



Recent developments in epigenetics of pediatric asthma

Hani Harb^a, Bilal Alashkar Alhamwe^a, Holger Garn^a, Harald Renz^a,
and Daniel P. Potaczek^{a,b}

Purpose of review

The goal of this review was to systematically analyze recent studies updating our knowledge on the role of epigenetic mechanisms in childhood asthma.

Recent findings

A systematic literature search was conducted that identified 23 fresh articles published within the last 5 years reporting the results of human studies on the relationships between epigenetic modifications and childhood asthma or its/related phenotypes. In almost all these studies, meaningful associations between levels of epigenetic marks (DNA methylation and/or histone modifications) and pediatric asthma or its/related phenotypes have been observed. In addition, many studies identified by our screening analyzed those associations in the context of environmental factors, such as pollution, tobacco smoke, farming, or diet, showing in a huge majority a modifying effect of those exposures.

Summary

The results of our systematic literature search provide a strong support for the role of epigenetic mechanisms in (mediating the effects of environmental exposure on) pediatric asthma. This knowledge may possibly be translated into diagnostic and/or therapeutic approaches.

Keywords

childhood (pediatric, early-onset) asthma, DNA methylation, environmental, epigenetic, histone acetylation (modification)

INTRODUCTION

Chronic inflammatory disorders including allergies and asthma are the result of the complex interactions between genetic predisposition and the environmental factors [1,2]. Childhood (pediatric, early-onset) asthma is considered to be one of the most common chronic diseases in adolescents, and in many cases it persists into adulthood. Genetic predisposition and environmental factors, such as (early or even prenatal) exposure to pathogens (viruses, harmful or noninfectious bacteria, or fungi), pollution, tobacco smoke, dietary factors, and others, play a crucial role in the development of the disease [3,4⁵,5,6⁷]. These environmental influences are thought to be, at least partly, mediated by so-called epigenetic mechanisms, that is, biochemical reactions directly modifying DNA or its accessory proteins (histones) and thus altering the availability of genes for transcription machinery and, as a result, gene expression [7⁸,8⁹].

In this review, based on a systematic literature search, we focus on the recent advances in the

understanding of the role of epigenetic modifications in pathogenesis of childhood asthma.

THE MECHANISMS OF EPIGENETIC REGULATION

Epigenetics is the branch of biomedicine that deals with environmentally induced, functionally relevant alterations of the genome not affecting its nucleotide sequence. In principle, epigenetic modifications change the expressional potential of the

^aInstitute for Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps University Marburg, Marburg, Germany and ^bJohn Paul II Hospital, Krakow, Poland

Correspondence to Dr Daniel P. Potaczek, Institute for Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps University Marburg, Hans-Meerwein Str. 3, 35043 Marburg, Germany. Tel: +49 6421 28 66085; fax: +49 6421 28 66086; e-mail: potaczek@staff.uni-marburg.de

Curr Opin Pediatr 2016, 28:000–000

DOI:10.1097/MOP.0000000000000424

KEY POINTS

- Systematic literature search identified 23 fresh articles describing the associations between epigenetic marks and childhood asthma and/or its/related phenotypes.
- Almost all those studies have demonstrated meaningful correlations.
- Many of those studies analyzed those relationships in the context of environmental influences almost uniformly reporting modifying effects of these exposures.
- Knowledge on the role of epigenetic mechanisms in childhood asthma may possibly be translated into therapeutic and/or diagnostic approaches.

cells making certain loci either more or less (causing even full gene silencing) accessible to transcription-stimulating factors and transcriptional machinery but they seem to play a role also in other processes, such as for example, DNA repair in response to damage. They have a character of biochemical reactions, including methylation of DNA or various types of histone alterations, for example, methylation, acetylation, and phosphorylation. It has been also suggested that epigenetic regulation can be mediated by other types of mechanisms, such as for example, some small RNA molecules [8¹¹].

DNA methylation is a process during which a methyl group is enzymatically added to cytosine nucleotides belonging to CpG sites, that is, DNA regions with a cytosine (C) nucleotide being directly followed by a guanine (G) nucleotide bridged via a phosphate (p). CpG sites, often clustered in form of so-called CpG islands, are frequently located in gene promoters and other transcription-regulating elements, such as for example, enhancers [9,10]. High CpG methylation is associated with inhibition of gene transcription, even up to a complete silencing of the gene. Lower CpG methylation (higher demethylation) in turn is related to an increase in promoter transcriptional activity [11].

DNA methyltransferases (DNMTs) are major enzymes catalyzing DNA methylation. DNMT1 is the maintenance DNMT that adds methylation to hemimethylated DNA, that is, double-stranded DNA having one of its strands already methylated. It is thought to be the primary enzyme responsible for maintaining DNA methylation patterns after DNA replication, including those established by *de novo* methyltransferases, DNMT3A and DNMT3B. These two are in turn enzymes with an equal preference to hemimethylated and unmethylated double-stranded DNA, responsible for the *de novo* DNA methylation, mainly during early embryogenesis [12,13].

