



Epigenetics in asthma and allergy

Michael Kabesch^{a,b}

Purpose of review

Epigenetic mechanisms such as DNA methylation, histone modification and microRNA control the accessibility of the genome and manage gene transcription in response to the environment in a heritable fashion. Recent evidence suggests that these mechanisms play a role in allergy and asthma.

Recent findings

Here, we give an overview on recent developments in the field of asthma and allergy epigenetics with a special focus on the role of DNA methylation in these diseases, where finally, first pilot studies investigating differences in methylation pattern in patients have been published. Although these studies have to be interpreted with caution, it seems that methylation is affected by environmental stimuli such as prenatal smoke exposure and farming environments, whereas asthma status is associated with change in methylation in early childhood.

Summary

Early stage data from population studies indicate a role of methylation differences in asthma and allergy, whereas the exact impact of these epigenetic mechanisms on disease development needs to be elucidated further.

Keywords

allergy, asthma, epigenetics, methylation

INTRODUCTION

'Epigenetics', a term that can be interpreted as 'the mechanisms above or in addition to genetics', is nowadays defined as the study of heritable changes in gene expression or cellular phenotype caused by mechanisms that do not alter the nucleotide sequence [1]. Epigenetic mechanisms in humans are posttranslational modifications of histones, DNA methylation and the expression of noncoding RNAs [2,3]. On the contrary, sequence mutation may affect epigenetic signatures, for example by creating or destroying methylation sites. Environmental influences such as cigarette smoke may lead to permanent but modifiable changes in epigenetic patterns. Thus, both genetics and the environment influence epigenetic mechanism that in turn can contribute to the development of complex diseases [4–6].

This review builds upon and extends our previous reviews [4,5] and will concentrate on assessing the current knowledge and recent publications in the last 12 months (Table 1) [7^{••},8,9^{••},10,11^{••},12[•],13,14^{••},15[•]] on the association of DNA methylation with asthma and allergy, whereas further reviews in this special issue of *Current Opinion in Allergy and Clinical Immunology* deal with Histone modification and miRNA in asthma.

DNA methylation is the major, but not the only modification of DNA so far identified in mammals. Methylation occurs after replication and is almost exclusively affects position five of the pyrimidine ring of cytosines in the context of the dinucleotide sequence CpG [16]. Approximately 75% of mammalian CpG dinucleotides are methylated. Clusters enriched in CpGs (CpG islands) are found in locus control regions, promoter regions and first exons of many genes. In contrast to heavily methylated single CpGs throughout the genome, CpG islands are generally much less methylated, potentially allowing transcription [17]. DNA methylation is tightly controlled in a tissue and development-specific manner. Possible trigger mechanisms initiating methylation

^aHannover Medical School, Department of Pediatric Pneumology, Allergy and Neonatology, Hannover, Member of the German Lung Research Center and ^bDepartment of Pediatric Pneumology and Allergy, KUNO University Children's Hospital Regensburg, Regensburg, Germany

Correspondence to Michael Kabesch, MD, KUNO University Children's Hospital Regensburg, Department of Pediatric Pneumology and Allergy, Campus St. Hedwig, Steinmetzstr. 1-3, D-93049 Regensburg, Germany. Tel: +49 941 369 5801; fax: +49 941 369 5802; e-mail: Michael.Kabesch@ukr.de

Curr Opin Allergy Clin Immunol 2014, 14:62–68

DOI:10.1097/ACI.0000000000000025

KEY POINTS

- Methylation signatures are affected during early life by the environment such as smoke exposure and growing up on farms.
- Genetic variations in asthma candidate genes influence methylation and methylation plays a role in regulating accessibility and expression of asthma candidate genes.
- Methylation between lung epithelial cells and peripheral blood cells differs in approximately 10% of measured CpG sites.
- Important transcription factors in T and B cells are affected by methylation, potentially affecting the development of an allergy skewed immune response.

include recognition of specific target DNA sequences, RNA interference, certain chromatin structures induced by histone modifications and other protein–protein interactions [18,19]. In general, active demethylation of a promoter region is necessary to allow gene transcription. Although CpG islands were within the focus of early methylation studies, specially in cancer research, it became more and more obvious that not only CpG islands but also regions attached to CpG islands (CpG shores) are functionally important in methylation control. At this point, it seems that in noncancer complex diseases, CpG shores are at least as important as CpG islands in transferring methylation effects.

