A, Stern DA, Lohman IC, et al. Epigenome-wide analysis links SMAD3 methylation at birth to asthma in children of asthmatic mothers. *J Allergy Clin Immunol*. 2017;140(2):534-42.
38. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13:86.
39. Arathimos R, Suderman M, Sharp GC, Burrows K, Granell R, Tilling K, et al. Epigenome-wide association study of asthma and wheeze in childhood and adolescence. *Clin Epigenetics*. 2017;9:112.

40. Rastogi D, Suzuki M, Grealley JM. Differential epigenome-wide DNA methylation patterns in childhood obesity-associated asthma. *Sci Rep*. 2013;3:2164.
41. Yang IV, Pedersen BS, Liu A, O'Connor GT, Teach SJ, Kattan M, et al. DNA methylation and childhood asthma in the inner city. *J Allergy Clin Immunol*. 2015;136(1):69-80.
42. Nicodemus-Johnson J, Myers RA, Sakabe NJ, Sobreira DR, Hogarth DK, Naureckas ET, et al. DNA methylation in lung cells is associated with asthma endotypes and genetic risk. *JCI Insight*. 2016;1(20):e90151.
43. Stefanowicz D, Hackett TL, Garmaroudi FS, Gunther OP, Neumann S, Sutanto EN, et al. DNA methylation profiles of airway epithelial cells and PBMCs from healthy, atopic and asthmatic children. *PLoS One*. 2012;7(9):e44213.
44. Rastogi D, Canfield SM, Andrade A, Isasi CR, Hall CB, Rubinstein A, et al. Obesity-associated asthma in children: a distinct entity. *Chest*. 2012;141(4):895-905.
45. Chan MA, Gigliotti NM, Aubin BG, Rosenwasser LJ. FCER2 (CD23) asthma-related single nucleotide polymorphisms yields increased IgE binding and Egr-1 expression in human B cells. *Am J Respir Cell Mol Biol*. 2014;50(2):263-9.
46. Laprise C. The Saguenay-Lac-Saint-Jean asthma familial collection: the genetics of asthma in a young founder population. *Genes Immun*. 2014;15(4):247-55.
47. Muljo SA, Ansel KM, Kanellopoulou C, Livingston DM, Rao A, Rajewsky K. Aberrant T cell differentiation in the absence of Dicer. *J Exp Med*. 2005;202(2):261-9.
48. Felix JF, Joubert BR, Baccarelli AA, Sharp GC, Almqvist C, Annesi-Maesano I, et al. Cohort Profile: Pregnancy And Childhood Epigenetics (PACE) Consortium. *Int J Epidemiol*. 2018;47(1):22-3u.
49. DeVries A, Wlasiuk G, Miller SJ, Bosco A, Stern DA, Lohman IC, et al. Epigenome-wide analysis links SMAD3 methylation at birth to asthma in children of asthmatic mothers. *J Allergy Clin Immunol*. 2017;140(2):534-42.
50. Wijga AH, Kerkhof M, Gehring U, de Jongste JC, Postma DS, Aalberse RC, et al. Cohort profile: the prevention and incidence of asthma and mite allergy (PIAMA) birth cohort. *Int J Epidemiol*. 2014;43(2):527-35.
51. Busse WW. The National Institutes of Allergy and Infectious Diseases networks on asthma in inner-city children: an approach to improved care. *J Allergy Clin Immunol*. 2010;125(3):529-37; quiz 38-9.
52. Lund RJ, Osmala M, Malonzo M, Lukkarinen M, Leino A, Salmi J, et al. Atopic asthma after rhinovirus-induced wheezing is associated with DNA methylation change in the SMAD3 gene promoter. *Allergy*. 2018;73(8):1735-40.
53. Yang IV, Pedersen BS, Liu AH, O'Connor GT, Pillai D, Kattan M, et al. The nasal methylome and childhood atopic asthma. *J Allergy Clin Immunol*. 2017;139(5):1478-88.
54. Zhang X, Biagini Myers JM, Burleson JD, Ulm A, Bryan KS, Chen X, et al. Nasal DNA methylation is associated with childhood asthma. *Epigenomics*. 2018;10(5):629-41.
55. Song YS, Yang EM, Kim SH, Jin HJ, Park HS. Effect of genetic polymorphism of ALOX15 on aspirin-exacerbated respiratory disease. *Int Arch Allergy Immunol*. 2012;159(2):157-61.
56. Choy DF, Modrek B, Abbas AR, Kummerfeld S, Clark HF, Wu LC, et al. Gene expression patterns of Th2 inflammation and intercellular communication in asthmatic airways. *J Immunol*. 2011;186(3):1861-9.
57. Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. *Nat Genet*. 2014;46(1):51-5.
58. Kogan V, Millstein J, London SJ, Ober C, White SR, Naureckas ET, et al. Genetic-Epigenetic Interactions in Asthma Revealed by a Genome-Wide Gene-Centric Search. *Hum Hered*. 2019;83(3):130-52.
59. Croteau-Chonka DC, Qiu W, Martinez FD, Strunk RC, Lemanske RF, Jr., Liu AH, et al. Gene Expression Profiling in Blood Provides Reproducible Molecular Insights into Asthma Control. *Am J Respir Crit Care Med*. 2017;195(2):179-88.