In addition to DNA methylation, also histone modifications are getting more and more attention as important epigenetic mechanisms of nucleosome dynamics, especially tightness of chromatin packaging and thus accessibility of DNA for transcription, involved in pathogenesis of multiple disorders, including allergies [8^{14,15}]. Generally, they are associated with either transcriptional gene activation or repression [16,17].

Possibly, all histone acetylation types determined by the targeted amino acid residues (H3K9, H3K14, H3K27, etc.) are correlated with gene activation [16]. Acetylation reaction is catalyzed by histone acetyltransferases (HATs), enzymes that transfer an acetyl group from acetyl-CoA to the ϵ -amino group of the target lysine side within histones but in some cases they also modify nonhistone proteins mediating other cellular activities, such as transcription factors, nuclear receptors, and enzymes [18,19,20²¹]. Thus, several families of HATs exist, including cytoplasmic HATs, Gcn5-related *N*-acetyltransferases, MYSTs (named after the members MOZ, YBF2/SAS3, SAS2, and TIP60), p300/CBP family, transcription coactivators, and steroid receptor coactivators [19,20²²]. Histone deacetylases (HDACs) are in turn enzymes removing acetyl groups from lysine residues on histones, which allows them more tight wrapping of DNA reducing its accessibility to transcriptional machinery [18,19,20²³]. So far, discovered HDACs are classified into four classes – I, II, III, and IV. Class II is additionally subdivided into subclasses IIa and IIb. HDACs from classes I, II, and IV contain zinc (Zn^{2+}) necessary for their catalytic activity [18,23,24].

Histone methylation is catalyzed by enzymes called histone methyltransferases. They transfer of one to three methyl groups to lysine or arginine residues of all four core histones, although the methylation of H3 and H4 histones is most common [25,26]. The effect of histone methylation on the chromatin structure and thus transcriptional activity of the gene (either promoting or suppressing transcription) depends on the amino acid residue number and on the degree of methylation (number of the methyl groups added) [26–28]. Although histone phosphorylation is best known for its role in DNA repair in response to damage, it also belongs, together with acetylation, methylation, and ubiquitination, to the important posttranslational modifications, sometimes called a ‘histone code’, directly or indirectly affecting chromatin structure and (transcriptional) function. Phosphorylation of serine, threonine, and tyrosine residues can be happen in core exons. However, phosphorylation of multiple amino acids in histone H1 has also been described [29–33].

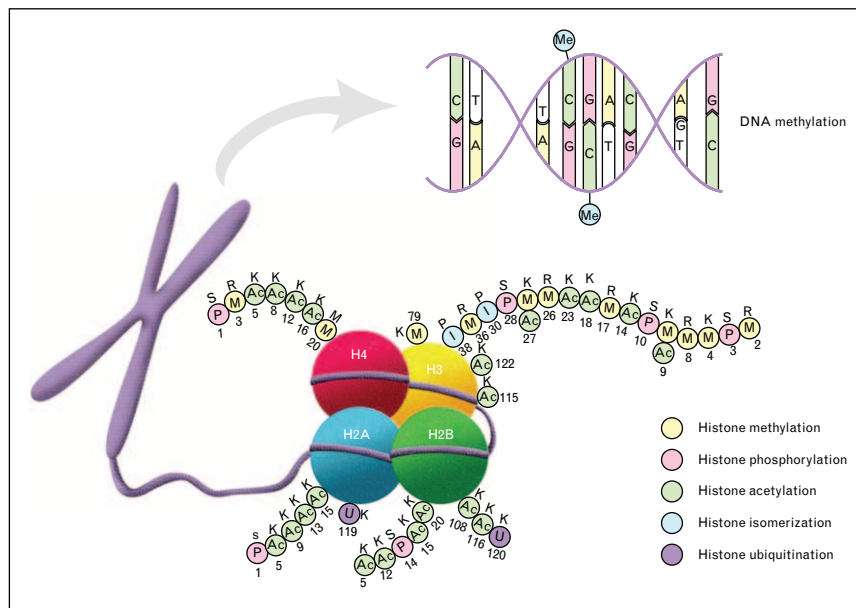


FIGURE 1. Types of epigenetic modifications. DNA methylation: cytosine nucleotides belonging to so-called CpG sites, that is, DNA regions with a cytosine nucleotide being directly followed by a guanine nucleotide, can be methylated. Histone modifications: amino acids in four core histones (H2A, H2B, H3, and H4) can undergo methylation, acetylation, phosphorylation, isomerization, or ubiquitination. This figure was inspired by several previously published images, especially by the one by Barber and Rastegar [39].

