Review Article

Progresses in epigenetic studies of asthma from the perspective of high-throughput analysis technologies: a narrative review

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Background and Objective: Asthma is the most common chronic respiratory disease in the world with an estimated heritability between 50% and 60%, and recent studies have shown that epigenetic mechanisms play an important role in its development. Many cutting-edge epigenetic research techniques have been applied to the study of the pathogenesis of asthma, which has promoted the development of asthma etiology and brought new possibilities for treatment. We summarized recent advances in epigenetic research of the pathogenesis of asthma, especially from the perspective of high-throughput analysis techniques, to find potential epigenetic biomarkers and possible molecular targets for the future intervention and treatment of the disease.

Methods: We reviewed and summarized recent progress in epigenomic studies of asthma on a "pre-transcriptional level", including DNA methylation, histone modification, and chromatin remodeling, and on a "post-transcriptional level" with a focus on non-coding RNA, from the perspective of high-throughput analysis technologies.

Key Content and Findings: We have summarized the progress of different kinds of recent epigenetic studies in asthma, including DNA methylation studies [candidate genes methylation studies and epigenome-wide association study (EWAS)], histone modification studies (histone acetylation/deacetylation studies and histone methylation studies), non-coding RNA studies [microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs)], to help the readers to gain a comprehensive insight into the epigenetic research fields for asthma. The application of high-throughput analysis techniques in asthma research, including EWAS (DNA methylation chips), chromatin immunoprecipitation sequencing (CHIP-seq), microRNA sequencing, whole transcriptome sequencing, co-expression network and competing endogenous RNA (ceRNA) analyses, were introduced accompany with the main findings. And the potential epigenetic biomarkers and possible molecular targets identified via high-throughput analyses were also discussed.

Conclusions: Epigenetic research has become a hotspot in research on the pathogenesis of asthma. The combination of high-throughput epigenetic analysis technologies and traditional biological function and clinical studies will bring new breakthrough in the pathogenesis study of asthma, which will improve the genetic interpretation of the disease and bring more possibilities for the development of precision medicine to treat it.

Keywords: Asthma; epigenetic research; EWAS; methylation; non-coding RNAs (ncRNAs)

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Introduction

Asthma is the most common chronic respiratory disease in the world, characterized by bronchospasm and reversible airflow obstruction, with common symptoms include wheezing, chest tightness, cough, and dyspnea (1). It is estimated that about 300 million people worldwide suffer from asthma, and the incidence rate is on the rise (2), placing an increasing burden on health systems. The pathogenesis of asthma is complicated and includes genetic factors, immune regulation, endocrine regulation, and environmental factors. And airway eosinophilic inflammation and structural remodelling are considered as the hallmark pathological features of asthma (3).

Genetic study has demonstrated asthma as an inherited disease with an estimated heritability between 50% and 60% (4). And more than 100 genes have been linked to asthma susceptibility (5). According to previous study (6), the asthma-susceptible genes were summarized into two categories as those participating in an inflammation and immune response pathway [e.g., *CCL5* (C-C motif chemokine ligand 5), *IL4* (interleukin 4), *STAT6* (signal transducer and activator of transcription 6), *TSLP* (thymic stromal lymphopoietin), *CD14* (cluster of differentiation 14), *ALOX5* (arachidonate 5-lipoxygenase) etc.], and those that are associated with airway structure and lung function [e.g., *ACE* (angiotensin I converting enzyme), *ADAM33* (a disintegrin and metalloproteinase domain 33), *AREG* (amphiregulin), *TGFB1* (transforming growth factor beta 1), *CMA1* (chymase 1) etc.]. The current treatment for asthma mainly depends on medications to control airway inflammation and hyperreactivity. Corticosteroids, β2-adrenergic agonists, and leukotrienes are three kinds of commonly used therapeutic agents for chronic management of asthma (7). Corticosteroids are common anti-inflammatory drugs that are used for asthma treatment. Variants on the gene *STIP1* (stress induced phosphoprotein 1) which activates the glucocorticoid receptor could affects the therapeutic effect of steroids. The short-acting and long-acting β2-adrenoceptor agonists are the most common bronchial dilatation prescription drugs. And the Arg16 homozygote mutation on the β2-adrenergic receptor gene *ADRB2* (adrenoceptor beta 2) could affect their efficacy in asthma treatment. Leukotriene agents, such as 5-lipoxygenase inhibitors, can significantly improve the lung function of asthma patients. And mutation in the promoter of their target gene *ALOX5* could affect their therapeutic effect (6).

