

The role of epigenetics in the developmental origins of allergic disease

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Objective: To review current research findings in the field of epigenetics pertaining to the developmental origins of allergic disease.

Data Sources: We examined original research and review articles identified from MEDLINE, OVID, and PubMed that addressed the topic of interest, using the search terms *atopy*, *allergy*, *asthma*, *development*, *IgE*, *origins*, and *cord blood* paired with *epigenetic(s)*. Relevant references from each article were also procured for review.

Study Selection: Articles were selected based on their relevance to the contributory role of epigenetic modifications in asthma and other atopic diseases.

Results: There is increasing evidence pointing to the influence of prenatal and early life exposures on the development of allergic disease. A growing body of literature supports the theory that transient environmental pressures can have permanent effects on gene regulation and expression through epigenetic mechanisms. Histone modifications have been associated with degree of bronchial hyperresponsiveness and corticosteroid resistance in asthma. Epigenetic mechanisms can operate independently in various cell types; recent studies have suggested a role in the differentiation of human T cells. Murine studies have revealed that a maternal diet rich in methyl donors can enhance susceptibility to allergic inflammation in the offspring, mediated through increased DNA methylation. Murine studies have also implicated epigenetically modified dendritic cells in the transmission of allergic risk from mothers to offspring.

Conclusion: The current literature offers exciting data to support a role for epigenetics in the development and persistence of asthma and allergic rhinitis. However, further human studies are necessary to explore these mechanisms and assess future clinical applicability.

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INTRODUCTION

Allergic diseases and asthma have increased significantly during the past quarter century and are associated with both a genetic predisposition and important environmental

triggers. Although twin studies have demonstrated a partial genetic component of allergic disease, differences in DNA sequence cannot fully explain the relatively sudden increase in the prevalence of allergic disorders or why only certain individuals are affected.^{1,2} Thus, researchers are now carefully evaluating gene-environment interactions, particularly during sensitive stages of development, to elucidate the complex factors responsible for the current allergy epidemic.

Researchers are now recognizing that many diseases arising later in life may have prenatal origins. Examples of disorders under such intense investigation include diabetes mellitus, cardiovascular disease, and cancer.³ *Epigenetics* is an emergent field of study that has begun to play a key role in explaining these key gene-environment interactions.⁴ Epigenetics refers to heritable changes in gene expression and gene expression potential that are not transmitted through

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changes in the actual genetic code.⁵ Mechanisms of epigenetic regulation have recently been shown to underlie many complex diseases and thus may hold some answers to explain the development and heritability of asthma.⁶ In this review, we provide an overview of epigenetic regulatory mechanisms, highlight key studies that have indicated an important role for epigenetic modifications in the developmental origins and pathogenesis of atopy and asthma, and consider the potential for future applications of these research findings to the clinical practice of allergy and immunology.

EPIGENICS

Progeny receive their biological inheritance from their parents via 2 main mechanisms: genetic and epigenetic.⁷ Genetic inheritance is determined when chromosomes from the individual's parents undergo crossover and genetic recombination during maternal and paternal gametogenesis and are subsequently combined during fertilization. Epigenetic inheritance is represented by DNA modifications, chromatin modifications, regulatory RNA molecules, and other processes that are independent of the genetic code but affect gene expression.⁵

Epigenetics can be thought of as the “clothing” of the genome because the DNA is covered with a knitted layer, akin to a sweater, that can be altered via epigenetic modifications. Some of this layer changes significantly during development or is modified throughout life, whereas other layers remain relatively permanent. Differences in this layer lead to differences in gene expression and regulation and can alter the clinical phenotype associated with the same genetic code. These changes to the “clothing” of DNA are potentially heritable from parent to offspring and usually conserved during somatic cell division, thus maintaining differentiated cell lineages.³ The epigenetic code that is established during development and changes that occur in specific cell types in response to the environment may each play a role in the development of allergies and asthma. Here we focus on the potential roles of 2 major classes of epigenetic markings: DNA methylation and histone modifications.

