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Epigenetic mechanisms and the development of asthma

Ivana V. Yang, PhD, and David A. Schwartz, MD *Aurora, Colo*

Asthma is heritable, influenced by the environment, and modified by *in utero* exposures and aging; all of these features are also common to epigenetic regulation. Furthermore, the transcription factors that are involved in the development of mature T cells that are critical to the T_H2 immune phenotype in asthmatic patients are regulated by epigenetic mechanisms. Epigenetic marks (DNA methylation, modifications of histone tails, and noncoding RNAs) work in concert with other components of the cellular regulatory machinery to control the spatial and temporal levels of expressed genes. Technology to measure epigenetic marks on a genomic scale and comprehensive approaches to data analysis have recently emerged and continue to improve. Alterations in epigenetic marks have been associated with exposures relevant to asthma, particularly air pollution and tobacco smoke, as well as asthma phenotypes, in a few population-based studies. On the other hand, animal studies have begun to decipher the role of epigenetic regulation of gene expression associated with the development of allergic airway disease. Epigenetic mechanisms represent a promising line of inquiry that might, in part, explain the inheritance and immunobiology of asthma. (J Allergy Clin Immunol 2012;130:1243-55.)

Key words: *Asthma, atopy, epigenetics, gene expression, DNA methylation, histone marks, noncoding RNAs*

Abbreviations used

AHR:	Airway hyperresponsiveness
CHARM:	Comprehensive Analysis of Relative DNA Methylation
ChIP:	Chromatin immunoprecipitation
DMR:	Differentially methylated region
DNMT:	DNA methyltransferase
FENO:	Fraction of exhaled nitric oxide
FOXP3:	Forkhead box protein 3
LCR:	Locus control region
miRNA:	MicroRNA
$PM_{2.5}$:	Particulate matter of 2.5 μm in diameter or less
QTL:	Quantitative trait locus
T-bet:	T-box transcription factor
Treg:	Regulatory T

Asthma is a complex heritable disease affecting more than 8% of the US population, approximately 7 million children and approximately 18.7 million adults.[1,2] This disease has been increasing in prevalence, incidence, and severity,[3] although recent evidence suggests that the prevalence of asthma and allergies might have reached a plateau in developed countries.[4] Asthma accounts for more than $10 billion of direct health care costs in the United States.[3] In 2008, persons with asthma missed 10.5 million school days and 14.2 million work days because of their disease.[1] Sex and ethnic differences exist for women and African American asthmatic patients, with both having a significantly higher rate of outpatient asthma visits, emergency department evaluations, and hospitalizations than non-Hispanic male subjects.[1] Consistent with these data is an increased ***mortality rate*** in women and African American asthmatic patients that is 45% and 200% higher, respectively, than that seen in non-Hispanic white male patients. What is most disturbing is that ongoing increases in disease prevalence, incidence, and severity are occurring despite the intense national and international investigation into the pathobiology, genetics, and treatment of asthma. Consequently, it is essential to consider alternative explanations for the growing health problems associated with the development and persistence of asthma.

Several separate lines of evidence support a role for epigenetics in asthma (Fig 1). First, asthma, like epigenetic mechanisms, is heritable. Although asthma is a strongly familial condition (36% to 79% ***heritability***) with a non-Mendelian pattern of inheritance and polymorphisms in more than 100 genes,[5-8] these associations have infrequently been replicated, and genetics has explained only a small portion of the cause of this disease.[6]

Second, asthma, like epigenetic mechanisms, shows a parent-of-origin transmission of inheritance, with an affected mother significantly more likely to transmit the disease than an affected father.[9] These parent-of-origin effects can result from immune interactions between the fetus and the mother.[10] Alternatively, the maternal effect might be the result of epigenetically regulated ***genomic*** imprinting.[11] Several known genes show parent-of-origin effects on allergic disease; these genes include the *FCERIB* locus,[12] and the ***Spink5*** gene.[13]

Third, asthma, like epigenetic mechanisms,[14,15] is affected by *in utero* exposures.[16,17] Prenatal exposure to maternal and

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Corresponding author: David A. Schwartz, MD, Department of Medicine, University of Colorado School of Medicine, 12631 E 17th Ave, B178, Aurora, CO 80045. E-mail: david.schwartz@ucdenver.edu.

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Terms in boldface and italics are defined in the glossary on page 1244.

grand-maternal cigarette smoke[18-20] and traffic-related air pollution[21,22] are among the *in utero* exposures that contribute to the development of this disease. On the other hand, higher maternal fruit and vegetable intake and ***oily fish*** consumption during gestation have been associated with a lower risk of asthma.[23]

Fourth, asthma, like epigenetics, is influenced by the general environment.[24] Environmental factors are known to play important roles in the pathogenesis of asthma, both in terms of main effects and those exerted indirectly through complex interactions with gene variants.[25] The dramatic increase in the prevalence, incidence, and severity of asthma over the last 20 years provides strong evidence that exposures, including diet, play an important role in the development of this disease; these changes have occurred too rapidly to be accounted for by changes in primary DNA sequences alone. Although allergens are classically associated with asthma,[26] many other exposures, including smoking behavior,[25,27] agents in the workplace,[28] indoor and outdoor air pollution,[29] viruses,[30] domestic[31] and occupational[32] exposure to endotoxin, and immunization against certain infectious diseases,[33] are associated with the development and progression of this disease, and several of these agents have been shown to alter epigenetic marks.

Finally, asthma is an immune-mediated disease characterized mainly by skewing toward a T_H2 phenotype, although other T-cell subtypes might be involved.[34] Epigenetic mechanisms regulate the expression of transcription factors that are involved in T-cell differentiation (T_H1, T_H2, and regulatory T [Treg] cells).[35-42]

EPIGENETIC MECHANISMS

Epigenetics is traditionally defined as the study of heritable changes in gene expression caused by molecules that bind to DNA rather than changes in the underlying DNA sequence (Table I)[43-45] Recent evidence suggests that the epigenome is dynamic and changes in response to the environment, diet, and aging.[46] In addition to a set of inherited epigenetic marks, there are likely nonheritable epigenetic marks that are more dynamic and change in response to environmental stimuli. Three main classes of epigenetic marks are DNA methylation, modifications of histone tails, and noncoding RNAs (Fig 2).

Methylation of cytosine residues in ***CpG dinucleotides*** (5-methylcytosine) within the context of CpG islands is the simplest form of epigenetic regulation in eukaryotes, with hypermethylation of CpG islands in gene promoters leading to gene silencing and hypomethylation leading to active transcription. CpG island methylation has long been studied in patients with cancer, with findings that hypermethylation of tumor

GLOSSARY

3′: A single strand of DNA is oriented based on the direction of the phosphodiester bonds that join each nucleotide. A 5′-3′ phosphodiester bond involves a bond between the fifth carbon of one pentose ring and the third carbon of the next pentose ring. DNA strands have opposite chemical polarity, with one strand running in a 5′ to 3′ direction and the other in a 3′ to 5′ direction.

CHROMATIN: Chromatin is a substance within a chromosome consisting of DNA and protein. The major proteins in chromatin are histones. Changes in chromatin structure are associated with DNA replication and gene expression.

CpG DINUCLEOTIDES: Shorthand for cytosine-phosphate-guanine or a cytosine base located adjacent to a guanine base in a linear sequence (as opposed to cytosine forming chemical bonds to guanine on the opposite DNA strand).

CYTOTOXIC T LYMPHOCYTE–ASSOCIATED ANTIGEN 4 (CTLA-4): A transmembrane protein containing immune tyrosine inhibitory motifs. CTLA-4 is expressed on peripheral T cells after activation and plays an important role in terminating T-cell responses *in vivo*.

GENOMIC IMPRINTING: Differential expression of genes depending on whether they are inherited from the maternal or paternal parent.

HERITABILITY: The proportion of observed variation in a particular trait that can be attributed to inherited genetic factors in contrast to environmental factors.

INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS): An enzyme that converts L-arginine into nitric oxide. iNOS is found in macrophages, fibroblasts, neutrophils, and smooth muscle cells. Many proinflammatory cytokines increase the expression of iNOS.

MICROARRAY: A technology used to study the expression, methylation, noncoding RNAs, and chromatin marks of many genes at once. It involves placing thousands of gene sequences in known locations on a glass slide called a gene chip. A sample containing DNA or RNA is placed in contact with the gene chip. Complementary base pairing between the sample and the gene sequences on the chip produces light that is measured. Areas on the chip producing light identify genes that are expressed in the sample.

MORTALITY RATE: The ratio of deaths to the number of subjects in a population usually expressed as the number of deaths per thousand subjects per year.

MyD88: MyD88 is key downstream adapter for most Toll-like receptors. MyD88 deficiency is an autosomal recessive immune deficiency involving life-threatening, often recurrent pyogenic bacterial and mycobacterial infections, including invasive pneumococcal disease.

NUCLEOSOME: A subunit of chromatin containing DNA and histone complex.

OILY FISH: Oily fish include salmon, trout, mackerel, sardines, pilchards, herring, kipper, eel, whitebait, and fresh tuna. Omega-3 fatty acids are found in these fish.

$PM_{2.5}$: Particles, such as those found in smoke and haze, that are 2.5 μm in diameter or less. These particles can be directly emitted from sources such as forest fires, or they can form when gases emitted from power plants, industries, and automobiles react in the air.

POLYCYCLIC AROMATIC HYDROCARBON (PAH): PAH compounds are a generally hazardous class of organic compounds found in petroleum and emissions from fossil fuel use and conversion processes. PAHs are comprised of 2 or more benzene rings arranged in various configurations.

RETROTRANSPOSON: An intermediate RNA transcript that has a copy of the DNA of the transposable element made by using a reverse transcriptase. This DNA is then inserted into the genome at a new location.

Spink5: A gene that encodes proteins important for epidermal integrity. Mutations in *Spink5* are associated with Netherton syndrome. *Spink5* mutations have also been found in children with atopic dermatitis and food allergy.

UBIQUITYLATION: Ubiquitin is a 76-amino-acid peptide. Ubiquitylation involves ubiquitin conjugation to a lysine residue of a target protein or another ubiquitin molecule, thereby forming a branching structure. This conjugation serves to modulate protein signaling.