However, to date, the single nucleotide polymorphisms (SNPs) found to explain asthma susceptibility are much less than originally expected (8). Therefore, more and more attention has been paid to pathogenic factors besides susceptibility variations, among which epigenetic factors, as the interplay between genomic and environmental factors, have become a hotspot in the research field of the pathogenesis of asthma (9). The recent report also showed that epigenetic mechanisms played an important role in the development of the disease (10). And powerful genetic and environmental drivers of asthma have been shown to interconnect through epigenetic mechanisms (11). Environmental factors (such as viruses or allergens) may cause injury to the epithelial cells of the airway, and the differential expression of some susceptibility genes [such as *ADAM33*, *HLA-G* (major histocompatibility complex, class I, G) and *IRAKM* (interleukin 1 receptor associated kinase 3)] in the epithelial cells may contribute to the airway re-modelling and activation of inflammatory responses which leading to the development of asthma. During this process, epigenetic mechanisms such as DNA methylation and miRNA suppression have regulated the expression of some susceptibility genes (e.g., hyper-methylation of ADAM33, and miRNA suppression of HLA-G) and thus modulated the interconnect between genetic and environmental drivers of asthma (12). Epigenetics refers to the biological mechanism that causes changes in gene expression under the premise of unchanged DNA sequence, and its transmission process to offspring (13). Epigenetics mainly affects gene expression through two types of mechanisms. One is the regulation of gene expression before and during transcription, including DNA methylation, histone modification, and chromatin remodeling, and the other is the post-transcriptional regulation of genes, which mainly affects the abundance and translation of transcripts through various non-coding RNAs, including miRNAs, lncRNAs, and circRNAs (14).

With the rapid development of epigenetic research in recent years, many cutting-edge techniques have been applied to the study of the pathogenesis of asthma, which has promoted the development of asthma etiology and brought new possibilities for treatment. Compare to the previous studies on asthma and epigenetic research (6-10,12,14), our study has summarized the recent progress in different epigenetic research fields for asthma, including the progress in DNA methylation research field(candidate genes methylation research and EWAS), histone modification research field (histone acetylation/

Table 1 The search strategy summary

Items	Specification
Date of search (specified to date, month and year)	June 5, 2021 to October 26, 2021
Databases and other sources searched	All from the PubMed database
Search terms used (including MeSH and free text search terms and filters). Note: please use an independent supplement table to present detailed search strategy of one database as an example	See Table S1 for details
Timeframe	April 1, 2001 to August 4, 2021
Inclusion and exclusion criteria (study type, language restrictions etc.)	English literatures including clinical trial, meta-analysis and review were collected for reviewing
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Ting Zhang and Peide Huang collected the literatures and extracted the relevant information. All the authors jointly discussed and selected the literatures to obtain the consensus of the review
Any additional considerations, if applicable	None

MeSH, medical subject headings.

deacetylation research and histone methylation research), non-coding RNA research (miRNAs, lncRNAs and circRNAs), which will help the readers to gain a globe and more comprehensive insight into epigenetic research for asthma, and may help the readers to compare the methodologies and new findings in different epigenetic research fields and choose suitable strategy in their research.

Different from the previous studies (6-10,12,14), we especially emphasize the application of high-throughput analysis techniques in asthma research, including EWAS (DNA methylation chips), CHIP-seq, microRNA sequencing, whole transcriptome sequencing, co-expression network and ceRNA analyses, which represent the cutting-edge techniques in epigenetic studies of asthma, and has added to the innovation of our study. And the potential epigenetic biomarkers and possible molecular targets identified via high-throughput analyses also contribute to the innovation of this review. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-929/rc).

Methods

Literatures published between 2001 and 2021 related to epigenomic studies of asthma on the "pre-transcriptional level", including DNA methylation (EWAS), histone modification, and chromatin remodeling and on the "post-transcriptional level" mainly focusing on non-coding RNA, were surveyed at the PubMed database (https://pubmed.ncbi.nlm.nih.gov/). The researching technologies and major findings are summarized and discussed. Sources were listed in *Table 1* and Table S1.