DNA METHYLATION

DNA methylation is the process by which a methyl group, donated from S-adenosyl methionine, is added to the cytosine residue of a cytosine-guanine (CpG) dinucleotide (Fig 1). This reaction is catalyzed by a family of DNA methyltransferases (Dnmt1, Dnmt2, and Dnmt3).⁷ Dnmt1 is referred to as a maintenance methyltransferase because this isoform adds methyl groups to hemimethylated sites during replication.⁸ The role of Dnmt2 is not as clear because this isoform exhibits only low levels of activity *in vitro*.⁴ Dnmt3a and 3b are *de novo* methyltransferases.⁹ The role of active demethylation is currently under debate. A mechanism has been identified in plants, but whether or not similar mechanisms operate in mammals is not fully understood.¹⁰ The effect of DNA methylation is generally *transcriptional repression*, and this occurs by altering recognition sites for transcription

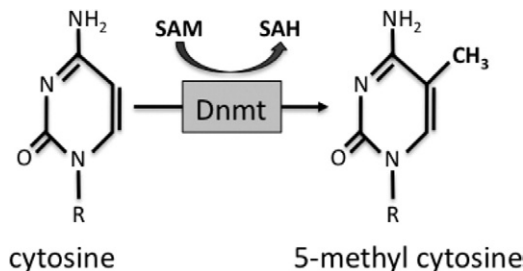


Figure 1. Conversion of cytosine to 5-methyl cytosine by DNA methyltransferase (Dnmt). The methyl group (CH₃) is derived from the cosubstrate S-adenosyl methionine (SAM), which is converted to S-adenosyl homocysteine (SAH).

factors and reducing the accessibility of DNA to the transcriptional machinery⁷ and hence a *lower expression* of the relevant encoded proteins.

HISTONE MODIFICATIONS

Histones are highly conserved basic proteins that interact with the nucleic acid chain, helping to package the meters-long human genome efficiently into tightly wound nucleosomes. The octameric core of a nucleosome is composed of 2 H2A-H2B heterodimers and a H3-H4 tetramer.¹¹ Linker histone H1 binds DNA between the nucleosomes.¹¹ Histone proteins have flexible N-terminal tails that can undergo a variety of posttranslational modifications, including methylation, acetylation, phosphorylation, ubiquitination, sumoylation, ribosylation, and deimination.⁷ Arginine and lysine residues can be methylated up to 2 and 3 times, respectively, and combined with the wide variety of other modifications that are possible, the histone code can become extremely complex.⁹

Histone acetylation, in particular, has been studied extensively during the past decade, and it has generally been associated with *transcriptional activation*, particularly when present on H3 or H4 (Fig 2). Histone acetyltransferase (HAT) enzymes covalently attach acetyl groups to lysine residues. This promotes transcription, possibly by 2 mechanisms; reducing the strength of the noncovalent interactions through neutralization of the negative charge on lysine and recruiting activator proteins to the DNA.⁴ This leads to increased transcription of the relevant encoded protein. Therefore, increased HAT activity leads to increased expression of the relevant gene. Conversely, acetyl groups are removed from histones via the histone deacetylase (HDAC) enzymes, leading to decreased gene expression. Transcriptional activators and repressors often possess HAT or HDAC activity, respectively, or recruit other proteins with such activity to the DNA.⁹ In addition, the binding of methyl CpG-binding protein to methylated sites recruits HDACs.³ Thus, DNA methylation and histone deacetylation can often act in concert to silence gene expression.