Considering the role of T-cell subsets in the pathogenesis of asthma, a main topic of this review, it is worth mentioning that, early in life, DNA methylation/demethylation plays a crucial role also in their development [34]. For example, the lineage commitment of immature T-cell precursors (thymocytes) to either the CD4⁺ helper or CD8⁺ killer T-cells have been found in mice to be epigenetically controlled by the zinc finger transcription factor *Th-POK* (T-helper-inducing POZ/Krüppel-like factor), one of the major players in regulation of CD4⁺ vs. CD8⁺ cells development [35,36]. Furthermore, animal and cell line studies have also shown *Th-POK* to recruit class II HDACs deacetylating (histones in) *Cd8* genes and thus silencing them during the development of the CD4⁺ lineage [37]. Finally, the correlations of histone methylation and expression of lineage-specific genes in both CD4⁺ and CD8⁺ T-cells have also been reported [35,38].

Types of epigenetic modifications are illustrated schematically in Fig. 1.

SYSTEMATIC SEARCH: METHODOLOGY

To cover the area of interest, a systematic literature search was conducted (Fig. 2). On 17 August 2016, PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed>) was searched using the '(childhood OR pediatric OR early OR children OR child) AND asthma AND (epigenetic OR epigenetics OR methylation OR histone acetylation OR histone

modifications)' as an input and limiting the results with '5 years' (publication dates) and 'Humans' (species) filters, which led to the identification of 138 records. Those were subsequently subjected to full text-based screening aiming to exclude articles not reporting original data (reviews and editorials), which resulted in elimination of 75 publications. From the remaining 63 articles, further 40 were excluded as reporting no epigenetic data on associations with childhood asthma (or its/related phenotypes) and/or reporting epigenetic data not obtained in asthmatic children or cohorts including them ($n=14$), comprising individuals of unidentified/unidentifiable age, adults, or mixed-age cohorts ($n=10$), reporting establishment/design of studies/cohorts ($n=5$), reporting epigenetic data obtained in animals or cell lines ($n=4$), reporting no epigenetic data ($n=5$), or due to a combination of those and/or other reasons ($n=2$). The final selection comprised 23 fresh (i.e., published within last 5 years) articles reporting the epigenetic data on associations with childhood asthma or its phenotypes or at least obtained in asthmatic children and (thus) relevant to the basic disease (Table 1).

SYSTEMATIC SEARCH: BRIEF ANALYSIS OF THE RESULTS

Our literature search identified 23 articles published in the last 5 years and reporting associations with childhood asthma or its/related phenotypes, such

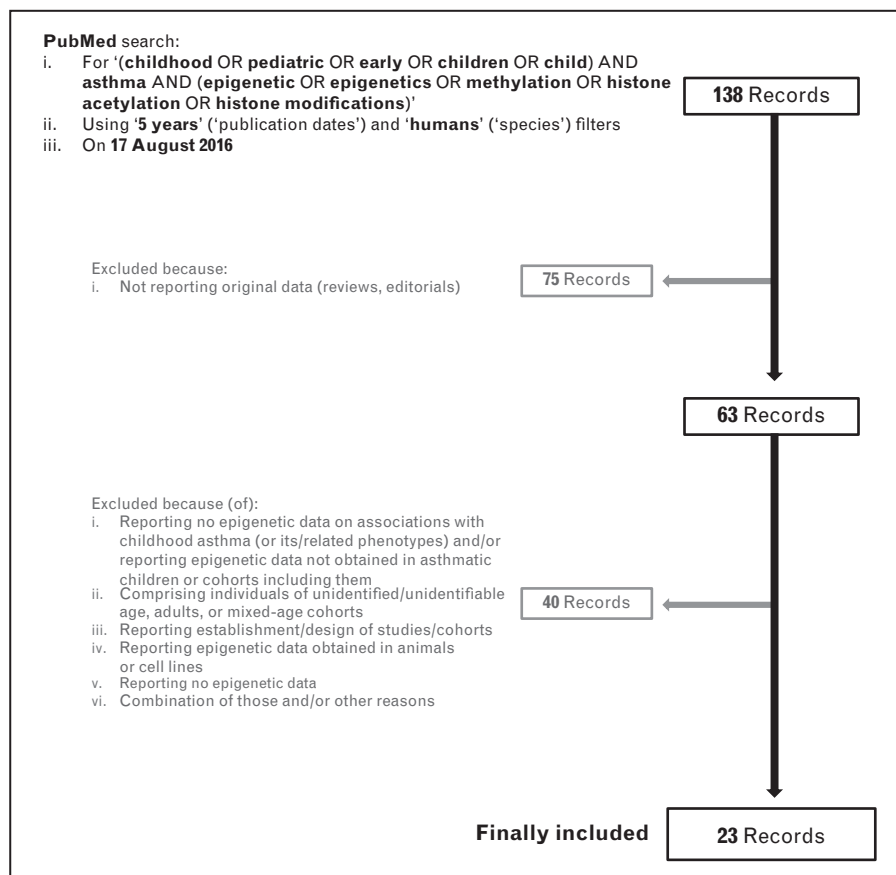


FIGURE 2. Strategy of systematic literature search and its results.

severity of the disease, wheezing, atopic asthma, atopy in asthmatics, or lung function parameters (Table 1). Some of those articles were fully focused on asthmatic children, whereas from the others only the data relevant for this systematic review with strict criteria were extracted.