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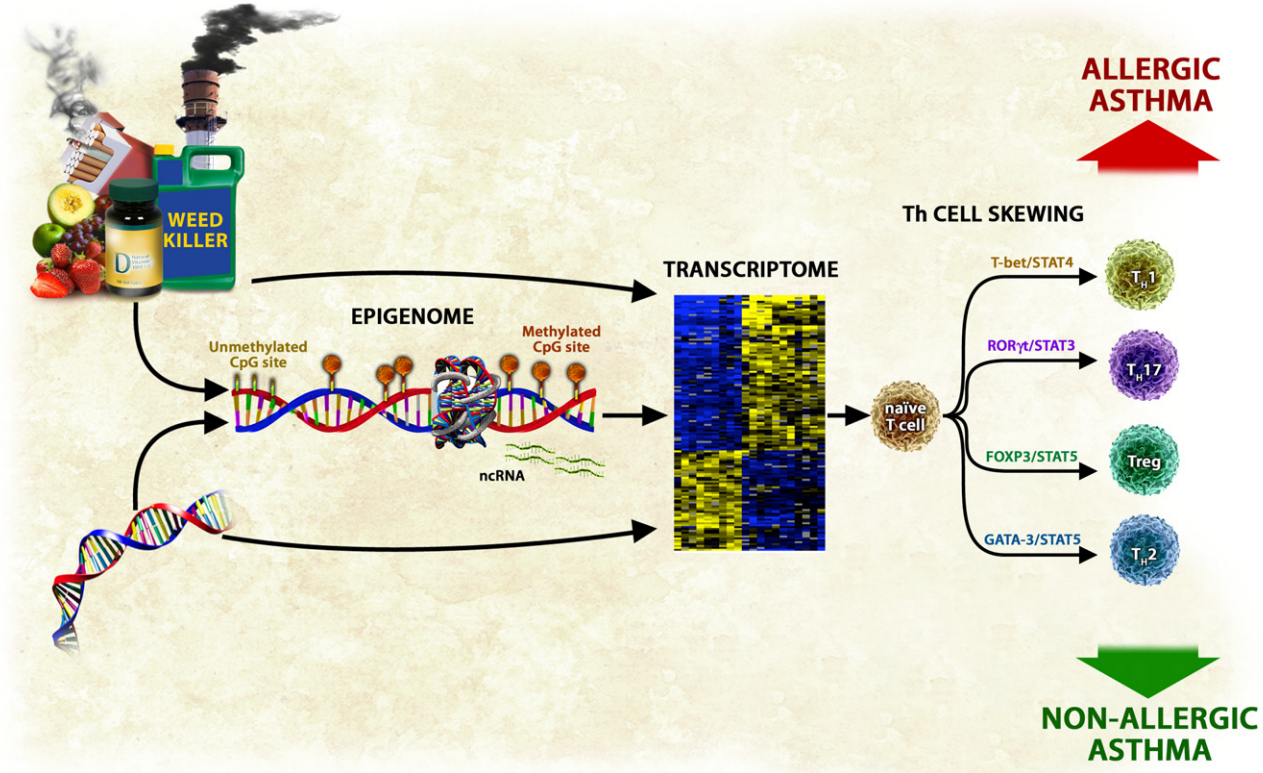

FIG 1. An overview of epigenetic regulation of gene expression in asthmatic patients. Environmental exposures and dietary factors to which a subject is exposed *in utero* and postnatally influence epigenetic marks, which in turn regulate genes expression. Underlying genetic variation can regulate gene expression by affecting epigenetic marks or through other mechanisms (eg, alteration of transcription factor binding sites). Alterations in epigenetic marks have consequences on expression of key immune genes that regulate T_H subtype cell skewing, which in turn leads to the development of disease. It is likely that distinct epigenomic profiles are associated with the development of allergic and nonallergic forms of asthma. *ncRNA,* Noncoding RNA; *RORγt,* retinoic acid–related orphan receptor; *STAT,* signal transducer and activator of transcription.

TABLE I. Key components of the epigenetic regulation of gene expression

Epigenetics	The word epigenetics is derived from the Greek word "epi-" for over or above and "genetics" for the science of heredity. Two key components of epigenetics (DNA methylation and histone modifications) together define the chromatin state beyond the information that is encoded in the genomic DNA sequence. Together, they control the degree of accessibility of the genomic DNA fraction of chromatin. The tightly bound "closed" chromatin state is less accessible to transcription machinery and other regulatory proteins, whereas the more accessible open chromatin state leads to active gene transcription. Epigenetic marks work in concert with other components of cellular regulatory machinery (transcription factors, enhancer, and repressors) to control the spatial and temporal level of expressed genes.
DNA methylation	DNA methylation is the extent of methylation of cytosines in approximately 30 million CpG sites in the human genome. Sixty percent to 90% of CpGs across the human genome are methylated, whereas regulatory regions containing more dense areas of CpG motifs (CpG islands; stretches of DNA >200 bp in length with >50% GC content and observed/expected CpG >0.6[44]) are generally unmethylated. DNA methylation also correlates with spatial organization of the chromatin (proximity of chromosomal loci).[45]
Histone modifications	In eukaryotes DNA is packaged and ordered into nucleosomes, the basic structural unit of chromatin, by wrapping around the octamer, which consists of 2 copies each of histone proteins (H2A, H2B, H3, and H4). Histone modifications include methylation, acetylation, phosphorylation, and other modifications of specific amino acids in nucleosomal histones. Generally, histone marks are described as "permissive" (active promoters), "repressive" (inactive promoters), or "poised" (accessible promoters).
Noncoding RNA	The term noncoding RNA is commonly used for RNA that does not encode a protein. Noncoding RNAs include both small and large classes of RNA molecules that control gene expression through a variety of mechanisms.

suppressor genes and hypomethylation of oncogenes contribute to the process of carcinogenesis.[11,47] More recent studies have demonstrated that methylation of less CpG-dense regions near CpG islands (CpG island shores) controls expression of tissue-specific genes, as well as genes relevant to carcinogenesis and lineage-specific cell differentiation,[48,49] suggesting that DNA

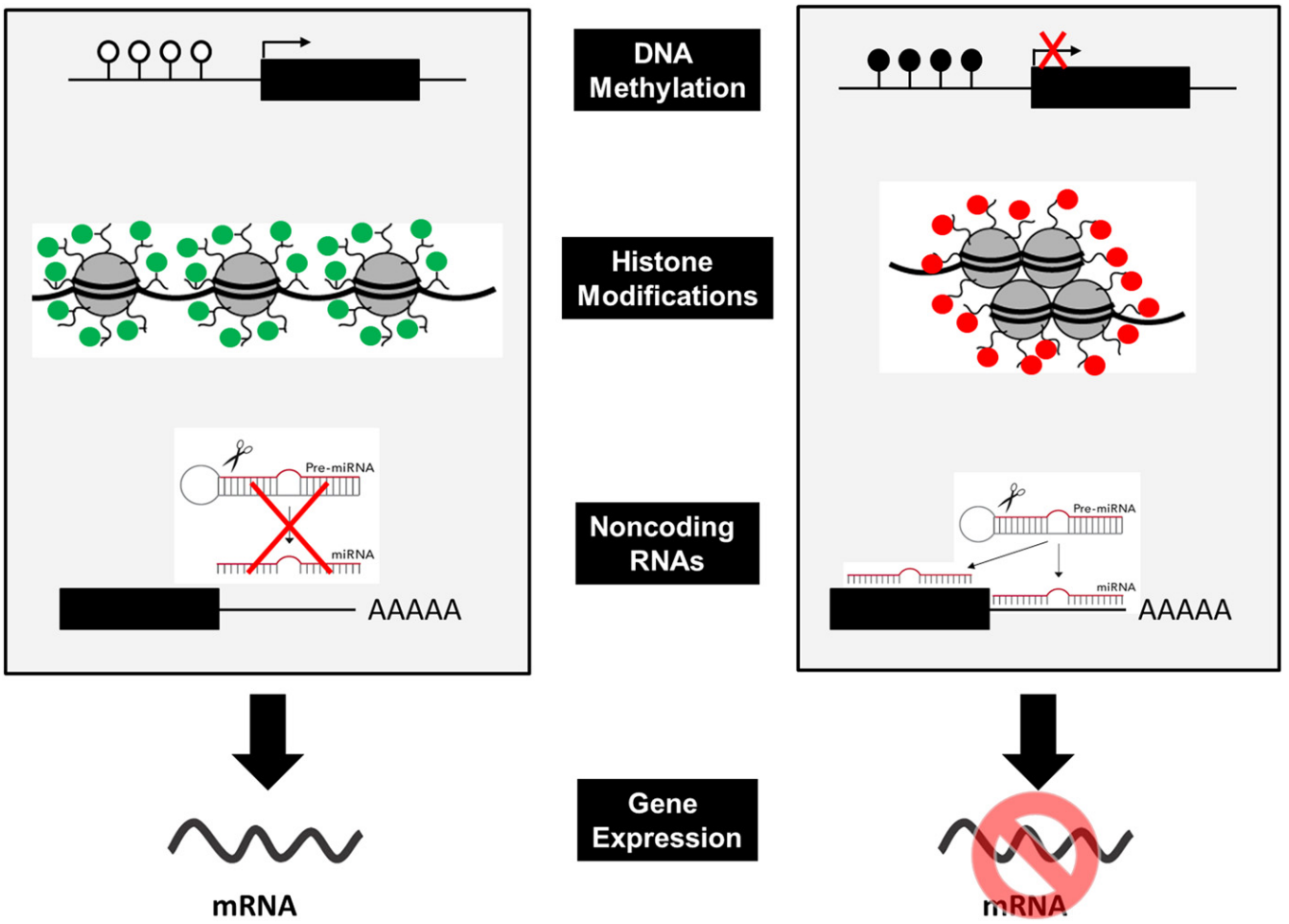

FIG 2. Effect of epigenetic marks (DNA methylation, histone modifications, and miRNAs) on gene expression. *White circles* denote unmethylated CpGs, and *black circles* denote methylated CpGs. *Green circles* refer to permissive histone modifications, and *red circles* indicate repressive histone marks. miRNAs can affect gene expression through either RNA degradation (perfect complementarity and binding) or inhibition of protein translation (imperfect complementarity and partial binding).