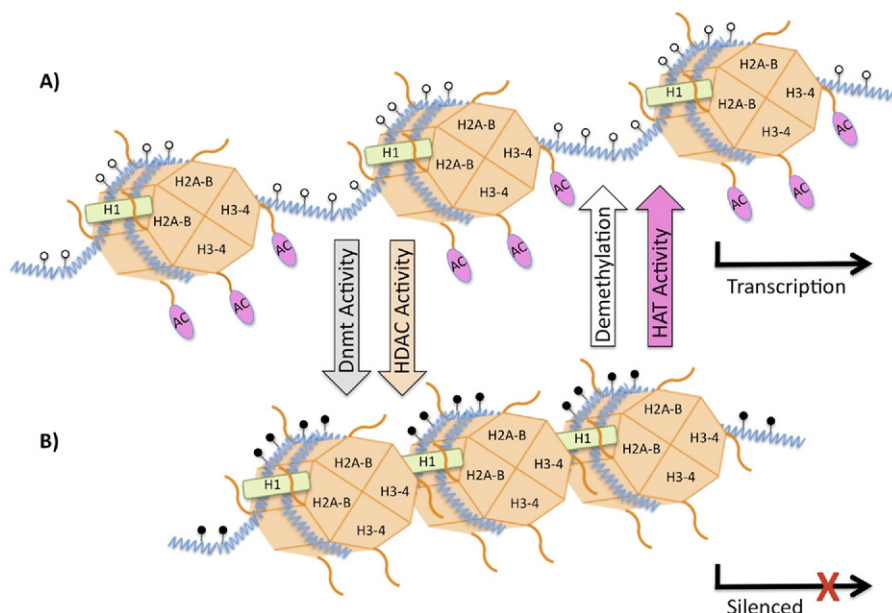


Figure 2. The octameric core of a nucleosome is composed of 2 H2A-H2B heterodimers and an H3-H4 tetramer. A, In the transcriptionally active state, CpG dinucleotides are unmethylated (○) and histones H3 and H4 are acetylated (AC) by histone acetyltransferase (HAT). B, When transcription is silenced, CpG dinucleotides are methylated (●) and histones are deacetylated through histone deacetylase (HDAC) activity.

EPIGENETICS AND GENOMIC IMPRINTING

A prime example of the complex coordination between DNA methylation and changes in DNA-associated proteins is the intricate chromosomal modifications that occur during fetal development. The early studies of DNA methylation in mammalian development examined the inactivation of 1 of the 2 X chromosomes in the somatic cells of females.^{12,13} From these studies, it is now known that DNA undergoes tightly regulated and sweeping changes in methylation during development. Beginning with gametogenesis, there are marked differences between the chromatin packaging of paternal vs maternal DNA. Within the sperm, paternal DNA is highly methylated and condensed with protamine proteins, rather than histone proteins.¹⁴ After fertilization, the paternal genome undergoes rapid demethylation before cell division.¹⁵ In contrast, the maternal genome is passively demethylated over several cycles of replication.¹⁵ After the paternal and maternal waves of demethylation, de novo methylation begins to restore methyl groups to many of the aforementioned CpG sites, excluding the CpG islands, which contain high concentrations of CpG sites but low levels of methylation throughout life.⁸ During this epigenetic reprogramming process, allele-specific imprinting occurs, which may help to explain intriguing parent-of-origin effects in complex diseases, such as asthma.^{16,17}

Epigenetic imprinting is a process by which a gene from one parent is suppressed through DNA methylation and histone alterations, and yet the other is fully expressed. Early studies probing the interactions between epigenetic changes induced in utero and subsequent adult gene expression were

performed using the viable yellow agouti mouse as a model. The agouti mouse model played an important role in establishing our understanding of metastable epialleles, which are alleles that can be variably expressed in individuals despite identical genotype, due to epigenetic modifications established during development.¹⁸ In the agouti model, coat color varies based on the level of methylation of the *A^{vy}* gene, from a yellow color if the gene is hypomethylated to pseudoagouti (individual fur strands with variable light and dark brown bands) if the gene is hypermethylated.¹⁸ In this model it has been demonstrated that a maternal diet supplemented with methyl donors, including folic acid, vitamin B₁₂, betaine, and choline, affects the imprinting of the *A^{vy}* gene, resulting in a shift toward pseudoagouti coloring.¹⁹ Thus, there is the potential for environmental factors, such as maternal nutrition, to affect epigenetic imprinting and significantly shift the phenotypes of offspring.