60. Castillo-Fernandez JE, Spector TD, Bell JT. Epigenetics of discordant monozygotic twins: implications for disease. *Genome Med.* 2014;6(7):60.
61. Knox J, Jarvis D, Walter EH, Committee EIS. The European Community Respiratory Health Survey II. *Eur Respir J.* 2003;21(3):556; author reply
62. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* 2010;38(Web Server issue):W214-20.
63. Dunican EM, Fahy JV. Asthma and corticosteroids: time for a more precise approach to treatment. *Eur Respir J.* 2017;49(6).
64. Tantisira KG, Silverman ES, Mariani TJ, Xu J, Richter BG, Klanderman BJ, et al. FCER2: a pharmacogenetic basis for severe exacerbations in children with asthma. *J Allergy Clin Immunol.* 2007;120(6):1285-91.
65. Koster ES, Maitland-van der Zee AH, Tavendale R, Mukhopadhyay S, Vijverberg SJ, Raaijmakers JA, et al. FCER2 T2206C variant associated with chronic symptoms and exacerbations in steroid-treated asthmatic children. *Allergy.* 2011;66(12):1546-52.
66. Lima JJ, Blake KV, Tantisira KG, Weiss ST. Pharmacogenetics of asthma. *Curr Opin Pulm Med.* 2009;15(1):57-62.
67. Farzan N, Vijverberg SJ, Arets HG, Raaijmakers JA, Maitland-van der Zee AH. Pharmacogenomics of inhaled corticosteroids and leukotriene modifiers: a systematic review. *Clin Exp Allergy.* 2017;47(2):271-93.
68. Sagoo GS, Little J, Higgins JP. Systematic reviews of genetic association studies. *Human Genome Epidemiology Network. PLoS Med.* 2009;6(3):e28.
69. Cerveri I, Cazzoletti L, Corsico AG, Marcon A, Niniano R, Grosso A, et al. The impact of cigarette smoking on asthma: a population-based international cohort study. *Int Arch Allergy Immunol.* 2012;158(2):175-83.
70. Zacharasiewicz A. Maternal smoking in pregnancy and its influence on childhood asthma. *ERJ Open Res.* 2016;2(3).
71. Magnus MC, Haberg SE, Karlstad O, Nafstad P, London SJ, Nystad W. Grandmother's smoking when pregnant with the mother and asthma in the grandchild: the Norwegian Mother and Child Cohort Study. *Thorax.* 2015;70(3):237-43.
72. Cohen RT, Raby BA, Van Steen K, Fuhlbrigge AL, Celedon JC, Rosner BA, et al. In utero smoke exposure and impaired response to inhaled corticosteroids in children with asthma. *J Allergy Clin Immunol.* 2010;126(3):491-7.
73. Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SI, et al. Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. *Hum Mol Genet.* 2012;21(13):3073-82.
74. Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect.* 2012;120(10):1425-31.
75. Nicole W. Pattern of clues: evidence of distinct DNA methylation in newborns of smoking women. *Environ Health Perspect.* 2012;120(10):a402.
76. Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, et al. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. *Am J Hum Genet.* 2016;98(4):680-96.
77. Wang IJ, Chen SL, Lu TP, Chuang EY, Chen PC. Prenatal smoke exposure, DNA methylation, and childhood atopic dermatitis. *Clin Exp Allergy.* 2013;43(5):535-43.
78. Zhang X, Biagini Myers JM, Yadagiri VK, Ulm A, Chen X, Weirauch MT, et al. Nasal DNA methylation differentiates corticosteroid treatment response in pediatric asthma: A pilot study. *PLoS One.* 2017;12(10):e0186150.
79. Xu CJ, Soderhall C, Bustamante M, Baiz N, Gruziova O, Gehring U, et al. DNA methylation in childhood asthma: an epigenome-wide meta-analysis. *Lancet Respir Med.* 2018;6(5):379-88.

80. Fabregat A, Sidiropoulos K, Viteri G, Forner O, Marin-Garcia P, Arnau V, et al. Reactome pathway analysis: a high-performance in-memory approach. *BMC Bioinformatics*. 2017;18(1):142.
81. Guo Q, Zheng R, Huang J, He M, Wang Y, Guo Z, et al. Using Integrative Analysis of DNA Methylation and Gene Expression Data in Multiple Tissue Types to Prioritize Candidate Genes for Drug Development in Obesity. *Front Genet*. 2018;9:663.
82. Yoo S, Takikawa S, Geraghty P, Argmann C, Campbell J, Lin L, et al. Integrative analysis of DNA methylation and gene expression data identifies EPAS1 as a key regulator of COPD. *PLoS Genet*. 2015;11(1):e1004898.
83. Bock C. Analysing and interpreting DNA methylation data. *Nat Rev Genet*. 2012;13(10):705-19.
84. Weinhold B. Epigenetics: the science of change. *Environ Health Perspect*. 2006;114(3):A160-7.
85. Brook PO, Perry MM, Adcock IM, Durham AL. Epigenome-modifying tools in asthma. *Epigenomics*. 2015;7(6):1017-32.
86. Kidd CD, Thompson PJ, Barrett L, Baltic S. Histone Modifications and Asthma. The Interface of the Epigenetic and Genetic Landscapes. *Am J Respir Cell Mol Biol*. 2016;54(1):3-12.
87. Begin P, Nadeau KC. Epigenetic regulation of asthma and allergic disease. *Allergy Asthma Clin Immunol*. 2014;10(1):27.
88. Dedeurwaerder S, Defrance M, Calonne E, Denis H, Sotiriou C, Fuks F. Evaluation of the Infinium Methylation 450K technology. *Epigenomics-Uk*. 2011;3(6):771-84.
89. McLean CY, Bristor D, Hiller M, Clarke SL, Schaar BT, Lowe CB, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nat Biotechnol*. 2010;28(5):495-501.
90. Elango N, Yi SV. Functional relevance of CpG island length for regulation of gene expression. *Genetics*. 2011;187(4):1077-83.
91. Tsai PC, Bell JT. Power and sample size estimation for epigenome-wide association scans to detect differential DNA methylation. *Int J Epidemiol*. 2015;44(4):1429-41.
92. Melen E, Barouki R, Barry M, Boezen HM, Hoffmann B, Krauss-Etschmann S, et al. Promoting respiratory public health through epigenetics research: an ERS Environment Health Committee workshop report. *Eur Respir J*. 2018;51(4).
93. Stefanowicz D, Ullah J, Lee K, Shaheen F, Olumese E, Fishbane N, et al. Epigenetic modifying enzyme expression in asthmatic airway epithelial cells and fibroblasts. *BMC Pulm Med*. 2017;17(1):24.
94. Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. *Epigenetics*. 2014;9(9):1302-16.