Discussion

Studies on pre-transcriptional epigenetics in asthma

DNA methylation

To date, DNA methylation has been the most studied epigenetic mechanism in asthma. DNA methylation refers to the process in which the methyl group (CH3) covalently binds to the 5' carbon of the cytosine residue in the DNA sequence to form 5-methylcytosine (5mC), changing gene expression (15). In mammals, DNA methylation occurs mainly on cytosine-phosphate-guanine (CpG) islands, which are regions of DNA sequence approximately 300–3,000 base pairs (bp) in length, rich in cytosine (C) and guanine (G) linked by phosphate ester bonds, and commonly distributed in the promoter region of genes. DNA methylation occurring in the promoter regions often causes down-regulation or silencing of the host genes, which is its most widely known effect. However, when DNA methylation occurs in the internal region of the gene, it is possible to increase transcriptional activity (16). DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which include DNMT1 (DNA methyltransferase 1), DNMT3A (DNA methyltransferase 3 alpha), and DNMT3B (DNA

methyltransferase 3 beta). The function of DNMT1 is to copy the methylation pattern of the template chain to the new chain after DNA replication, so as to transfer the existing methylation pattern, while DNMT3A and DNMT3B mainly participate in the generation of new methylation pattern on the DNA chain (15).

The two major strategies in DNA methylation studies are candidate genes study and EWAS. Candidate gene methylation study is a classical research method in epigenetics which primarily hypothesizes that the methylation status of certain candidate gene regions will affect the disease phenotype, and methylation analyses are carried out on these potential pathogenic genes to validate the primary hypothesis. According to previous study (17), $CD4^+$ (cluster of differentiation 4 positive) T cells differentiates into helper T 1 cells (T helper 1, Th1) and helper T 2 cells (T helper 2, Th2) under antigen stimulation, and the imbalance of Th1 and Th2 cells in the immune response is considered to be an important mechanism for the initiation of asthma. Th1 and Th2 cells secrete IFN (interferon)-lymphocytes and IL-4 to participate in different immune responses. In a candidate gene study, Kwon *et al.* (18) found that while CpG islands in the promoter regions of *IL-4* and *IFN*-γ (interferon gamma) genes were highly methylated in naive $CD4^+$ T lymphocytes, under the stimulus of allergens, the methylation of *IL-4* gene promoter decreased and the gene expression increased obviously, which suggested that prompting the demethylation of *IL-4* gene promoter could promote the differentiation of $CD4^+$ T lymphocytes in the development of asthma. Another candidate gene study found that hypermethylation of CpG sites of *IFN*-γ and *FOXP3* (forkhead box P3) genes reduced the genes' expression and impaired the function of regulatory and effector T cells in asthma patients (19). Similarly, methylation of interleukin 2 promoter CpG site1 (site1 *IL-2*) has been found to increase the risk of severe asthma in children under 8 years old (20). These studies (18-20) have focused on methylation of candidate genes, revealing the effects of changes in methylation status of the genes (especially cytokines and transcription factors) on gene expression and their function in the development of asthma.

However, the candidate gene research method has been limited by its low efficacy and failure to provide a comprehensive view on the research objects. Nowadays, owing to the rapid development of high throughput analysis technologies, researchers are taking more efficient 'data-driven' approaches which are capable of detecting thousands of targets simultaneously in pathogenic research of complex disease (21). EWAS is a high throughput epigenetic research method which developed from the "Genome-wide association study (GWAS)" and has been increasingly adopted in epigenetic studies of asthma. This research method based on the technologies of whole genome DNA bisulfite conversion and chip hybridization, enables researchers to compare tens of thousands of methylation sites across the whole genome between patients and healthy people, thereby identifying epigenetic changes associated with certain disease. Moreover, EWAS can be combined with other high-throughput analysis techniques, such as RNA sequencing, to study the correlation between genomic methylation changes and gene expression, as well as disease phenotypes and environmental exposure factors (22).

Many international research teams have conducted EWAS in asthma studies and these can be divided into two main types according to the age of the samples: (I) EWAS of childhood asthma (pediatric asthma) and (II) EWAS of adult-onset asthma. EWAS of childhood asthma mainly focus on the effects of parental and perinatal exposures on the epigenome of offspring and their relationships to the risk of asthma in offspring.