Overall, in absolutely huge majority, the studies identified by our systematic search show meaningful associations between the levels of the epigenetic marks and asthma or its/related phenotypes, in many cases additionally modified by environmental influences.

Most of the articles focused on DNA methylation, whereas only two, rather recent articles, addressed other epigenetic modifications, specifically those of the histone proteins [41[■],43[■]]. In one of them, a kind of global histone acetylation (of H3K18ac) or methylation (of H3K9me3) was compared between asthmatics and nonasthmatics first, then disease-related differences in histone modifications of specific genes were investigated [41[■]]. The other study, in which some of us actually participated, analyzed the differences in H3 and H4 histone pan-acetylation between several genes specific for different disease-relevant populations

of T-cells in well defined groups of allergic asthmatics and healthy controls [43[■]]. In addition, it is the first article reporting the results obtained with our novel miniaturized method assessing histone acetylation in relatively small numbers of cells very recently described elsewhere [63[■]].

The remaining articles reported generally two types of DNA methylation studies, including those targeting selected genes or studies applying hypothesis-free approaches to DNA methylation. The latter varied in the epigenome coverage, ranging from ~1.8 to 2 million CpG sites [49], through more than 450 000 CpG sites [40[■],44[■],45[■]] and more than 27 000 CpG sites [47,51], to 1505 CpG sites [58,62]. In some of those studies, initial hypothesis-free approach was in principle followed by the analysis of the loci with the top ranks in a screening phase (e.g., [44[■],47]). From those, however, the final targets were frequently selected with help of hypothesis-driven strategy, that is, based on the potential of the genes to contribute to the pathophysiology and/or pathogenesis of asthma and/or related phenotypes suggested by previous studies of different types, immune character of the loci, and others (e.g., [40[■],62]).

Table 1. Major epigenetic childhood asthma-oriented goals and results reported in 23 articles identified by our literature search^a