EPIGENETICS AND THE DEVELOPMENTAL ORIGINS OF DISEASE HYPOTHESIS

The developmental origins of disease hypothesis proposes that environmental factors can induce permanent changes during prenatal development, which subsequently affect an individual's predisposition to disease later in life.²⁰ Another way to view this hypothesis is from the perspective of developmental plasticity, whereby environmental cues, such as maternal nutrition and hormones, can influence the way the genome is expressed in the offspring.³ Although the developmental origins of disease hypothesis is not particularly new, the discovery of epigenetic modifications and the

emerging field of epigenetics in general have led to a greater acceptance of the role of the developmental environment in mediating the risk of future illnesses, by providing concrete mechanisms through which these influences may act.²⁰ Such an example of how in utero exposures can affect an offspring's development and future disease risk is the consequences of treating pregnant women with the synthetic estrogen diethylstilbestrol to prevent miscarriage.²¹ Strong associations were determined between in utero exposure to diethylstilbestrol and abnormalities in the reproductive system development of female offspring, with resultant reduced fertility later in life, and a significantly increased risk of developing a typically rare clear cell adenocarcinoma of the vagina or cervix at a young age.²¹ Subsequent murine studies have revealed links between persistent demethylation of the *c-fos*, lactoferrin, and nucleosomal binding protein 1 (*Nsbp1*) genes and in utero diethylstilbestrol exposure, suggesting estrogen imprinting as a potential mechanism behind the health effects observed in exposed humans.²² Thus, there is evidence that prenatal exposures can affect phenotypes and disease risk in animal and human studies.

EPIGENETIC DYSREGULATION IN ALLERGIC DISEASE

In Utero and Early Life

There are likely windows of vulnerability during prenatal development and early life when environmental influences lead to changes to the epigenetic code, with subsequent important phenotypic effects. In murine studies, placing pregnant dams on a methyl donor-enriched diet, including increased folic acid, genistein, zinc, methionine, choline, and betaine, results in progeny that are more susceptible to the development of ovalbumin-induced allergic airway inflammation.²³ The proallergic effect of gestational exposure to a maternal diet with increased methyl donors was also inherited through the male line to the next generation.²³ Potential epigenetic mechanisms explaining the increased allergic risk may involve the silencing through methylation of anti-inflammatory genes, such as the runt-related transcription factor 3 (*Runx3*), a gene known to suppress allergic airway disease.²³

As a consequence of this study, concern has been raised about current North American recommendations for pregnant women to receive routine folic acid supplementation to prevent neural tube defects.²⁴ Others have begun to examine the possibility that maternal supplementation with methyl donors may affect the incidence of asthma in children.^{25,26} In a Japanese cohort, no significant relationships were found between maternal folate or vitamin B intake and the risk of wheeze or eczema in the offspring.²⁵ However, Whitrow et al²⁶ examined folate supplement intake both early and late in pregnancy in an Australian birth cohort and found an association, with a relative risk of 1.26, between supplementation in late pregnancy and physician-diagnosed asthma in the offspring. Further studies are necessary to determine whether

there is indeed an increased risk and to examine the risk-benefit relationship.

There is also evidence that certain prenatal exposures that are well-established risk factors for childhood atopy and asthma, such as maternal smoking, may act at least partly through epigenetic mechanisms.²⁷ Global and gene-specific changes in methylation have been demonstrated in children exposed to tobacco smoke in utero.²⁸ In addition, grandmaternal smoking during pregnancy has recently been associated with asthma in the grandchildren, suggesting that some environmental effects lead to a trans-generational inheritance legacy.²⁸ There is also emerging evidence that prenatal exposure to maternal stress may increase asthma risk in the offspring, although the mechanisms are not currently known.²⁹ An epigenetic alteration recently associated with maternal stress during in utero development is increased methylation of the human glucocorticoid receptor gene (*NR3C1*).³⁰ In addition, maternal allergic rhinitis symptoms during early pregnancy have been associated with allergic rhinitis in the offspring but not asthma, allergic dermatitis, or food allergy in a retrospective cohort.³¹ Therefore, gene-environment interactions modify the ensuing risk of allergic disease in the offspring, and epigenetic dysregulation may be one of the key players in the developmental origins of disease.

