

Epigenetic regulation of immune function in asthma



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Asthma is a common complex respiratory disease characterized by chronic airway inflammation and partially reversible airflow obstruction resulting from genetic and environmental determinants. Because epigenetic marks influence gene expression and can be modified by both environmental exposures and genetic variation, they are increasingly recognized as relevant to the pathogenesis of asthma and may be a key link between environmental exposures and asthma susceptibility. Unlike changes to DNA sequence, epigenetic signatures are dynamic and reversible, creating an opportunity for not only therapeutic targets but may serve as biomarkers to follow disease course and identify molecular subtypes in heterogeneous diseases such as asthma. In this review, we will examine the relationship between asthma and 3 key epigenetic processes that modify gene expression: DNA methylation, modification of histone tails, and noncoding RNAs. In addition to presenting a comprehensive assessment of the existing epigenetic studies focusing on immune regulation in asthma, we will discuss future directions for epigenetic investigation in allergic airway disease. (*J Allergy Clin Immunol* 2022;150:259-65.)

Key words: Asthma, epigenetics, DNA methylation, histone modifications, noncoding RNA, microRNA

Asthma is a heterogeneous disease characterized by airway inflammation and partially reversible airflow obstruction.¹ It is a common chronic respiratory disease defined by a history of shortness of breath, wheeze, chest tightness, and cough that vary over time and intensity.¹ Asthma is a complex disease resulting from genetic, epigenetic, and environmental determinants. Genetic studies have identified numerous genetic polymorphisms associated with asthma susceptibility, but these genetic variants alone are unable to explain a large proportion of the variability in asthma risk.²⁻⁴ Environmental exposures are

Abbreviations used

HDAC: Histone deacetylase
lncRNA: Long noncoding RNA
miRNA: MicroRNA
mRNA: Messenger RNA
ncRNA: Noncoding RNA

thought to contribute to the increased prevalence of asthma; however, environmental exposures further compound the complexity of this disease by causing airway inflammation and obstruction through various mechanisms. Notably, epigenetic modifications are increasingly recognized as critical in the pathogenesis of asthma and may be a key link between environmental exposures and asthma endotypes.^{5,6} In this review, we will present a comprehensive assessment of the existing epigenetic studies focusing on immune regulation in asthma and discuss future directions for epigenetic investigation in allergic airway disease.

EPIGENETICS

Epigenetics refers to potentially heritable changes in gene expression that do not involve specific changes in nucleotide sequence. The epigenome, the collection of epigenetic modifications across an individual's genome, is influenced primarily by environmental exposures and genetic variation. Environmental exposures of high relevance to asthma, including specific allergens,⁷ cigarette smoke,⁸ and air pollution,⁹ have all been associated with changes in epigenetic modifications. This is also true of dietary factors that have been associated with asthma, such as folate and vitamin D.¹⁰ Environmental influences on the epigenome have been documented both prenatally and postnatally.^{11,12} An individual's genetic background can impact epigenetic modifications by genetic variants that are directly associated with changes in epigenetic marks.^{11,13-16} Importantly, epigenetic modifications can also be inherited, classically involving imprinted loci.¹⁷ Although there is a global loss of most known epigenetic modifications on parental chromosomes during primordial germ cell development and after fertilization, potential mechanisms of transgenerational epigenetic inheritance are being unraveled.¹⁸ Recently, transcription factors were identified to be carriers of epigenetic information during germ cell and preimplantation development.¹⁹ Through this, epigenetic modifications are likely to be a key mechanism by which traits are passed from parent or grandparent to offspring, such as the influence of maternal and grandmaternal cigarette smoking on risk of asthma in the offspring.²⁰ Unlike changes to DNA sequence,