methylation outside of CpG islands is an important mechanism that controls gene transcription. Additionally, recent evidence suggests that DNA methylation is more prevalent within gene bodies than in promoters.[50] Intragenic DNA methylation functions at least in part by regulating transcription from alternative promoters,[51] but it is likely that other mechanisms are also involved. The DNA "methylome" of the H1 human embryonic stem cell line uniquely revealed that nearly one quarter of all methylation is in non-CpG context,[52] suggesting that embryonic stem cells might use different methylation mechanisms to control gene expression. 5-Methylcytosine can be oxidized to 5-hydroxymethylcytosine by the recently discovered TET family of enzymes.[53] Although the role of 5-hydroxymethylcytosine in epigenetic regulation of gene expression is not fully elucidated, it has been suggested that 5-hyroxymethycytosine is a mark of demethylation[53] and that it potentially plays a role in the regulation of specific promoters and enhancers.[54]

Methylation, acetylation, phosphorylation, and ***ubiquitylation***[43] of histone tails occur at specific sites and residues and control gene expression by regulating DNA accessibility to RNA polymerase II and transcription factors. H3K4 trimethylation (H3K4me3), for example, is strongly associated with transcriptional activation, whereas H3K27 trimethylation (H3K27me3) is frequently associated with gene silencing.[55] Similarly, histone tail acetylation leads to active gene transcription, whereas deacetylation is a repressive mark and leads to gene silencing. Histone acetyltransferases are enzymes that acetylate histone tails, whereas histone deacetylases remove acetyl groups from histone tails. Analogous to DNA methylation, deregulation of these histone modifications has been linked to misregulation of gene expression in patients with cancer.[56]

MicroRNAs (miRNAs) are the most studied class of noncoding RNAs and control gene expression by binding to the ***3′*** untranslated regions of mRNA, which leads to either mRNA degradation or inhibition of protein translation.[57] Almost 2000 mature miRNAs have been identified in the human genome (http://www.mirbase.org/), but it is expected that more miRNAs will be identified in the near future. Alterations of expression of miRNAs contribute to the pathogenesis of most malignancies, with miRNAs acting as both oncogenes and tumor suppressor genes,[58] but miRNAs also have well-established roles and are therapeutic targets in cardiovascular disease[59] and liver injury.[60] More recently, noncoding RNAs, such as PIWI-interacting RNAs, small nucleolar RNAs, transcribed ultraconserved regions, and large

intergenic noncoding RNAs are emerging as a key component of deregulated transcription not only in tumorigenesis but also in patients with many nonmalignant diseases.[61]

An emerging paradigm for epigenetic regulation of gene expression is the relationship between DNA methylation and histone modification. One example of these interactions is binding of DNA methyltransferase (DNMT) 3L, a regulatory factor related in sequence to mammalian *de novo* methyltransferases DNMT3A and DNMT3B, to the N-terminus of histone H3 tail.[62,63] DNMT3L recognizes unmethylated H3 tails at lysine 4 and induces *de novo* DNA methylation by using recruitment or activation of DNMT3A2; these findings establish the N-terminus of the histone H3 tail with an unmethylated lysine 4 as a ***chromatin*** determinant for DNA methylation. Similarly, DNMTs preferentially target ***nucleosome***-bound DNA.[64] The relationship of histones and DNA methylation is bidirectional; in addition to histones playing a role in the establishment of DNA methylation patterns, DNA methylation is important for maintaining patterns of histone modification through cell division.[65] Cross-talk between DNA methylation and miRNAs has also been identified.[66,67]

In addition to cross-talk between different epigenetic marks, it is becoming evident that underlying genetic variation and epigenetic marks work together. The best example is allele-specific gene expression, in which differences can arise because of sequence variation that might be marked by differences in DNA methylation,[68-70] histone modifications, or chromatin structure.

Epigenetic marks (DNA methylation and histone marks) are a key component of cell-specific gene expression and, as such, are erased during germ cell development (meiosis) and re-established after fertilization. This process is referred to as epigenetic reprogramming[71] and constitutes of comprehensive erasure and re-establishment of DNA methylation and extensive remodeling of histone modifications in 2 steps. Epigenetic reprogramming is a key feature of inheritance of epigenetic marks. Genes that are expressed from only 1 parental allele, known as imprinted genes, are protected during the second reprogramming step by mechanisms that are being unraveled.[72]

EPIGENOMIC STUDY DESIGN

The first step in epigenomic analysis is experimental design, including the choice of tissues/cells to be profiled and study design. Challenges in selection of the material to be used include limited availability of lung tissue for asthma studies and cell heterogeneity in available samples (eg, DNA from whole blood). One way to address the first challenge will be to analyze paired lung-blood samples to identify epigenetic marks that carry over from the lung to the peripheral blood and test whether surrogate tissues (eg, PBMCs, nasal epithelia, and sputum) adequately reflect activity in the lung. The second challenge can be addressed by collecting white blood cell count data and including the constituent cell counts in the analysis. If this information is not available, established epigenomic profiles for constituent cells (eg, data generated by the Roadmap Epigenomic project, http://www.roadmapepigenomics.org/) can be used to estimate the relative abundance of different cell types.[73]

Study design and power calculations based on previously collected data are important in designing studies that are able to identify significant epigenetic changes after adjustment for genome-wide comparisons. The most powerful study design for epigenomic analysis uses monozygotic twins who are essentially identical genetically so that all differences in phenotype can be attributed to environmental factors, with a paired-sample design allowing for better statistical power.[74] Another design with reasonably high power includes siblings (not necessarily twins) discordant for disease phenotypes with parental DNA also available for estimates of heritability. A case-control design with a large enough number of subjects included in the analysis is often used because of availability of samples. The final considerations in the study design are clinical and immune phenotypes of interest. Before sample selection from available specimens for epigenomic profiling, clinical/immune variables must be analyzed to identify those who have reliable measurements, normal distribution, and a strong clinical or biological rationale to be included in statistical models. Once epigenomic profiles are collected and data are normalized, principal components analysis can be used to prioritize variables based on the amount of variance in the dataset for which they account.

EPIGENOMIC PROFILING

Epigenetic marks can be studied by using focused and genome-wide approaches (Table II).[75] Generally, studies begin with genome-wide approaches to identify targets, followed by focused approaches to internally (confirmation in the same cohort) or externally (independent cohort) validate the initial findings. ***Microarrays*** have been the tool of choice for profiling epigenetic marks on a genomic scale, with several platforms and protocols available for DNA methylation (Table II).[76] The most commonly used array platforms for DNA methylation are the Illumina 450k BeadChip (Illumina, San Diego, Calif), the Comprehensive Analysis of Relative DNA Methylation (CHARM) platform,[77] and the Methylated DNA immunoprecipitation (MeDIP) arrays (Agilent Technologies, Santa Clara, Calif, and Roche NimbleGen, Madison, Wis). Array platforms have also been used to examine histone modifications by using chromatin immunoprecipitation (ChIP) followed by hybridization on microarrays (ChIP-chip),[76] as well as for miRNAs.[78]

However, the most substantial advance in the area of technologies for the assessment of epigenetic marks on the genome scale in recent years has been the introduction of next-generation sequencing technologies.[79] Application of next-generation sequencing to epigenomic research has been recently reviewed.[80] These technologies have been widely used for the study of histone marks (ChIP-seq) and miRNAs (miRNA-seq) because they provide superb-quality data compared with array platforms. They have also been used to identify open chromatin areas of the genome (FAIRE-seq)[81] and spatial chromatin organization (3C-seq).[82] The majority of methylation profiling is still done on array platforms because bisulfite-converted DNA sequencing (BS-seq) on the genomic scale is expensive. However, a number of techniques that examine only regions of the genome enriched for methylation marks have been developed and are being increasingly used.[83] Recent advances in the development of techniques for epigenomic profiling include attempts to define genome-wide patterns of DNA hydroxymethylation[84,85] and to study DNA methylation and histone modifications in one experiment.[86,87]

Pyrosequencing[88] and EpiTYPER assays on the Sequenom MassARRAY platform (Sequenom, San Diego, Calif) are commonly used techniques for interrogation of a small number of CpG sites, whereas quantitative PCR methods are typically

TABLE II. Summary of commonly used techniques for epigenetic profiling

Type of epigenetic mark	Type of sample preparation approach	Type of profiling approach
DNA methylation	Bisulfite conversion	Microarray (Illumina), high-throughput sequencing (BS-seq and RRBS-seq), EpiTYPER (mass spectrometry; focused), or pyrosequencing (focused)
	Methylated DNA immunoprecipitation (MeDIP)	Microarray or high-throughput sequencing (MeDIP-seq)
	Methyl-binding domain (MBD) precipitation	Microarray or high-throughput sequencing (MBD-seq)
	Restriction digest with methylation-sensitive restriction enzymes (eg, *Mcr*BC, *Msp*I, *Hpa*II, and *Msp*JI)	Microarray (CHARM, HELP) or high-throughput sequencing (MRE-seq)
Histone modifications	ChIP	Microarray (ChIP-chip), high-throughput sequencing (ChIP-seq), or quantitative PCR (focused)
DNA methylation associated with chromatin modifications	ChIP followed by bisulfite conversion	High-throughput sequencing (ChIP-BS-seq and Bis-ChIP-seq)
Noncoding RNAs	Size selection of appropriate RNA molecules	Microarray, high-throughput sequencing (miRNA-seq and RNA-seq) or quantitative RT-PCR (focused)
Chromatin accessibility	DNAse I cleavage	High-throughput sequencing (DNase sensitivity-seq)
Chromatin accessibility, chromatin spatial organization	Formaldehyde cross-linking	High-throughput sequencing (FAIRE-seq)
	Chromosome conformation capture	High-throughput sequencing (3C-seq, 4C-seq, and 5C-seq)

used for focused studies of histone modifications and miRNAs. In addition to site-specific methods for assessment of DNA methylation, some studies assess overall level of methylation in each sample (global methylation); this is often measured by assessing methylation in repeat regions of the genome (Alu, LINE-1, and Sat2), mass spectrometric methods, or the luminometric methylation assay.[89]

EPIGENOMIC DATA ANALYSIS

The first step in analysis of collected epigenomic data is to identify statistically significant differences between disease states. Statistical methods used for microarray analysis have generally been applicable to epigenomic profiles collected on arrays or sequence data after alignments and tag counts have been performed.[80] Strategies for analyzing tiling arrays have also been used in epigenomic analyses (eg, the CHARM platform or ChIP-chip).[90] One of the problems with this type of analysis is that it is only associative and does not demonstrate causality. Methods used in epidemiology and genetical genomics are beginning to be applied to epigenomes to identify causal relationships.[91]

