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Epigenetics in allergic diseases

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Abstract

Purpose of review—Allergic diseases are among the most prevalent chronic diseases of childhood, affecting more than 7 million children in the United States. Epidemiological evidence supports the idea that the inception of allergic diseases is typically before the pre-school years, even when chronic symptoms do not emerge until adulthood. The role of epigenetic mechanisms (particularly DNA methylation) in allergic disease is under active investigation because these mechanisms are known to be at the interface among gene regulation, environmental stimuli and developmental processes, all of which are essential for the pathogenesis for asthma and allergy. This article specifically reviews genome-wide DNA methylation studies in allergic disease.

Recent findings—Differential DNA methylation at specific regions appears to be associated with concurrent allergic disease. A few studies have identified methylation signatures predictive of disease.

Summary—DNA methylation signatures have been shown be associated with several allergic disease phenotypes, typically concurrently with disease. The few that have been found to precede diagnosis are especially interesting because they highlight an early trajectory to disease.

Keywords

Epigenetics; Allergic disease; DNA methylation

Introduction

Asthma and other allergic diseases are among the most prevalent chronic non-communicable diseases of childhood [1, 2]. For instance, according to the World Health Organization,

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Conflicts of interest

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asthma affects > 7.0 million children under 18 in the United States, with an economic burden that is estimated to exceed that of tuberculosis and HIV/AIDS combined [3].

Despite much research, asthma is still in many ways elusive. While epidemiologic studies indicate that the disease begins in the pre-school years even when chronic symptoms appear in early adulthood [4, 5], firm diagnostic criteria to distinguish children who will wheeze transiently during early life lower respiratory illnesses from children who will wheeze persistently, and then develop asthma, are still lacking. Yet, such criteria are urgently needed, because at least at present asthma can be treated but not cured, and therefore the focus must be on prevention. A number of asthma predictive algorithms have been proposed and refined over time [6–8], but their performance in terms of sensitivity, specificity and predictive value remains suboptimal. Interestingly, these tools rely on family history and the child's clinical characteristics in early life but do not incorporate variables that can be measured already at birth – a significant drawback, considering that the trajectory to asthma may well begin at birth if not earlier.

It is in this context that the role of epigenetics in regulating the susceptibility to and the severity of asthma and allergic disease is drawing more and more attention, as shown by a continuous and steep rise in the number of publications [9]. Remarkably, certain years saw reviews actually outnumber primary research papers - a pattern that points to an interest of unusual urgency. Such interest conceivably stems from multiple considerations. An important one is the strong functional link between epigenetic processes, environmental stimuli and developmental programs. That asthma has a strong environmental component was clearly illustrated by the seminal epidemiologic studies that revealed major differences in asthma prevalence among countries with more or less Westernized life styles [2, 10], while the critical role of early life exposures as determinants of asthma during adulthood has been repeatedly emphasized [2]. To the extent that epigenetic mechanisms faithfully and sensitively transduce environmental signals and preside over the time-dependent unfolding of developmental differentiation programs, their involvement in asthma and allergy is both possible and probable. Genetic factors are also important, but despite high expectations and high costs, genome-wide association studies have been unable to explain more than a limited proportion of the total phenotypic variability in allergy and asthma [11]. On the other hand, interrogating the epigenome, particularly the methylome, in order to explore its ability to influence complex disease phenotypes has recently become more feasible and is increasingly pursued as a tool to assess the contribution of epigenetic mechanisms to allergic disease pathogenesis.

Studying epigenetics

Epigenetics studies heritable changes in gene activity that are independent of alterations in the underlying DNA sequence [12–14]. Among epigenetic modifications, studies in human asthma and allergic diseases have primarily focused on DNA methylation, a process with intimate albeit complex connections with the regulation of gene expression. DNA methylation is a robust epigenetic mark, and user-friendly, quantitative methods to extensively survey the methylome are now widely available and are replacing the candidate gene studies that were initially the approach of choice. These genome-wide methods rely on

straightforward assays and streamlined analytical pipelines, and require DNA rather than chromatin isolation procedures [15]. Therefore DNA methylation studies, unlike the more challenging analyses of post-translational histone modifications, are flourishing and currently represent the totality of the epigenetic studies performed in human populations with asthma and allergy.