ROLE AND FUNCTION OF EPIGENETICS IN ASTHMA AND ALLERGY

The development of asthma is strongly influenced by the environment; in addition, these diseases also have a strong genetic component [20]. Air pollution such as active and passive cigarette smoke and exposure to diesel exhausts increase the risk for asthma, although being born into and growing up in families dedicated to traditional farming protects from disease development. Inflammation, airway remodeling and abnormal epithelial cell responsiveness are key features of asthma [21]. In all these mechanisms, timed and tissue-specific gene expression is important and, such as in the case of inflammation, the balance of proinflammatory and anti-inflammatory mechanisms may be influenced by differential and delicately regulated gene expression. Thus, epigenetic mechanisms may play an important role in the pathogenesis and heritability of asthma and allergy. Epigenetics may contribute to heritability [2,6,22] and it has a great potential to

answer many open questions in the pathogenesis of asthma. Phenomenons in allergy and asthma potentially explained by epigenetics are as follows:

- (1) Phenotypic discordance (25% in asthma)
- (2) Tissue-specific effects and symptoms
- (3) Difference in age of onset of symptoms
- (4) Fluctuation of symptoms
- (5) Sex effects
- (6) Parent-of-origin effects
- (7) Sporadic occurrence of disease
- (8) Transgenerational effects of environmental exposures

In a simple, one-dimensional model, inflammatory genes are shut down by methylation of their promoter regions and, thus, increased methylation leads to decreased inflammation and less disease. However, not only proinflammatory genes are affected by methylation but also regulatory regions within the genome (such as locus control regions) and genes inhibiting inflammation. Thus, epigenetics works in a delicately balanced network of gene networks, tissue-specific effects and subtle timing of epigenetic events. As a consequence, it seems important to investigate the methylation state of larger stretches within genes instead of single CpG islands in the promoter of a single gene; to study methylation extensive loci containing a gene region rather than one gene of interest within its narrow intron/exon boundaries or even methylation patterns of gene networks or the whole genome; to assess cell and tissue specific effects of methylation; to take environmental effects and disease-specific changes of methylation into account when studying epigenetic effects; and finally to consider the dynamic nature of methylation over time (Table 1).

DNA METHYLATION AFFECTS T AND B-CELL DIFFERENTIATION

Both T cells and B cells are thought to be involved in allergy and asthma pathogenesis. The differentiation and activation of type 2 T helper cells (TH2) is a hallmark of experimental allergy and asthma. DNA methylation plays an important role in T-cell differentiation and regulation [23,24]. Methylation of the intergenic region between *IL4* and *IL13* [25] suppresses the expression of TH2 signature cytokines in TH1 cells. In TH2 cells, methylation of *IFNG* is increased [26], for example inhibiting the binding of the activation AP-1 transcription factor in the *IFNG* promoter [27]. At the same time, the *IL13/IL4* region is demethylated allowing the binding of activating transcription factors [25]. Simultaneously, histones in the *IL13/IL4* region

Table 1. Recent epigenetic population studies in asthma and allergy highlighting study characteristics and major findings