In a large childhood asthma epigenetic study published in 2018 (23), researchers used Illumina HumanMethylation450K chips (capable to detect more than 450,000 CpG loci in a sample simultaneously, which covers most of the CpG islands and promoter areas in the human genome) to conduct an EWAS study on eight cohorts containing 668 asthmatic neonates and 2,904 controls. In that study, nine new CpG sites and 35 differentially methylated regions (DMRs) were found to be significantly associated with the development of asthma [false discovery rate (FDR) <0.05]. Moreover, 179 new CpG sites and 36 DMRs were found to be associated with asthma in nine cohorts containing 631 asthmatic children and 2,231 control samples. The signaling pathway enrichment analyses revealed genes with newly discovered CpG sites were enriched in immune pathways associated with asthma, and the enrichment results were highly consistent in neonates and children. Gene expression analysis revealed the methylation of CpG sites affected the expression of asthma-related genes, and several, including *IL5RA* (interleukin 5 receptor subunit alpha) and *KCNH2* (potassium voltage-gated channel subfamily h member 2), may be potential targets for new drugs to treat asthma. In another large pediatric EWAS study published in 2019 (24), researchers also used an Illumina HumanMethylation450K chip to

study the relationship between exposure to air pollution in pregnant women and DNA methylation in newborns. The study analyzed the correlation between prenatal exposure to different air pollution particles [$PM_{2.5}$ (particulate matter 2.5, n=1,551) and PM_{10} (particulate matter 10, n=1,949)] and the DNA methylation in several thousands of newborns from nine European and American populations. The results showed that six CpG sites in neonates were significantly correlated with maternal exposure to PM_{10} during pregnancy, and 14 CpG sites were significantly correlated with maternal exposure to $PM_{2.5}$ during pregnancy (FDR <0.05), within which, two methylation sites, cg00905156 and cg06849931, were located in the known asthma-related genes *FAM13A* (family with sequence similarity 13 member A) and *NOTCH4* (notch receptor 4), respectively. Notably, mRNA expression analysis also found that the up-regulation of *NOTCH4* expression was significantly correlated with prenatal exposure to PM_{10}. These findings suggest genomic methylation changes and the development of asthma in children may be associated with prenatal exposure to air pollution. Moreover, previous study has found that tobacco exposure during pregnancy increased the risk of asthma in children (25). Recent EWAS studies in children found that parental smoking history and tobacco exposure during pregnancy could affect the genomic methylation of the offspring, thereby affecting the expression of related genes and the changes of signaling pathways, and the genomic methylation changes in offspring could be maintained into adulthood (26-28).

EWAS of adult asthma has focused on the genomic methylation changes in adults and their associations with the risk of asthma. Nicodemus-Johnson *et al.* (29) used airway epithelial cells obtained by bronchoscopy from 74 adult asthma patients and 41 normal adults to conduct an EWAS and transcriptome sequencing study. They found the methylation landscapes of CpG loci on the genome region 17q12-21 were associated with the risk of asthma, which may represent a special molecular subtype of the disease. This suggests DNA methylation may affect the risk and clinical heterogeneity of asthma through different molecular signaling pathways in its pathogenesis. In another study published in 2019 (30), researchers used the Illumina Infinium Human Methylation27 chip to conduct a genomic-epigenomic association study in 356 adult asthmatic patients and 220 normal adults, looking for the "genomic-epigenomic interactive genes" associated with the disease. They found that twelve genes including *UPK1B* (uroplakin 1b), *LHX6* (LIM homeobox 6), *CHMP4B* (charged multivesicular body protein 4b), *HOPX* (HOP homeobox), *SCARNA18* (small cajal body-specific RNA 18), *PF4* (platelet factor 4), *ATF3* (activating transcription factor 3), *STC1* (stanniocalcin 1), *TPRA1* (transmembrane protein adipocyte associated 1), *OR10K1* (olfactory receptor family 10 subfamily k member 1), *LOC101928523*, and *LANCL1* (lanC like 1), showed significant SNP-CpG interactions, within which three genes, *PF4*, *ATF3*, and *TPRA1*, had previously been linked to an increased risk of asthma. This study suggests that the synergistic effects of genetic and epigenetic variations may play an important role in the development of asthma.