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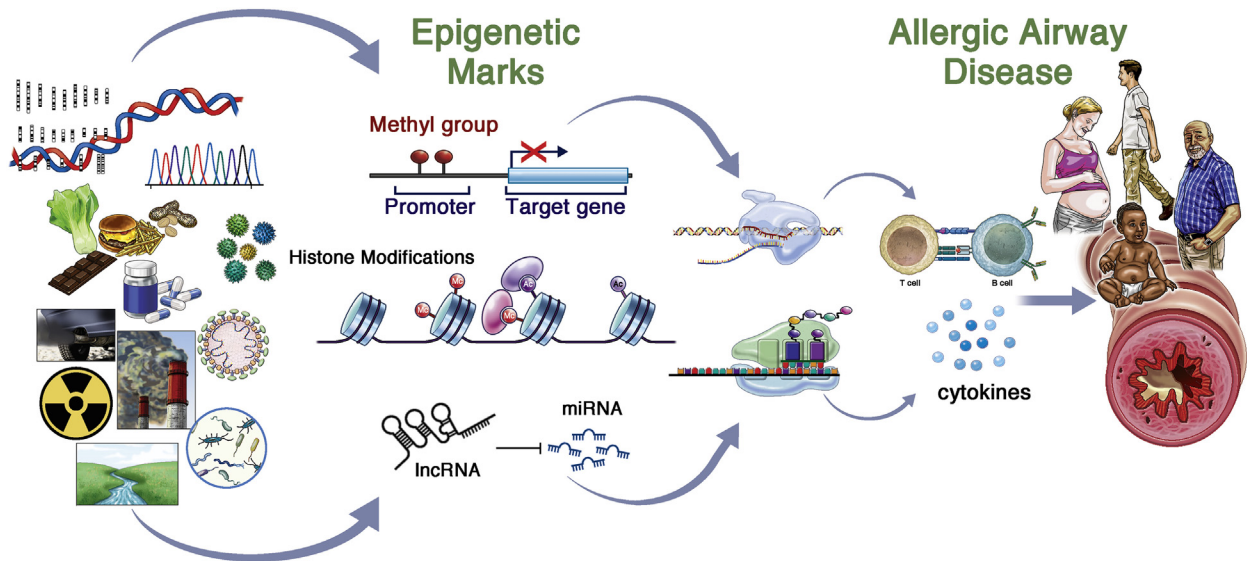


FIG 1. The relationship between asthma and 3 key epigenetic processes that modify gene expression: DNA methylation, modification of histone tails, and noncoding RNAs.

epigenetic marks are dynamic and can be reversible, creating an opportunity for not only therapeutic targets but also biomarkers to follow disease course and identify molecular subtypes in heterogeneous diseases. We will examine the relationship between asthma and 3 key epigenetic processes that modify gene expression: DNA methylation, modification of histone tails, and noncoding RNAs (Fig 1).

DNA METHYLATION

Addition of a methyl group to cytosine bases in DNA, most often in the context of cytosine followed by guanine (CpG), alters DNA accessibility by transcriptional machinery and results in recruitment of downstream proteins for chromatin remodeling.²¹ In the human genome, gene bodies, repeat sequences, and intergenic regions are generally methylated, whereas promoters of housekeeping genes are not methylated to allow for gene transcription to occur.²² The extent of DNA methylation at gene promoters and enhancers is associated with the level of gene expression, with increased DNA methylation often associated with decreased accessibility and repressed transcription. However, recent studies have identified transcription factors that bind methylated DNA²³ and suggest that this canonical relationship of DNA methylation and gene expression is not always followed. Although DNA methyl transferase enzymes catalyze the addition of methyl group to CpGs, demethylation of CpGs can occur either actively through successive oxidation reactions catalyzed by the ten-eleven translocase family of enzymes and 5-hydroxymethylcytosine intermediate, resulting in eventual replacement with cytosine through base excision repair,²⁴ or passively, in which newly synthesized DNA is not methylated by DNA methyl transferases. Sodium bisulfite conversion is the key technique used to assess the level of CpG methylation. DNA treatment with bisulfite results in deamination of cytosine to uracil (which becomes thymine during DNA amplification by PCR), but a methyl group protects this from occurring so that the ratio of C to T at any CpG reflects the extent of DNA methylation.²⁵ Next-generation sequencing and array-based genotyping can then be performed.

