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Enhancing our understanding of asthma

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Allergic diseases, particularly asthma, pose a significant and growing medical challenge worldwide. CD4⁺ T cells, which are critical for host defense against infection, can also instigate a detrimental inflammatory response to harmless environmental substances such as pollens. This misdirected immune response may then lead to allergic disease. A strong genetic basis has been established for asthma, but despite stunning advances in genomics identifying associated loci, a substantial proportion of the causality remains unexplained. In addition to genetics, the environment also greatly impacts disease, with asthma and allergies serving as profound examples of this influence. In this issue, Seumois et al establish the feasibility of measuring modifications of DNA-packaging proteins in populations of primary human CD4⁺ T cells and utilizing this information to better understand the foundations of asthma. The authors choose to focus on a marker for genomic enhancers, and the study therefore represents an important step in deciphering how genetic risk and environmental impact on the T cell epigenome come together to affect disease.

More than a decade ago, geneticists envisioned principles for genetic mapping using populations rather than families to study common diseases such as asthma. This approach, coined genome-wide association study (GWAS), tests a comprehensive catalogue of common genetic variants in cases and controls from a population to identify variants associated with a particular disease. Although GWASs have played key roles in discovering a few major genes and pathways driving immune mediated disorders¹, the outcomes in asthma² and many other diseases have been largely sobering: much susceptibility cannot be explained by common genetic variants, and of those diseases-associated variants identified, the majority are non-coding, complicating their functional assessment.

Besides genetics, environment is also a key player in the pathogenesis of asthma. Moreover, the dramatic increase in asthma prevalence over the last few decades, especially in developing countries, suggests that environmental factors also influence disease development. Although allergens are classically associated with asthma, many other exposures including smoking and pollution, as well as hygiene and antibiotic use, appear associated with development and progression of this disease³.

The link between genetic and environmental influences on asthma can be captured by epigenetic profiling. Epigenetics refers to the study of DNA packaging into a structure

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called ‘chromatin’ and the effects of variable chromatin organization on activity of genetic regulatory elements. In addition to a set of inherited epigenetic marks, there are likely nonheritable epigenetic marks that are more dynamic and change in response to environmental stimuli^{3,4}.

During the last few years, advances in the epigenomic annotation of functional noncoding elements, commonly referred to as “enhancers”, have begun to shed light on gene regulation at the genetic and epigenetic levels. Enhancers are associated with distinct chromatin structures that display unique histone modifications. Recent developments in genomic technology such as chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), enable us to construct genome-wide maps of enhancers using global patterns of these histone modifications. Comparisons of enhancer maps in a variety of cell types have revealed incredible levels of cell type specificity. Particularly important for understanding genetic associations with disease was the realization that a DNA variant can disrupt or generate a transcription factor binding site within an enhancer. This suggests that with the enhancer map of a disease-relevant cell type, we may be able to assess the functionality of non-coding variants associated with the disease.

ChIP-seq experiments begin with immunoprecipitation of genomic fragments followed by library preparation for high-throughput sequencing to identify enriched genomic regions⁵. Due to inefficiencies at the immunoprecipitation and library preparation steps, this procedure requires large amounts of starting material, preventing its application in rare, biologically important cell types.

Several groups have developed small-scale immunoprecipitation protocols to overcome the challenges related to inefficiencies at the ChIP step⁵, but Vijayanand and colleagues are the first to tackle this problem in primary immune cell populations from patients. The chromatin landscapes of T helper cells have been extensively studied for in vitro-generated cells in both humans and mice^{4,6,7}, however, Seumois et al present the first enhancer maps of in vivo-generated T cells. Their work focuses on a subset of CD4+ T cells known as T helper (Th)2 cells, which are key players in asthma. The authors isolated circulating naive and memory CD4+ T cells from peripheral blood of healthy controls and asthma patients, and then further subdivided the CD4+ memory T cells based on their surface expression of the chemokine receptor CCR4 to enrich for Th2 cells. The histone modification H3K4me2 was chosen for ChIP as a reliable indicator of enhancer elements, following a number of studies highlighting the value of H3K4me2 in identifying poised and active enhancer domains across the genome.