The second and most complex step in the analysis of epigenomic data is understanding how different epigenetic mechanisms together influence gene expression. Each of the 3 epigenetic mechanisms is independently complex, but when combined, the complexity of these interactions presents unique analytic challenges. We are just beginning to understand how one type of epigenetic mark affects gene expression.[92] However, the evidence for cross-talk among different types of epigenetics marks is accumulating. The complexity of epigenetic regulation of gene expression is high, even when one is interested in examining only 1 gene or locus, and there are considerable challenges associated with understanding these interactions and the effect on gene regulation genome wide. Analytic strategies for these types of integrative epigenomic analyses have not reached maturity but are starting to be applied to disease datasets.[93] Two types of integrative analysis will be important to apply to epigenomic data: mapping strategies and network analysis. Expression quantitative trait locus (QTL) mapping approaches[94] can be applied to identify genetic variants that underlie methylation status (methyl-QTL) or methylation marks that control expression changes (methyl-expression QTL). Similarly, coexpression network analysis strategies that have been applied to expression analysis can be applied to epigenomic analysis.[95]

EPIGENETIC MARKS AND THE IMMUNE SYSTEM

A substantial body of evidence suggests that epigenetic mechanisms affect the expression of cytokines and binding of transcription factors that control the lineage of T_H1, T_H2, and Treg cells. In the context of T_H1/T_H2 differentiation, the most extensively studied are the T_H1 cytokine IFN-γ and the T_H2 cytokines IL-4 and IL-13. It has been shown that *de novo* DNMT3A methylates CpG-53 in the *Ifng* promoter[35] and cord blood $CD4^+$ cells enhance the development of the T_H1 (but not T_H2) lineage through progressive demethylation of the *Ifng* promoter.[36] Methylation of the *Ifng* promoter was reduced in $CD8^+$ cells from atopic children in the age range during which hyperproduction of IFN-γ occurs, suggesting that DNA methylation at this locus might be a contributing factor in the development of atopy in children. Differentiation of human $CD4^+$ cells into the T_H2 subtype is accompanied by the appearance of DNase I hypersensitive sites and CpG demethylation around these DNase I hypersensitive sites within IL-4 and IL-13 promoters.[37-39] Extensive studies of the T_H2 cytokine locus control region (LCR)[40] have shown that RAD50-hypersensitive site 7 within the T_H2 cytokine LCR undergoes rapid demethylation during T_H2 differentiation.[41]

In addition to DNA methylation, histone modifications are also important in guiding T-cell differentiation. The T-box transcription factor (T-bet) and GATA-3 transcription factor control lineage-specific histone acetylation of *Ifng* and *Il4* loci during T_H1/T_H2 differentiation.[42] Rapid methylation of H3K9 and H3K27 residues (repressive marks) at the *Ifng* locus are associated with differentiating T_H1 cells, whereas demethylation of H3K9 and methylation of H3K27 were associated with T_H2 differentiation. In a study of human cord blood $CD4^+$ cells, histone acetylation marks at the proximal *Il13* promoter were selectively observed in T_H2 cells,[39] suggesting that permissive histone marks together with DNA demethylation lead to expression of IL-13 in T_H2 cells. In aggregate, these studies suggest that DNA

methylation and histone modifications are highly dynamic and represent important determinants of T_H1 and T_H2 cell lineages.

Although miRNAs were discovered relatively recently, there is already a substantial body of evidence for the role of miRNAs in the development and function of the immune system.[96-98] A number of differentially expressed miRNAs have been identified in response to innate and adaptive immune stimuli, with many commonalities in miRNA expression (miR-21, miR-103, miR-155, and miR-204).[96] miR-155 is the most often identified differentially regulated noncoding RNA in studies involving the immune system[96,97]; a recent study revealed that miR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting ***cytotoxic T lymphocyte–associated antigen*** 4.[99]

Epigenetic mechanisms controlling Treg cell development are also beginning to be explored. Treg cells are a unique T-cell lineage with an important role in immunologic tolerance the development of which is primarily regulated by the transcription factor forkhead box protein 3 (FOXP3). Evidence for the role of DNA methylation[100] and histone modifications[101] in the regulation of FOXP3 expression are summarized in 2 recent reviews.[102,103] There is also clear evidence that miRNAs are involved in Treg cell development and function.[98]

ROLE OF THE ENVIRONMENT AND *IN UTERO* EXPOSURES IN MODULATING THE EPIGENOME

Unlike a patient's genetic make-up, epigenetic marks can be influenced much more easily by exposures, diet, and aging. Randy Jirtle's seminal experiments showed that a maternal diet supplemented with methyl donors (folic acid, vitamin B12, choline, and betaine) shifts coat color distribution of progeny toward the brown pseudoagouti phenotype and that this shift in coat color resulted from an increase in DNA methylation in a ***retrotransposon*** adjacent to the agouti gene.[14,15] These studies also revealed that mice with yellow coat color are obese and develop cancer, suggesting for the first time that changes in DNA methylation caused by diet *in utero* might be linked to disease development. Other studies have shown that pesticides and fungicides can alter the methylome, resulting in changes in male fertility,[104] and that aging is also associated with changes in DNA methylation and gene expression.[105] The concepts associated with environmental epigenetics were reviewed recently elsewhere.[46]

More recent evidence suggests that environmental exposures relevant to the development of asthma, such as air pollution and cigarette smoke, also affect the epigenome. Decreased DNA methylation in peripheral blood (as measured by LINE-1 repeats) was found to be associated with exposure to particulate matter of 2.5 μm in diameter or less ***($PM_{2.5}$)*** among 718 elderly subjects in the Boston area,[106] and although this correlated with time-dependent variables, such as day of the week and season, there was no association with air pollution–related health effects. Another study demonstrated that hypomethylation of the ***inducible nitric oxide synthase*** *(iNOS [Nos2])* promoter in buccal cells was associated with exhaled nitric oxide levels and $PM_{2.5}$ exposure among 940 participants in the Children's Health Study.[107]

Several epidemiologic studies have examined the relationship between exposure to cigarette smoke and epigenetic marks. Among 384 children, a global reduction in DNA methylation, as measured by the extent of methylation of Alu repeats, and differential methylation of 8 specific CpG motifs was found to be associated with *in utero* smoke exposure.[108] Fifteen specific genomic loci were significantly associated with current smoking, 2 with cumulative smoke exposure, and 3 with time since quitting cigarettes in 1085 subjects enrolled in the International COPD Genetics Network and validated in the Boston Early-Onset COPD study (n = 369).[109] Cigarette smoke exposure has also been shown to have a significant influence on the expression of miRNAs.[110-112] Comparing current with never smokers, 28 miRNAs were differentially expressed and mostly downregulated in the human bronchial airway epithelia of smokers.[110] miR-218 was found to be one of the strongly associated miRNAs with cigarette smoke exposure, and it was further shown that a change in miR-218 expression in primary bronchial epithelial cells and the H1299 cell line resulted in a corresponding anticorrelated change in the expression of predicted mRNA targets for miR-218.

Other studies have examined the influence of cigarette smoke exposure on epigenetic marks *in vitro* or in animal models. Normal human airway epithelial cells and immortalized bronchial epithelial cells exposed to cigarette smoke condensate identified time- and dose-dependent changes in histone modifications (decrease in H4K16Ac and H4K20Me3 and increase in H3K27Me3) accompanied by decreased DNMT1 and increased DNMT3b expression; these changes are characteristic of lung cancer progression.[113] Two other studies also demonstrated changes in miRNA expression in lungs of mice[112] and rats[111] exposed to cigarette smoke, with substantial overlap between mice and rats and some overlap of rodent miRNA expression changes in the lung with those observed in human airway epithelium.[110]

In addition to influencing epigenetic marks as a result of direct exposure, *in utero* exposure to components of air pollution or cigarette smoke results in changes in global and site-specific DNA methylation. Maternal exposure to benzo(a)pyrene, a representative airborne ***polycyclic aromatic hydrocarbon***, was associated with hypermethylation of IFN-γ in cord blood DNA from 53 participants in the Columbia Center for Children's Environmental Health cohort.[114] In another study global hypomethylation has been associated with maternal smoking and cotinine levels in umbilical cord blood from 30 newborns.[115] In a birth cohort of 90 women born from 1959 to 1963 in New York City, prenatal tobacco exposure, measured at the time of pregnancy and not retrospectively reported, was associated with a decrease in Sat2 methylation but not LINE-1 or Alu methylation.[116] Examination of 2 differentially methylated regions (DMRs) regulating 2 imprinted loci (*H19* and *Igf2*) in infants born to 418 pregnant women demonstrated that infants born to smokers had higher methylation at the *Igf2* DMR than those born to never smokers or those who quit during pregnancy (no differences were seen in the *H19* DMR).[117] Similarly, DNA methylation in *Axl*, a receptor tyrosine kinase relevant in cancer and immune function, was 2.3% higher in peripheral blood of children exposed to maternal smoking *in utero*.[118] Finally, one study has demonstrated association of maternal cigarette smoking during pregnancy with downregulation of several miRNAs in the placenta; expression of one of the miRNAs (miR-146a) was downregulated in a dose-dependent manner in immortalized placental cell lines exposed to nicotine and benzo(a)pyrene.[119]

ASTHMA EPIGENETICS: ANIMAL STUDIES

Given the evidence for the strong influence of environmental exposures on epigenetic marks and the role of epigenetic

regulation in T-cell differentiation, it is becoming clear that epigenetic changes might be one of the factors to explain the increasing prevalence of asthma. Our group hypothesized that these dietary influences are, at least in part, mediated by the epigenome. To test this hypothesis, we conducted a study in which pregnant female mice were fed either a low- or high-methylation diet and progeny were sensitized and challenged with ovalbumin.[17] We observed an increase in airway inflammation, serum IgE levels, and airway hyperresponsiveness (AHR) in pups of mothers who were fed a high-methylation diet compared with those of mothers on a low-methylation diet. Furthermore, we demonstrated hypermethylation of 82 gene-associated CpG islands throughout the genome, including extensive hypermethylation of the promoter and decreased expression of *Runx3*, a gene known to regulate allergic airway disease in mice. Importantly, we reversed the immune phenotype by treatment with a demethylating agent (5-aza-deoxycytidine). Epidemiologic evidence for association of folic acid with the development of asthma in children has been mixed,[120-124] but it might be that folate, together with other methyl donors in the diet, plays a role in this disease.