Table 1: Study characteristics of included studies:

First Author, Year	Number of DMRs or CpGs detected	Age range (Discovery)	Number of subjects	Methylation method	Samples	Outcome assessment	Top gene (s) annotated	Discovery P-value
		Design						
Kogan et al. 2019[58]	12 genomic regions with CpG-SNP interaction	Adults (including 142 subjects <20 years old)	356 asthmatics 220 non-asthmatics	Illumina Infinium Human Methylation27 Bead Chip	Whole blood samples	Asthma was defined via a questionnaire by the presence of asthma symptoms, use of an inhaled bronchodilator at least twice per week, or use of a daily asthma medication for the 6 months before the screening interview.	<i>PF4, ATF3, TPRA1, HOPX, SCARNA18, STC1, OR10K1, UPK1B, LOC101928523, LHX6, CHMP4B, and LANCL1</i>	(FDR-adjusted P value <0.05)
		Cross-sectional case control						
Reese et al., 2018	Newborn : 9 CpGs, 35 DMRs Childhood: 179 CpGs, 36 DMRs	school age, defined as age 5 years	Newborn Discovery: 668 cases /2,904 non-cases Children Discovery : 631 cases	Illumina Infinium HumanMethylation 450k BeadChip	Peripheral blood mononuclear cells	Asthma was defined by doctor diagnosis of asthma and the report of at least one of the following: (a) current asthma, (b) asthma in the past year, or (c) asthma medication use in the last year.	Newborn: <i>CLNS1A, MAML2/Mir_548, GPATCH2/STATA17, SCOC/LOC100129858, AK091866, SUB1, WDR20</i> Childhood <i>ACOT7, 456 LOC100189589, IL5RA, SLC25A26/LRIG1,</i>	(FDR-adjusted P value <0.05)
		Cross-sectional cohort						

							<i>RPS6KA2, KCNH2, ZNF862/BC045757, AK096249, PRG2, 457 EVL/AX747103, KIAA0182, ZFPM1, and EPX</i>	
Xu et al., 2018[32]	14 CpG	4-5 years	Discovery: 392 cases /1156 controls Replication: 247 cases / 2,949 controls	Illumina Infinium HumanMethylation 450k BeadChips iPlex (Agena Biosciences) to validate and replicate	Whole blood	2/3 criteria present: 1) doctor diagnosis of asthma ever 2) use of asthma medication in the past 12 months and 3) wheezing/breathing difficulties in the past 12 months.	<i>SERPINC1, PROK1, KCNA10, MXD3, LMAN2, AMD1, RAPGEF1, MED27, STX3, MRPL16</i>	<i>P</i> < 1.14×10 ⁻⁷
		Cross-sectional case control						
Lund et al, 2018 [51]	17 genomic regions	Children 3-23 months	124 initially enrolled (only 48 most representative)	(RRBS)reduced representation bisulfite sequencing	Whole blood samples	Children were diagnosed to have current asthma at the 4-year follow-up visit if they met one or more of the subsequent criteria during the preceding 12 months: doctor-diagnosed asthma, regular use of doctor-prescribed corticosteroid asthma therapy, use of oral corticosteroids for asthma exacerbations, acute asthma attack relieved by repeated use of bronchodilator	<i>SMAD3, PTGDS</i>	FDR ≤0.05
		Longitudinal Cohort						
Zhang et al. 2018[54]	6 CpGs	Children 5-18 years old	29 asthma-control African American sibling pairs	Illumina Infinium HumanMethylation 450k BeadChip	Nasal epithelial cells	Asthma diagnosis was obtained from the parental report, and confirmed via the electronic medical record that the diagnosis is current.	<i>OR2B11, NLRP3, LAMA5, ATP9B</i>	<i>P</i> < 0.05, <i>q</i> values for genome-wide Significance
		Cross-sectional discordant sibling pairs						
Forno et al., 2018 [33]	30 CpGs	15(mean)	Discovery: 169 asthmatics /104 no-asthma or atopy. Validation: 72 African American children	Illumina Infinium Human Methylation 450k BeadChip	Nasal epithelial cells	Asthma was defined as a physician's diagnosis plus at least one episode of wheeze in the previous year	<i>CDHR3, GRK5, PDE6A, FBXL7, LINC00704, ANKRD31, SUCNR1,</i>	(FDR p<0.01).
		Cross-sectional cohort						

			432 16-year-old Dutch subjects				<i>PLEC, PCSK6, CAPN14, NCF2, GJA4, C22orf31</i>	
De Vries et al., 2017[37]	589 DMRs	9-year-old children	60 cases / 58 controls	Methylated CpG Island Recovery Assay (MIRA)-chip	Cord blood mononuclear cells	Physician-diagnosed, with symptoms or medication use for asthma in the past year reported at least once on the age 2, 3, 5 or 9-year questionnaires	<i>SMAD3</i>	p=0.00018
		Nested case-control						
Arathimos et al., 2017 [39]	302 CpG sites	7.5 years 16 years	149 cases / 632 controls 194 cases / 554 controls	Infinium HumanMethylation 450k BeadChip	Peripheral blood and cord blood samples	Questionnaires completed by the mothers of the study children on 7.5 and 16.5 years	<i>ZFPM1 (Top locus)</i>	(FDR-adjusted <i>P</i> value <0.05)
		Cross-sectional case control						
Yang et al., 2017[35]	119 DMRs 118 CpGs	11 years	Discovery: 32 cases / 32 controls Validation: 12 cases/ 12 controls	Illumina Infinium Human Methylation 450k BeadChip	Nasal epithelial cells	1) a physician diagnosis of asthma; 2)persistent or uncontrolled disease as defined by the National Asthma Education and Prevention Program(5); 3) physiologic evidence of asthma (FEV1 < 85% predicted or FEV1/FVC ratio < 85%; and bronchodilator responsiveness (≥ 12%) or PC20 < 8 mg/ml of methacholine); and 4) positive skin prick test to at least one of a panel of indoor aeroallergens	<i>ALOX15, CAPN14, IFNGR2, LAG3, HLKA-DPA1, NFIL3, PRF1, TNFSF13</i>	FDR calculated by Benjamini and Hochberg
		Cross-sectional cohort						
Yang et al., 2015[41]	81 DMRs (55 after adjustment for cell counts)	6-12 years	97 cases /97 controls 101 for validation	Illumina infinium human methylation 450k beadchip, pyrosequencing for	Peripheral blood mononuclear	1) a physician diagnosis of asthma; 2) persistent or uncontrolled disease 3) physiologic evidence of asthma (FEV ₁ , FEV ₁ /FVC or	<i>IL13, RUNX3</i>	p < 0.05
		Cross-sectional						