Investigations of DNA methylation changes are the most common epigenetic studies in asthma. Identification of DNA methylation profiles using both candidate gene and epigenome-wide association studies in asthmatic populations is being used to elucidate biologic mechanisms that influence asthma susceptibility and severity. Importantly, DNA methylation patterns are cell and tissue-specific and reveal how methylation changes are distinct based on the biological site being investigated.

DNA methylation studies in the developmental origin of asthma

Epigenetic modifications have been studied in lung development and may provide mechanistic insight into the impact of intrauterine environmental perturbations on subsequent disease risk. The developmental origins of health and disease hypothesis proposes that environmental exposures during critical periods of development can induce permanent changes in fetal physiology and metabolism that impact disease susceptibility later in life.²⁶ Alterations in the epigenome are being increasingly recognized as mechanisms by which intrauterine exposures enhance post-natal disease risk.²⁷ Initial studies in the agouti mouse model demonstrated that prenatal nutrition could result in transgenerational changes in DNA methylation, altering the phenotype of subsequent generations.²⁸ We and others have shown in murine models that maternal diet supplemented with methyl donors and alterations in folate metabolism enhance the severity of allergic airway disease that is inherited in a transgenerational fashion.^{29,30} These data suggest a causal link between maternal diet and fetal epigenetic changes that subsequently influence post-natal disease.

Other common prenatal exposures including *in utero* exposure to maternal smoking have been shown to result in differential methylation of genes in children.³¹ We have previously shown that *in utero* smoke exposure results in differential fetal lung tissue and placental methylation,³² and others have demonstrated widespread DNA methylation changes in the blood of children exposed to maternal smoking during pregnancy.^{31,33} Previous

studies have also identified the intrauterine period as a key developmental time frame during which preventive strategies can be implemented. For example, vitamin C supplementation in pregnant smokers was found to decrease the impact of maternal smoking on offspring pulmonary function and incidence of wheeze at 1 year of age.³⁴ Improvements in lung function were mediated by the resolution of DNA methylation changes identified in the placenta of the treated individuals. The reduction in DNA methylation in the placenta occurred at loci in pathways associated with oxidative stress, fetal development, and lung function.³⁵ Furthermore, normalization of *in utero* smoke-induced methylation changes was noted across multiple additional tissues in the offspring, suggesting a more systemic impact of vitamin C supplementation on blunting the impact of maternal smoke exposure.³⁶ These data demonstrate the benefit of understanding the epigenetic impact of environmental exposures during human lung development to identify novel preventive and therapeutic targets.

Intrauterine exposures have also been shown to impact postnatal susceptibility to allergic disease through regulation of T_H1 and T_H2 polarization. A study of prenatal exposure to traffic-related airborne polycyclic aromatic hydrocarbons demonstrated an association between methylation of acyl-CoA synthetase long-chain family member 3 (*ASCL3*) and asthma symptoms before age 5 years.³⁷ These changes resulted in increased T_H1 polarization. Interestingly, animal models have demonstrated that a combination of other environmental exposures including diesel exhaust particles and allergen exposure differentially modulate T_H1 and T_H2 gene methylation, gene expression, and subsequent IgE production.³⁸ These data suggest that asthma endotypes may be influenced by epigenetic changes that result from environmental exposures during fetal development.