Study	N assessed population	N methylation sites assessed	Cell type assessed	Gene expression measured	Environment assessed	Changes over time assessed	Replication of major findings	Major finding
Joubert <i>et al.</i> [7**]	1062	450K (Illumina chip)	Cord blood	No	Maternal smoking by cotinine level	No	Yes (small sample)	26 CpGs in 10 genes associated with maternal smoking in pregnancy, 3 genes replicated
Naumova <i>et al.</i> [8]	80	>100	PBMCs	No	Sex	Yes; but not in the same individuals	No	
Michel <i>et al.</i> [9**]	67	151	Cord blood and whole blood	No	Farming exposure	Yes; cord blood vs. 4.5 years	Yes (small sample)	Effects of farming, especially in CB, effects of asthma over time
Kim [10]	24	27k methylation bead chip	Airway mucosa	No	No	No	No	55 loci in 54 genes differentially methylated in atopic vs. nonatopic asthmatics
Morales <i>et al.</i> [11**]	122	1505 CpGs/Illumina cancer panel I	Whole blood	No	No	No	Yes large sample, 4 CPGs)	ALOX12 hypomethylation associated with persistent wheezing
Fu <i>et al.</i> [12*]	182	1 CpG in the 5'UTR of ADRB2	Whole blood	No	No exposure level	No	No	ADRB2 methylation is associated with severe asthma
Runyon <i>et al.</i> [13]	42 (21 twin pairs)	18 CpG sites in FOXP3 and INFG	Treg and Teff	Yes	No	No	No	Difference in methylation of FOXP3 and INFG in T cell subtypes of MZ twins discordant for asthma
Stefanowicz <i>et al.</i> [14**]	25	1505 CpGs/Illumina cancer panel I	Airway epithelial cells and PBMCs	Yes, but in different data set	No	No	Gene expression of candidate genes	Approximately 10% difference in methylation between airway epithelial cells and PBMCs, two atopic asthma candidate genes identified
Martino <i>et al.</i> [15*]	60	450K (Illumina chip)	CBMC and PBMCs	Yes	No	Birth vs. 12 months	No	Differences in methylation after T-cell stimulation change over time

CBMC, cord blood mononuclear cells; PBMCs, peripheral blood mononuclear cells; Teff, effector T cells; Treg, regulatory T cells.

become acetylated [28] and the chromatin structure of the locus opens [29]. CpG methylation in the *IL4* and *IFNG* promoters was reported to differ in patients with asthma and without asthma after stimulation with phytohaemagglutinin in a small first study [30] including three cases and three controls. When 21 monozygotic twin pairs discordant for asthma were assessed for the methylation of *FOXP3* (13 CpGs) and six promoter CpGs in *IFNG* in T cells, increased methylation of these loci in regulatory T cells (Treg) and effector T cells (Teff) were found to correspond to a decreased gene expression, respectively [13]. Assessing patients with established asthma, it was not possible to discriminate if methylation differences are a prerequisite or consequence of asthma. Reproducible differences in DNA methylation profiles at specific loci in B lymphocytes between participants with house dust mite allergy, aspirin-intolerant asthma and controls were also reported [31].

In a genome-wide analysis of methylation (GWAM) analysis linked to gene expression profiling in blood, Martino *et al.* [15[•]] were able to demonstrate that 1188 differentially methylated loci were associated with a change in expression in 599 genes in children. When T cells were stimulated, over 630 genes were induced but this was not associated with any significant change in DNA methylation. This suggests that DNA methylation has a role in the consolidation of T-cell-specific gene expression but is of limited importance in short-term T-cell responsiveness.

TISSUE-SPECIFIC METHYLATION IN ASTHMA AND ALLERGY

Methylation signatures can either be tissue-specific or globally affect all cells. For allergy, as a systemic condition, studying epigenetic and methylation patterns in blood immune cells may be feasible but for asthma, as a lung disease with a systemic component, the target of epigenetic mechanisms is less clear. Although disease-specific methylation signatures may be found in lung epithelial or immune cells only, these cells are difficult to collect, specially in children. A small but interesting study [14^{••}] has now compared tissue-specific DNA methylation of airway epithelial cells to peripheral blood mononuclear cells (PBMCs) in 25 children categorized as atopic ($n=9$), atopic asthmatic ($n=4$), nonatopic asthmatic ($n=5$) or healthy controls ($n=7$). Differences in methylation between airway epithelial cells and PBMCs were found for 80 (out of 1027) CpG sites across 67 out of 671 investigated genes. DNA methylation signatures of PBMCs from asthmatic, atopic and healthy individuals were compared, but no CpG sites were found to be differentially methylated between these groups.

Also in airway epithelial cells, DNA methylation profiles of patients with asthma compared with healthy controls and atopics compared with healthy controls did not differ. Only a comparison between methylation of airway epithelial cells from asthmatic and atopic children showed eight differentially methylated CpG sites from eight different genes. Overall, this study is explorative in nature with few individuals using a methylation panel that is not tailored for studying complex diseases. However, it shows that methylation differs between PBMCs and airway epithelial cells in a limited number of loci and genes (less than 10%) and one may conclude that PBMCs may have some, but not the final, value in studying methylation effects in asthma.