		cohort		validation	cells	bronchodilator response)		
Nicodemus-Johnson et al., 2016 [42]	40,892 DMRs	Adults 39±12	74 asthmatics	Illumina Infinium Human Methylation 450k Bead Chip	Airway epithelial cells	Doctor's diagnosis of asthma and current use of asthma medications	ORMDL3/GSDMB	(FDR-adjusted P value <0.05)
		Cross-sectional cohort	41 non-asthmatics					
Stefanowicz et al., 2015 [93]	One acetylation site and one trimethylation site	Adults 11-25	5 asthmatics	EpiTect ChIP Kit (Qiagen)	Airway epithelial cells	Not Mentioned	EGFR STAT6	p=0.05
		Cross-sectional cohort	5 non-asthmatics					
Murphy et al., 2015 [30]	11 CpGs (nominal significance)	Children 5 years old (followed up to 18 years)	37 discordant Monzygotic twins	Illumina Infinium HumanMethylation 450k BeadChip	Buccal cell samples	Event history calendar for recording children's history of chronic health conditions Private individual interview conducted by trained professionals when 18 years old.	AFF4, DACT2, DLX6AS, IKBKE, HSPC15, HGSNAT	p<0.0001
		Longitudinal discordant MZ twins study						
Gunawardhana et al., 2014 [94]	Eosinophilic asthma: 413 CpGs Paucigranulocytic asthma: 495 CpGs Neutrophilic asthma : 89 CpGs	Adults (33-69 years)	52 asthmatics	Illumina Infinium Human Methylation27 Bead Chip	Peripheral blood mononuclear cells	Asthma was defined according to ATS guidelines based upon current (past 12 mo) episodic respiratory symptoms, doctor's diagnosis (ever) and demonstrated reversible airflow obstruction with a FEV1 bronchodilator response of ≥12% (and >200 ml) or airway hyper-responsiveness (AHR) to hypertonic (4.5%) saline with a PD15 < 15 ml.	RBP1, NRG1, KCNQ4, PYY2, FAM19A4, SYNM, ME1, AK5	p < 0.05
		Cross-sectional cohort	10 healthy controls					

Rastogi et al., 2013[40]	215 DMRs (between obese asthmatics and the three other groups)	8-9 years	Cases : 8 obese/ 8 non obese asthmatics Controls : 8 obese/ 8 non obese non asthmatics	massively-parallel sequencing (MPS)-based HELP-tagging assay	Peripheral blood mononuclear cells	Diagnosis by primary care physician	<i>ADAM17, LIF, NHEJ1, AHR, IL2RA, TBX21</i> <i>FCER2</i>	p = 0.05
		Cross-sectional cohort						
Stefanowicz et al. 2012[43]	No differential CpGs detected	Children 2-14 years old	25 children (5 asthmatics)	Illumina GoldenGate Methylation Cancer Panel I	Airway epithelial cells and Peripheral blood mononuclear cells	Physician-diagnosed asthma plus documented wheeze by a physician in the past 12 months. Positive responses to relevant questions on the ISAAC and ATS respiratory questionnaires as reported by the parents or subject were used to corroborate the diagnoses and negative responses were used to validate absence of respiratory symptoms.	-	p < 0.05
		Cross-sectional cohort						