DNA methylation typically occurs at cytosines within CpG dinucleotides, the methylation status of which is assessed by techniques that rely on bisulfite conversion. In this reaction, unmethylated cytosines are converted to thymines and only methylated cytosines are preserved as such. The most widely used platform for genome-wide DNA methylation profiling, the 450K Illumina Human **Methylation** BeadChip, interrogates approximately 450,000 CpG sites throughout the genome at single CpG resolution and provides an output that can be readily understood as the percentage of DNA methylation at each site. The array covers 99% of RefSeq genes with an average of 17 probes per gene region [16] but cannot discriminate between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). Genome-wide DNA methylation surveys are the focus of this review.

Most genome-wide studies performed in asthma and allergy have targeted DNA methylation in peripheral blood cells, and only a few have surveyed airway tissues. Even though the lung is a major target organ in asthma and allergy, routinely obtaining lung tissue is often problematic in adults and virtually impossible in children. On the other hand, immune alterations accompany and often precede a clinical diagnosis of allergic diseases [2, 17–20]. Therefore, surveying peripheral blood immune cells provides information potentially relevant to disease pathogenesis.

Epigenetic alterations in concurrent allergic disease

So far, most DNA methylation studies in allergy and asthma have focused on subjects with concurrent disease and/or positive for the phenotype of interest. Among the most recent ones, a study surveyed associations between serum IgE concentrations and DNA methylation in 95 nuclear pedigrees. Positive results were validated in additional families and in subjects from the general population. Replicated associations between IgE and low methylation were found at 36 loci. Genes annotated to these loci encode known eosinophil products, and also implicate phospholipid inflammatory mediators, specific transcription factors and mitochondrial proteins [21].

Another recent study compared DNA methylation patterns and gene expression in 6–12 year old inner-city children with persistent atopic asthma versus healthy control subjects. Results were validated in an independent population of asthmatic patients. Eighty-one differentially methylated regions were identified. Several immune genes, including IL13, RUNX3, and TIGIT, were hypomethylated in asthma. Among asthmatic patients, 11 differentially methylated regions were associated with higher serum IgE concentrations, and 16 were associated with percent predicted FEV₁. Methylation marks involved in T-cell maturation (RUNX3), Th2 immunity (IL4), and oxidative stress (catalase) were validated in an independent asthmatic cohort of children living in the inner city [22].

Food allergy was the theme of an analysis that was performed in 11–15 month old children and relied on a supervised learning approach to discover a 96-CpG signature that best distinguished food allergic and food sensitized individuals [23]. Using a composite score derived from these 96 methylation site, this study was able to distinguish between food-allergic and food-sensitized infants, and between food-allergic and non-allergic infants. The authors also showed that their methylation signature outperformed both egg- and peanut-specific serum IgE levels as a predictor of clinical allergy. Of note, food allergy status was correctly predicted in a replication cohort of 48 individuals with an accuracy of 79.2%.

The most recent genome-wide study in allergy examined the association of DNA methylation with yet another phenotype, eczema, in the first generation of the Isle of Wight birth cohort at age 18 years, seeking confirmation in cord blood from the second generation of the same cohort. Using a recursive random forest approach, this work identified 88 CpGs associated with eczema at age 18 in the first generation. In the second generation, about half of these CpGs showed the same direction of association with eczema risk. Two CpGs in novel putative eczema genes (PROZ and NEU1) emerged from this analysis [24].

Epigenetic predictors of allergic disease

Despite their potential clinical relevance, the interpretation of studies focused on concurrent asthma and allergic disease is problematic because it is impossible to determine whether a given disease-associated alteration is a cause or a consequence of that disease. More insightful and promising is a study design in which the epigenome is surveyed to discover *predictors* of disease in early life or even at birth, that is, *prior* to the emergence of disease symptoms. Thus this design is aligned with epidemiologic evidence indicating that asthma begins much before symptoms occur [4, 5].

Wang et al. relied on the Illumina 27K platform to unbiasedly survey the methylome for an impact of prenatal smoke exposure [25]. After several candidates were identified in 14 cord blood cell samples and these candidates were validated by methylation-dependent fragment separation in 150 additional samples, only *TSLP* methylation remained significantly associated with prenatal smoke exposure (OR=3.17) [25].

