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Early predictors of asthma and allergy in children: The role of epigenetics

Avery DeVries^{1,2} and Donata Vercelli^{2,3,4,5}

¹Graduate Program in Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA

²Functional Genomics Laboratory, Arizona Respiratory Center, University of Arizona, Tucson, Arizona, USA

³Arizona Center for the Biology of Complex Diseases, University of Arizona, Tucson, Arizona, USA

⁴Department of Cellular and Molecular Medicine, University of Arizona, Tucson, Arizona, USA

⁵The Bio5 Institute, University of Arizona, Tucson, Arizona, USA

Abstract

Purpose of the review—Asthma and allergic diseases are among the most prevalent chronic non-communicable diseases of childhood. While epidemiologic studies suggest that asthma begins in the pre-school years, the lack of firm diagnostic criteria to distinguish children who will wheeze only transiently during early life lower respiratory illnesses from children who will wheeze persistently and develop asthma prevents pinpointing the time at which disease truly begins. Epigenetic mechanisms link gene regulation to environmental cues and developmental trajectories. This article reviews the search for epigenetic predictors of asthma and/or allergy that can be identified already at birth and/or in early life.

Recent findings—DNA methylation signatures associated with asthma and/or allergy at birth, and time-dependent DNA methylation signatures associated with allergic disease phenotypes in early life have been identified.

Summary—The identification of early epigenetic predictors of allergic diseases points to a potential role of epigenetic mechanisms in regulating the inception of, and the susceptibility to these diseases. Predictive signatures to more accurately estimate a child's risk for asthma and allergy may improve childhood asthma diagnosis. Moreover, understanding the biological implications of these signatures may help elucidate novel disease pathways and endotypes.

Keywords

Epigenetic predictors; Childhood allergic disease; DNA methylation

Introduction

Asthma and other allergic diseases are among the most prevalent chronic non-communicable diseases of childhood [1,2]. Epidemiologic studies indicate that asthma begins in the pre-school years even when chronic symptoms do not appear until early adulthood [3,4]. However, the lack of firm diagnostic criteria to distinguish children who will wheeze only transiently during early life lower respiratory illnesses from children who will wheeze persistently and then develop asthma prevents pinpointing the true inception of a child's trajectory to the disease. The role of epigenetic mechanisms in regulating susceptibility to and severity of asthma and allergic disease is under active investigation because of the inability of genetic factors to explain more than a limited proportion of total phenotypic variability, and the functional relationships between epigenetic processes, environmental stimuli and developmental programs. Nevertheless, current studies of the epigenetics of human allergy and asthma have primarily focused on concurrent disease [5] and only a few have surveyed the epigenome to discover predictors of disease at birth and/or in early life. The latter studies, arguably the most innovative and transformational ones, are the focus of this review.

Epigenetic mechanisms are defined as heritable changes in gene activity that are independent of alterations in the underlying DNA sequence [6–8]. These epigenetic modifications include DNA methylation and post-translational histone modifications. Epigenetic studies of allergy and asthma in human populations have primarily focused on DNA methylation, because 1) there is a close functional relationship between DNA methylation and gene expression, 2) DNA methylation is a stable epigenetic mark, and 3) methods that survey the methylome do not require chromatin isolation. DNA methylation occurs at cytosines in individual CpG dinucleotides and in clusters of CpG sites called CpG islands (CGIs). The relationship between DNA methylation and gene expression is context-dependent. The canonical pattern typically involves CGI promoter methylation that leads to gene silencing; conversely, an unmethylated CGI promoter is necessary but not sufficient for gene expression, and the relevant gene is said to be poised for expression. However, methylation in gene bodies is often associated with gene transcription [9].

Current techniques to study DNA methylation rely on bisulfite conversion of DNA, a reaction in which unmethylated cytosines are converted to thymines and only methylated cytosines are preserved as such in the sequence. While the decreasing cost and higher quality of next-generation sequencing are improving research in many areas of genomics, whole-genome bisulfite sequencing remains problematic because of the reduced complexity of the DNA sequence that results from this sequence conversion process, thus making it difficult to unambiguously map 100–200 bp reads to the genome. Therefore, targeted (gene- and/or pathway-specific) bisulfite sequencing or genome-wide microarrays are still widely used. Presently, the most widely used platform for genome-wide DNA methylation profiling is the Illumina Human Methylation BeadChip, particularly the one that interrogates approximately 450,000 CpG sites throughout the genome. This platform interrogates individual CpG sites (at single CpG resolution) and its output can be intuitively understood as the percentage of DNA methylation at each site [10]. The Illumina 450k array was designed to cover 99% of RefSeq genes with a global average of 17.2 probes per gene