Importantly, a direct link between epigenetic control of the T_H2 cytokine locus and development of allergic airway diseases was further demonstrated in mice with deficiency in the T_H2 LCR.[125] A more recent study also identified a DNase I hypersensitive site 2 element in the second intron of the *Il4* gene as the strongest of all known *Il4* enhancers and showed that this enhancer is strictly controlled by GATA-3 binding.[126] Moreover, Tanaka et al[126] propose a new model in which independent recruitment of GATA-3 to locus-specific regulatory elements controls the status of the expression of genes encoding T_H2 cytokines.[127]

A number of other animal studies have since examined DNA methylation in the context of allergic airway disease. Fedulov et al[128] demonstrated DNA methylation changes in splenic $CD11c^+$ dendritic cells from neonate mice born to allergic mothers (mothers sensitized and challenged with ovalbumin). Brand et al[129] observed increased methylation of the *Ifng* promoter (and increased IFN-γ cytokine production) in $CD4^+$ T lymphocytes after ovalbumin sensitization challenge and demonstrated that methylation of the *Ifng* promoter is required for development of allergic airway disease by using 5-aza-deoxycytidine (demethylating agent) and adoptive transfer experiments transferring $CD4^+$ T cells from sensitized/challenged to naive animals and the reverse. Although both demethylation and adoptive transfer experiments clearly demonstrate the importance of methylation marks in $CD4^+$ cells in the development of allergic airway disease, loci other than *Ifng* might be important in this process and should be examined. Finally, DNMT3A, but not DNMT3B, deficiency in $CD4^+$ lymphocytes (conditional mutant mice) was shown to result in increased expression of IL-13 (and other T_H2 cytokines), decreased DNA methylation and changes in H3K27 acetylation/methylation in the IL-13 promoter, increased airway inflammation, and AHR in the ovalbumin model of allergic airway disease.[129] This study clearly demonstrates the role of DNA methylation in controlling the expression of T_H2 cytokines and the development of allergic airway disease in mice.

Several recent studies have also begun to shed light on the role several miRNAs play in the development of allergic airway disease in animal models.[130] Selective miR-126 blockade resulted in a diminished T_H2 response, inflammation, and AHR in the house dust mite model; these effects were shown to be mediated by activation of the ***MyD88*** innate immune signaling pathway. By using the same house dust mite model, this group also demonstrated that inhibition of miR-145 inhibited eosinophilic inflammation, mucus hypersecretion, T_H2 cytokine production, and AHR and that the anti-inflammatory effects of miR-145 antagonism were comparable with those of glucocorticoid treatment.[131] Two studies identified a controversial role for the let-7 family of miRs in the ovalbumin model of allergic airway disease.[132,133] The first study showed that multiple members of the highly conserved let-7 miRNA family are the most increased lung miRNAs in response to allergen.[132] The authors confirmed that IL-13 is regulated by let-7a *in vitro* and demonstrated that inhibition of let-7 miRNAs *in vivo* using a locked nucleic acid profoundly inhibited allergic inflammation and AHR, suggesting a proinflammatory role for let-7d. The second independent study demonstrated that let-7 miRNAs regulate IL-13 production in A549 cells and primary cultured T cells and that intranasal administration of mature let-7 mimic to the lungs of mice with allergic inflammation resulted in decreased IL-13 levels, AHR, and mucus metaplasia, implying an anti-inflammatory role for let-7.[133] More studies are needed to understand the discrepancy in these findings, but this illustrates the complexity of miRNA regulation of gene expression.

Finally, 3 recent studies have demonstrated how miRNAs play a crucial role in the regulation of IFN-γ and therefore T-cell polarization. Targeted ablation of miR-21 led to reduced lung eosinophilia after ovalbumin sensitization and challenge, with a broadly reprogrammed immunoactivation transcriptome and significantly increased levels of the T_H1 cytokine IFN-γ.[134] Consistent with the miR-21 binding site in *IL-12p35*, dendritic cells from miR-21–deficient mice produced more IL-12 after LPS stimulation, and OVA-challenged $CD4^+$ T cells from the same mice produced more IFN-γ and less IL-4. Two studies showed that miR-29 suppresses IFN-γ production.[135,136] Steiner et al[136] performed gene expression profiling of cells that do not produce miRNAs (DGCR8-deficient cells[137]) transfected with a synthetic miR-29 and wild-type cells with antisense inhibitors of miR-29, respectively. In this elegant experiment they found reduced expression of 2 transcription factors that regulate IFN-γ production (*Tbx21*/T-bet and *Eomes*) under gain-of-function conditions and increased expression of these 2 transcription factors under loss-of-function conditions. They further proved the role of miR-29 regulation of the expression of these transcription factors in $CD4^+$ lymphocytes *in vitro* and in both $CD4^+$ and $CD8^+$ T cells in an *in vivo* viral infection model. Ma et al[135] demonstrated an inverse correlation between IFN-γ production and levels of miR-29 in natural killer cells and T cells from mice infected with *Listeria monocytogenes* or *Mycobacterium bovis*. Mice lacking miR-29 infected with *M bovis* showed less inflammation, lower bacterial burden, and increased numbers of IFN-γ–producing $CD4^+$ T cells in their lungs compared with control mice.

ASTHMA EPIGENETICS: HUMAN STUDIES

Although animal studies have begun to decipher the role of epigenetic regulation of gene expression associated with the development of allergic airway disease in the lung, several recent publications in human cohorts have examined DNA methylation in cells outside of the lung: peripheral blood cells,[138] buccal cells,[139,140] and nasal cells.[141] These early studies have only

demonstrated the statistical association of DNA methylation and specific exposure or asthma phenotype but have not elucidated the role of DNA methylation in the control of gene expression in human asthma. Breton et al[139] demonstrated that DNA methylation in promoters of 2 arginase genes (*Arg1* and *Arg2*) is associated with exhaled nitric oxide levels in children with asthma from the Children's Health Study and indicates a role for epigenetic regulation of nitric oxide production. In a pilot study in the Columbia Center for Children's Environmental Health cohort, Kuriakose et al[140] found that iNOS methylation was not significantly associated with fraction of exhaled nitric oxide (FENO) but was associated inversely with bronchial nitric oxide flux. This latter study emphasizes the importance of careful selection of clinical parameters used in the association study. A more recent study of DNA methylation in nasal cells from 35 asthmatic children 8 to 11 years old identified inverse association of FENO levels and promoter methylation of both *Il6* and iNOS.[141] Finally, data from 2 independent pregnancy cohorts in Spain (discovery and validation)[138] showed that DNA hypomethylation in *Alox12* in peripheral blood of children was associated with a higher risk of persistent wheezing at age 4 years. In aggregate, these studies suggest that DNA methylation in easily obtained samples (buccal, nasal, or peripheral blood cells) might be a useful biomarker for airway inflammation in pediatric research.

A recent study has also examined DNA methylation in *Foxp3* and Treg cell function in peripheral blood from children with and without asthma and with high and low exposures to air pollution.[142] Treg cell suppression was impaired, and Treg cell chemotaxis was reduced as a result of exposure to air pollution. Changes in DNA methylation have also been associated with the development of asthma among older smokers in the Lovelace Smokers Cohort. Comparison of 184 smokers with asthma with 511 control subjects with a similar smoking history (patients with chronic obstructive pulmonary disease were excluded) identified an association of DNA methylation in the protocadherin-20 gene in sputum DNA with asthma, as well as a significant synergistic interaction between methylation of protocadherin-20 and paired box protein transcription factor 5α on the odds of having asthma.[143]

A set of earlier studies suggested that acetylation of histones can also play a role in asthma. Increased acetylation of H4 has been demonstrated in asthmatic patients and is associated with an increase in the expression of several inflammatory genes in the lung.[144] It has also been shown that increased acetylation of histones results in decreased histone deacetylase activity, which might be responsible for enhanced expression of inflammatory genes. In addition, glucocorticoids appear to suppress inflammation by altering acetylation of histones that regulate inflammatory and anti-inflammatory genes; these studies are described in detail in a review[145] and suggest that targeting histone acetylation (and possibly other epigenetic marks) might lead to novel anti-inflammatory therapies, especially in corticosteroid-resistant cases of asthma. A more recent study found that TGF-β2 suppresses expression of a disintegrin and metalloprotease 33 (ADAM33), one of the most replicated asthma susceptibility genes, in fibroblasts from healthy subject or asthmatic patients and that this occurs by altering chromatin structure (deacetylation of histone H3, demethylation of lysine 4 on H3, and hypermethylation of lysine9 on H3) and not by gene silencing through DNA methylation, as in epithelial cells.[146]

The role of miRNAs in asthma and atopy in human subjects is also emerging. Although no detectable differences in the expression of miRNAs from airway biopsy specimens were observed between patients with mild asthma and healthy subjects in an early study,[147] only patients with mild asthma were included in this study, and the number of miRNAs examined was limited. However, this study demonstrated cell type–specific expression of miRNAs in cells isolated from airways and lung tissue, suggesting a possible role for miRNAs in asthmatic patients. A more recent study has indeed identified miRNAs that play a role in specific cells in asthmatic patients. In a study of 8 control subjects, 4 patients with mild asthma, and 12 patients with severe asthma, widespread changes in mRNA and noncoding RNA expression in circulating $CD8^+$ but not $CD4^+$ T cells were associated with severe asthma.[148] miRNA expression profiles showed selective downregulation of miR-28-5p in $CD8^+$ lymphocytes and reduction of miR-146a and miR-146b in both $CD4^+$ and $CD8^+$ T cells. It is likely that some of the other miRNAs identified in animal models play a yet uncovered role in the development of asthma in human subjects.