Epigenetics in Established Allergic Disease

In addition to the role of epigenetic modification and dysregulation in the developmental origins of asthma and other atopic conditions, there is mounting evidence that epigenetic alterations are contributory to established asthma. Ito et al³² examined HATs and HDACs in bronchial biopsy specimens from patients with asthma and healthy controls. Patients with asthma exhibited lower HDAC1 and HDAC2 protein expression, along with reduced total HDAC activity.³² HAT activity was also found to be perturbed because the authors demonstrated greater HAT activity in the asthmatic patients.³² Similar alterations were also observed by Cosio et al³³ in alveolar macrophages collected via bronchoalveolar lavage. The authors demonstrated decreased HDAC activity and increased HAT activity in asthmatic patients compared with controls.³³ This would lead to an increase in histone acetylation and a decrease in deacetylation, potentially resulting in the activation of genes relevant to the asthmatic phenotype.

Alterations in histone-modifying enzymes have also been demonstrated in peripheral blood mononuclear cells (PBMCs). Su et al³⁴ examined the ratio of HDAC to HAT activity in PBMCs from asthmatic children with bronchial hyperreactivity and nonasthmatic controls. The authors demonstrated significantly decreased HDAC activity, increased HAT activity, and a consequent skewing of the HDAC:HAT ratio in the asthmatic children.³⁴ The authors also determined that the HDAC:HAT ratio significantly correlated with the degree of bronchial hyperreactivity.³⁴ However, this is merely a documentation of an association between HDAC:HAT ratio and asthma severity and as of yet cannot be shown to prove causality.

Epigenetics and T-Cell Phenotype

The activation and lineage commitment of T cells involve epigenetic modifications and chromatin remodeling in specific regions of the genome. For example, the expression of interleukin 4 (IL-4) messenger RNA is low in naive T cells and on antigen presentation is silenced under T_H1-polarizing conditions and overexpressed during T_H2 polarization.³⁵ It has been established that T_H2 polarization involves demethylation and increased histone acetylation of the *IL-4* gene, mediating the transition from closed to open chromatin conformation.³⁶ The maintenance of the open chromatin conformation and increased transcription levels are then dependent on the binding of polarizing transcription factors, such as STAT-6 and GATA-3, which upregulate IL-4 production, or alternatively STAT-4 and T-bet, which in turn upregulate interferon γ production.³⁶ Interactions between Dnmts and methyl CpG-binding proteins are integral in silencing appropriate cytokine genes during differentiation. For example, Dnmt1 and MBD2 work in concert to silence *IL-4* expression in cells of the T_H1 lineage, whereas GATA-3 binding to the *IL-4* promoter prevents the silencing effects of Dnmt1 and MBD2 in the T_H2 lineage.³⁵

What is most relevant to determine, however, is whether the overall reduction in HDAC activity reported in asthmatic patients is actually involved in initiating or maintaining the T_H2 phenotype. To address this, Su et al have demonstrated that inhibition of HDAC activity in PBMCs increases the ratio of T_H2 cytokine production to T_H1 cytokine production on stimulation ex vivo.³⁷ The authors also demonstrated that cytokine secretion was largely derived from antigen-experienced memory T cells.³⁷ Recently, Grausenburger et al³⁸ selectively deleted HDAC1 in murine T cells and demonstrated increased T_H2-mediated inflammation in vivo. T cells lacking HDAC1 demonstrated increased cytokine production regardless of T_H1 vs T_H2 polarization,³⁸ indicating a role for HDAC1 in controlling cytokine expression levels in T cells. These findings appear to be consistent with the observation of decreased HDAC activity in asthmatic patients; however, more studies are needed to understand the roles of these enzymes in T-cell phenotypes.

