Epigenetic Mechanisms in Asthma

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Abstract

Asthma and allergic diseases are among the most prevalent chronic noncommunicable diseases of childhood, but the underlying pathogenetic mechanisms are poorly understood. Because epigenetic mechanisms link gene regulation to environmental cues and developmental trajectories, their contribution to asthma and allergy pathogenesis is under active investigation. DNA methylation signatures associated with concurrent disease and with the development of asthma during childhood asthma have been identified, but their significance is not easily interpretable. On the other hand, the characterization of early epigenetic predictors of asthma points to a potential role of epigenetic mechanisms in regulating the inception of, and the susceptibility to, this disease.

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The role of epigenetic processes in asthma is receiving an increasing degree of attention that is reflected in the continuous and steep rise in the number of publications. Remarkably, in certain years the reviews about asthma and allergy epigenetics actually outnumbered primary research papers—a pattern that suggests an unprecedented interest. This unusual situation begs several questions. Specifically, why is asthma epigenetics such a hot topic? What have we learned from the studies performed so far? Where is the field going next?

Why Is Asthma Epigenetics a Hot Topic?

Epigenetic mechanisms are defined as heritable changes in gene activity that are independent of alterations in the underlying DNA sequence (1, 2). Among epigenetic modifications, studies in human asthma have primarily focused on DNA methylation, because there is a close functional relationship between DNA methylation and gene expression. Moreover, DNA methylation is a robust epigenetic mark, and user-friendly, quantitative methods to survey the methylome have recently become widely available and only require DNA rather than more cumbersome chromatin isolation procedures (3).

The reasons asthma epigenetics has become a hot topic are likely multiple and complex. A major one is the inability genome-wide association studies (GWAS) have demonstrated to account for more than a limited proportion of the total phenotypic variance in asthma (4), despite the fact that this disease is well known to have a strong genetic component (5). Current studies focused on rare variants (as opposed to the common ones that GWAS typically interrogate) will probably improve the situation only marginally. The realization that genetics cannot "explain" asthma and allergic disease has shifted interest toward alternative potential sources of phenotypic variance, primarily the environment and development. That asthma has a strong environmental component has been known for several decades and was clearly illustrated by the seminal epidemiologic studies that revealed major differences in asthma prevalence among countries with more or less Westernized life styles (5). The point was reinforced by the discovery of asthma protection in the farming communities of Alpine Europe (6). The critical role of early life exposures in determining risk for asthma later in life is also well established (7). Thus, much effort currently focuses on assessing the extent to which integrating environmental and developmental factors into studies of asthma susceptibility and severity can further account for the phenotypes of interest.

It is against this backdrop that the current interest in asthma and allergy epigenetics can be best understood. Indeed, epigenetic mechanisms are exquisitely designed to faithfully and sensitively transduce environmental signals and preside over the time-dependent unfolding of developmental differentiation programs. On the other hand, interrogating the

epigenome, particularly the methylome, to highlight its ability to influence complex disease phenotypes has been made more feasible by the advent of technologies that allow one to simultaneously sample relatively large numbers of potential DNA methylation sites (CpG dinucleotides) throughout the genome using straightforward assays and streamlined analytical pipelines. 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.

What Have We Learned from Asthma Epigenetics?

We and others have recently reviewed the results obtained by both candidate gene and genome-wide DNA methylation studies in human asthma and allergic disease (8–10). At first glance, the scenario emerging from these data is not encouraging. Indeed, the regions where disease-associated differential methylation was detected are spread throughout the genome, with no immediately discernible pattern and no internal consistency. Only two regions (MRI1 and FAM181A) replicated in two distinct studies, and neither of them harbors genes seemingly related to asthma and/or allergy pathogenesis. Moreover, even when significant phenotype-related differences in DNA methylation were detected, these differences were often modest, of the order of a few percent, raising questions about their biological significance and consequences.

There are, however, some extenuating circumstances. Even though the studies in question examined "asthma" or "atopic dermatitis," the phenotypic heterogeneity among study populations is so striking that few if any studies can be directly compared with one another. Moreover, because of the relatively simple technical requirements of genome-wide DNA methylation analyses, oftentimes these studies were a byproduct of existing data collections. As a result, the questions they asked (and the answers they got) often appear contrived. The numbers of cases and control subjects in each study also vary greatly, reflecting the lack of firm criteria to define population sizes adequate to generate robust results, but these numbers are overall relatively small.

The tissues/cells on which these studies were performed also deserve a comment. Because of availability and ease of access, many studies relied on DNA isolated from unfractionated peripheral blood leukocytes or peripheral blood mononuclear cells. Such an approach can create problems if epigenetic marks are tissue/cell type–specific and the cells bearing the mark of interest are present in different proportions among cases and control subjects. On the other hand, allergic diseases have a major immunologic component, and therefore focusing on DNA methylation signatures of peripheral immune blood cells may be informative. Moreover, methods have been recently developed to infer the proportion of immune cell populations in peripheral blood from the DNA methylation data themselves (11).