References	Objectives	Results
Somineni <i>et al.</i> [40 [■]]	First epigenome-wide (>450 000 CpG sites) screening, then comparison of <i>TET1</i> (selected gene) DNA methylation levels in nasal AECs between asthmatic and nonasthmatic siblings or unrelated patients in connection with traffic-related air pollution. Verification of <i>TET1</i> methylation patterns in saliva, PBMCs, and human bronchial epithelial cells	Loss of methylation at a single CpG site located in the <i>TET1</i> promoter (g23602092) associated with asthma. Increased methylation at the same site in response to traffic-related air pollution exposure at participants' current homes. <i>TET1</i> promoter methylation patterns conserved between different tissue types
Stefanowicz <i>et al.</i> [41 [■]]	Comparison of global and gene-specific [<i>TP63</i> (Δ Np63 isoform), <i>EGFR</i> , and <i>STAT6</i>] histone acetylation and methylation status in AECs obtained from asthmatic and nonasthmatic nontransplantable teenage human lung donors	Higher global H3K18 acetylation (H3K18ac) and H3K9 trimethylation (H3K9me3) in asthmatic patients. Higher association of H3K18ac (but not H3K9me3) around TSSs of <i>TP63</i> (Δ Np63 isoform), <i>EGFR</i> , and <i>STAT6</i> in asthmatics
Xiao <i>et al.</i> [42 [■]]	Comparative analysis of <i>VNN1</i> promoter DNA methylation in NECs of children/teenagers with acute asthma exacerbation on admission and after systemic corticosteroid treatment (18–24-h interval)	A tendency of the methylation levels of one CpG site in <i>VNN1</i> promoter to decrease in the poor responders but to increase the good responders after systemic corticosteroid treatment
Harb <i>et al.</i> [43 [■]]	Comparison of promoter H3 and H4 histone pan-acetylation in several Th1-specific (<i>IFNG</i> and <i>TBX21</i>), Th2-specific (<i>IL4</i> , <i>IL5</i> , and <i>IL13</i>), or Treg-specific (<i>FOXP3</i>) genes between PBMC-isolated CD4 ⁺ T-cells obtained from allergic asthmatic and healthy control children	Higher H3 pan-acetylation at <i>IL13</i> promoter in allergic asthmatics. A tendency toward a higher H4 pan-acetylation at <i>IL13</i> and a higher H3 pan-acetylation at <i>FOXP3</i> promoters in asthmatics
Yang <i>et al.</i> [44 [■]]	Comparison of epigenome-wide (>450 000 CpG sites) DNA methylation levels between PBMCs obtained from persistent atopic asthmatic and healthy inner city children	Eighty-one DNA regions differentially methylated between asthmatics and controls detected. Hypomethylation of several immune genes (<i>IL13</i> , <i>RUNX3</i> , <i>TIGIT</i> , and others) in asthmatic patients. In analysis of asthmatics only, 11 differentially methylated DNA regions associated with total serum IgE, and 16 with percentage predicted FEV ₁
Ji <i>et al.</i> [45 [■]]	Generation of induced pluripotent stem cells from NECs of asthmatic children and comparative analysis of their methylome (>450 000 CpG sites) vs. cultured NECs or embryonic stem cells	Methylome of NEC-derived induced pluripotent stem cells similar to that of embryonic stem cells but with some persisted marks in epithelium-specific or asthma-related genes suggesting the retention of an epigenetic memory of the tissue of origin
Hew <i>et al.</i> [46 [■]]	Analysis of the relationship between DNA methylation of 13 CpG sites within the <i>FOXP3</i> locus and preceding ambient PAHs exposure studied in Tregs isolated from PBMCs of asthmatic and nonasthmatic children	Higher average PAHs exposure associated with increased methylation in the <i>FOXP3</i> locus. The effect more pronounced in asthmatics and increasing with the exposure averaging time from 24 h to 1 year, the latter suggesting more of chronic response
Breton <i>et al.</i> [47]	Comparison of DNA methylation patterns (>27 000 CpG sites) between whole blood samples obtained from asthmatic children with and without prenatal tobacco smoke exposure	Mean methylation levels associated with prenatal tobacco smoke exposure for 19 CpG loci, including, among others, <i>FRMD4A</i> and <i>C11orf52</i> but also <i>XPNPEP1</i> , <i>PPEF2</i> , <i>SMPD3</i> , and <i>CRYGN</i>
Gaffin <i>et al.</i> [48]	Analysis of the relationship between DNA methylation of multiple CpG sites in <i>ADRB2</i> promoter and disease phenotypes in blood or saliva samples obtained from a cohort of inner city school-aged asthmatic children	Higher average <i>ADRB2</i> methylation associated with lower asthma severity measured by a report of dyspnea. Tendency toward less rescue medication use, nighttime symptoms, school absences, activity limitation due to asthma, and improved lung function measurements with higher methylation
Rastogi <i>et al.</i> [49]	Comparison of epigenome-wide (~1.8–2 million CpG sites) DNA methylation patterns between PBMCs obtained from preadolescent obese asthmatics with those in children with asthma or obesity alone, and healthy controls	Distinctive DNA methylation patterns in obese asthmatic children with lower promoter methylation of <i>CCL5</i> , <i>IL2RA</i> , and <i>TBX21</i> , and higher promoter methylation of <i>FCER2</i> and <i>TGFB1</i> . Identification of T-cell signaling and macrophage activation as the two primary pathways selectively hypomethylated in obese asthmatics
Naumova <i>et al.</i> [50]	Comparison of DNA methylation levels of the <i>ZBP2</i> promoter (11 CpG sites) between PBMCs obtained from asthmatic and nonasthmatic (male or female) children	DNA methylation levels of the <i>ZBP2</i> similar between asthmatic and nonasthmatic female children and slightly tending to differ between affected and nonaffected male adolescents
Rossnerova <i>et al.</i> [51]	Analysis of DNA methylation patterns (>27 000 CpG sites) in whole blood samples obtained from asthmatic or healthy children from highly polluted or control geographic region	No asthma-related difference of methylation patterns in both highly polluted and control locations. Methylation of almost 10 000 CpG sites different between the regions. Differences also related to sex or urinary cotinine levels
Curtin <i>et al.</i> [52]	The effect of DNA methylation of a functional CpG site at <i>IL2</i> promoter (<i>IL2 Site1</i>) analyzed in CBMC samples on asthma exacerbations prospectively followed in a population-based cohort	Methylation of <i>IL2 Site1</i> independently associated with the risk of asthma exacerbation or hospital admission. Methylation levels of <i>IL2 Site7</i> (another <i>IL2</i> site not important for its transcription) or <i>LINE-1</i> (both used as controls) not predictive for asthma exacerbations or hospital admissions
Reinius <i>et al.</i> [53]	Analysis of the association between DNA methylation of five CpG sites in <i>NPSR1</i> promoter and asthma, lifestyle, or environmental factors in blood samples obtained from birth cohort-deriving (asthmatic and healthy) children	Lower <i>NPSR1</i> promoter methylation in children with the doctor's diagnosis of asthma ever up to age of 8 years (CpG site 4) or allergic asthma (CpG sites 1 and 4). Associations of <i>NPSR1</i> promoter methylation with parental smoking during infancy (CpG site 1), current dietary folate intake (CpG sites 1 and 4), month of sample collection (CpG site 5), and sensitization to (some) inhalant allergens (CpG site 1)
Michel <i>et al.</i> [54]	Combined effect of farming and DNA methylation of a total of 149 CpG sites in 23 regions of 10 genes (<i>ORMDL1</i> , <i>ORMDL2</i> , <i>ORMDL3</i> , <i>CHI3L1</i> , <i>RAD50</i> , <i>IL13</i> , <i>IL4</i> , <i>STAT6</i> , <i>FOXP3</i> , and <i>RUNX3</i>) analyzed in cord blood or whole blood collected at the age of 4.5 years on early asthma development	No differences in methylation between early asthmatics and nonasthmatics except for a region in intron 1 of <i>ORMDL3</i> (cord blood) and TSS of <i>ORMDL2</i> (4.5 years of age). No relationship with regard to early asthma status in children grown on farm (cord blood and 4.5 years of age). Differences in methylation over time between early asthmatics and nonasthmatics for CpG islands in several genes, such as <i>ORMDL3</i> , <i>ORMDL1</i> , <i>RAD50</i> , <i>IL13</i> , or <i>STAT6</i>