CHALLENGES IN UNDERSTANDING THE ASTHMA EPIGENOME

Some of the key questions in regard to future studies in asthma epigenetics revolve around understanding how the epigenome contributes to inheritance of asthma, developmental vulnerability of the epigenome, effect of the environment/diet/aging, and influence of asthma (and other diseases) on the epigenome. Although sorting out these factors will be challenging, it is absolutely essential that the proper tissue be chosen to study the effects of the epigenome on asthma. The more pure and relevant the cell population is to the disease state, the more likely the epigenetic marks will regulate the expression of key genes involved in the pathogenesis of asthma. In the absence of airway biopsy specimens in pediatric asthma, nasal epithelial cells or sputum might be the closest surrogate for disease-relevant cells. Specific cell populations, such as $CD4^+$ and $CD8^+$ T lymphocytes isolated from peripheral blood, might also be informative in identifying immune genes with expression that is dysregulated by epigenetic marks in the disease state. Despite these concerns, epigenetic marks in peripheral blood might provide biomarkers to identify those at risk, responses to different forms of environmental stress, or the likelihood of responding to specific therapeutic agents.

Analogous to asthma genetics studies, the choice of the study population will be crucial to the success of future epigenetic studies. The ancestry of study subjects will likely need to be taken into account given the early evidence for the role of genetic variation and DNA methylation at asthma-associated loci, such as *Ormdl3*.[149] Moreover, a recent study suggests that DNA methylation is highly divergent between populations of European and African descent and that this divergence might be due to a combination of differences in allele frequencies and complex epistasis or gene-environment interactions.[150] Based on this, population stratification might be a confounder in population-based genome-wide DNA methylation studies and might have to be accounted for by using principal components from the methylation profile, genome-wide association studies, or ancestry-specific marker panels. Given the strong influence of the environment on epigenetic marks, environmental and dietary exposures, as well as medication use, must be measured/recorded in the study not only for exposures of interest but also for any confounding

exposures that need to be adjusted for in the analysis. Despite the differences between disease phenotypes in human cohorts and animal models of allergic airway disease, animal models with a fixed genetic background and controlled exposures are likely to remain a crucial component of future studies in the field.

One of the major hurdles to overcome in future asthma epigenetics research will be the validation component. Necessary components of the validation process include internal validation of epigenetic marks in the same samples by using a different technique, association of epigenetic marks with changes in gene expression in the study population, and external validation of epigenetic marks in an independent cohort (Fig 3). Some of the difficulties encountered in the validation process are platform differences in technologies; differences in DNA methylation measurements are encountered based on the approach used to capture methylated marks (restriction digest, immunoprecipitation, and bisulfite conversion) and probes used to measure the extent of methylation (single CpG site vs a region covered by overlapping probes). Another major challenge in validations studies is interpretation of epigenetic marks in the context of changes in gene expression. Both *cis* and *trans* effects of methylation marks are likely to be important in gene regulation, and this process is very complex. Depending on the site of methylation (promoter vs intron), epigenetic marks might play different roles in control of gene expression in *cis*. Mapping studies of methylation marks on gene expression (methyl-expression QTL) will be essential in the identification of *cis* and *trans* effects. The final major challenge will be identification of cohorts with comparable genetic background and environmental exposures to use as an independent validation step. It is likely that cohorts with similar exposures and phenotypes will be of most utility for broad validation of a large number of epigenetics marks and identification of specific phenotype- and exposure-driven epigenetic changes, whereas more divergent cohorts might still be useful in validation of a small number of epigenetic marks associated with disease, regardless of other factors.

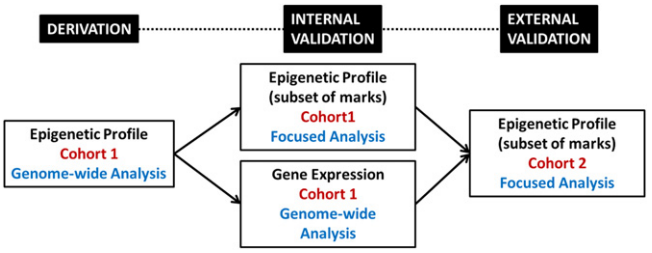

FIG 3. An overview of the validation process for discovery of epigenetic marks associated with the development of asthma. *Internal validation* refers to confirmation in the same cohort by using a different technique and association of epigenetic marks with changes in gene expression, whereas *external validation* refers to validation of epigenetic marks in an independent cohort. Genome-wide analysis of gene expression is preferable to focused approaches because of complexities in the relationship of epigenetic marks and gene expression alterations.

POTENTIAL EFFECT OF EPIGENETICS RESEARCH ON ASTHMA

Although we know that inheritance, parent of origin, environment, *in utero* exposures, and T_H2 immunity play important roles in the cause of asthma, there is no well-developed unifying mechanism accounting for these causative events/triggers. Although the hygiene hypothesis is appealing conceptually[3] and ties a number of these basic causative events together, there are several competing hypotheses (eg, T-cell skewing, infection, diet, and obesity), and none of them fully account for the complex interaction between host and environmental determinants of asthma. For example, the hygiene hypothesis suggests that a decrease of exposure to microbes would, through enhanced atopic immune responses, increase the incidence of allergies and allergic asthma.[3] However, the prevalence of atopy and asthma are not concordant, allergic mechanisms account for at most 50% of asthma cases, very high asthma rates are present in some countries in which hygienic conditions are less than ideal, and although the prevalence and incidence of asthma continue to increase in inner cities in the United States, housing conditions in these communities are becoming more hygienic.

Although epigenetic mechanisms provide a unique cause of asthma, these basic transcriptional controls potentially serve to explain some of the prevailing hypotheses underlying the development of asthma. For example, the hygiene hypothesis is dependent on activation of innate immune genes, including genes activated by the Toll-like receptors; importantly, epigenetic mechanisms control the activation of these innate immune genes and, consequently, the extent of the inflammatory response.[151,152] Moreover, a recent study demonstrated that microbes can also operate by means of epigenetic mechanisms.[153] In this animal study prenatal administration of the farm-derived gram-negative bacterium *Acinetobacter lwoffii* F78A prevented the development of an asthmatic phenotype in progeny, and this effect was IFN-γ dependent. Prenatal microbial exposure was also associated with a significant protection against loss of H4 acetylation in the *Ifng* promoter, which was closely associated with IFN-γ expression in $CD4^+$ lymphocytes, as well as a decrease in H4 acetylation at the *Il4* promoter. Pharmacologic inhibition of H4 acetylation in offspring abolished the asthma-protective phenotype. Therefore although epigenetic mechanisms have the potential of changing our basic concepts about asthma, these mechanisms might not only account for the causative events/triggers related to asthma but also help explain some of the prevailing hypotheses attributed to this disease.

Furthermore, identification of key epigenetic marks has the potential to transform asthma therapy from palliative to preventive and might alter our recommendations for pregnancy throughout the world. Currently, other than avoidance of cigarette smoke, we are simply unable to prevent asthma. Most patients with asthma rely on chronic medications to reduce the severity of their symptoms. Understanding the importance of epigenetic mechanisms in the development of asthma and the periods of vulnerability in establishing epigenetic marks has the potential to prevent the development of this disease not only in our offspring but also in their children. Identification of critical epigenetic marks associated with the development of asthma and influenced by specific environmental factors at certain time points, *in utero* or postnatally, would allow us to advise our patients on intake of dietary supplements and limiting harmful exposures during the critical windows when these dietary and environmental factors have the strongest influence on the development of disease. Understanding the complex interactions between *in utero* exposures and epigenetic vulnerability will provide insight into future interventions for subjects at risk for allergic asthma and might lead to the prevention of this disease altogether.

However, asthma is a complex disease, and although epigenetic mechanisms might contribute to the cause and pathogenesis of

this disease, there are multiple pieces to the asthma puzzle. The challenge will be to understand how genetic variation, the transcriptome, epigenetic marks, the environment, and the immune system interface with each other to result in the development of allergic and nonallergic forms of asthma.