Regulatory T cells (Tregs) are a special subset of T cells that suppress immune responses and promote tolerance to self-antigens. Treg numbers have been shown to be reduced in the bronchoalveolar lavage fluid of asthmatic patients, suggesting that their ability to suppress the T_H2 response may be impaired.³⁹ The transcription factor forkhead box p3 (FOXP3) is essential for the development of these cells, which produce the anti-inflammatory cytokines transforming growth factor β and IL-10.⁴⁰ It has recently been shown that FOXP3 expression is controlled by methylation at a CpG island containing the binding site of cyclic adenosine monophosphate response element binding protein/activating transcription factor.⁴¹ Environmental exposures may also alter Treg function through epigenetic modifications at the *FOXP3* promoter. Recently, Nadeau et al⁴² examined Treg function

and *FOXP3* methylation in relation to ambient air pollution exposure. They demonstrated hypermethylation of the *FOXP3* locus with associated Treg functional impairment in asthmatic patients from Fresno, California, an area with high annual ambient air pollution levels, compared with asthmatic patients from Stanford, California, an area with relatively low air pollution levels.⁴² Thus, epigenetic modifications may play a key role in the development and functioning of Tregs and may also be modified by environmental factors.

Epigenetic Modifications in Dendritic Cells and Allergic Disease Risk

Another specific cell type that is known to be of importance in asthma and allergy and may be altered in function by epigenetic modifications is the dendritic cell. Dendritic cells play an important role in innate immunity and as antigen-presenting cells in the initiation of an adaptive immune response.⁴³ Mucosal dendritic cells in the lung encounter antigens from the external environment and migrate to the lymph nodes, where they can activate naive T cells.⁴³ It has been hypothesized that dendritic cells may be contributory to the increased asthmatic risk attributed to maternal atopy.⁴⁴ Fedulov and Kobzik⁴⁵ recently used a murine model to examine the potential role of dendritic cells in the increased risk of allergy to offspring born to allergic mothers. The authors demonstrated hypermethylation of DNA in splenic dendritic cells harvested from pups born to mothers previously sensitized and challenged with ovalbumin.⁴⁵ Notably, gene expression changes were not detected in these cells.⁴⁵ However, the dendritic cells from pups of allergic mothers exhibited increased antigen presentation activity, and adoptive transfer of these cells into offspring from nonallergic mothers transferred the increased susceptibility to allergic disease to the recipients.⁴⁵ This suggests that even when gene expression in dendritic cells is the same regardless of atopic status, altered epigenetics may have encoded changes in gene expression potential that translated into functional differences when these cells are exposed to allergens. However, these results await confirmation in human studies.

Epigenetics and Asthma Therapy

Although the field of epigenetic research is contributing to a better understanding of the mechanisms behind allergic disease, it is not yet certain if these discoveries will lead directly to novel antiallergic treatments. However, it is becoming apparent that some therapies already in use likely exert their effects at least partly through epigenetic mechanisms. For example, Ito et al³² found that HAT activities were elevated in asthmatic bronchial biopsy specimens of untreated asthmatic patients but were reduced to normal levels in the asthmatic patients receiving inhaled corticosteroids. Conversely, HDAC activity was increased in biopsy specimens from asthmatic patients treated with inhaled corticosteroids, compared with untreated asthmatic patients, although the activity still remained reduced compared with healthy controls.³² Corticosteroids have also been reported to attenuate

the repression of HDAC1 in alveolar macrophages.³³ Similar to the findings in bronchial biopsy specimens, the elevation of HAT activity was attenuated in macrophages from asthmatic patients receiving corticosteroid therapy.³³ In addition, Cosio et al demonstrated that a steroid treatment intervention produced an increase in HDAC activity in PBMCs of mild asthmatic patients; however, a similar treatment protocol in severe asthmatic patients failed to produce an effect.³³ In a subsequent study, HDAC activities were shown to be significantly lower in PBMCs isolated from the severe asthmatic patients compared with nonsevere asthmatic patients and to correlate directly to the degree of steroid hyporesponsiveness.⁴⁶ In patients with asthma who smoke, a group known to be less responsive in general to steroid treatment, a significantly greater reduction of HDAC activity in bronchial biopsy specimens has been reported.⁴⁷ Thus, there appears to be a relationship between HDAC activity and steroid responsiveness.