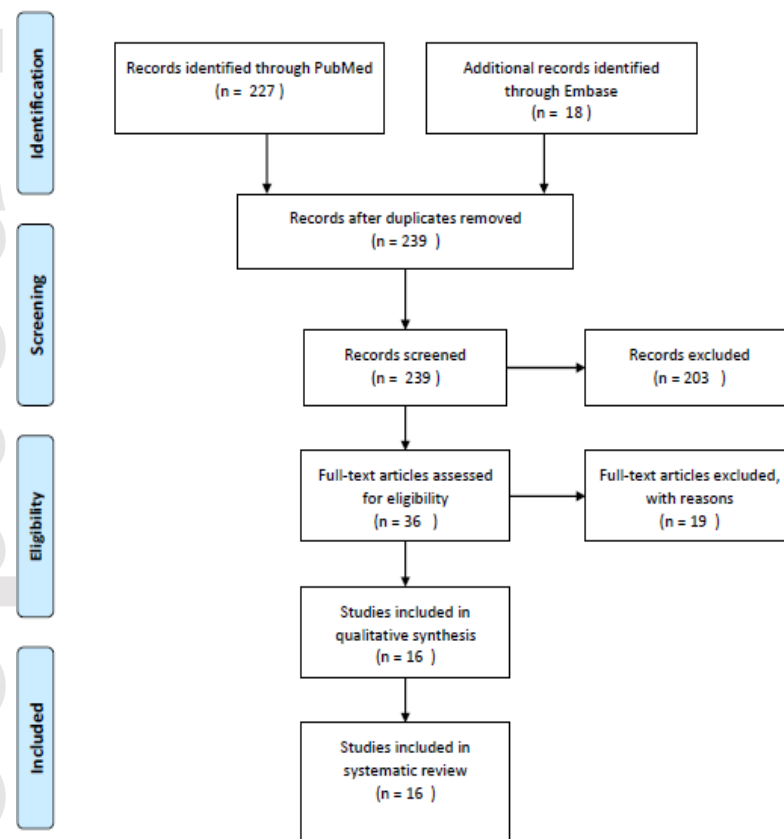
Accepted Article

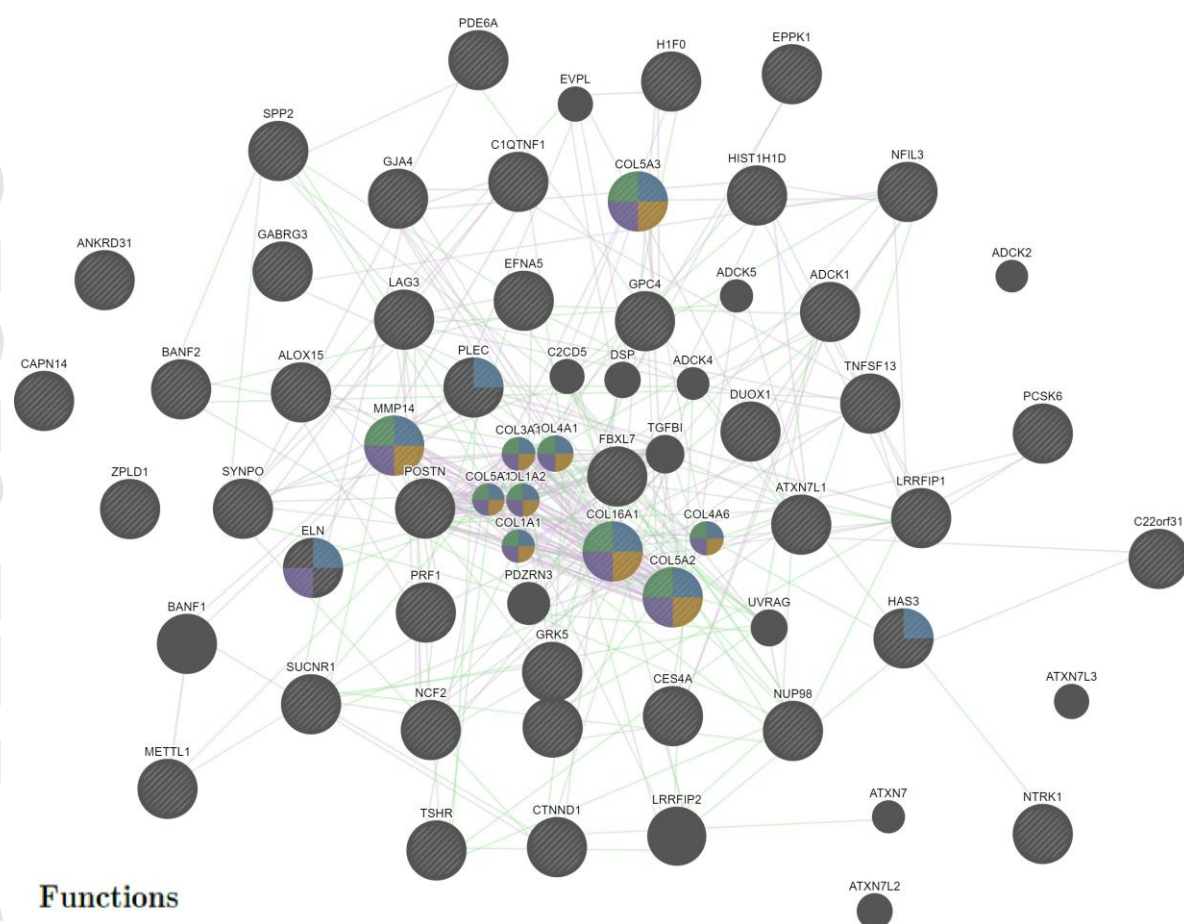
Figure Legends:

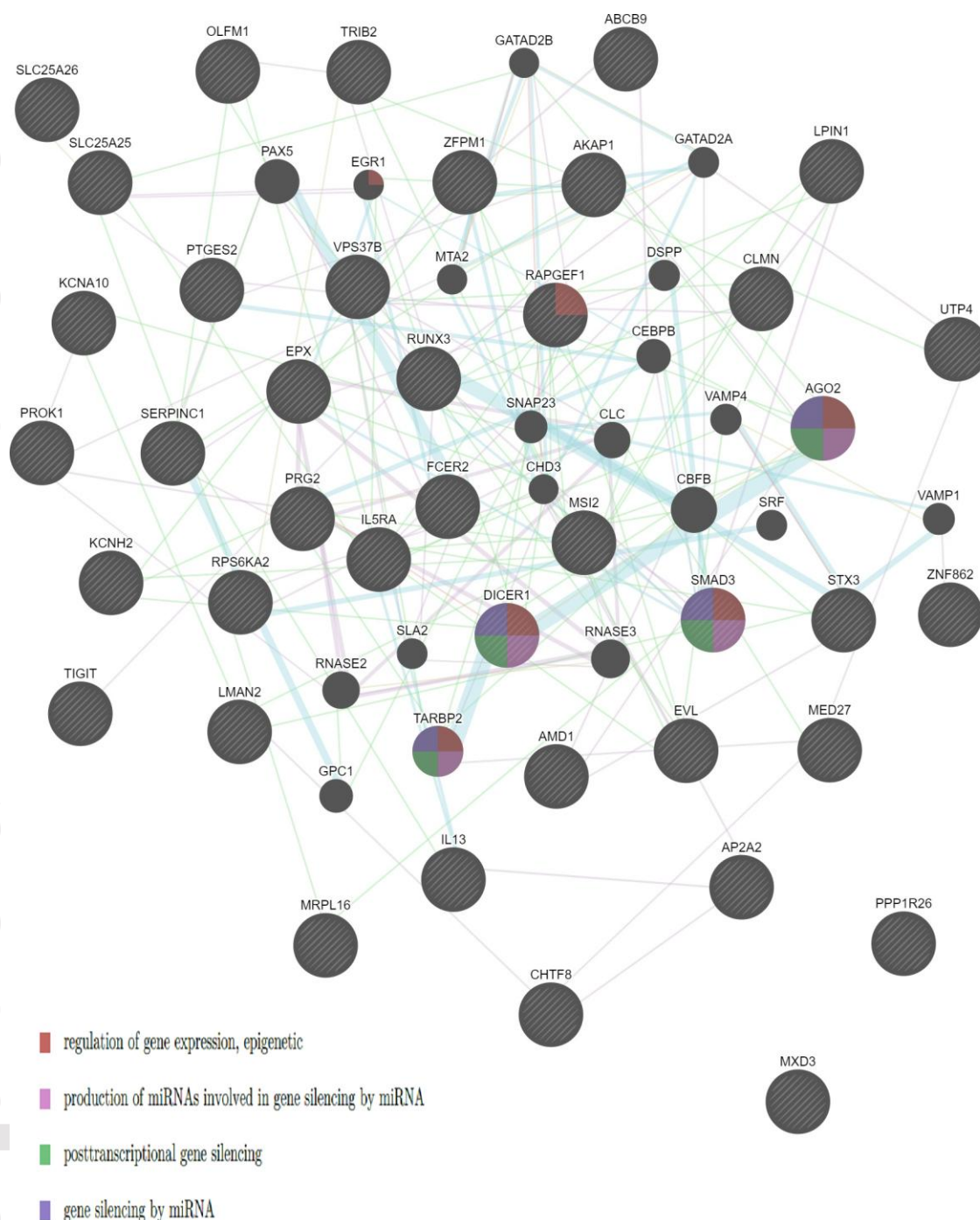
Figure 1 : Flowchart of Search and Selection Procedure.