Table 1 (Continued)

References	Objectives	Results
Chen <i>et al.</i> [55]	Analysis of an association between DNA methylation of a CpG site in <i>ADCYAP1R1</i> promoter (cg11218385) and asthma or exposure to violence in white blood cell samples obtained from asthmatic and nonasthmatic children	Increase in <i>ADCYAP1R1</i> cg11218385 site methylation associated with higher risk of asthma. In children aged 9 years and older (nonasthmatics alone or the whole cohort), increase in violence exposure associated with higher methylation
Brunst <i>et al.</i> [56]	The relationship between DNA methylation of the only CpG island in the 5' region of <i>FOXP3</i> (four CpG sites in the 3' end of the island) analyzed in saliva samples and asthma, wheezing, or chronic DEPs exposure in a cohort of children	Increase in <i>FOXP3</i> methylation with increased DEPs exposure. Higher risk of asthma, persistent wheezing, or early transient wheezing in children with higher <i>FOXP3</i> methylation
Munthe-Kaas <i>et al.</i> [57]	DNA methylation of an intragenic CpG island (consisting of 87 CpG sites) covering most of the <i>CD14</i> coding region in a birth cohort children (at 2 and 10 years) with substantial portion of asthmatics	The increase in <i>CD14</i> methylation from 2 to 10 years of age not correlated with asthma
Stefanowicz <i>et al.</i> [58]	Comparative DNA methylation analysis (1505 CpG sites across 807 genes) in PBMCs and bronchial AECs obtained from atopic, atopic asthmatic, nonatopic asthmatic, or healthy controls	A signature set of CpG sites differentially methylated in AECs vs. PBMCs regardless of disease phenotype, of which 13 CpG sites specific to healthy controls, eight found in atopics, and six unique to asthmatics. No differences in the methylation status of PBMCs between disease phenotypes. In AECs from asthmatics vs. atopics, eight differentially methylated sites identified, including, among others, those in <i>STAT5A</i> and <i>CRIP1</i>
Fu <i>et al.</i> [59]	Analysis of the effect of DNA methylation of a CpG island in 5'-UTR of <i>ADRB2</i> (three CpG sites) assessed in blood samples obtained from intermediate or severe asthmatic children on disease severity	Intermediate or high levels of <i>ADRB2</i> methylation associated with severe childhood asthma. Association between indoor exposure to NO ₂ (an air pollutant) and severe asthma in children with high (but not low) <i>ADRB2</i> methylation levels
Breton <i>et al.</i> [60]	DNA methylation of 12 different CpG sites in important regulatory regions of the three <i>NOS</i> genes (three in <i>NOS1</i> , seven in <i>NOS2</i> , and two in <i>NOS3</i>) assessed in buccal brushing samples obtained from a cohort of air pollutant-exposed children (comprising also asthmatics)	DNA methylation higher in the <i>NOS2</i> site 1, lower in the <i>NOS2</i> site 2, and lower in the <i>NOS2</i> site 3 in children with current wheeze vs. the remainders. No direct association between DNA methylation and asthma overall. However, among children with asthma, lower DNA methylation in the <i>NOS2</i> promoter region in those with current wheeze vs. the remainders or those taking asthma medications vs. the remainders. Fine particle air pollutant exposure associated with DNA methylation of several CpG sites in <i>NOS</i> genes
Baccarelli <i>et al.</i> [61]	The correlation of FE _{NO} , FEV ₁ , and wheezing with DNA methylation of the CpG island in the <i>NOS2</i> promoter (two CpG sites) and of the CpG island located downstream in the proximity of the <i>IL6</i> promoter (two CpG sites) analyzed in nasal cells obtained from asthmatic children on days 4 and 7 of the study week. Methylation of <i>Alu</i> and <i>L1NE-1</i> repetitive elements also assessed	Negative association of FE _{NO} with both <i>IL6</i> and <i>NOS2</i> methylation. A tendency toward increased risk of wheezing in children with lower <i>IL6</i> methylation
Morales <i>et al.</i> [62]	Analysis of DNA methylation (1505 CpG sites across 807 genes) in whole/cord blood samples obtained from children in the context of pediatric asthma-related phenotypes and the effects of prenatal exposures to DDE on these epigenetic marks	A substantial difference in methylation at age 4 years between children with persistent wheezing or no wheezing ever (by the age of 6 years; first cohort) observed for CpG sites in <i>ZNF264</i> , <i>ALOX12</i> , <i>EPO</i> , and <i>PDGFB</i> . In the analyses in the second cohort, <i>ALOX12</i> hypomethylation at birth (also) associated with higher risk of persistent wheezing by the age of 4 years. Higher levels of prenatal DDE (measured in cord blood or maternal serum) associated with DNA hypomethylation of <i>ALOX12</i> in the first cohort