DNA methylation studies in childhood asthma

Initial studies of DNA methylation in human subjects identified association of DNA methylation with candidate genes in the blood,³⁹ buccal mucosa,⁴⁰ and the nasal epithelium⁴¹ of asthmatic subjects. For example, increased methylation of the beta-2-adrenergic receptor (*ADRB2*) in the blood of childhood asthmatic patients was associated with increased asthma severity,⁴² whereas hypomethylation of genes in the lipoxygenase pathway in the blood of childhood asthmatic patients was associated with increased wheeze.³⁹

Epigenome-wide association study investigations identified DNA methylation signatures associated with markers of atopy including serum IgE and peripheral blood eosinophils. A study using data from the Avon Longitudinal Study of Parents and Children demonstrated that DNA methylation changes that were associated with asthma were largely driven by higher eosinophil counts in asthma cases.⁴³

The Pregnancy and Childhood Epigenetics consortium investigated the association of DNA methylation changes identified at birth with the development of childhood asthma. DNA methylation in newborn blood and measured during childhood identified differentially methylated regions associated with childhood asthma.⁴⁴ The genes identified as dysregulated included genes in the endothelial nitric oxide synthase pathways and biological processes associated with immune function.⁴⁴

We have previously identified 81 differentially methylated regions in PBMCs associated with allergic asthma in Black inner-

city children.⁴⁵ We found that several immune genes were hypomethylated, resulting in overexpression of IL-4 and IL-13 in asthma.⁴⁵ These findings demonstrate the potential importance of DNA methylation on the T_H2 immune response in allergic asthma and the impact of epigenetic regulation of T-cell differentiation.

Nasal epithelial DNA methylation changes have also been used to identify additional biological pathways that influence asthma susceptibility and severity. Previous investigation of differential DNA methylation in the nasal epithelium between children with and without atopic asthma identified methylation changes in genes involved in epithelial barrier function and immune regulation. We have previously identified extensive methylation changes in the nasal epithelium of inner-city Black children with asthma.⁴⁶ Notably, many of the DNA methylation changes that were identified in this cohort influenced the expression of numerous genes⁴⁶ including many that were also identified in the IL-13 DNA methylome signature of cultured airway epithelial cells of asthmatic patients, suggesting a potential mechanistic link to allergic asthma.⁴⁷ Importantly, replication of nasal epithelial methylation profiles across cohorts demonstrates consistency across top loci and similar effect sizes.^{46,48}

Notably, DNA methylation studies have also been used to classify subjects on the basis of atopic status, demonstrating their potential as predictive biomarkers of asthma. Previous studies have demonstrated that nasal epithelial methylation changes can be used to classify atopic asthmatic patients from those without asthma with a high level of accuracy (area under the curve = 0.93).⁴⁸

DNA methylation studies in childhood asthma have also established a link between early-life environmental exposures and asthma susceptibility and disease severity. Emerging literature suggests that air pollution exposure modulates DNA methylation during childhood, demonstrating the importance of investigation of the impact of environmental exposures throughout the life course. Prunicki et al⁴⁹ have demonstrated differential methylation of the *Foxp3* gene in asthmatic subjects with exposure to ambient air pollution compared with nonasthmatic control subjects with similar levels of ambient air pollution exposure. The DNA methylation changes identified in the promoter of the *Foxp3* gene were also shown to be associated with impairment in regulatory T-cell function.⁴⁹ *Foxp3* has been shown to be involved in immune regulation⁵⁰ and has been implicated in asthma pathogenesis.⁵¹ In addition, exposure to traffic-related air pollution including exposure to black carbon has been associated with changes in DNA methylation in the nitric oxide synthase 3 gene in the buccal mucosa and is also associated with increased fraction of expired nitric oxide in asthmatic children.⁵²

DNA methylation studies in adult asthma

Epigenome-wide studies of DNA methylation support a role for epigenetic mechanisms in childhood asthma, but DNA methylation studies in adults remain limited. Liang et al⁵³ identified an association of serum IgE and hypomethylation at 36 loci that were identified in 3 independent adult cohorts. DNA methylation patterns demonstrate biological differences between remitting and persistent asthma in adults. DNA methylation patterns in bronchial biopsy specimens between subjects with asthma in remission were compared with those with

persistent disease and healthy controls. Differentially methylated regions associated with remission include genes involved in the resolution of inflammation.⁵⁴