TOBACCO SMOKE EXPOSURE AND METHYLATION

Exposure to environmental tobacco smoke is the single most important risk factor for the development of asthma in childhood. Several studies associate in-utero tobacco smoke exposure, smoking of the mother previous to pregnancy and even smoking by the grandmother to occurrence of asthma in the child or the grandchild, respectively [32]. Parental smoking affects the methylation of CpGs in the vicinity of numerous genes investigated in DNA collected from buccal cells of children [33]. Methylation was increased for AluYb8 and LINE1 repetitive elements and decreased for the promoter regions of *AXL* and *PTPRO* genes. A genome-wide methylation analysis of approximately 27 000 CpGs in 14 000 gene promoter regions identified differences in DNA methylation related to tobacco smoking when smokers and nonsmokers were compared. The top association hit in this GWAM was located and replicated in the vicinity of *F2RL3* that codes for a protease-activated receptor-4. These findings were made in blood cells suggesting that epigenetic mechanisms of lung-related inflammation may not only be observed in pulmonary cells [34]. In a recent GWAM analysis of 473 844 CpG sites [7^{••}], in more than 1000 children, maternal plasma cotinine measured during pregnancy was associated with DNA methylation. Differential DNA methylation at epigenome-wide statistical significance was found for 26 CpGs in 10 genes. Of these, methylation differences in *AHRR*, *CYP1A1* and *GFI1* were replicated in a much smaller but independent sample of 36 individuals. Although *AHRR* and *CYP1A1* play a role in the aryl hydrocarbon receptor signaling pathway, which mediates the detoxification of the components of tobacco smoke, *GFI1* cannot easily be connected to smoke-related mechanisms.

FARM LIFE INFLUENCES METHYLATION

Pre and postnatal exposure to farm life reduces the risk of asthma and allergies during childhood in relationship to the amount of microbial diversity in these environments [35]. There is some evidence that gene by environment interactions occur in this setting and it has been reported that endotoxin levels affect the association between *CD14* promoter polymorphisms and IgE levels in a dose-dependent manner [36]. Methylation of the gene locus seems to influence *CD14* expression and levels of soluble *CD14* [37] and hypermethylation alters the effect of promoter polymorphisms in *CD14* on gene expression during childhood. In a recent pilot study [9^{***}] from our laboratory in the European Protection Against Allergy: Study in Rural Environments birth cohort we assessed in 67 children if epigenetic patterns in asthma candidate genes are influenced by farm exposure, and change over the first years of life and whether these changes may contribute to the development of asthma. DNA methylation status of 10 asthma and allergy candidate genes was analyzed in cord blood and DNA extracted from whole blood at the age of 4.5 years in children living on farms and a rural control group not living on farms as well as in patients with asthma and without asthma from the same population. Three groups of genes were analyzed: genes that were associated with asthma in the first genome-wide association studies on asthma and IgE, genes involved in Th2 development and genes involved in T regulatory effects. In cord blood, regions in *ORMDL1* and *STAT6* showed hypomethylation whereas regions in *RAD50* and *IL13* were hypermethylated when DNA from farmers' was compared to nonfarmers' children. Changes in methylation over time occurred in 14 gene regions. Interestingly, these differences clustered in the genes highly associated with asthma (*ORMDL* family) and IgE regulation (*RAD50*, *IL13* and *IL4*), but not in the T-regulatory genes (*FOXP3*, *RUNX3*). Although acknowledging the explorative nature of this small study, there is some evidence that DNA methylation patterns in early childhood change significantly in specific asthma and allergy-related genes in peripheral blood cells. Early exposure to farm environment seems to influence methylation patterns of distinct genes in cord blood.