region, 96% of CGIs, 92% of CpG shores, and 86% of CpG shelves [10]. This platform detects differences in DNA methylation by using the Infinium assay on bisulfite-converted DNA and incorporates both Infinium I and Infinium II probe types. Infinium I probes are designed in pairs—one against the methylated locus and the other against the unmethylated locus—whereas Infinium II probes are designed to bind both the methylated and the unmethylated locus, and the methylation state is detected upon a single base extension and a distinct signal is given from labeled nucleotides [10]. One specific limitation of this approach is its inability to distinguish between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). However, 5hmC appears to occur most commonly in the brain and in embryonic stem cells, and as such, its analysis is a concern primarily for studies focused on these tissues.

To date, a number of genome-wide and candidate gene DNA methylation studies have been performed in populations with current asthma and/or allergy [5]. Most of these studies 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, obtaining lung tissue is often problematic in adults and impossible in children. On the other hand, allergic diseases have an immune component that accompanies and often precedes the clinical diagnosis [11–14]. Therefore, surveying peripheral blood immune cells is appropriate albeit perhaps not optimal for identifying the full spectrum of epigenetic predictors of disease. Here we will review the most recent studies aimed at the identification of DNA methylation signatures detected in peripheral blood immune cell within the first year of life that *predict* asthma and/or allergy during childhood.

DNA methylation predictive of allergic disease

As we are writing, only a few studies have surveyed the DNA methylome for signatures predictive of allergic disease. Not surprisingly, all of these predictive DNA methylation signatures have been identified in peripheral blood immune cells. Based on their design and results, these studies can be separated into two groups: 1) work that seeks altered DNA methylation signatures detectable at birth that are associated with allergic disease later in childhood, and 2) analysis of time-dependent DNA methylation signatures detectable throughout early life. While the former work explores the impact that distinct pre- and perinatal exposures may have on a child's path to disease later in life, thus contributing to the child's overall risk for disease, the latter analyses seek signatures that may be used in clinical settings to identify children who are *already* on a path to allergic disease.

Allergy-associated DNA methylation signatures at birth

Studies aimed at understanding the contribution of epigenetic mechanisms to disease pathogenesis prior to the emergence of disease symptoms are highly informative. Some of the studies we will discuss have used cord blood immune cells to identify predictive DNA methylation signatures associated with (and possibly involved in) disease *at birth*. This prospective design eliminates the confounding effects that potentially arise when searching for epigenetic signatures associated with concurrent disease, a situation in which one cannot tease out whether a given pattern is a cause or a consequence of the disease. In contrast, epigenetic signatures identified *at birth* imply that there is an epigenetic component to

disease pathogenesis and the genes harboring differential methylation contribute to placing the child on a trajectory to disease long before symptoms develop.

The initial studies suggesting that DNA methylation signatures in cord blood are associated with risk for asthma and allergy during childhood focused on candidate genes. In a pioneering analysis of 20 subjects at birth, with results replicated in an additional group (n=56), Perera et al. used methylation-sensitive restriction fingerprinting and methylation-specific PCR to show that prenatal exposure to airborne polycyclic aromatic hydrocarbons altered *ACSL3* DNA methylation and was significantly associated with parental reports of childhood asthma symptoms before age 5 (odds ratio, OR = 3.9) [15]. These results suggested that prenatal exposures act through epigenetic processes early in life to modify the trajectory to disease. In a later study by Curtin et al., pyrosequencing of 303 cord blood samples revealed that higher *IL2* promoter methylation was associated with a 1.07-fold increase in the likelihood of severe asthma exacerbations and a 1.12-fold increase in the likelihood of hospital admissions for asthma/whoop during childhood [16].