REFERENCES

1. Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, health care use, and mortality: United States, 2005-2009. Natl Health Stat Rep 2011;(32):1-14.
2. Bloom B, Cohen R, Freeman G. Summary health statistics for U.S. children: National Health Interview Survey, 2010. Hyatsville (MD): National Center for Health Statistics; 2011.
3. Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med 2006;355: 2226-35.
4. Asher MI, Stewart AW, Wong G, Strachan DP, Garcia-Marcos L, Anderson HR. Changes over time in the relationship between symptoms of asthma, rhinoconjunctivitis and eczema: a global perspective from the International Study of Asthma and Allergies in Childhood (ISAAC). Allergol Immunopathol (Madr) 2012 [Epub ahead of print].
5. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SA, et al. Variants of DENND1B associated with asthma in children. N Engl J Med 2010;362:36-44.
6. Vercelli D. Discovering susceptibility genes for asthma and allergy. Nat Rev Immunol 2008;8:169-82.
7. March ME, Sleiman PM, Hakonarson H. The genetics of asthma and allergic disorders. Discov Med 2011;11:35-45.
8. Mathias RA, Grant AV, Rafaels N, Hand T, Gao L, Vergara C, et al. A genome-wide association study on African-ancestry populations for asthma. J Allergy Clin Immunol 2010;125:336-46, e4.
9. Moffatt MF, Cookson WO. The genetics of asthma. Maternal effects in atopic disease. Clin Exp Allergy 1998;28(suppl 1):56-61; discussion 65-6.
10. Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. Nature 1999;402:B12-7.
11. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer 2004;4: 143-53.
12. Cookson WO, Young RP, Sandford AJ, Moffatt MF, Shirakawa T, Sharp PA, et al. Maternal inheritance of atopic IgE responsiveness on chromosome 11q. Lancet 1992;340:381-4.
13. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. Nat Genet 2001;29:175-8.
14. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007;8:253-62.
15. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23:5293-300.
16. Cohen RT, Raby BA, Van Steen K, Fuhlbrigge AL, Celedon JC, Rosner BA, et al. In utero smoke exposure and impaired response to inhaled corticosteroids in children with asthma. J Allergy Clin Immunol 2010;126:491-7.
17. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 2008;118:3462-9.
18. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. Chest 2005;127:1232-41.
19. Henderson AJ, Newson RB, Rose-Zerilli M, Ring SM, Holloway JW, Shaheen SO. Maternal Nrf2 and glutathione-S-transferase polymorphisms do not modify associations of prenatal tobacco smoke exposure with asthma and lung function in school-aged children. Thorax 2010;65:897-902.
20. Hylkema MN, Blacquiere MJ. Intrauterine effects of maternal smoking on sensitization, asthma, and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009;6:660-2.
21. Wang L, Pinkerton KE. Air pollutant effects on fetal and early postnatal development. Birth Defects Res C Embryo Today 2007;81:144-54.
22. Clark NA, Demers PA, Karr CJ, Koehoorn M, Lencar C, Tamburic L, et al. Effect of early life exposure to air pollution on development of childhood asthma. Environ Health Perspect 2010;118:284-90.
23. Fitzsimon N, Fallon U, O'Mahony D, Loftus BG, Bury G, Murphy AW, et al. Mothers' dietary patterns during pregnancy and risk of asthma symptoms in children at 3 years. Ir Med J 2007;100(suppl):27-32.
24. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. Nat Immunol 2010;11:577-84.
25. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med 2008;359:1985-94.
26. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study [see comments]. N Engl J Med 1990;323:502-7.
27. Oh SS, Tcheurekdjian H, Roth LA, Nguyen EA, Sen S, Galanter JM, et al. Effect of secondhand smoke on asthma control among black and Latino children. J Allergy Clin Immunol 2012;129:1478-83, e7.
28. Chan-Yeung M, Malo JL. Occupational asthma. N Engl J Med 1995;333: 107-12.
29. Samet JM, Lambert WE. Epidemiologic approaches for assessing health risks from complex mixtures in indoor air. Environ Health Perspect 1991;95:71-4.
30. Folkerts G, Busse W, Nijkamp F, Sorkness R, Gern J. State of the art: virus-induced airway hyperresponsiveness and asthma. Am J Respir Crit Care Med 1998;157:1708-20.
31. Gereda J, Leung D, Thatayatikom A, Streib J, Price M, Klinnert M, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000;355:1680-3.
32. Schwartz DA, Donham KJ, Olenchock SA, Popendorf WJ, Van Fossen DS, Burmeister LF, et al. Determinants of longitudinal changes in spirometric function among swine confinement operators and farmers. Am J Respir Crit Care Med 1995;151:47-53.
33. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science 1997;275:77-9.
34. Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. Nat Rev Immunol 2010;10:838-48.
35. Jones B, Chen J. Inhibition of IFN-gamma transcription by site-specific methylation during T helper cell development. EMBO J 2006;25:2443-52.
36. White GP, Hollams EM, Yerkovich ST, Bosco A, Holt BJ, Bassami MR, et al. CpG methylation patterns in the IFNgamma promoter in naive T cells: variations during Th1 and Th2 differentiation and between atopics and non-atopics. Pediatr Allergy Immunol 2006;17:557-64.
37. Santangelo S, Cousins DJ, Winkelmann NE, Staynov DZ. DNA methylation changes at human Th2 cytokine genes coincide with DNase I hypersensitive site formation during CD4(+) T cell differentiation. J Immunol 2002;169: 1893-903.
38. Lee DU, Agarwal S, Rao A. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. Immunity 2002;16: 649-60.
39. Webster RB, Rodriguez Y, Klimecki WT, Vercelli D. The human IL-13 locus in neonatal CD4+ T cells is refractory to the acquisition of a repressive chromatin architecture. J Biol Chem 2007;282:700-9.
40. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by cis elements and epigenetics. Immunity 2006;24:369-79.
41. Kim ST, Fields PE, Flavell RA. Demethylation of a specific hypersensitive site in the Th2 locus control region. Proc Natl Acad Sci U S A 2007;104:17052-7.
42. Fields PE, Kim ST, Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. J Immunol 2002;169:647-50.
43. Allis CD, Jenuwein T, Reinberg D, editors. Epigenetics. Woodbury (NY): Cold Spring Harbor Laboratory Press; 2009.
44. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. J Mol Biol 1987;196:261-82.
45. Espada J, Esteller M. DNA methylation and the functional organization of the nuclear compartment. Semin Cell Dev Biol 2010;21:238-46.
46. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet 2011;13:97-109.
47. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature 2007;447:433-40.
48. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nat Genet 2009;41:1350-3.
49. Ji H, Ehrlich LI, Seita J, Murakami P, Doi A, Lindau P, et al. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. Nature 2010;467:338-42.
50. Shenker N, Flanagan JM. Intragenic DNA methylation: implications of this epigenetic mechanism for cancer research. Br J Cancer 2012;106:248-53.
51. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature 2010;466:253-7.
52. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 2009;462:315-22.
53. Branco MR, Ficz G, Reik W. Uncovering the role of 5-hydroxymethylcytosine in the epigenome. Nat Rev Genet 2012;13:7-13.

54. Szulwach KE, Li X, Li Y, Song CX, Han JW, Kim S, et al. Integrating 5-hydroxymethylcytosine into the epigenomic landscape of human embryonic stem cells. PLoS Genet 2011;7:e1002154.
55. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet 2012;13:343-57.
56. Chi P, Allis CD, Wang GG. Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. Nat Rev Cancer 2010;10:457-69.
57. Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 2008;9:831-42.
58. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009;10:704-14.
59. Papageorgiou N, Tousoulis D, Androulakis E, Siasos G, Briasoulis A, Vogiatzi G, et al. The role of microRNAs in cardiovascular disease. Curr Med Chem 2012;19: 2605-10.
60. Jopling C. Liver-specific microRNA-122: biogenesis and function. RNA Biol 2012;9:137-42.
61. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011;12:861-74.
62. Hu JL, Zhou BO, Zhang RR, Zhang KL, Zhou JQ, Xu GL. The N-terminus of histone H3 is required for de novo DNA methylation in chromatin. Proc Natl Acad Sci U S A 2009;106:22187-92.
63. Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature 2007; 448:714-7.
64. Chodavarapu RK, Feng S, Bernatavichute YV, Chen PY, Stroud H, Yu Y, et al. Relationship between nucleosome positioning and DNA methylation. Nature 2010;466:388-92.
65. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009;10:295-304.
66. Pan W, Zhu S, Yuan M, Cui H, Wang L, Luo X, et al. MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. J Immunol 2010; 184:6773-81.
67. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A 2007;104:15805-10.
68. Tycko B. Allele-specific DNA methylation: beyond imprinting. Hum Mol Genet 2010;19:R210-20.
69. Fang F, Hodges E, Molaro A, Dean M, Hannon GJ, Smith AD. Genomic landscape of human allele-specific DNA methylation. Proc Natl Acad Sci U S A 2012;109:7332-7.
70. Li Y, Zhu J, Tian G, Li N, Li Q, Ye M, et al. The DNA methylome of human peripheral blood mononuclear cells. PLoS Biol 2010;8:e1000533.
71. Smallwood SA, Kelsey G. De novo DNA methylation: a germ cell perspective. Trends Genet 2012;28:33-42.
72. Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, et al. PGC7/Stella protects against DNA demethylation in early embryogenesis. Nat Cell Biol 2007;9:64-71.
73. Milosavljevic A. Emerging patterns of epigenomic variation. Trends Genet 2011; 27:242-50.
74. Bell JT, Spector TD. A twin approach to unraveling epigenetics. Trends Genet 2011;27:116-25.
75. Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. Am J Respir Crit Care Med 2011;183:1295-301.
76. Schones DE, Zhao K. Genome-wide approaches to studying chromatin modifications. Nat Rev Genet 2008;9:179-91.
77. Ladd-Acosta C, Aryee MJ, Ordway JM, Feinberg AP. Comprehensive high-throughput arrays for relative methylation (CHARM). Curr Protoc Hum Genet 2010;Chapter 20(Unit 20.1):1-19.
78. Liu CG, Spizzo R, Calin GA, Croce CM. Expression profiling of microRNA using oligo DNA arrays. Methods 2008;44:22-30.
79. Metzker ML. Sequencing technologies—the next generation. Nat Rev Genet 2010;11:31-46.
80. Ku CS, Naidoo N, Wu M, Soong R. Studying the epigenome using next generation sequencing. J Med Genet 2011;48:721-30.
81. Gaulton KJ, Nammo T, Pasquali L, Simon JM, Giresi PG, Fogarty MP, et al. A map of open chromatin in human pancreatic islets. Nat Genet 2010;42:255-9.
82. Tanizawa H, Noma K. Unravelling global genome organization by 3C-seq. Semin Cell Dev Biol 2012;23:213-21.
83. Harris RA, Wang T, Coarfa C, Nagarajan RP, Hong C, Downey SL, et al. Comparison of sequencing-based methods to profile DNA methylation and identification of monoallelic epigenetic modifications. Nat Biotechnol 2010;28:1097-105.
84. Song CX, Clark TA, Lu XY, Kislyuk A, Dai Q, Turner SW, et al. Sensitive and specific single-molecule sequencing of 5-hydroxymethylcytosine. Nat Methods 2012;9:75-7.
85. Robertson AB, Dahl JA, Ougland R, Klungland A. Pull-down of 5-hydroxymethylcytosine DNA using JBP1-coated magnetic beads. Nat Protoc 2012;7:340-50.
86. Statham AL, Robinson MD, Song JZ, Coolen MW, Stirzaker C, Clark SJ. Bisulfite sequencing of chromatin immunoprecipitated DNA (BisChIP-seq) directly informs methylation status of histone-modified DNA. Genome Res 2012;22: 1120-7.
87. Brinkman AB, Gu H, Bartels SJ, Zhang Y, Matarese F, Simmer F, et al. Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk. Genome Res 2012;22:1128-38.
88. Tost J, Gut IG. DNA methylation analysis by pyrosequencing. Nat Protoc 2007;2: 2265-75.
89. Karimi M, Johansson S, Stach D, Corcoran M, Grander D, Schalling M, et al. LUMA (LUminometric Methylation Assay)—a high throughput method to the analysis of genomic DNA methylation. Exp Cell Res 2006;312:1989-95.
90. Kechris KJ, Biehs B, Kornberg TB. Generalizing moving averages for tiling arrays using combined p-value statistics. Stat Appl Genet Mol Biol 2010;9: Article29.
91. Relton CL, Davey Smith G. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. Int J Epidemiol 2012;41:161-76.
92. Knight JC. Resolving the variable genome and epigenome in human disease. J Intern Med 2012;271:379-91.
93. Kang HP, Yang X, Chen R, Zhang B, Corona E, Schadt EE, et al. Integration of disease-specific single nucleotide polymorphisms, expression quantitative trait loci and coexpression networks reveal novel candidate genes for type 2 diabetes. Diabetologia 2012;55:2205-13.
94. Montgomery SB, Dermitzakis ET. From expression QTLs to personalized transcriptomics. Nat Rev Genet 2011;12:277-82.
95. Allen JD, Xie Y, Chen M, Girard L, Xiao G. Comparing statistical methods for constructing large scale gene networks. PLoS One 2012;7:e29348.
96. Nana-Sinkam SP, Hunter MG, Nuovo GJ, Schmittgen TD, Gelinas R, Galas D, et al. Integrating the MicroRNome into the study of lung disease. Am J Respir Crit Care Med 2009;179:4-10.
97. Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. Cell 2009;136:26-36.
98. Cobb BS, Hertweck A, Smith J, O'Connor E, Graf D, Cook T, et al. A role for Dicer in immune regulation. J Exp Med 2006;203:2519-27.
99. Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, et al. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. J Allergy Clin Immunol 2010;126:581-9, e1-20.
100. Kim HP, Leonard WJ. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. J Exp Med 2007;204:1543-51.
101. Floess S, Freyer J, Siewert C, Baron U, Olek S, Polansky J, et al. Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol 2007;5:e38.
102. Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? Nat Rev Immunol 2009;9:83-9.
103. Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. Blood 2009;114:3727-35.
104. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 2005;308:1466-9.
105. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A 2005;102:10604-9.
106. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, et al. Rapid DNA methylation changes after exposure to traffic particles. Am J Respir Crit Care Med 2009;179:572-8.
107. Salam MT, Byun HM, Lurmann F, Breton CV, Wang X, Eckel SP, et al. Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. J Allergy Clin Immunol 2012;129:232-9, e7.
108. Breton CV, Byun HM, Wenten M, Pan F, Yang A, Gilliland FD. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. Am J Respir Crit Care Med 2009;180:462-7.
109. Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SI, et al. Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. Hum Mol Genet 2012;21:3073-82.
110. Schembri F, Sridhar S, Perdomo C, Gustafson AM, Zhang X, Ergun A, et al. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. Proc Natl Acad Sci U S A 2009;106:2319-24.
111. Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S. Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. FASEB J 2009;23:806-12.