It is important to carefully assess the differentially methylated sites (DMSs) or DMRs associated with human asthma, which may potentially lead to the new discovery of pathogenesis or clinical utility for asthma.

Several approaches have been employed in the assessment of differentially methylated sites and regions. Firstly, gene annotation can be performed to uncover the possible functions of the DMSs and DMRs. For examples, in Reese *et al.*'s study (23), the DMSs and DMRs were annotation based on UCSC (University of California Santa Cruz) Known Gene and fills in nearest gene within 10 MB (megabase). And the DMSs cg13427149 and cg16792002 were annotated to gene *GPATCH2* (G-patch domain containing 2) and *MAML2* (mastermind like transcriptional coactivator 2), which had previously been associated with obesity phenotypes related to childhood asthma. Secondly, the correlation of differentially methylated sites with expression of nearby genes can be analyzed when the gene expression data are available. Such as the DMSs annotation and mRNA expression analysis for the known asthma-related genes *NOTCH4* in (24). Thirdly, Base on gene annotation, pathways enrichment and gene interactions network can also be performed to uncover the underlying biologic mechanism (22,23). Moreover, some new approach such as machine learning also has been applied to select CpGs predicting asthma, which has showed better performance compared to two negative controls (31).

Histone modification and chromatin remodeling

The basic unit for the human genetic material chromatin is the nucleosome, which is a complex of four pairs of histones (each two of H2A and H2B, H3, and H4) that form the core of the octamer and its tangled DNA, and a histone H1 that binds the DNA between the two nucleosomes. Previous study has shown various forms of chemical modification, including acetylation, methylation, phosphorylation, and

ubiquitination, can occur at specific sites of histones (32), and that different histone modifications regulate the structural remodeling of chromatin through different mechanisms. Some types of modifications can change chromatin into active forms of euchromatin that are easily transcribed, while others can change it into heterochromatin that are not easy to be transcribed, regulating the expression of related genes (33). As summarized in (32), histone modification research in asthma has mainly been focused on histone acetylation and histone methylation.

The acetylation and deacetylation of histone are a pair of dynamic-equilibrium processes catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Acetylation can add negative charges to the N-term lysine residue of the histone, which causes mutex with the negatively charged DNA, leading to the relaxation of chromatin for the contact of transcription factors and DNA to activate the expression of certain genes. On the contrary, histone deacetylation can reverse the abovementioned process and stabilize the chromatin structure, preventing the transcription of related genes (14,32). Histone acetylation and deacetylation are widely involved in the processes of DNA replication and repair, cell cycle regulation, cell proliferation, and apoptosis, and play an important role in regulating the differentiation of Th1 and Th2 cells in the immune response (34). Harb *et al.* (35) compared the H3 and H4 histone acetylation levels of genome regions associated with Th1, Th2, and Treg cells between allergic asthmatic children and healthy controls through ChIP (chromatin immune co-precipitation) assay. They found acetylation levels of histones H3 and H4 in the gene region of *IL-13* (interleukin 13) were higher in children with allergic asthma, and the high H3 and H4 histone acetylation was associated with the overexpression of IL-13 protein in peripheral blood monocytes. In addition, levels of H3 histone acetylation in the gene region of *FOXP3* were significantly higher in patients with allergic asthma than in healthy controls. Moreover, multiple studies based on asthma animal models also showed the activities of histone acetylation enzymes were associated with the severity of the disease. Histone deacetylases inhibitors such as trichostatin (TSA) and budesonide (BUD) controlled the secretion of inflammatory factors and the T cell proportion balance in asthma models via regulating the activity of histone deacetylases, and finally alleviated the symptoms of asthma (36,37).

Histone methylation is another important histone modification pattern, which is catalyzed by histone methyltransferases (HMTs), including protein arginine methyltransferases (PRMTs) and lysine methyltransferases (KMTs), while histone demethylation is catalyzed by histone demethylases (HDMs). Unlike the acetylation of histones, which affects their interaction with DNA via changing the charges of the former, the methylation of lysine or arginine in histone does not affect its electrostatic binding to DNA, but indirectly affects the recruiting and binding of different regulatory proteins to the chromatin (38). Previous study has shown histone methylation status was closely related with the activities of the gene expression regulators on DNA such as promoter, enhancer, and silencer. By using histone methylation analysis technology, such as CHIP-seq, researchers can distinguish the regulators on DNA and predict their activation state (39). For example, most H3K4me2 (dimethylation of histone H3 at lysine 4) and H3K4me3 (trimethylation of histone H3 at lysine 4) modifications are enriched near the promoters to activate the expression of related genes, while the enrichments of H3K27me2 (dimethylation of histone H3 at lysine 27) and H3K27me3 (trimethylation of histone H3 at lysine 27) are often associated with gene expression inhibition.