INTERPLAY BETWEEN GENETIC VARIATION AND METHYLATION

It seems possible that epigenetic patterns are inherited, challenging the dogma that DNA alone is the inherited blueprint of life. Recent studies in rats suggest that effects of nicotine exposure during pregnancy in F0 animals reduce lung function not

only in the F1 but also the F2 generation of offspring [38^{***}]. At the same time, overall histone acetylation in the lungs and DNA methylation in the testis and ovaries of these animals are altered. Almost all these effects were prevented when F0 rats received the 'nicotine neutralizer' rosiglitazone. These data may shed new light on epidemiological observations that maternal smoking in grandmothers increases the asthma risk in grandchildren irrespective of the smoking status of the child's mother [32]. However, these results cannot finally discriminate between epigenetic and genetic effects. Recent studies in plants indicate that 30% of all differentially methylated regions in the (plant) genome are influenced by genetic variants in the locus [39]. As suggested by Eichten and Borevitz [40] '*... there are two extreme possibilities as to the potential relevance of heritable epigenetic information. The first is that epigenetic information is fully controlled by the genetic content of an individual. In this case, epigenetic profiling would be largely redundant because any epigenetic status is correlated with the individual's genotype. The second case is that epigenetic variation forms within a uniform genetic background, allowing an additional level of heritable information to exist in an individual. These new 'epialleles' may be akin to single nucleotide polymorphisms in the genome that are rare in a population, but may be common in a family.*' At this point, both hypotheses seem possible as well as their parallel existence.

When methylation patterns of approximately 1500 CpG sites were studied in the blood of children with wheeze before the age of 6, methylation signatures differed compared with healthy individuals [11^{***}]. Specifically, CpG methylation in the *ALOX12* gene was associated with wheezing in the discovery and replication cohort. In addition, genetic variation in the locus influenced *ALOX12* methylation [11^{***}]. The interplay between genetic variants and methylation was also assessed for the major asthma susceptibility locus in 17q21, where polymorphism and methylation seem to interact in determining expression of multiple genes in the locus [41]. In a further small study [8] it was suggested that methylation in the 17q21 locus is age and sex-specific, correlation with sex and age specific effects of genetic associations of the locus. In the *ADRB2* gene, coding for the major receptor for bronchodilator therapy in asthma, methylation of a CpG site in the 5'untranslated region was associated with asthma severity in 182 children [12^{***}]. However, as in many of the current explorative studies, neither gene expression analysis nor replication was performed.

At this point, research groups and consortia in the United States, the United Kingdom and Europe, including ours, have GWAM data available from

large cohorts of patients with allergy and asthma, which are not yet published. However, also these studies will leave open questions such as how methylation patterns affect global gene expression or how genes, environmental stimuli and methylation interact. It will be crucial to answer the question of which tissue to analyze at which time point for a disease like asthma. Most likely, there will be small steps forward but it can be expected that completely new disease mechanisms may be discovered in the process. It will be the task of next generation system biology analyses to put all these data into perspective.

THERAPEUTIC IMPLICATIONS IN ALLERGY AND ASTHMA

As epigenetic mechanisms are inducible and reversible, it would be highly appreciated if drugs were available to explicitly reprogram disease-specific epigenetic changes. Controlling epigenetic programming even before disease occurs may be achievable. Studies in a mouse model indicate that prenatal epigenetic modulation could prevent the development of asthma later in life. Prenatal administration of the Gram-negative, farm-derived *Acinetobacter lwoffii* isolate F78 prevented the development of airway inflammation in an Ovalbumin (OVA) mouse model [42]. F78 administration also affected H4 acetylation at *INFG* and *IL4* loci and pharmacological inhibition of histone acetylation abolished protective effects. Interestingly, the *INFG* promoter region also showed increased methylation after OVA challenge in CD4⁺ T cells and inhibition of DNA methyl transferases prevented the methylation of the *IFNG* locus, decreased Th2-like cytokine production and abrogated the development of an asthma like phenotype in the OVA model [43[■]].

Pharmaceutical industry massively funds the development of novel epigenetic targets [44[■]], not only for cancer but but also for complex diseases. Together, more than 30 epigenetic drugs are in phase I and phase II studies, whereas only one has reached phase III so far. Of these, only one drug is aimed at DNA methylation whereas all others target histone-related mechanisms [44[■]]. Because modulation of the epigenome has the potential to reprogram all cells, adverse effects may only become apparent over longer periods of time or even over generations. Strategies to investigate and avoid such effects will be needed.