AECs, airway epithelial cells; CBMCs, cord blood mononuclear cells; DDE, dichlorodiphenyldichloroethylene; DEPs, diesel exhaust particulates; FE_{NO}, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; H3, histone H3; H4, histone H4; NECs, nasal epithelial cells; PAHs, polycyclic aromatic hydrocarbons; PBMCs, peripheral blood mononuclear cells; Tregs, regulatory T-cells; TSS, transcription start site; UTR, untranslated region. Genes/genomic elements: *TET1*, tet methylcytosine dioxygenase 1 gene; *TP63*, tumor protein p63 gene; *EGFR*, epidermal growth factor receptor gene; *STAT6*, signal transducer and activator of transcription 6 gene; *VNN1*, vanin 1 gene; *IFNG*, interferon gamma gene; *TBX21*, T-box 21 gene; *IL4*, interleukin 4 gene; *IL5*, interleukin 5 gene; *IL13*, interleukin 13 gene; *FOXP3*, forkhead box P3 gene; *RUNX3*, runt related transcription factor 3 gene; *TIGIT*, T-cell immunoreceptor with lg and ITIM domains gene; *FRMD4A*, FERM domain containing 4A gene; *C11orf52*, chromosome 11 open reading frame 52; *XPNPPE1*, X-prolyl aminopeptidase 1 gene; *PPEF2*, protein phosphatase with EF-hand domain 2 gene; *SMPD3*, sphingomyelin phosphodiesterase 3 gene; *CRYGN*, crystallin gamma N gene; *ADRB2*, adrenoceptor beta 2 gene; *CCL5*, C-C motif chemokine ligand 5 gene; *IL2RA*, interleukin 2 receptor subunit alpha gene; *FCER2*, Fc fragment of IgE receptor II gene; *TGFB1*, transforming growth factor beta 1 gene; *ZBP2*, zona pellucida binding protein 2 gene; *IL2*, interleukin 2 gene; *L1NE-1*, long interspersed nucleotide element-1; *NPSR1*, neuropeptide S receptor 1 gene; *ORMDL1*, ORM DL sphingolipid biosynthesis regulator 1 gene; *ORMDL2*, ORM DL sphingolipid biosynthesis regulator 2 gene; *ORMDL3*, ORM DL sphingolipid biosynthesis regulator 3 gene; *CHI3L1*, chitinase 3 like 1 gene; *RAD50*, RAD50 double strand break repair protein gene; *ADCYAP1R1*, ADCYAP receptor type I gene; *CD14*, CD14 molecule gene; *STAT5A*, signal transducer and activator of transcription 5A gene; *CRIP1*, cysteine rich protein 1 gene; *NOS1*, nitric oxide synthase 1 gene; *NOS2*, nitric oxide synthase 2 gene; *NOS3*, nitric oxide synthase 3 gene; *IL6*, interleukin 6 gene; *ZNF264*, zinc finger protein 264 gene; *ALOX12*, arachidonate 12-lipoxygenase, 12S type gene; *EPO*, erythropoietin gene; *PDGFB*, platelet derived growth factor subunit B gene.

°Only studies on epigenetic associations with childhood asthma and/or its/related phenotypes (severity, wheezing, allergic asthma, etc.) conducted in pure and clearly defined cohorts of human asthmatic (at least in part) children/adolescents were selected (see also Fig. 2 and 'SYSTEMATIC SEARCH: METHODOLOGY' section). In case of the studies reporting also the other sorts of investigations, (almost) only the data fulfilling the abovementioned conditions are given. Ethnicity or genetics is not taken into account (see also 'SYSTEMATIC SEARCH: BRIEF ANALYSIS OF THE RESULTS' section).

Candidate gene DNA methylation studies focused on different genes, several of which were targeted by more than one group. These include *FOXP3* present in three articles [46[■],54,56] (and in addition in one of the articles reporting the data on histone modifications [43[■]]) and *ADRB2* [48,59] or *NOS2* [60,61], both analyzed in two studies. In addition, *IL13* or *STAT6*, both assessed by one research group for its DNA methylation [54], were also the target of two different histone modification studies [41[■],43[■]]. *IL13*, a gene analyzed by hypothesis-driven approaches in one DNA methylation [54] and one histone acetylation study [43[■]], appeared among top hits of one genome-wide screening [44[■]]. This was also a case of *RUNX3* [44[■],54] and *TBX21* [43[■],49]. All these cannot be surprising considering the fact that the associations between polymorphisms in a huge majority of those genes and asthma, atopic sensitization, and/or serum IgE have been reported [64–66].