Using epigenome-wide association studies from the peripheral blood of adult participants in the Agricultural Lung Health Study, 524 differentially methylated sites were identified in adult subjects with nonatopic asthma compared with controls.⁵⁵ These differentially methylated CpG sites were enriched for genes in the asthma and sphingolipid metabolism pathways.⁵⁵ In this cohort, 1086 DNA methylation sites were identified to be differentially methylated in subjects with atopic asthma compared with nonasthmatic controls at a false-discovery rate (<0.05) including genes involved in insulin signaling. Notably, more CpG sites were differentially methylated in those with atopic asthma compared with those with nonatopic asthma, with approximately 10% overlapping between the 2 conditions. These results highlight the need to differentiate between atopic and nonatopic asthma. Furthermore, these results highlight the need to investigate epigenetic mechanisms across the life course because asthma risk factors and pathobiological mechanisms may differ during development, in childhood, and into adulthood.

HISTONE MODIFICATIONS

Chromatin accessibility is directly regulated by chemical modifications to tails of histone proteins that form octamers around which DNA is wrapped to form nucleosomes.⁵⁶ Histone tails can be modified in many ways, including methylation, acetylation, ubiquitylation, and SUMOylation.⁵⁷ As an example, acetylation of histone tails is often associated with gene activation, whereas deacetylation is associated with gene repression. A common acetylation mark, lysine 27 on the histone H3 (H3K27ac), is a mark of active enhancers and active promoters, whereas trimethylation of that same lysine residue (H3K27me3) is a repressive mark on both promoters and enhancers.⁵⁸ Histone acetyltransferases acetylate histone tails, histone deacetylases (HDACs) remove acetyl groups from histone tails, and bromodomain (Brd) proteins are chromatin readers that recognize and bind acetylated histones and play a key role in transmission of epigenetic memory across cell divisions and transcription regulation.⁵⁹ Similarly, histone methyltransferases add the methyl groups to histone tails, whereas histone demethylases remove them. The Encyclopedia of DNA Elements, Roadmap Epigenomics, and the International Human Epigenome Consortium projects have created maps of histone modifications in cell lines and primary cells/tissues, providing important public resources for the research community.⁶⁰⁻⁶²

Histone modifications in asthma

Although studied less frequently than DNA methylation, histone modifications have been implicated in asthma susceptibility, disease severity, and treatment response. The level of histone acetylation, which is associated with increased gene expression, is dependent on the balance of HDAC and histone acetyltransferase activity. Evidence demonstrates a role for histone modifications in immune cell differentiation including those involved in T_H2 -mediated inflammation that are crucial to the development of asthma.^{7,63} Acetylation of histones has been implicated in the differentiation and function of T_H cells, with

increased histone acetylation being identified at T_H2 cytokine loci in response to T_H2 differentiation, resulting in increased T_H2 cytokine production.⁶⁴ Previous animal models have also demonstrated the impact of histone acetylation on asthma by demonstrating that a loss of histone acetylation at the IL-4/IL-13 locus greatly reduces T_H2 effector functions.⁶⁵ A study characterizing genome-wide histone modifications in T-cell subsets from patients with asthma and healthy controls showed asthma-specific differences in enhancers involved in T-cell differentiation.⁶⁶

There is also increasing evidence for the role of HDACs in asthma severity and treatment response. Glucocorticoids remain the most effective anti-inflammatory medications for the treatment of chronic airway inflammation in asthma. Glucocorticoids are known to cause activation of anti-inflammatory gene expression through acetylation of histone H4. Furthermore, activated glucocorticoid receptors reduce histone acetylation through recruitment of HDAC2, resulting in suppression of activated inflammatory genes.⁶⁷ Notably, HDAC2 deficiency results in decreased corticosteroid treatment response in those with severe asthma.^{68,69} Furthermore, exposure to passive smoke diminished corticosteroid sensitivity of alveolar macrophages obtained from children with severe asthma due to decreased HDAC2 expression.⁷⁰

HDAC1 is integral to airway epithelial repair and remodeling after injury. Increased HDAC1 has been previously associated with severe asthma and airway remodeling in those with severe asthma compared with those with mild disease.^{71,72} HDAC1 is associated with T-cell development, and previous murine models have demonstrated that HDAC1-deficient T cells result in increased eosinophil recruitment and enhancing T_H2 polarization.⁷³ The data linking histone modifications to asthma remain limited. Given the importance of these mechanisms in glucocorticoid handling, additional investigation of the role of histone modifications in asthma susceptibility and treatment response are warranted.

