Epigenetics of human asthma and allergy: promises to keep

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Summary

Objective: **The interest in asthma epigenetics is high because epigenetic mechanisms likely contribute to the environmental origins of the disease and its phenotypic variability. This review presents the main findings of asthma epigenetics and the challenges that still delay progress.**

Data Sources: **We examined the current literature on asthma epigenetics (31 reviews and 25 original data publications).**

Study Selections: **We focused on DNA methylation studies in populations.**

Results: **Both genome-wide and candidate gene studies have explored DNA methylation in allergic disease. Genome-wide studies ask whether and which regions of the genome are differentially methylated in relation to the phenotype of interest. Identification of such regions provides clues about the identity of the genes, pathways and networks underpinning a phenotype and connects these networks to the phenotype through epigenetic mechanisms. Candidate gene studies examine DNA methylation in genes chosen because of their known or hypothesized role in immunity, responses to environmental stimuli or disease pathogenesis. Most existing studies in asthma and allergy focused on candidate genes involved in the response to environmental pollutants.**

Conclusion: **Asthma epigenetics is still in its infancy. The paucity of primary literature originates from methodological and analytical challenges of genome-wide studies, the difficulties in interpreting small differences in DNA methylation, and the need to develop robust bioinformatic tools for pathway, network and system analyses of epigenetic data. Once these challenges have been overcome, epigenetic studies will likely provide important insights about the inception and pathogenesis of allergic disease and will help define disease endotypes.** ***(Asian Pac J Allergy Immunol 2013;31:183-9)***

Key words: *epigenetics, DNA methylation, asthma, allergy*

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Introduction

The epigenetics of human asthma and allergy is currently entangled in an intriguing paradox. As we are writing (early Summer 2013), a literature search lists 31 reviews but only 25 original data publications, a disproportion which reflects great demand but also great challenges. The reasons why the demand for asthma epigenetics is high and keeps rising are rather transparent. Complex diseases in general and asthma in particular are widely recognized as conditions in which both genes and the environment play critical roles. The environmental component of asthma is highlighted by the steep increase in its prevalence over just a few decades[1] – a trend incompatible with purely genetic mechanisms. To add to the complexity, asthma is also strongly influenced by developmental factors, well underscored by the detection of subtle but eloquent harbingers of disease in early life, regardless of the age at which an asthma diagnosis is actually made.[2] In such a context, epigenetics, which studies heritable changes in gene expression or cellular phenotypes that do not involve changes in the underlying DNA sequence,[3-5] has emerged as a promising field of investigation. Indeed, epigenetic mechanisms are essential for the plastic response elicited by environmental exposures and the timely unfolding of developmental processes. To the extent

that environmental and developmental factors are essential for asthma pathogenesis, epigenetics, which sits squarely at the mechanistic intersection between these factors, is a likely contributor to the origins of the disease and a plausible source of phenotypic variability.

The current interest in asthma epigenetics also stems from the mixed results generated by asthma genetics. Both classical single gene association studies[6] and the more recent genome-wide association studies have succeeded in identifying a number of candidate genes of suggestive biological significance,[7-10] but have failed to account for more than a modest proportion of phenotypic variance – a situation that has come to be known as the problem of the missing or hidden heritability.[11,12] This realization motivates the search for additional sources of phenotypic variance that are DNA-based but not purely genetic.