CONCLUSION

This review highlights the potential involvement of epigenetics in allergy and asthma. Recent studies

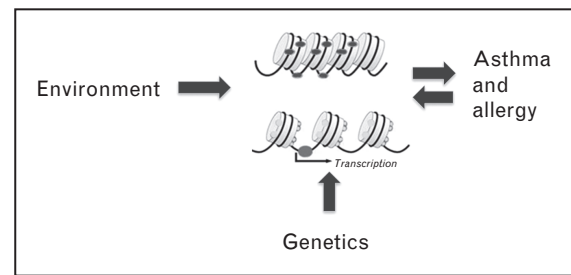


FIGURE 1. Relationship of environment, genetics and disease and epigenetics. Note that methylation may influence disease development but that disease status may also impact on epigenetic mechanisms and change methylation.

indicate that environmental influences leave marks on methylation signatures that may even be inherited over generations. How the genome and the epigenome interact is yet unclear. First studies in humans indicate that changes in methylation patterns over time are associated with disease development but that disease itself can also impact on methylation status and other epigenetic mechanisms (Fig. 1). There is a potential for epigenetics to discover completely novel disease mechanisms in allergy and to understand those mechanisms better that we have already identified such as the development of allergen tolerance.

Acknowledgements

The author has expressed the gratitude to Sven Michel and Jörg Tost for their continuous epigenetic input and Gerard Koppelman for fruitful discussions on the topic.

Conflicts of interest

The Kabesch lab is supported by grants from the EU project HEALS and the German Lung Research Center (DZL). The author has received lecture fees from medical and scientific societies (ERS, ATS, EAACI, GPP, and DGI) and pharmaceutical industry (Glaxo, Novartis). The authors have no conflicts of interest to declare.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. *Genes Dev* 2009; 23:781–783.
2. Koppelman GH, Nawijn MC. Recent advances in the epigenetics and genomics of asthma. *Curr Opin Allergy Clin Immunol* 2011; 11:414–419.
3. Lovinsky-Desir S, Miller RL. Epigenetics, asthma, and allergic diseases: a review of the latest advancements. *Curr Allergy Asthma Rep* 2012; 12:211–220.
4. Kabesch M, Michel S, Tost J. Epigenetic mechanisms and the relationship to childhood asthma. *Eur Respir J* 2010; 36:950–961.
5. Kabesch M, Adcock IM. Epigenetics in asthma and COPD. *Biochimie* 2012; 94:2231–2241.
6. Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. *Am J Respir Crit Care Med* 2011; 183:1295–1301.