On balance, we would argue that the search for epigenetic biomarkers of allergic disease is still in its infancy, and the studies performed so far provide food for thought in terms of fundamental variables such as study design, population size, depth of phenotypic assessment, and choice of DNA source. Some studies have reported hits in plausible genes (e.g., TSLP, IL2RA, TBX21, FCER2, TGFB1), but the vast majority of results point to regions that hold no clear mechanistic relationship to asthma pathogenesis and will require further functional explorations, particularly in relation to the possibility that differential methylation resides in regulatory elements that control the expression of distant genes. Moreover, interestingly enough, none of the hits generated in peripheral blood replicated in more "physiologic" airway tissues (for asthma) or skin (for atopic dermatitis), and vice versa. This discrepancy may reflect the inability of peripheral blood to elucidate tissue-based disease mechanisms but may also point to distinct components of disease pathogenesis.

So far, most if not all DNA methylation studies in allergy and asthma were aimed at the identification of the epigenetic components of allergic diseases and thus they sought DNA methylation signatures in patients with concurrent disease. For instance, a recent study surveyed associations between serum IgE concentrations and DNA methylation in 95 nuclear pedigrees from individuals in their twenties. 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 (12). Another recent study compared DNA methylation patterns and gene expression in 6- to 12-year-old inner-city children with persistent atopic asthma versus healthy control subjects. Results were validated in an independent population of patients with asthma. Eighty-one differentially methylated regions were identified. Several immune genes were hypomethylated in asthma, including IL13, RUNX3, and TIGIT. Among patients with asthma, 11 differentially methylated regions were associated with higher serum IgE concentrations, and 16 were associated with percent predicted FEV_1. Methylation marks involved in T-cell maturation (RUNX3), Th2 immunity (IL4), and oxidative stress (catalase) were validated in an independent cohort of children with asthma living in the inner city (13).

Despite their potential clinical relevance, the interpretation of studies focused on concurrent asthma is problematic because it is impossible to determine whether a given alteration associated with disease is a cause or a consequence of that disease. Perhaps more insightful is a different study design in which the epigenome is surveyed to discover predictors of disease in early life or even at birth, that is, before the emergence of disease symptoms. This design is particularly compelling because epidemiologic studies indicate that asthma begins in the preschool years even when chronic symptoms do not appear until early adulthood (14, 15). 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.

Wang and colleagues relied on the Illumina 27K platform to unbiasedly survey the methylome for an impact of prenatal smoke exposure (16). 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 (odds ratio = 3.17) (16). 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 without asthma, 18 with asthma by age 2–9 yr) enrolled in the

Tucson Infant Immune Study, an unselected birth cohort closely monitored for asthma. 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 proinflammatory pathways (17). The identification of epigenetic signatures at birth implies 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. A composite DNA methylation signature associated with food sensitization has also been reported (18).

Conclusions: Where Is the Field Going?

There is undoubtedly much need (and room) for improvement in asthma epigenetics. One unavoidable hurdle of the initial genome-wide studies is that even the most extensive ones relied on platforms that provide limited genomic coverage and were often designed primarily to address cancer-related differential DNA methylation. Collection of more robust information will require the development of more inclusive array platforms and/or the coming of age of next-generation sequencing methods that can efficiently handle data from bisulfite-converted DNA.

Another major hurdle is study design. For epigenetics to make a difference in the asthma field, future studies will need to incorporate the notion that epigenetics explores mechanisms potentially influencing disease susceptibility and severity and that such mechanisms are typically triggered in response to environmental signals and developmental programs. In this respect, well-phenotyped, longitudinal mother–child cohorts with cord blood and early infancy samples and in-depth assessments of environmental exposures (to chemicals, diet, and microbes) will likely prove transformational for the field. Although obtaining samples other than cord and peripheral blood may be impossible in such populations for obvious logistical reasons, such cohorts will allow for the analysis of epigenetic trajectories over time, a theme that is exquisitely relevant to the developmental processes 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. Indeed, the link between environmental exposures, epigenetic marks, and immune phenotypes appears to be robust (19). Several such birth cohorts (e.g., the Tucson Infant Immune Study [IIS] [20], the Wisconsin Childhood Origins of Asthma [COAST] study, the Danish Copenhagen Prospective Study of Asthma in Childhood [COPSAC], and the UK Manchester Asthma and Allergy Study [MAAS]) exist in the United States and Europe, and research along these lines is already beginning. Because the unique time- and environment-dependent nature of epigenetic marks appears better suited for the analysis of prenatal and early postnatal trajectories to disease than for conventional, static assessments of disease risk, we expect this second 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. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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