Seumois *et al.* (40) compared H3K4me2 histone methylation profiles in Th1 and Th2 cells in asthmatic patients and normal subjects by using CHIP-seq technology, and found H3K4me2 histone methylation enriched in 200 enhancer regions in asthmatic patients compared with normal people. Among these asthma related enhancers, 163 were Th2 cell-specific, and 84 contained binding sites for transcription factors related to T-cell differentiation, such as RUNX3 (runt-related transcription factor 3), TBX21 (T-Box transcription factor 21), and GATA3 (GATA binding protein 3). These findings suggest histone methylation modifications in T cells of asthmatic patients are involved in the transcriptional regulation of genes related to T cell differentiation, affecting the pathogenesis of asthma.

Post-transcriptional regulation in asthma: non-coding RNA and the overexpression and silencing of genes

Study have shown that while most regions of the human genome are transcriptable (41), only about 2% of the human genome encodes proteins, and most genomes (75–90%) transcribe ncRNAs (42). ncRNAs can be divided into small non-coding RNA (small ncRNAs, length ≤200 nt) and long non-coding RNA (lncRNAs, length >200 nt). Although the biological functions of most ncRNAs are still unclear, many have been shown to play important roles

in the post-transcriptional regulation of genes and are an important part of epigenetics.

To date, ncRNA study in asthma have mainly focused on microRNA (miRNA), which are a class of highly conserved single-strand RNA molecules with length around 22 nt, which prompt the translation inhibition or degradation of mRNA in cells via inducing the formation of silent compounds (RISC). Previous report has shown that miRNAs are widely involved in the controlling of cell signaling pathways, and play an important role in the regulation of the inflammatory response and development of immune cells (43). Several studies to date have focused on the differential expression of asthma-related miRNA through *in vitro* or animal model experiments. The most studied asthma-related miRNAs include miR-155, miR-221, miR-145, miR-21, miR-146a/b, miR-126, let-7a, miR-133, miR-135a, miR-150, miR-20, and miR-19a (44), and multiple studies have shown that the up-regulation of certain miRNA is closely related to the development of asthma. For instant, miR-21 was shown to be up-regulated in the bronchial epithelial cells of asthma patients and involved in regulating the level of cytokines IL-13 and the Th1/Th2 cells balance, playing an important role in the pathogenesis of the disease (45,46). Similarly, miR-155 was found to be over-expressed in human bronchial epithelial cells, which promoted the secretion of inflammatory cytokines including IL-8 (interleukin 8), activating the JNK (c-Jun N-terminal kinase) signaling pathway and triggering the asthmatic inflammatory response in lung tissue (47). *In vitro* experiments also showed that miR-155 was involved in the regulation of Th2 cell differentiation, and targeting it could inhibit the development of clinical manifestations of asthma (48). Moreover, some studies have also shown the down-regulation of certain miRNA was associated with asthma. CD38 (cluster of differentiation 38) is a membrane protein highly expressed on the surface of airway smooth muscle cells in asthmatic patients, and is a target of miR-140-3p. Study has shown that in asthmatic airway smooth muscle cells, cytokine TNF-α (tumor necrosis factor alpha) induced up-regulation of CD38 expression through down-regulating the expression of miR-140-3p (49). A recent miRNA study showed that, compared with normal children, miR-29c was significantly decreased in children with asthma, while functional studies showed that miR-29c plays an important role in the occurrence of asthma in children by targeting the B7-H3 (B7 homolog 3) protein and regulating Th2/Th17 cell differentiation (50).