Reduced sensitivity to the anti-inflammatory effects of corticosteroids also represents a clinical challenge in the management of chronic obstructive pulmonary disease (COPD).⁴⁸ The activity and expression of HDAC2 have been shown to be reduced in COPD and correlated to disease severity.⁴⁹ Oxidative stress may reduce HDAC2 activity through a phosphoinositide-3-kinase-delta-mediated mechanism, which is potentially reversed by treatment with theophylline. Recent clinical trials in both COPD and asthma have demonstrated that low-dose theophylline in combination with inhaled corticosteroids may improve pulmonary function and reduce inflammation in both groups.⁵⁰

CONCLUSIONS

Epigenetic changes, including DNA methylation and histone modifications, play an essential role in fetal development, gene-environment interactions, and the maintenance of cell lineage. It has been shown that the prenatal environment can have lasting effects on the phenotype of the offspring, mediated at least in part through epigenetic modifications. Murine studies have demonstrated a link between epigenetics and the developmental origins of increased allergic risk. However, evidence in humans is limited at the present time. There is current evidence that histone acetylation/deacetylation is dysregulated in asthma and COPD and that DNA methylation plays an important role in T-cell differentiation. Although further studies are clearly necessary to determine the specific disease processes mediated by epigenetic modifications, the currently available literature offers exciting data to support the importance of epigenetic alterations in the development and manifestations of atopic diseases, such as asthma and allergic rhinitis.

REFERENCES

- Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res*. 2001;4:81–93.
- Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. *Am J Respir Crit Care Med*. 2008;177:567–573.
- Burdge GC, Lillycrop KA. Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease. *Annu Rev Nutr*. 2010;30:315–339.
- Adcock IM, Ford P, Ito K, Barnes PJ. Epigenetics and airways disease. *Respir Res*. 2006;7:21.
- Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr*. 2007;27:363–388.
- Martino DJ, Prescott SL. Silent mysteries: epigenetic paradigms could hold the key to conquering the epidemic of allergy and immune disease. *Allergy*. 2010;65:7–15.
- Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*. 2003;33(suppl):245–254.
- Geiman TM, Muegge K. DNA methylation in early development. *Mol Reprod Dev*. 2010;77:105–113.
- Kabesch M, Michel S, Tost J. Epigenetic mechanisms and the relationship to childhood asthma. *Eur Respir J*. 2010;36:950–961.
- Ooi SK, Bestor TH. The colorful history of active DNA demethylation. *Cell*. 2008;133:1145–1148.
- Marino-Ramirez L, Kann MG, Shoemaker BA, Landsman D. Histone structure and nucleosome stability. *Expert Rev Proteomics*. 2005;2:719–729.
- Riggs AD. X inactivation, differentiation, and DNA methylation. *Cytogenet Cell Genet*. 1975;14:9–25.
- Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. *Science*. 1975;187:226–232.
- Kimmins S, Sassone-Corsi P. Chromatin remodelling and epigenetic features of germ cells. *Nature*. 2005;434:583–589.
- Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. *Nature*. 2000;403:501–502.
- Strauch K, Bogdanow M, Fimmers R, Baur MP, Wienker TF. Linkage analysis of asthma and atopy including models with genomic imprinting. *Genet Epidemiol*. 2001;21(suppl 1):S204–S209.
- Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Parental history and the risk for childhood asthma: does mother confer more risk than father? *Am J Respir Crit Care Med*. 1998;158:176–181.
- Dolinoy DC. The agouti mouse model: an epigenetic biosensor for nutritional and environmental alterations on the fetal epigenome. *Nutr Rev*. 2008;66(suppl 1):S7–S11.
- Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J*. 1998;12:949–957.
- Shaheen SO, Adcock IM. The developmental origins of asthma: does epigenetics hold the key? *Am J Respir Crit Care Med*. 2009;180:690–691.
- Li S, Hursting SD, Davis BJ, McLachlan JA, Barrett JC. Environmental exposure, DNA methylation, and gene regulation: lessons from diethylstilbesterol-induced cancers. *Ann N Y Acad Sci*. 2003;983:161–169.
- Prins GS. Estrogen imprinting: when your epigenetic memories come back to haunt you. *Endocrinology*. 2008;149:5919–5921.
- Hollingsworth JW, Maruoka S, Boon K, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. *J Clin Invest*. 2008;118:3462–3469.
- Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. *Int J Epidemiol*. 2010;39(suppl 1):i110–i121.
- Miyake Y, Sasaki S, Tanaka K, Hirota Y. Maternal B vitamin intake during pregnancy and wheeze and eczema in Japanese infants aged 16–24 months: The Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol*. 2010;22(1 pt 1):69–74.
- 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;170:1486–1493.
- Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. *Chest*. 2005;127:1232–1241.