With the advent of user-friendly, affordable platforms that allow interrogating thousands and even hundreds of thousand potential DNA methylation sites, the focus shifted towards genome-wide analyses of DNA methylation [5]. Wang et al. relied on the Illumina 27K platform to unbiasedly survey the methylome for an impact of prenatal smoke exposure [17]. After identifying several candidates in 14 cord blood cell samples and validating these candidates by methylation-dependent fragment separation in 150 additional samples, only *TSLP* methylation remained significantly associated with prenatal smoke exposure (OR=3.17) and was also associated with atopic dermatitis (OR=2.32) [17]. 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 at age 2–9 years) enrolled in the Tucson Infant Immune Study, an unselected birth cohort closely monitored for asthma. Cord blood cells were found to harbor several hundred differentially methylated regions associated with childhood asthma. Network and upstream regulator analysis showed that a subset of these regions mapped to genes that clustered in immunoregulatory and pro-inflammatory pathways [18]. The fact that epigenetic differences associated with allergic disease are detectable at birth is remarkable in itself, and the biological implications of these differences are compelling enough to deserve follow-up studies.

Time-dependent DNA methylation signatures associated with allergic disease in early life

In addition to DNA methylation profiling at birth, recent studies have pursued time-dependent epigenetic predictors of asthma and allergy-related phenotypes. Both Michel et al. [19] and Martino et al. [20] assessed whether DNA methylation signatures associated with asthma and allergy change during early childhood. Michel et al. chose to focus on ten candidate genes from blood obtained at birth and age 4.5 years to assess the relationship between a traditional farming lifestyle, DNA methylation and childhood asthma. Using pyrosequencing, this study found that in cord blood, *ORMDL1* and *STAT6* were hypomethylated in DNA from farmers' as compared to non-farmers' children, while regions in *RAD50* and *IL13* were hypermethylated. Time-dependent methylation differences

occurred in 15 genomic regions that clustered in genes highly associated with asthma (ORMDL family) and IgE regulation (RAD50, IL13, and IL4), but not in T-regulatory cell genes (FOXP3, RUNX3) [19]. On the other hand, using the Illumina 450K platform, Martino et al. examined the methylome of 24 children at two time points (birth and 12 months) and identified 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 [20]. Importantly, this 92 CpG 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. A later study by the same group focused on peripheral blood mononuclear cells from 11–15 months old children and relied on a 96 CpG signature that best distinguished food allergic and food sensitized individuals to develop statistical prediction models at a defined level of sensitivity and specificity [21]. Using a composite methylation score, this study was able to distinguish between food allergic and food sensitized infants with an area under the curve (AUC) of 0.9774, and food allergic vs non-allergic infants with an AUC of 0.8408. A methylation score >47.49 had a specificity of 96.55% and sensitivity of 89.66%. The authors also showed that this methylation signature outperformed serum IgE to both egg and peanut food allergens as a predictor of clinical allergy. Finally, food allergy status was correctly predicted in a replication cohort of 48 individuals with an accuracy of 79.2%.

Conclusion

While it remains to be seen whether predictive epigenetic marks will become useful therapeutic targets, current evidence suggests that these marks may be leveraged for screening or diagnosis, and more generally to estimate a child's risk for asthma and allergy and/or identify the epigenetic trajectory a child is on. Such an application would decisively improve childhood asthma diagnosis and management, which still struggle because of our inability to distinguish between early viral wheezing and early asthma symptoms. Moreover, the genes containing differential methylation robustly associated with asthma and allergy are likely to play a role in allergic disease pathogenesis, and understanding the biological implications of these epigenetic signatures may help elucidate mechanistic pathways and define disease endotypes.

However, in this era of high throughput analytical methods, several challenges remain:

1. Replication of findings in independent populations, to define robust signatures.
2. Rigorous statistical analyses (including specificity and sensitivity), in order to understand the extent to which these epigenetic predictors improve current clinical assessment.
3. Stability of the epigenetic marks over time (i.e. in which developmental window does a signature present, and how long is the signature maintained).
4. Development of diagnostic methods to include a broad range of differentially methylated loci.

Because the characterization of epigenetic predictors of childhood allergic disease is still in its infancy, future studies will need to address these issues to achieve a thorough understanding of the role epigenetic predictors and the underlying mechanisms play in the genesis of and/or trajectory to allergic disease.

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Key points

- Allergy and asthma are complex diseases with strong genetic, environmental and developmental determinants.
- The ability of epigenetic processes (particularly DNA methylation) to regulate developmental programs in responses to environmental signals suggests these processes may contribute to allergic disease pathogenesis.
- DNA methylation signatures associated with asthma and/or allergy have been identified both at birth and time-dependently in early life.