In addition to small ncRNA, lncRNA is also an important subject in epigenetic research. It has been reported that lncRNAs make-up a large part of the transcripts of the human genome and widely influence the processing and post-transcriptional regulation of mRNAs (51). The influence of lncRNAs on target genes depends on positional relationships between the lncRNAs and regulated genes in the genome (52). Antisense lncRNAs are transcribed by antisense DNA strands in the same gene loci with the regulated genes. Such lncRNAs often complementary bind to mRNA precursors generated by the gene in the sense strands, causing expression silencing or regulating the editing and splicing of mRNA precursors, ultimately affecting the expression of mature mRNAs (53). In addition, lncRNAs located in the regulatory region of genes also play an important regulatory role in gene expression. Many lncRNAs from enhancer regions can recruit various transcription factors and RNA polymerases, greatly improving gene transcription (54). Moreover, some lncRNAs also function as miRNA sponges, blocking the regulation effects of certain miRNAs on target mRNAs through the adsorption of these miRNAs (55). To date, few asthma-related lncRNAs have been reported, and these are a new research field in the pathogenesis of the disease. In 2019, Wang *et al.* (56) conducted a whole transcriptome sequencing study in acute asthma mice models and analyzed the differential expression of lncRNAs in $CD4^+$ T cells between acute asthma mice and control mice. They identified 98 up-regulated lncRNAs and 36 down-regulated lncRNAs in the acute asthma mice models compared with the control group, and further constructed a lncRNA-mRNA co-expression network based on the differentially expressed lncRNAs and mRNAs. Two lncRNAs, fantom3_4933428M03 and fantom3_9230106C11, were found to be located in the center of the co-expression network, indicating they might play a key role in signal regulation. The following bioinformatics analysis showed that lncRNA fantom3_9230106C11 may regulate Th2 cell differentiation through interaction with the transcription factors of Akr1b7 (aldo-keto reductase family 1 member b7), Gata1 (GATA binding protein 1), and the miRNA miR-19. In another transcriptome sequencing study in 2019, Zhu *et al.* (57) compared the expression differences of lncRNA in inflammatory cells of asthma patients with eosinophilic (Eos) and neutrophil (Neu) phenotypes. They found that compared with control samples, Eos samples contained 190 specifically differentially expressed lncRNAs, and Neu samples contained 166 specifically differentially expressed lncRNAs. Notably, the lncRNA LNC_000127

was specifically over-expressed in Eos samples, and the subsequent analyses showed that it was involved in regulation of the Th2 inflammatory pathway. This study indicates different subtypes of asthma may present different lncRNA profiles, and some specific lncRNAs may be used as biomarkers to identify different them. In addition, targeting LNC_000127 may be a potential strategy to alleviate the Th2 inflammatory response in Eos asthma patients.

In recent years, ceRNA has also become a hotspot in epigenetic research, and refers to the biological phenomenon that various long-stranded RNA molecules, including lncRNA, circRNA, and mRNA, competitively bind to the same miRNA to regulate the expression of the target genes of miRNA (58). Recently, some researchers have also taken the advantages of high-throughput analysis technology to study the interaction and regulation network of various RNAs in asthma from the perspective of ceRNAs. Qiu *et al.* (59) analyzed lncRNA and miRNA profiles in peripheral blood $CD4^+$ T cells of asthma patients and healthy controls using expression microarray technology, and focused on those which may participate in regulating the Th17/Treg cells balance. They found that lncRNA-MEG3 acted as a ceRNA of miRNA-17 which regulated the expression of miRNA-17's target gene *RORC* (RAR related orphan receptor c), and ultimately affected the Treg/Th17 cells balance in asthma patients. Moreover, in 2020, Liao *et al.* (60) downloaded the RNA expression data of 108 samples (including 88 asthmatic patients and 20 normal controls) from the Gene Expression Omnibus (GEO) database. They performed RNA differential expression analyses and built an asthma-related lncRNA-miRNA-mRNA interaction network based on the starBase RNA database. This network included 378 miRNAs, 163 mRNAs, and five key lncRNAs [MIR17HG (miR-17-92a-1 cluster host gene), MALAT1 (metastasis associated lung adenocarcinoma transcript 1), MAGI2-AS3 (MAGI2 antisense RNA 3), CASC2 (cancer susceptibility 2), and DAPK1-IT1 (DAPK1 intronic transcript 1)]. Two lncRNAs in the network, MALAT1 and MIR17HG, were found to be associated with several confirmed asthma-related mRNAs and miRNAs, and the subsequent functional analysis showed that these two lncRNAs may be involved in the differentiation of T cells and bronchial epithelial cells. In addition, a ceRNA-drug association network was established by combining the DrugBank and Therapeutic Target Database (TTD), and eight drugs including tamoxifen, ruxolitinib, retinoic acid, quercetin, dasatinib, levocarnitine, niflumic acid, and glybenclamide were found to be associated with MALAT1 and MIR17HG, which may provide new possibilities for the treatment of asthma.