112. Izzotti A, Calin GA, Steele VE, Croce CM, De Flora S. Relationships of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J 2009;23:3243-50.
113. Liu F, Killian JK, Yang M, Walker RL, Hong JA, Zhang M, et al. Epigenomic alterations and gene expression profiles in respiratory epithelia exposed to cigarette smoke condensate. Oncogene 2010;29:3650-64.
114. Tang WY, Levin L, Talaska G, Cheung YY, Herbstman J, Tang D, et al. Maternal exposure to polycyclic aromatic hydrocarbons and 5'-CpG methylation of interferon-gamma in cord white blood cells. Environ Health Perspect 2012;120:1195-200.
115. Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Ili-Gangas C, Lebron C, Witter FR, et al. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. Epigenetics 2010;5:539-46.
116. Flom JD, Ferris JS, Liao Y, Tehranifar P, Richards CB, Cho YH, et al. Prenatal smoke exposure and genomic DNA methylation in a multiethnic birth cohort. Cancer Epidemiol Biomarkers Prev 2011;20:2518-23.
117. Murphy SK, Adigun A, Huang Z, Overcash F, Wang F, Jirtle RL, et al. Gender-specific methylation differences in relation to prenatal exposure to cigarette smoke. Gene 2012;494:36-43.
118. Breton CV, Salam MT, Gilliland FD. Heritability and role for the environment in DNA methylation in AXL receptor tyrosine kinase. Epigenetics 2011;6:895-8.
119. Maccani MA, Avissar-Whiting M, Banister CE, McGonnigal B, Padbury JF, Marsit CJ. Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21, and miR-146a in the placenta. Epigenetics 2010;5:583-9.
120. Whitrow MJ, Moore VM, Rumbold AR, Davies MJ. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. Am J Epidemiol 2009;170:1486-93.
121. Haberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. Arch Dis Child 2009;94:180-4.
122. Martinussen MP, Risnes KR, Jacobsen GW, Bracken MB. Folic acid supplementation in early pregnancy and asthma in children aged 6 years. Am J Obstet Gynecol 2012;206:72, e1-7.
123. Magdelijns FJ, Mommers M, Penders J, Smits L, Thijs C. Folic acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. Pediatrics 2011;128:e135-44.
124. Bekkers MB, Elstgeest LE, Scholtens S, Haveman A, de Jongste JC, Kerkhof M, et al. Maternal use of folic acid supplements during pregnancy and childhood respiratory health and atopy: the PIAMA birth cohort study. Eur Respir J 2012;39:1468-74.
125. Koh BH, Hwang SS, Kim JY, Lee W, Kang MJ, Lee CG, et al. Th2 LCR is essential for regulation of Th2 cytokine genes and for pathogenesis of allergic asthma. Proc Natl Acad Sci U S A 2010;107:10614-9.
126. Tanaka S, Motomura Y, Suzuki Y, Yagi R, Inoue H, Miyatake S, et al. The enhancer HS2 critically regulates GATA-3-mediated Il4 transcription in T(H)2 cells. Nat Immunol 2011;12:77-85.
127. Van Stry M, Bix M. Explaining discordant coordination. Nat Immunol 2011;12:16-7.
128. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. Am J Respir Cell Mol Biol 2011;44:285-92.
129. Brand S, Kesper DA, Teich R, Kilic-Niebergall E, Pinkenburg O, Bothur E, et al. DNA methylation of T(H)1/T(H)2 cytokine genes affects sensitization and progress of experimental asthma. J Allergy Clin Immunol 2012;129:1602-10, e6.
130. Ariel D, Upadhyay D. The role and regulation of microRNAs in asthma. Curr Opin Allergy Clin Immunol 2012;12:49-52.
131. Collison A, Mattes J, Plank M, Foster PS. Inhibition of house dust mite-induced allergic airways disease by antagonism of microRNA-145 is comparable to glucocorticoid treatment. J Allergy Clin Immunol 2011;128:160-7, e4.
132. Polikepahad S, Knight JM, Naghavi AO, Oplt T, Creighton CJ, Shaw C, et al. Proinflammatory role for let-7 microRNAS in experimental asthma. J Biol Chem 2010;285:30139-49.
133. Kumar M, Ahmad T, Sharma A, Mabalirajan U, Kulshreshtha A, Agrawal A, et al. Let-7 microRNA-mediated regulation of IL-13 and allergic airway inflammation. J Allergy Clin Immunol 2011;128:1077-85, e1-10.
134. Lu TX, Hartner J, Lim EJ, Fabry V, Mingler MK, Cole ET, et al. MicroRNA-21 limits in vivo immune response-mediated activation of the IL-12/IFN-gamma pathway, Th1 polarization, and the severity of delayed-type hypersensitivity. J Immunol 2011;187:3362-73.
135. Ma F, Xu S, Liu X, Zhang Q, Xu X, Liu M, et al. The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-gamma. Nat Immunol 2011;12:861-9.
136. Steiner DF, Thomas MF, Hu JK, Yang Z, Babiarz JE, Allen CD, et al. MicroRNA-29 regulates T-box transcription factors and interferon-gamma production in helper T cells. Immunity 2011;35:169-81.
137. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. Nat Genet 2007;39:380-5.
138. Morales E, Bustamante M, Vilahur N, Escaramis G, Montfort M, de Cid R, et al. DNA hypomethylation at ALOX12 is associated with persistent wheezing in childhood. Am J Respir Crit Care Med 2012;185:937-43.
139. Breton CV, Byun HM, Wang X, Salam MT, Siegmund K, Gilliland FD. DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. Am J Respir Crit Care Med 2011;184:191-7.
140. Kuriakose J, Rosa MJ, Perzanowski M, Miller R. Bronchial nitric oxide flux may be better associated with inducible nitric oxide synthase promoter methylation. Am J Respir Crit Care Med 2012;185:460-1; author reply 1.
141. Baccarelli A, Rusconi F, Bollati V, Catelan D, Accetta G, Hou L, et al. Nasal cell DNA methylation, inflammation, lung function and wheezing in children with asthma. Epigenomics 2012;4:91-100.
142. Nadeau K, McDonald-Hyman C, Noth EM, Pratt B, Hammond SK, Balmes J, et al. Ambient air pollution impairs regulatory T-cell function in asthma. J Allergy Clin Immunol 2010;126:845-52, e10.
143. Sood A, Petersen H, Blanchette CM, Meek P, Picchi MA, Belinsky SA, et al. Methylated genes in sputum among older smokers with asthma. Chest 2012 [Epub ahead of print].
144. Ito K, Lim S, Caramori G, Cosio B, Chung KF, Adcock IM, et al. A molecular mechanism of action of theophylline: induction of histone deacetylase activity to decrease inflammatory gene expression. Proc Natl Acad Sci U S A 2002;99:8921-6.
145. Barnes PJ. Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009;6:693-6.
146. Yang Y, Wicks J, Haitchi HM, Powell RM, Manuyakorn W, Howarth PH, et al. Regulation of a disintegrin and metalloprotease-33 expression by transforming growth factor-beta. Am J Respir Cell Mol Biol 2012;46:633-40.
147. Williams AE, Larner-Svensson H, Perry MM, Campbell GA, Herrick SE, Adcock IM, et al. MicroRNA expression profiling in mild asthmatic human airways and effect of corticosteroid therapy. PLoS One 2009;4:e5889.
148. Tsitsiou E, Williams AE, Moschos SA, Patel K, Rossios C, Jiang X, et al. Transcriptome analysis shows activation of circulating CD8+ T cells in patients with severe asthma. J Allergy Clin Immunol 2012;129:95-103.
149. Berlivet S, Moussette S, Ouimet M, Verlaan DJ, Koka V, Al Tuwaijri A, et al. Interaction between genetic and epigenetic variation defines gene expression patterns at the asthma-associated locus 17q12-q21 in lymphoblastoid cell lines. Hum Genet 2012;131:1161-71.
150. Fraser HB, Lam LL, Neumann SM, Kobor MS. Population-specificity of human DNA methylation. Genome Biol 2012;13:R8.
151. Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature 2007;447:972-8.
152. Qin H, Roberts KL, Niyongere SA, Cong Y, Elson CO, Benveniste EN. Molecular mechanism of lipopolysaccharide-induced SOCS-3 gene expression in macrophages and microglia. J Immunol 2007;179:5966-76.
153. Brand S, Teich R, Dicke T, Harb H, Yildirim AO, Tost J, et al. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. J Allergy Clin Immunol 2011;128:618-25, e1-7.