The emphasis on epigenetics has been made possible by major technological advances. Large, population-scale epigenetic studies are becoming more and more feasible thanks to the coming of age of genome-wide methods for the analysis of the main classes of epigenetic marks: DNA methylation and post-translational histone modifications.[13,14] As in the realm of genetics, these high-throughput approaches provide hypothesis-generating data and go beyond the slow candidate gene studies that were the only choice available just a few years ago. DNA methylation has emerged as a particularly tractable epigenetic readout in populations because (unlike histone modifications, the study of which requires large numbers of cells and cumbersome procedures for chromatin isolation), it can be reliably assessed using low amounts of DNA, even if stored for long periods of time. Moreover, high resolution methods such as bisulfite sequencing provide a golden standard for the validation of array-based genome-wide results, strengthening the robustness to DNA methylation analyses.[15,16]

Thus, the asthma community is actively pursuing epigenetics because these studies must and can be done. Here we will discuss the main findings of asthma epigenetics, but also the challenges that still delay progress in this field. We will focus exclusively on DNA methylation studies in populations, because other aspects of epigenetic regulation (particularly those related to histone modifications) are not yet readily amenable to large scale analyses.

DNA methylation

DNA methylation is an ancient adaptation used to distinguish an organism's own DNA from that of invaders, such as viruses.[17] In eukaryotes, DNA methylation has further evolved into an important mechanism for controlling endogenous gene activity.[18] Methylation targets the C5 position of cytosine in CpGd inucleotides and can increase the repressive nature of chromatin by providing docking sites for methyl binding proteins (e.g., MeCP2 and MBD2) that in turn recruit complexes with histone deacetylase and histone methyltransferase activity.[18] Interactions with histone-modifying enzymes underpin the functional cross-talk between DNA methylation-based and histone-based regulation of chromatin architecture. DNA methylation can also repress gene expression by directly interfering with transcription factor binding. These functions are common in parent-of-origin-specific expression of imprinted genes and X chromosome inactivation.[19] However, the regulatory role of DNA methylation is made more complex by the fact that position of the methylation site relative to the transcription unit influences its effect on gene expression. Methylation in the immediate vicinity of the transcription start site blocks initiation, but methylation in gene bodies does not, and might even stimulate transcriptional elongation and splicing.[20]

Upon DNA replication, the DNA methyltransferase Dnmt1 copies each methylation mark of the parental DNA strand to the newly synthesized DNA, ensuring faithful transmission of the genomic patterns of cytosine methylation to both daughter cells. Thus, cytosine methylation has an important role in mitotically heritable gene silencing.[21] If 5-methylcytosine (5mc) is the fifth base of DNA, 5-hydroxymethylcytosine (5hmC) is the sixth. This recently discovered type of methylation is most abundant in the brain and in embryonic stem cells. Ten-Eleven-Translocation (TET) proteins oxidize 5mC converting it to 5hmC.In murine embryonic stem cells, 5hmC is enriched at transcription start sites and 5' untranslated regions of genes. Enhancers are relatively more enriched in 5hmC than 5mC.[22] Important insights about the role of 5hmC were recently provided by studies in mouse primordial germ cells, which undergo sequential epigenetic changes and genome-wide DNA demethylation to reset the epigenome for totipotency. Erasure of CpG methylation in 5mC in these cells occurs via conversion to 5hmC, driven by high levels of TET1 and TET2. Global conversion to 5hmC initiates

asynchronously at embryonic day 9.5 to 10.5 and accounts for the unique process of imprint erasure. Nonetheless, rare regulatory elements escape systematic DNA demethylation. This process may provide a long-sought mechanism for trans-generational epigenetic inheritance.[23] It is noteworthy that while all these results imply that 5hmC can regulate gene transcription differently than 5mC, conventional techniques do not distinguish between 5mC and 5hmC.[24] As a consequence, the results of most genome-wide analyses of DNA methylation should be interpreted with caution.