Conclusions

This review has focused on recent advances in the epigenetic research of the pathogenesis of asthma, especially from the perspective of high-throughput analysis techniques. We have summarized recent progress in epigenomic studies of asthma on the "pre-transcriptional level", including DNA methylation (EWAS), histone modification, and chromatin remodeling, and on the "post-transcriptional level" mainly focusing on non-coding RNA.

Thanks to the rapid development of high-throughput analysis technology in recent years we can now conduct more effective and comprehensive epigenetic research on asthma, further uncovering its pathogenesis and finding potential epigenetic biomarkers and possible molecular targets for future intervention and treatment. For example, the discovery of non-coding RNAs related to asthma, including LNC_000127 and MALAT1, may bring new inspiration for the clinical classification and targeted therapy of the disease.

In this review, we have summarized the EWAS of childhood asthma (pediatric asthma) which mainly focus on the effects of parental and perinatal exposures on the epigenome of offspring and their relationships to the risk of asthma in offspring. The blood DNA methylation data in newborns were used to evaluate whether methylation patterns at birth relate to asthma development. In Reese *et al.* 's study (23), 9 new CpG sites and 35 differentially methylated regions were found to be significantly associated with the development of asthma in childhood, which may be used to develop a risk assessment model for the evaluation of risk score for asthma, and providing new basis for the early diagnosis of asthma. Moreover, the methylation patterns in newborns also correlated with maternal exposure to air pollution and tobacco smoking (24,26-28), which may also serve as risk factor or biomarker for early diagnosis of pediatric asthma. Furthermore, DNA methylation landscapes and lncRNA expression patterns were reported to represent different subtypes of asthma (29,57). So, these specific DNA methylation patterns or lncRNA expression patterns may also serve as biomarkers for the subtle classification of asthma and contribute to the evaluation and prognosis judgment of this disease.

Nowadays, more and more breakthrough technologies have been introduced into the epigenetic research field.

Such as Hi-C (high-throughput chromosome conformation capture), ATAC-seq (assay for transposase-accessible chromatin with high throughput sequencing) and single-cell sequencing have been applied to generate high-resolution 3D epigenomic maps for cells in different diseases (61-63). We believe that the future research on epigenetic mechanisms of asthma will also benefit from the implementation of such new technologies, which may help to efficiently decipher the correlations between the genome-wide epigenetic changes and the susceptibility gene expression for asthma, and lead to the new insights into the pathogenesis for asthma. Moreover, machine learning, the breakthrough method of data analysis also has been applied in the epigenetic study of asthma (31), which is supposed to benefit the future research on epigenetic mechanisms of asthma.

Currently, the number of high-throughput epigenetic studies of asthma (such as EWAS and transcriptomic sequencing) is relatively small, and most studies involve small sample sizes. The statistical significances of many newly discovered CpG markers or noncoding RNAs associated with asthma still need to be further validated in studies with larger sample sizes. Moreover, the actual biological function of many putative biomarkers or therapeutic targets of asthma identified by high-throughput techniques also require further study. We believe that the future research on epigenetic mechanisms of asthma will benefit from the improvement of the sample sizes and the conducting of biological function studies. And the combination of high-throughput epigenetic analysis technologies and traditional biological function and clinical studies will bring new breakthroughs in the pathogenesis study of asthma, which will improve the genetic interpretation of the disease and bring more possibilities for the development of precision medicine to treat it.

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Footnote

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Supplementary

Table S1 Detailed search strategy of PubMed database

Terms	Database	Number of results
Epigenetic research of asthma	PubMed	751
EWAS and asthma	PubMed	23
Methylation and asthma	PubMed	1,388
Histone modification and asthma	PubMed	148
Non-coding RNAs and asthma	PubMed	140

EWAS, epigenome-wide association study.

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