NONCODING RNAs

Noncoding RNAs (ncRNAs) comprise a much larger portion of the human genome than protein-coding RNAs. They are broadly classified into long noncoding RNAs (lncRNAs) and small RNAs (<200 nucleotides). lncRNAs comprise a large family consisting of long intergenic noncoding RNAs (linkRNAs), enhancer RNAs, and antisense RNAs, to name a few. They perform various functions, including regulation of transcriptional, posttranscriptional control, structural function, and genome integrity.⁷⁴ Most lncRNAs are polyadenylated and captured with standard messenger RNA (mRNA) library preparation; however, lncRNAs are less abundant and more cell/tissue-specific, therefore requiring deeper sequencing than mRNAs. As an alternative to mRNA sequencing, library preparation protocols that remove ribosomal RNA (rRNA depletion) capture all lncRNAs. The most well-studied class of small RNAs is microRNAs (miRNAs), which are approximately 22 nucleotide long regulatory RNAs that control gene expression by binding to the 3' untranslated regions of mRNA, which leads to either mRNA degradation or inhibition of protein translation.⁷⁵ Although immature forms of miRNAs are detectable in standard RNA-sequencing protocols, the fully mature form can only be detected by doing small RNA-sequencing library preparation.

TABLE I. Human epigenetic studies in asthma

Epigenetic mark	Age	Tissue type	Trait association	Reference*
DNA methylation	Fetal	Fetal lung	Asthma	32
		Blood	Asthma	39,42-44
	Children	Buccal mucosa	Asthma	40
		Nasal epithelium	Asthma	41,46,48
		PBMC	Asthma	45
	Adult	Blood	Asthma	53,54
			Atopy/IgE	53
			Atopic asthma	55
			Asthma	68,69
Histone modifications	Adult	Blood	Asthma	79
miRNA	Fetal	Fetal lung	Asthma	80,81
	Children	Blood	Asthma	90
			Asthma treatment response	87
			Asthma/lung function	85
	Adult	Blood	Asthma	89
			Asthma/treatment response	

*These studies have been highlighted in the review.

Noncoding RNAs in asthma

Although ncRNAs are known to regulate gene transcription, an understanding of their role in asthma susceptibility remains limited. Murine models have identified the role of several lncRNAs with biological mechanisms tied to asthma pathogenesis. For example, lncRNA PTPRE-AS1 has been shown to modulate M2 macrophage activation and has been associated with allergic inflammation.⁷⁶ In addition, the expression of PTPRE was found to be significantly lower in PBMCs from patients with allergic asthma, suggesting a role in asthma pathogenesis.⁷⁶ lncRNA MALAT1 has also been previously shown to be upregulated in airway smooth muscle cells of patients with asthma and has been shown to promote proliferation of airway smooth muscle cells by downregulating miR-216a.⁷⁷ Together, these data demonstrate the cell-specific nature of the impact of noncoding RNAs and demonstrates that these lncRNAs impact underlying pathobiology through complex mechanisms that involve microRNAs and other epigenetic and genomic features.⁷⁸ Further research using integrative methods to investigate these epigenetic mechanisms is necessary to elucidate the full impact of ncRNAs on asthma pathobiology.

Role of miRNAs in asthma

The role of miRNAs in asthma pathogenesis has been more widely investigated. miRNAs are integral to normal lung development, and murine models have shown that aberrant miRNA expression during lung development has been implicated in subsequent postnatal respiratory disease. We have previously demonstrated the involvement of miRNA-15a with the developmental origin of asthma through its differential expression by intrauterine smoke exposure in our murine model, demonstrating that miR-15a was associated with the intrauterine expression of gasdermin B (*GSDMB*), a known childhood asthma gene in the human fetal lung, and demonstrating an association of miR-15a expression with asthma exacerbations in children participating in the Childhood Asthma Management Program.⁷⁹ These results suggest that miRNAs impact the expression of known asthma susceptibility loci, impacting subsequent disease development.