Many of the studies identified by our search analyzed asthma and/or its/related phenotypes in the direct [40[■],46[■],51,56,59,60] or indirect (inner city children) [44[■],48] context of pollution exposure. One article analyzed the effects of tobacco smoke exposure [47]. This direction of investigation is fully in line with the environment-induced character of epigenetic modifications [8[■],67], which is also a case of dietary [34,68,69[■]] or farming (microbial) [70–72] influences, both also addressed by some articles identified by our search [53,54]. Two articles from our systematic selection focused on in-utero toxic exposures [47,62], which corresponds to the concept that epigenetic modifications in the prenatal period play a crucial role in the development of allergic disorders [7[■],34,73[■]]. Interestingly, one study investigated the influence of violence [55], whereas the other analyzed DNA methylation patterns related to obese asthmatic phenotype [49]. The latter approach fits to the idea of the functional overlap between allergic diseases, mechanisms of allergic inflammation, or IgE system and lipid metabolism [74–76]. This concept seems to be nicely further supported by the presence of *FCER2* among top hits of the abovementioned DNA methylation study of obese asthmatics [49]. It is because *FCER2* encodes low-affinity IgE receptor, a very important regulatory member of IgE system [65,77,78]. One article from our systematic literature search directly deals with pharmacoepigenetics and identifies *VNN1* promoter methylation to correlate with response of acute asthma exacerbations to the treatment with systemic corticosteroids [42[■]]. Another article addresses the same topic in a more indirect way by associating *ADRB2* methylation with a tendency toward less rescue medication

use, which is actually more appropriately reported by the authors as a parameter of asthma severity [48]. It is worth mentioning here that *ADRB2* genetic variants are known to affect the response to therapy with β 2-adrenoreceptor agonists [64,79].

Various kinds of the input biological materials were used in the studies identified by our literature screening. Those included different types of airway epithelial cells, such as nasal epithelium [40[■],42[■]], bronchial/lung epithelium [40[■],41[■],58], or even induced pluripotent stem cells [45[■]]. Most of the remaining studies were conducted using peripheral blood or its derivatives, including whole (e.g., [47,51,62]), peripheral blood mononuclear cells (PBMCs) (e.g., [40[■],44[■],50]), or subpopulations of cells isolated from PBMCs [43[■],46[■]]. Cord blood [54,62] and cord blood mononuclear cells [52] were also tested in some cases. Several DNA methylation studies used saliva [40[■],48,56] or buccal brushing [60] samples.

Some articles identified by our search attempted to address also the role of genetic variants in modulation of the epigenetic associations by more complex analyses, elimination of the genetic influences in the experimental design, or at least discussing them theoretically, whereas in some others reported genetic analyses were conducted in adults or mixed-age cohorts [44[■],48,50,53–55,57,58,60,62]. Nevertheless, as we decided that such data are beyond the scope of the present review, the summary of the literature given in Table 1 does not take them into account. Also, ethnicity of the study participants was not addressed in our tabular literature extract.

CONCLUSION AND PERSPECTIVES

In this review, we focused on the recent developments in epigenetics of childhood asthma. For this purpose, we conducted a systematic search identifying relevant articles published in the last 5 years. Almost without exception, the studies identified by our literature screening demonstrated meaningful and important associations between the levels of the epigenetic modifications and pediatric asthma or its/related phenotypes. Most of those studies focused on DNA methylation but two very recent articles addressed histone modifications, the role of which in the pathogenesis of chronic inflammatory disorders is lately getting more and more recognition. Multiple identified articles analyzed the correlations between the levels of epigenetic marks and childhood asthma in the context of environmental factors, such as toxic pollutants, tobacco smoke, farming, or diet, with an absolute majority of them showing the modifying effect of those exposures. In conclusion, (environmentally induced) epigenetic

modifications play an important role in childhood asthma.

Although this issue is generally beyond the topic of the present review, it is worth mentioning that, potentially, there might be some practical applications of the proven importance of epigenetic mechanisms in asthma. One such possibility would be a usage of epigenetic marks for diagnostic approaches aiming to improve asthma predictive algorithms or the assessment of treatment responsiveness. Although obtaining lung tissue, a major target organ, may be not so easy, some relevant information might be provided also by peripheral blood immune cells [80[■]]. Cord blood [63[■],68] or placenta [81] samples, both not requiring any invasive methods of collection, could possibly be useful here as well. Another practical application of this knowledge would be medications interfering with epigenetic regulation pathways, such as for example, HDAC [82[■],83,84] or DNMT [85,86] inhibitors. The role of epigenetic mechanism in allergic diseases beyond childhood asthma is comprehensively described in another recent review article, to which some of us also contributed [8[■]].

Acknowledgements

We would like to thank Eng Osama Salti for his artistic contribution to the illustration of different epigenetic modifications in Fig. 1.

Financial support and sponsorship

The work was supported by the Universities Giessen and Marburg Lung Centre (UGMLC), the German Centre for Lung Research (DZL; 82DZL00502), and the Von Behring-Röntgen-Foundation (Von Behring-Röntgen-Stiftung). B.A.A. is the German Academic Exchange Service (DAAD) fellow (personal reference number: 91559386).

Conflicts of interest

There are no conflicts of interest.

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