28. 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;180:462–467.
29. Cookson H, Granell R, Joinson C, Ben-Shlomo Y, Henderson AJ. Mothers' anxiety during pregnancy is associated with asthma in their children. *J Allergy Clin Immunol*. 2009;123:847–853.
30. Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 2008;3:97–106.
31. Shinohara M, Wakiguchi H, Saito H, Matsumoto K. Symptoms of allergic rhinitis in women during early pregnancy are associated with higher prevalence of allergic rhinitis in their offspring. *Allergol Int*. 2007;56(4):411–417.
32. Ito K, Caramori G, Lim S, et al. Expression and activity of histone deacetylases in human asthmatic airways. *Am J Respir Crit Care Med*. 2002;166(3):392–396.
33. Cosio BG, Mann B, Ito K, et al. Histone acetylase and deacetylase activity in alveolar macrophages and blood mononocytes in asthma. *Am J Respir Crit Care Med*. 2004;170:141–147.
34. Su RC, Becker AB, Kozyskyj AL, Hayglass KT. Altered epigenetic regulation and increasing severity of bronchial hyperresponsiveness in atopic asthmatic children. *J Allergy Clin Immunol*. 2009;124:1116–1118.
35. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). *Annu Rev Immunol*. 2010;28:445–489.
36. Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by cis elements and epigenetics. *Immunity*. 2006;24:369–379.
37. Su RC, Becker AB, Kozyskyj AL, Hayglass KT. Epigenetic regulation of established human type 1 versus type 2 cytokine responses. *J Allergy Clin Immunol*. 2008;121:57–63.
38. Grausenburger R, Bilic I, Boucheron N, et al. Conditional deletion of histone deacetylase 1 in T cells leads to enhanced airway inflammation and increased Th2 cytokine production. *J Immunol*. 2010;185:3489–3497.
39. Hartl D, Koller B, Mehlhorn AT, et al. Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T cells in pediatric asthma. *J Allergy Clin Immunol*. 2007;119:1258–1266.
40. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity*. 2009;31:438–449.
41. Kim HP, Leonard WJ. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. *J Exp Med*. 2007;204:1543–1551.
42. Nadeau K, McDonald-Hyman C, Noth EM, et al. Ambient air pollution impairs regulatory T-cell function in asthma. *J Allergy Clin Immunol*. 2010;126:845–852.
43. Wen H, Schaller MA, Dou Y, Hogaboam CM, Kunkel SL. Dendritic cells at the interface of innate and acquired immunity: the role for epigenetic changes. *J Leukoc Biol*. 2008;83:439–446.
44. Lim RH, Kobzik L. Maternal transmission of asthma risk. *Am J Reprod Immunol*. 2009;61:1–10.
45. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. *Am J Respir Cell Mol Biol*. Epub ahead of print. January 29, 2011.
46. Hew M, Bhavsar P, Torrego A, et al. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. *Am J Respir Crit Care Med*. 2006;174:134–141.
47. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med*. 2003;168:1308–1311.
48. Barnes PJ. Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2009;6:693–696.
49. Ito K, Ito M, Elliott WM, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med*. 2005;352:1967–1976.
50. To Y, Ito K, Kizawa Y, et al. Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2010;182:897–904.

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