Figure 2 : Pathway and gene interactions network for a selected list of genes annotated to differentially methylated regions in nasal studies.

Figure 3 : Pathway and gene interactions network for a selected list of genes annotated to differentially methylated regions in blood studies.







QUALITY ASSESMENT FORM

Sample selection

- 1. Was a power calculation performed?**
Yes, before initiation of study; Yes, but retrospectively; No
- 2. If a power calculation was performed (Yes to #2), were the number of inclusions required for a power of 80 met?**
Yes; No; n/a (No to #2)
- 3. Were in- and exclusion criteria clearly described?**
Yes; No
- 4. Were sufficient basic characteristics reported for the study population?***
Yes; No
- 5. Was a validation cohort selected (in this study) to test reproducibility of findings in the original cohort?**
Yes; No

Methylation detection

- 6. What enrichment/discrimination method has been used?**
- 7. Have the relevant controls been used?**
- 8. Were clinical data collected prospectively or retrospectively?**
Prospectively; Retrospectively; NR
- 9. What was the overall percentage of missing data for the association between the differential methylation and asthma?**
Specify percentage; NR; n/a
- 10. Specify epigenetic method(s) used.**
PCR; Sanger sequencing; allelic discrimination assays; GWA array; other (specify); NR
- 11. Have reactions been done in replicate ?**
Yes; No; NR; n/a (for GWAS)
- 12. If reactions have been done in replicate (Yes to #16), what was the duplication concordance?**
Specify percentage; n/a

Statistical analyses

13. How were missing data handled (select all that apply)?

Excluded (complete case analysis)

Single imputation (specify method)

Multiple imputation (specify method)

Other (specify)

NR

n/a

14. Were analyses performed to assess the effect of confounders?

Yes (specify); No; n/a

15. What statistical tests were used?

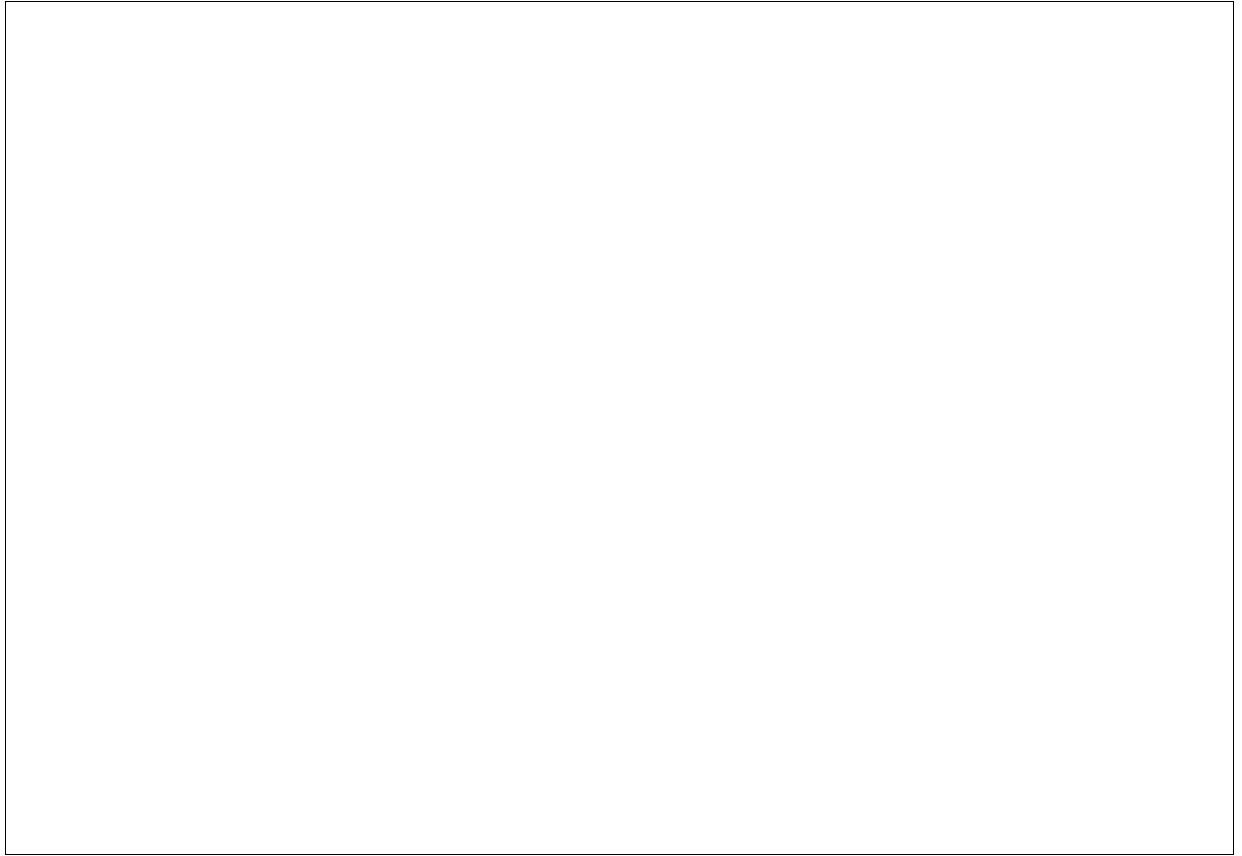
16. What magnitude of difference is being reported?