The complex relationship between the DNA methylation landscape and genetic variation is also attracting more and more attention, because the discovery of pervasive allele-specific methylation modifies our understanding of the nexus between genetic variants and phenotypes. CpG-SNPs (single nucleotide polymorphisms that abolish or create a CpG site) often modify DNA methylation not only at the site itself but also throughout the neighboring region.[25] Allele-specific methylation could disturb the cooperative interactions that underpin the binding of CpGs to the methylation machinery.[26] CpG SNPs could also influence the binding of specific transcription factors, either positively or negatively. As discussed above, the functional implications of altered methylation extend not only to promoters but also to gene bodies and exons. Recent genome-wide surveys have demonstrated that allele-specific methylation, far from being restricted to imprinted genes as originally thought, can be detected at ≈40% of heterozygous SNPs in any given cell line.[25,27] Notably, in 40-90% of the relevant regions allele-specific methylation depends on the presence of CpG-SNPs.[25] According to the snp129 database, 225,659 SNPs locate to CpG sites.[25]

Analyses of DNA methylation in human asthma and allergy

Both genome-wide and candidate gene studies have been performed to explore patterns of DNA methylation in asthma and allergy. These studies answer distinct biological questions and produce results of different biological significance. Unbiased, genome-wide studies are hypothesis-generating and ask whether and which regions of the genome are differentially methylated in relation to the phenotype of interest. Identification of such regions achieves two important goals: it provides clues about the identity of the genes, pathways and networks underpinning a phenotype, and it connects these networks to the phenotype through epigenetic mechanisms. Studies of this kind are likely to provide novel insights into disease pathogenesis, and thus justify the current interest in asthma epigenetics. The results of genome-wide analyses of DNA methylation in asthma and allergy[28-34] are presented in Table 1.

In contrast to genome-wide studies, candidate gene studies are more limited in their scope and goals, and examine DNA methylation in genes chosen because of their known or hypothesized role in immunity, responses to environmental stimuli or disease pathogenesis. Most existing studies in asthma and allergy focused on candidate genes.[35-48] Their results are presented in Table 2. It is important to reflect on the biological significance of candidate gene epigenetic studies. Because of the known strong functional links between DNA methylation and regulation of gene expression, it is an educated guess that differential methylation will be found in a gene already known to be differentially expressed in asthmatic and non-asthmatics. Thus such a finding will simply confirm the expectation that differences in gene expression are mediated by differential epigenetic remodeling of the relevant locus. The significance and novelty of such studies lies elsewhere, in identifying specific regions and specific regulatory sites in the locus that harbor differential DNA methylation and thus may be responsible for the differential regulation of transcriptional activity at the molecular level.

Challenges

The remarkable paucity of primary literature on asthma epigenetics likely originates from challenges related to the methodological and analytical complexity of genome-wide DNA methylation studies and the lack of effective methods to characterize other epigenetic signatures. Some of these challenges are:

- DNA methylation throughout the genome can be studied using a variety of approaches: microarrays, methylated DNA immunoprecipitation/ capture or next generation sequencing. Each of these techniques has advantages and disadvantages in terms of coverage, specificity, biases, statistical power, analytical requirements and cost.[13] Because these methods are distinct in their properties and biological targets, they are likely to provide results that are not readily comparable and may in fact be