Previous studies have also demonstrated a link between miRNAs and allergic asthma by altering T_H1/T_H2 polarization. The let-7 family of miRNAs has been associated with T_H2 inflammation,^{80,81} which is critical to the development of allergic asthma. Two additional miRNAs, mir-155 and miR-221, have been associated with T_H2 response and eosinophilia in asthma.⁸² miR-155 targets CTLA-4 expression and enhances the secretion of IL-4, IL-5, and IL-13, increasing T_H2-mediated inflammation.⁸³ miR1 has been shown to have an opposite effect, resulting in the inhibition of IL-4 and IL-5 secretion, thereby regulating the T_H1/T_H2 balance in asthma.⁸⁴

miRNAs are also involved in airway smooth muscle proliferation in asthma. miR-221 has also been shown to play a role in smooth muscle activation in asthma by increasing proliferation of smooth muscle cells in patients with severe disease.⁸⁵ Similarly, miR26 promotes airway smooth muscle hypertrophy through regulation of the expression of GSK-3 β .⁸⁶ These miRNAs are involved in smooth muscle hypertrophy and contraction seen in the airway of patients with asthma.

miRNAs have been previously associated with asthma susceptibility and respiratory outcomes in asthmatic subjects. Studies in children with mild to moderate persistent asthma participating in the CAMP study identified 74 serum miRNAs to be associated with asthma susceptibility and 34 miRNAs to be associated with lung function in asthma.⁸⁷ Eight serum miRNAs were also associated with airway responsiveness in the CAMP cohort.⁸⁸ Although these data demonstrate the association of miRNAs with asthma and asthma outcomes, additional functional validation of these loci is necessary to understand the mechanism of action of these miRNAs in asthma pathobiology.

miRNAs have also been implicated in asthma treatment response. Budesonide, an inhaled corticosteroid used in the treatment of asthma, has also been shown to impact the miRNA signature in the bronchial epithelium of patients with asthma. Solberg et al⁸⁹ showed that inhaled budesonide had modest effect on the miRNA profile in the bronchial epithelium of steroid-naïve patients after initiation of inhaled corticosteroid treatment. In addition, several circulating miRNAs have been associated with inhaled budesonide treatment response in CAMP.⁹⁰ Together, these observations demonstrate a role for miRNAs in asthma susceptibility and treatment response by highlighting their impact on

the control of inflammatory responses, T_H1/T_H2 polarization, disease outcomes, and response to therapy.

FUTURE DIRECTIONS IN EPIGENETIC INVESTIGATION

Cross-sectional studies of epigenetic signatures in asthmatic subjects have identified some of the biologic mechanisms underlying asthma susceptibility and disease heterogeneity (Table I). However, epigenetic studies in extensively phenotyped subjects with asthma followed prospectively remain limited. In addition to better characterization of environmental exposures that impact tissue-specific epigenetic signatures, systems-based approaches that incorporate additional genomic data types in asthma cohorts will identify additional biological pathways that impact disease susceptibility and help to identify novel therapeutic targets for this common disease.⁹¹⁻⁹³

Furthermore, technological advances including the assessment of chromatin accessibility⁹⁴ using the Assay for Transposase-Accessible Chromatin using sequencing, Cleavage Under Targets & Tagmentation, and single-cell sequencing technologies have yet to be extensively applied in asthma. In addition, recent advances in molecular imaging provide novel noninvasive methods for visualization of epigenetic changes *in vivo* in a tissue-specific manner.⁹⁵ Application of these novel technologies in at-risk and asthmatic cohorts is necessary to elucidate new biological mechanisms that impact asthma susceptibility.

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