17. Do the differences reported exceed the sensitivity of the platform?

Yes (specify); No; n/a

Other

18. Please provide any additional remarks (either positive or negative) on quality.



File 2. Risk of bias assessment according to the Newcastle-Ottawa Scale (NOS) for cohort studies.

Study (Ref)	<u>Selection</u>				<u>Comparabilit</u> <u>y</u>	<u>Outcome</u>			Quality judgement
	S1	S2	S3	S4	C1	O1	O2	O3	
<i>Devries et al , 2016</i>	(★)	★		★		★	★	★	Moderate Risk 6
<i>Xu et al , 2018</i>	★	★	★	★	★	★	★		Low Risk 7
<i>Arathimos et al , 2017</i>	★	★			★		★	★	High Risk 4
<i>Yang et al , 2015</i>	★	★	★	★	★	★	★	★	Low Risk 8
<i>Rastogi et al., 2018</i>		★	★	★		★	★		High Risk 4
<i>Nicodemus-Johnson et al., 2016</i>	(★)	★	★	★	★	★	★	★	Low Risk 8
<i>Rees et al, 2018</i>	★	★	★	★	★	★	★	★	Low Risk 8
<i>Forno et al.</i>	★	★	★	★	★	★	★	★	Low Risk 8
<i>Yang et al.,2017</i>		★	★	★		★	★	★	Moderate Risk 6
<i>Stefanowicz et al.2015</i>								★	Very high Risk 1
<i>Murphy et al. 2015</i>		★			★		★	★	High Risk 4
<i>Gunawardhana et al., 2014</i>	★			★			★	★	High Risk 4
<i>Stefanowicz et al.2015</i>	★		★	★			★	★	High Risk 5
<i>Zhang et al., 2018</i>		★	★	★		★	★	★	Moderate Risk 6

[illegible]

QUALITY ASSESMENT FORM

Sample selection

- 1. Was a power calculation performed?**
Yes, before initiation of study; Yes, but retrospectively; No
- 2. If a power calculation was performed (Yes to #2), were the number of inclusions required for a power of 80 met?**
Yes; No; n/a (No to #2)
- 3. Were in- and exclusion criteria clearly described?**
Yes; No
- 4. Were sufficient basic characteristics reported for the study population?***
Yes; No
- 5. Was a validation cohort selected (in this study) to test reproducibility of findings in the original cohort?**
Yes; No

Methylation detection

- 6. What enrichment/discrimination method has been used?**
- 7. Have the relevant controls been used?**
- 8. Were clinical data collected prospectively or retrospectively?**
Prospectively; Retrospectively; NR
- 9. What was the overall percentage of missing data for the association between the differential methylation and asthma?**
Specify percentage; NR; n/a
- 10. Specify epigenetic method(s) used.**
PCR; Sanger sequencing; allelic discrimination assays; GWA array; other (specify); NR
- 11. Have reactions been done in replicate ?**
Yes; No; NR; n/a (for GWAS)
- 12. If reactions have been done in replicate (Yes to #16), what was the duplication concordance?**
Specify percentage; n/a

Statistical analyses

13. How were missing data handled (select all that apply)?

Excluded (complete case analysis)

Single imputation (specify method)

Multiple imputation (specify method)

Other (specify)

NR

n/a

14. Were analyses performed to assess the effect of confounders?

Yes (specify); No; n/a

15. What statistical tests were used?

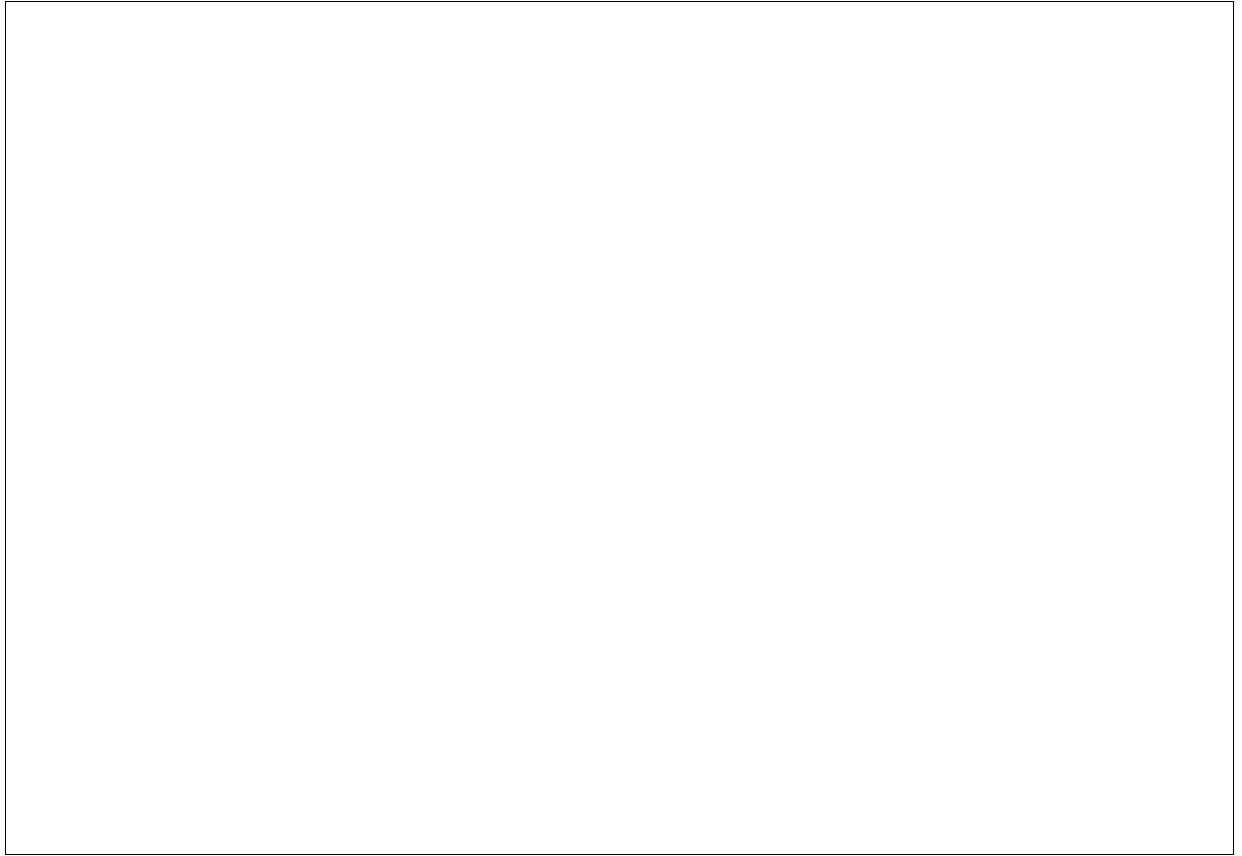
16. What magnitude of difference is being reported?