7. Joubert BR, Haberg SE, Nilsen RM, *et al*. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 2012; 120:1425–1431.
- Large-scale methylation analysis of prenatal nicotine exposure with more than 1000 individuals and 450k coverage of the genome.
8. Naumova AK, Al Tuwaijri A, Morin A, *et al*. Sex- and age-dependent DNA methylation at the 17q12-q21 locus associated with childhood asthma. *Hum Genet* 2013; 132:811–822.
9. Michel S, Busato F, Genuneit J, *et al*. Farm exposure and time trends in early childhood may influence DNA methylation in genes related to asthma and allergy. *Allergy* 2013; 68:355–364.
- Pilot study investigating methylation from different perspectives addressing time trends, disease development and environmental influences.
10. Kim YJ, Park SW, Kim TH. Genome-wide methylation profiling of the bronchial mucosa of asthmatics: relationship to atopy. *BMC Med Genet* 2013; 14:39.
11. Morales E, Bustamante M, Vilahur N, *et al*. DNA hypomethylation at ALOX12 is associated with persistent wheezing in childhood. *Am J Respir Crit Care Med* 2012; 185:937–943.
- Study investigates genetic variation and methylation at the ALOX12 locus and their interaction.
12. Fu A, Leaderer BP, Gent JF, *et al*. An environmental epigenetic study of ADRB2 5'-UTR methylation and childhood asthma severity. *Clin Exp Allergy* 2012; 42:1575–1581.
- Interesting findings from a major therapeutic target gene.
13. Runyon RS, Cachola LM, Rajeshuni N, *et al*. Asthma discordance in twins is linked to epigenetic modifications of T cells. *PLoS One* 2012; 7:e48796.
14. Stefanowicz D, Hackett TL, Garmaroudi FS, *et al*. DNA methylation profiles of airway epithelial cells and PBMCs from healthy, atopic and asthmatic children. *PLoS One* 2012; 7:e44213.
- Small but important article comparing methylation profiles in airway epithelial cells and peripheral blood. First insight into tissue-specific methylation in asthma.
15. Martino D, Maksimovic J, Joo JH, *et al*. Genome-scale profiling reveals a subset of genes regulated by DNA methylation that program somatic T-cell phenotypes in humans. *Genes Immun* 2012; 13:388–398.
- State-of-the-art GWAM related to gene expression in an experimental setting.
16. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002; 16:6–21.
17. Antequera F. Structure, function and evolution of CpG island promoters. *Cell Mol Life Sci* 2003; 60:1647–1658.
18. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 2006; 31:89–97.
19. Cheng X, Blumenthal RM. Mammalian DNA methyltransferases: a structural perspective. *Structure* 2008; 16:341–350.
20. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev* 2011; 242:10–30.
21. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008; 118:3546–3556.
22. Schwartz DA. Epigenetics and environmental lung disease. *Proc Am Thorac Soc* 2010; 7:123–125.
23. Larche M. Regulatory T cells in allergy and asthma. *Chest* 2007; 132:1007–1014.
24. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol* 2009; 9:91–105.
25. Lee DU, Agarwal S, Rao A. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. *Immunity* 2002; 16:649–660.
26. Jones B, Chen J. Inhibition of IFN-gamma transcription by site-specific methylation during T helper cell development. *EMBO J* 2006; 25:2443–2452.
27. Ho SM. Environmental epigenetics of asthma: an update. *J Allergy Clin Immunol* 2010; 126:453–465.
28. Avni O, Lee D, Macian F, *et al*. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 2002; 3:643–651.
29. Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and IL4 locus accessibility. *Annu Rev Immunol* 2006; 24:607–656.
30. Kwon NH, Kim JS, Lee JY, *et al*. DNA methylation and the expression of IL-4 and IFN-gamma promoter genes in patients with bronchial asthma. *J Clin Immunol* 2008; 28:139–146.
31. Pascual M, Suzuki M, Isidoro-Garcia M, *et al*. Epigenetic changes in B lymphocytes associated with house dust mite allergic asthma. *Epigenetics* 2011; 6:1131–1137.
32. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. *Chest* 2005; 127:1232–1241.
33. Breton CV, Byun HM, Wenten M, *et al*. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *Am J Respir Crit Care Med* 2009; 180:462–467.
34. Breitling LP, Yang R, Korn B, *et al*. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *Am J Hum Genet* 2013; 88:450–457.
35. Ege MJ, Mayer M, Normand AC, *et al*. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011; 364:701–709.
36. Simpson A, John SL, Jury F, *et al*. Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. *Am J Respir Crit Care Med* 2006; 174:386–392.
37. Munthe-Kaas MC, Torjussen TM, Gervin K, *et al*. CD14 polymorphisms and serum CD14 levels through childhood: a role for gene methylation? *J Allergy Clin Immunol* 2010; 125:1361–1368.
38. Rehan VK, Liu J, Naeem E, *et al*. Perinatal nicotine exposure induces asthma in second generation offspring. *BMC Med* 2013; 10:129.
- Experimental study investigating heritability of epigenetic mechanisms and epigenetic effects on lung function after prenatal nicotine exposure.
39. Schmitz RJ, Schultz MD, Ulrich MA, *et al*. Patterns of population epigenomic diversity. *Nature* 2013; 495:193–198.
40. Eichten S, Borevitz J. Epigenomics: methylation's mark on inheritance. *Nature* 2013; 495:181–182.
41. Berlivet S, Moussette S, Ouimet M, *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; 131:1161–1171.
42. Brand S, Teich R, Dicke T, *et al*. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. *J Allergy Clin Immunol* 2011; 128:618–625.
43. Brand S, Kesper DA, Teich R, *et al*. DNA methylation of TH1/TH2 cytokine genes affects sensitization and progress of experimental asthma. *J Allergy Clin Immunol* 2012; 129:1602–1610.
- This proof-of-concept study investigates methylation and the effect of its reversibility in a mouse model.
44. Dewoskin VA, Million RP. The epigenetics pipeline. *Nat Rev Drug Discov* 2013; 12:661–662.
- Up-to-date information on epigenetic drug discovery.