Table 1. DNA methylation in human asthma and allergy: genome-wide studies

Method/Platform	Phenotype	Exposures/Variables	Outcome/Results	Ref.
Illumina Infinium 27K	Atopic asthma	Sensitization to Dermatophagoides	One CpG site in the LCN6 promoter was differentially methylated in asthmatics and controls. In the bronchial mucosa of atopic asthmatics, hypermethylation was detected at 6 loci in 6 genes, while hypomethylation was detected at 49 loci in 48 genes.	28
Illumina Infinium 27K	Asthma	Pollution levels	Of 9916 CpG sites differentially methylated in children from Ostrava (high pollution area) and Prachatice (low pollution area), 58 had a difference >10% and were consistently hypomethylated in Ostrava.	29
Illumina Infinium 450K	Childhood and adult illness	Maternal smoking during pregnancy	26 CpG sites in 10 genes were differentially methylated. Findings were replicated for AHRR, CYP1A1 and GFI1.Cotinine levels were inversely associated with AHRR and GFI1 methylation and positively associated with CYP1A1 methylation.	30
Illumina Golden Gate array, Pyrosequencing	Asthma-related persistent wheeze	Prenatal di-chlorodiphenyl-dichloroethylene (DDE)	ALOX12 hypomethylation was associated with risk of persistent wheezing in the Menorca and Sabadell studies. High prenatal DEE levels were associated with ALOX12 hypomethylation in the Menorca study.	31
HELP assay, NimbleGen 2.1M array, MassArray	Allergic asthma, aspirin-exacerbated respiratory disease (AERD)	Dermatophagoides pteronyssinus allergy	CYP26A1 promoter was hypermethylated in allergic asthmatics. The allergic group showed a tendency towards global hypomethylation relative to the control and AERD groups.	32
Bisulfite-PCR pyrosequencing, Illumina Golden Gate array	---	Prenatal tobacco smoke exposure	Exposed children exhibited hypomethylation of AluYb8 repeats and hypermethylation of AXL and PTPRO. LINE1 methylation differed in children with common GSTM1 null genotypes, and CpG-specific methylation differed in children with the common GSTP1 haplotype.	33
Methylation-sensitive Restriction Fingerprinting	Asthma symptoms before age 5	Maternal polycyclic aromatic hydrocarbon (PAH) exposure	Methylation of the ACSL3 5' CpG island was positively associated with asthma. Hypermethylation of the CpG island was associated with maternal PAH exposure.	34

Keywords used for literature search: Asthma, Allergy, DNA methylation, Epigenetics.

complementary. Therefore, the choice of a method and platform is a defining moment in DNA methylation studies.

- Many studies use unfractionated blood cells, rather than isolated and homogeneous cell populations, as their source of DNA. The use of unfractionated cells is a limitation often imposed by the population study design and the logistical difficulties inherent to isolating individual cell subsets, especially when samples derive from small children and contain low numbers of cells. Because cell type heterogeneity may affect results, especially for tissue-specific DNA methylation differences, these results need to be interpreted with caution.[49] On the other hand, epigenetic modifications signal a permissive as well as an active chromatin architecture, and thus are not necessarily tissue-specific. Moreover, tissue specificity (of epigenetic modifications or even gene expression) is a relative concept, a continuum more than an all-or-none event. For instance, most genes associated with lung function in a recent genome-wide association study were found to be expressed in peripheral blood, albeit at moderate levels.[50]
- It is also important to recognize that despite their limitations, DNA methylation studies in unfractionated cell populations (typically from blood) provide a unique tool to explore gene regulatory events at the genome level in population studies in which samples were not adequately collected and/or preserved for RNA expression analyses.
- Beyond technical considerations, perhaps the most demanding challenge in the field is how to interpret DNA methylation differences that, albeit statistically significant, are extremely small (e.g., 1-5%). These are the exception in diseases such as cancer, in which in most cases hypo- or hyper-