17. Do the differences reported exceed the sensitivity of the platform?

Yes (specify); No; n/a

Other

18. Please provide any additional remarks (either positive or negative) on quality.



Review Protocol: Systematic Review of Epigenome Wide Association Studies In Asthma

Team Information

Project Lead	Prof. Lies Lahousse
Research Team Members	Ahmed Edris
Date	18/10/2018
Institution(s)	Ghent University

Background

Describe the population and condition or phenomenon of interest and contextualize it. In other words, describe what this review is about.

Epigenetics are generally defined as the study of changes in gene activity that are not due to underlying changes in genetic sequences. The genetic makeup of an individual (e.g. inheritance of an imprinted loci) and its interaction with the environment can both affect epigenetics .Mechanisms of epigenetics generally include : DNA methylation , posttranslational modifications of histone and the expression of non-coding RNAs.

Interest in the field of asthma epigenetics is growing , and several epigenome wide association studies have been conducted. A role has been suggested for environmental interactions (e.g.: Smoking) and their effects on DNA , leading to altered gene expression in the pathogenesis of asthma, referred to by the term epigenetics.

Epigenome wide association studies are a relatively novel type of studies conducted to evaluate the association of epigenetic variants (e.g. : methylation sites) with incidence of certain conditions. Several epigenome wide association studies of asthma have been conducted with differing results but no systematic review of the current evidence has been conducted.



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Objective

Describe the justification for this review. In other words, describe why this review/the information it collects is important.

- 1- To identify all epigenome wide association studies evaluating the risk of asthma in a humans
- 2- Asses the validity of evidence and determine if an association exists
- 3- Assess consistency of epigenetic markers associated with asthma across different studies.

Review Question

Full Review Question

Provide the full review question in sentence format, and then break up the question according to the PICO framework (or other frameworks as appropriate).

Population	Adult and pediatric
Intervention	Epigenetic changes in DNA
Comparison	No Epigenetic changes in DNA
Outcome	Risk of asthma

Search Strategy

Databases

List the bibliographic databases to be searched.

Embase : terms : Epigenome wide association AND Asthma
, Pubmed : (((“Asthma” [MeSH Terms] OR “asthma” [Title]) AND (“Epigenetics” [MeSH Terms] OR “methylation” [All Fields]))

Hand Searching

List journals or websites that will be hand searched for relevant articles.

Experts or Stakeholders

If experts or key stakeholders are being contacted for additional grey literature or research, list them and how they will be contacted.

Reference Searches

If forward or backward citations will be performed (also known as chain or snowball searching), detail them here.



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Eligibility Criteria

Operationalize your PICO (or other framework) concepts by explicitly stating what would and would not meet inclusion. Wherever possible, provide definitions, ICD codes or other identifiers to be as clear as possible.

PICO	Inclusion Criteria	Exclusion Criteria
Population	• Adult and Pediatric • Any age • Both males and Females	• Models of asthma cells • In vitro or In silico
Intervention	• Epigenetic changes	•
Comparison	• Reference datasets	• Low statistical threshold for marker significance
Outcomes	• Incidence of asthma • Asthma defined as at least physician-diagnosed asthma	• Asthma incidence not an outcome
Study Design	• Epigenome wide Association study	•
Language	• English	•



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Data Extraction

Provide a description of methods used to collect data from included studies (e.g. categories of data you intend to collect, how many people will conduct extraction, how disagreements will be resolved, etc).

- **Source** : study ID , author , citation
- **Eligibility** : Confirm eligibility or reason for exclusion
- **Methods** : Design , Statistical analysis tools , quality control , method of epigenetic analysis , definition of asthma
- **Participants** : Number , Setting , age , sex , ethnicity
- **Outcomes** : methylation sites associated with asthma , quality control , imputation
- **Results** : Sample sizes , patient characteristics , P-value ,
- **Conclusions** : key conclusions for the study authors (Key implicated methylation sites) , limitations of the study

Study Quality Assessment

If applicable, describe the tool(s) you will use to assess risk of bias.

A quality assessment questionnaire has been developed

And the Newcastle-Ottawa Quality Assessment Form for Non-Randomized Studies has been used to assess the risk of bias

Data Synthesis

Describe how you will analyze and summarize the included study results.

A diagram outlining the number of initially found studies , included and excluded studies.
Comparison table for the different epigenetic variants detected by the studies and the strength of association.



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