The message that emerges from this study is that enhancers marked in Th2-enriched cells harbor a large portion of asthma-associated genetic variants. Furthermore, comparison of enhancers identified in healthy controls versus asthma patients highlights that the majority of differences in H3K4me2 distribution appear in Th2-enriched as opposed to other memory or naïve CD4+ T cell populations.

The most intriguing aspect of this study is its proof of concept for generating histone modification-based enhancer maps in primary immune cells, particularly in small cell

populations from patient tissues. Although Vijayanand and colleagues restricted their focus to a particular histone mark and cell type, their approach can easily be extended to other histone modifications and cell populations, opening the door for epigenomic studies in asthma and other diseases.

To date, genomic studies addressing the role of regulatory elements in the human genome have generally taken one of two approaches. Studies such as that of Seumois et al aim to annotate functional enhancer elements in pathogenic cells using techniques such as ChIP-seq. These studies focus on identifying genomic loci with differential enrichment of histone modifications in cases and controls. In contrast, other studies strive to map various quantitative trait loci (QTL), such as those associated with gene expression (eQTLs)⁸, and thereby identify genetic variants that functionally impact gene regulation. Ultimately, it will be enormously valuable to have a resource of individuals who have been both comprehensively genotyped and epigenomically characterized across a wide range of tissue types. Without genetic variation data, a critical gap remains in efforts to explain the different histone modification patterns observed between asthma patients and healthy controls and it remains unclear whether those differences in the enhancer landscapes are footprints of the environment.

Even after identifying disease-associated enhancers, it is a considerable challenge to link these functional noncoding elements to target genes. Distal regulatory elements often physically interact with the promoters of their target genes, and technologies to detect these interactions, such as chromatin conformation capture (3C, Hi-C) and chromatin interaction paired-end tagging (ChIA-PET) are being rapidly developed. Nonetheless, technical limitations such as a requirement for very large quantities of DNA, currently hinder their use in primary human cells.

An additional complication to the present study is that while H3K4me2 modification is thought to mark active and poised enhancers, it does not capture a comprehensive map of regulatory elements. Given the combinatorial nature of gene expression regulation, it is now evident that no single modification, but rather integration of multiple layers of genomic information such as H3K27ac, binding of acetyltransferase p300 and DnaseI hypersensitivity, will provide the most complete annotation of enhancer elements⁹.

The ability to perform epigenomic profiling on cell-type-specific populations of patient samples dramatically widens the possibilities open to “omic” disease research. The potential combinations of cell types, diseases, and histone modifications seem endless, not to mention the transcription factors that could be studied by the same ChIP-seq procedure. Matching this data with cell-type-specific gene expression, DNA methylation, and DNA-enhancer interaction will illuminate processes specific to particular cell types and diseases. With such comprehensive maps, we can even start investigating how the newly characterized subset of enhancers, known as stretch or super-enhancers^{10,11}, plays a role in asthma and other diseases.

The potential for genomics to contribute to clinical care has long been recognized. With genomic characterization technologies, we may begin to subset common diseases by their

altered pathways. Genomic diagnostics could allow for a more personalized understanding of an individual's condition and thereby inform the choice of treatment. It is now becoming increasingly routine for doctors to perform whole exome sequencing. No doubt sooner than we expect, it will be reasonable for a patient to ask, "Hey Doc, should you check my epigenome?" Due to compositional heterogeneity of patient tissue samples, methods that use sorted cell populations will likely prove more effective for epigenomic approaches. Recently, the development of an assay for transposase-accessible chromatin using sequencing (ATAC-seq)¹², which enables open chromatin profiling from blood populations in a timeline on the order of days, has brought progress toward this goal. Now we may add to the list a technology for epigenome mapping in small cell populations from clinical samples. As these technologies emerge, it becomes increasingly important to understand how all of these "omic" measurements relate to each other and to downstream physiological consequences if we are to one day use such technology to determine a means of intervention.

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