Table 2. DNA methylation in human asthma and allergy: candidate gene studies

Candidate Genes	Phenotype	Exposures/Variables	Outcome/Results	Ref.
ORMDL1-3, CHI3L1, RAD50, IL13, IL4, STAT6, FOXP3, RUNX3	Childhood asthma	Farming, age	ORMDL1 and STAT6 were hypomethylated, and RAD50 and IL13 were hypermethylated, in cord blood from farmers. One region in ORMDL3 was hypermethylated in asthmatics. DNA methylation changes between birth and age 4.5 years occurred in genes associated with asthma (ORMDL family) and IgE regulation (RAD50, IL13, and IL4), but not T-regulatory cell activity (FOXP3, RUNX3).	35
NPSR1	Allergic asthma	Parental and current smoking, sampling season	NPSR1 promoter methylation was lower in peripheral blood from allergic asthmatic children. Significant but small decreases in DNA methylation were associated with adult severe asthma and childhood allergic asthma. DNA methylation was significantly associated with parental smoking and sampling season in children, and with current and former smoking in adults.	36
IL4R	Asthma at age 18	IL4R SNPs	Risk of asthma from IL4R rs3024685 was positively associated with methylation at cg09791102.	37
IL6, iNOS, Alu and LINE-1 repetitive elements	Childhood asthma	Fractional exhaled nitric oxide (FeNO), forced expiratory volume in 1 s (FEV_1), wheezing	Hypomethylation of the IL6 and the iNOS promoters was associated with increased FeNO.	38
NOS1, NOS2A, NOS3	Childhood respiratory disease	Particulate matter ≤2.5 μ and ≤10 μ aerodynamic diameter ($PM_{2.5}$ and PM_{10})	An increase in $PM_{2.5}$ was associated with NOS2A hypomethylation depending on the length of exposure and CpG locus. One-year $PM_{2.5}$ exposure was associated with hypermethylation of 4 loci in the NOS2A CpG island. An increase in 7-day and 1-year $PM_{2.5}$ was associated with higher NOS3 methylation. PM_{10} showed similar but weaker associations.	39
ADRB2	Childhood asthma	Nitrogen dioxide (NO_2)	ADRB2 methylation was positively associated with asthma severity. Indoor exposure to NO_2 and severe asthma were selectively associated among children with ADRB2 hypermethylation.	40
CD14	---	Farming	The CD14 promoter was significantly hypomethylated in placentas of mothers living on a farm.	41
IL4, IFNG	---	Maternal PAH exposure	Maternal PAH exposure was associated with IFNG hypermethylation in cord blood.	42
IFNG, iNOS	---	Reproducibility of DNA methylation over 4-7 days	Replicate and field duplicate samples were correlated strongly, while repeat samples demonstrated low within-subject correlations over a 4-7 day period.	43
ARG1, ARG2, NOS	Childhood asthma	FeNO	ARG2 hypermethylation was inversely associated with FeNO, particularly in asthmatic children. Differences in FeNO by asthma status were also observed for ARG1.	44
PTGDR	Allergic asthma	House dust mite allergy	PTGDR was hypomethylated in allergics. -613CC individuals exhibited higher DNA methylation than -613CT subjects.	45
MS4A2	Atopic asthma	Atopy	An AluSp repetitive element was highly methylated across all individuals regardless of atopic status.	46
CD14	Childhood asthma	Allergic sensitization	Decreasing effects of CD14 polymorphisms on sCD14 levels were paralleled by small but significant increases in CD14 methylation from 2 to 10 years of age.	47
FOXP3	Asthma severity	Ambient air pollution in Fresno, CA (high) and Stanford, CA (low); T regulatory cell function	Children exposed to high ambient air pollution exhibited FOXP3 hypermethylation, impaired T regulatory cell function and higher asthma severity scores.	48

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methylation in the affected tissue is readily and unambiguously detectable, but appear to be the rule in other complex diseases such as asthma. While it is not inconceivable that the regulatory properties of selected CpG sites may be modulated by threshold effects, such that small quantitative differences in DNA methylation might translate into larger qualitative differences in downstream events, biological validation by independent functional data (for instance, RNA and/or protein expression)

remains essential to reinforce confidence in the biological significance of modest differences in DNA methylation patterns.

- Finally, a common problem is how to make biological sense of a list of differentially methylated regions or CpG sites. Some useful bioinformatics tools allow annotation of these regions/sites, but the ultimate goal of bioinformatic efforts in this area should be the development of robust methods for pathway, network and system analyses of epigenetic data.

Conclusions

Asthma epigenetics is still in its infancy. However, once the existing challenges have been overcome, genome-wide (and then whole genome) epigenetic studies will likely keep their promises, bloom and provide critical insights about the inception and pathogenesis of asthma and allergy. Even at this early stage, despite the existing challenges, epigenetic analyses offer a powerful tool to explore disease mechanisms and better define disease endotypes.

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