A more recent study that relied on a high-coverage platform searched the genome for DNA methylation signatures predictive of childhood asthma in cord blood mononuclear cells from 36 children (18 non-asthmatics, 18 asthmatics by age 2–9 years) enrolled in the Tucson Infant Immune Study, an unselected birth cohort closely monitored for asthma for over a decade. Cord blood cells were found to harbor 589 differentially methylated regions associated with childhood asthma. Network and upstream regulator analysis showed that a subset of these regions mapped to genes that cluster in immunoregulatory and pro-inflammatory pathways [26]. The identification of epigenetic signatures *at birth* implies that there is an epigenetic component to disease pathogenesis, and suggests that the genes harboring differential methylation contribute to placing the child on a trajectory to disease.

This possibility is supported by another interesting study that examined the methylome of children with or without food sensitization at two time points (birth and 12 months). A supervised learning approach led to the identification of a novel 92-CpG signature in CD4+

T cells that distinguished children who developed clinical food allergy by age 12 months, and was enriched in genes encoding MAP kinase signaling molecules. Importantly, this signature was stable from birth until 12 months of age, suggesting that the children bearing that signature were on an epigenetic path to disease already at birth [27].

Conclusion: where is the field now and where is it heading?

Overall, the results of candidate gene and genome-wide DNA methylation studies in human asthma and allergic disease are not too encouraging [28, 29]. At a first glance, the scenario emerging from these data is not encouraging. Indeed, with few exceptions, the regions where disease-associated differential methylation was detected are spread throughout the genome, with no obvious functional links to asthma and/or allergy-related pathways and no or minimal replication across studies. Moreover, even when statistically significant, disease-associated differences in DNA methylation were typically modest, of the order of a few percent, raising questions about their biological significance.

There are, however, some extenuating circumstances. Studies are difficult to compare because of their high heterogeneity in design and phenotypic characterization. Moreover, because of the relatively simple technical requirements of genome-wide DNA methylation analyses, oftentimes these studies were a byproduct rather than a primary goal of previously existing data collections. As a result, the questions they asked (and the answers they got) often appear contrived. The numbers of cases and controls in each study also vary greatly, reflecting the lack of firm criteria to define population sizes adequate to generate robust results. The tissues/cells on which these studies were performed also deserve a comment. Because of availability and ease of access, most studies relied on DNA isolated from unfractionated peripheral blood leukocytes or peripheral blood mononuclear cells. Such an approach may be problematic if the cells bearing a given mark are present in different proportions among cases and controls.

On balance, the epigenetics of asthma and allergy is still in its infancy and some hurdles need to be overcome for the field to bloom. In terms of technology, collection of more robust information will require the development of array platforms with higher coverage and ultimately the coming of age of next-generation sequencing methods that can efficiently handle data from bisulfite-converted DNA.

For study design, it is desirable for the emphasis to shift more and more towards the search for marks predictive of, as opposed to associated with, the disease phenotype of interest. This move appears to be especially appropriate in allergic diseases, which are typically triggered in response to environmental signals and interact with the child's developmental programs. Especially transformational will be the findings provided by studies in well-phenotyped mother-child cohorts that collect samples from birth throughout the first decade of life and carefully assess environmental exposures to chemicals, diet and microbes for both mothers and children. While the ability to obtain samples other than cord and peripheral blood from such populations may remain limited, such cohorts will allow for the analysis of *epigenetic trajectories over time*, a theme that is exquisitely relevant to the developmental processes that epigenetic mechanisms primarily regulate. Such birth cohorts

will also allow investigating the relationships between the fetal methylome and the methylome and exposures of the mother (for instance, to smoking or to specific environmental microbial profiles), and simultaneously, early (and later) life allergic disease outcomes. We expect this second, more dynamic generation of epigenetic studies will avoid most of the early pitfalls, and will return exciting results highlighting the potential of epigenetic studies to foster a better understanding of asthma and allergy disease pathogenesis.

Finally, down the line allergic disease epigenetics will need to come to grips with a broader issue that looms large for all complex disease research: the necessity to integrate epigenetic data with those coming from other fields (genetics, microbiome and exposure studies, immunology, physiology, just to name some) that are technically distinct and disparate, and yet complementary. Novel bioinformatics and biostatistics strategies will have to be developed for the field to reach this goal, a true Holy Grail.

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Key points

- Asthma and allergy are complex diseases with strong genetic, environmental and developmental components.
- Because epigenetic processes (DNA methylation in particular) have the ability to regulate genetic and developmental processes in response to environmental signals, these mechanisms may contribute to allergic disease pathogenesis.
- DNA methylation signatures have been identified in the context of concurrent disease as well as before symptoms emerge, suggesting epigenetic mechanisms may contribute to both active disease processes and disease inception.