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Using epigenetic mechanisms to understand the impact of common disease causing alleles

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Abstract

Many common genetic variants have been identified to be associated with autoimmune diseases such as Type I diabetes. Methods to identify these genetic loci have become powerful, but deciphering the functional effects of these variants in disease progression remains a major challenge. Recent studies have shown that single nucleotide polymorphisms are associated with altered DNA methylation and chromatin accessibility, suggesting that genetic variants can alter epigenetic features and epigenetic variations can mediate genetic variability. In this review, we highlight recent studies that have examined the relationship between genetics and epigenetics, and how epigenetic studies may complement genetic studies in understanding the impact of common disease causing alleles.

Introduction

Genome-wide association studies (GWAS) have identified many loci that are associated with autoimmune diseases such as type 1 diabetes (T1D), multiple sclerosis, and rheumatoid arthritis [1–4]. While these studies have found common single-nucleotide polymorphisms (SNPs) that are associated with disease, understanding how these variants contribute to disease progression has been a major challenge. Furthermore, many of the characterized disease-associated SNPs are located in non-protein coding regions of the genome [5], making prediction of their functional effects difficult. We review here recent studies investigating the influence of genetic variants on epigenetic phenomenon, with a particular emphasis on Type 1 diabetes (T1D) and Type 2 diabetes (T2D). The results of these studies indicate that epigenetic studies performed in parallel with GWAS experiments are crucial to fully understanding the consequences of genetic variations.

Epigenetic Mechanisms

The classic definition of epigenetics is the study of heritable changes in phenotype that do not involve a change in genotype [6]. Recently, many researchers have relaxed the requirement that epigenetic phenomena be heritable and begun to refer to all information carried on the genome that is not encoded in the DNA sequence as epigenetic [7]. In this

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review, we consider DNA methylation, post-translational histone modifications, and alterations in chromatin structure as epigenetic mechanisms. Within the nucleus, chromosomes are highly regulated assemblies of DNA and proteins known as chromatin. At the most basic level, chromatin is comprised of DNA wrapping around an octamer of histone proteins to form nucleosomes. The chromatin structure is not static. Nucleosomes can be translocated along the DNA by ATP-dependent nucleosome remodelers, and histones can be displaced and replaced by protein complexes that are recruited to DNA during processes such as transcription [8]. Individual components of the nucleosome can be modified, affecting protein-DNA interactions. The modifications may influence chromatin accessibility, the binding of regulatory factors, and/or distal chromatin interactions [9]. The genomic profile of all possible epigenetic phenomena in a given cell is referred to as the epigenome of that cell. It is important to emphasize that while each cell has the same genotype, epigenomes are cell-type specific and can change in response to endogenous and exogenous stimuli.

The most well studied DNA modification is DNA methylation, which in humans predominantly occurs at cytosines of CpG dinucleotides. Mediated by DNA methyltransferases, DNA methylation is essential for development and for determining cell fate decisions in mammalian systems [10]. DNA methylation has been shown to be intimately involved in gene regulation (for a thorough review see [11]) and aberrant DNA methylation has also been associated with disease progression [12].

In addition to modifications to DNA, histone proteins can also be modified. These post-translational modifications (PTMs), including acetylation, methylation, phosphorylation, and ubiquitination among others, depend on enzymatic complexes that target particular sites in the genome. Recent studies have utilized the genomic positions of these modifications to identify specific functional sites and regulatory regions in the human genome [13–14]. As an example, monomethylation and trimethylation of histone H3 at lysine 4 (H3K4me1 and H3K4me3) have been used to identify enhancers and promoters, respectively, of transcribed genes [15–16]. Misregulation of histone PTMs can alter gene expression as well as promote disease [6–7]. In addition, the misregulation of histone modifiers themselves can have profound effects on human disease and gene expression. Loss of histone methyltransferase activity of the *MLL* gene is associated with the development of leukemia [17]. Histone modifiers can also be regulated by non-coding RNAs. For example, non-coding SNPs in the 9p21 locus that are associated with gliomas, coronary artery diseases, and T2D have been shown to affect the ability of a non-coding RNA, *ANRIL*, to recruit the polycomb repressive complex 2 (PRC2) which regulates histone H3 lysine 27 trimethylation, a chromatin modification typically associated with transcriptionally silenced genes. [18–22].

DNA methylation and genetic variants

In recent years, we have begun to appreciate how genetic variants may impact epigenetic phenomenon. In one of the first studies to investigate the relationship between genetic variation and DNA methylation, Kerkel and colleagues profiled DNA methylation at SNP regions in human tissues [23]. They identified allele-specific methylation outside of imprinted regions, some of which were associated with allele-specific expression of genes located proximal to the methylated region. This demonstrated that the variants may affect methylation status of local regions of the genome and ultimately the regulation and expression of nearby genes. These observations were confirmed by a more recent study profiling DNA methylation in peripheral blood leukocytes of individuals from three generations of the same family. Heterozygous SNPs were associated with differences in DNA methylation among individuals [24]. These methylation differences were correlated with local gene expression levels observed in lymphoblastoid cell lines. The allele-specific

methylation was also observed in unrelated individuals suggesting that these heritable regions of differential methylation are influenced by genotype.

DNA methylation studies can also be integrated with GWAS studies to further classify important disease-associated SNPs. Bell and colleagues identified differentially methylated regions of the genome in peripheral whole blood of control and T2D individuals that are in proximity to T2D SNPs identified by GWAS [25]. They characterized a differentially methylated region containing T2D-associated SNPs proximal to *FTO*, a gene known to be involved in diabetes and obesity. Analysis of histone H3K4me1 levels in human skeletal muscle cells suggested that this differentially methylated region is a putative enhancer region that may contribute to T2D. This study highlights the utility of identifying differentially methylated regions near important disease-associated genes in order to characterize disease relevant SNPs. A similar study assessing differential methylation between T1D patients and controls was able to identify differentially methylated regions proximal to 19 other genes, including *UNC13B*, a gene containing a SNP associated with diabetic nephropathy [26]. Altogether these studies suggest that genetic variants at critical DNA sites can influence DNA methylation in these specific regions (genotype-dependent epigenetic outcomes) and may lead to disease progression.

Chromatin accessibility, histone PTMs, and variants

There is now ample evidence that chromatin remodeling is a necessary step in the binding of transcription factors (TFs) to DNA. While some factors can compete directly with nucleosomes for access to underlying DNA, other factors require mediators of chromatin remodeling [27]. Furthermore, both the binding of proteins to DNA and the accessibility of chromatin can be allele-specific. Analysis of TF binding profiles revealed that regions bound by specific TFs can vary from individual to individual. For example, 7–10% of binding sites for the nuclear factor kappa-B (NF- κ B), an important TF regulating inflammation and autoimmune disease genes, were demonstrated to differ among individuals, with many of the differing binding sites containing polymorphisms [28]. In a complementary study, it was determined that approximately 11% of CCCTC-binding factor (CTCF) binding sites were allele-specific in cell lines derived from parents and children from two ancestries [29]. These allele-specific sites contained SNPs that likely alter binding of the protein to DNA. In the same study, chromatin accessibility profiling through DNase I hypersensitivity (DNase I HS) analysis, which enriches for regions of open chromatin including regulatory elements, revealed that a portion of the accessible chromatin regions was also allele-specific. While sequence variations to binding sites can alter TF binding, SNPs outside of canonical binding sites are also associated with differential binding of TFs [30].

Prediction of regulatory regions based on specific chromatin modifications has also been used to interpret the functional role of genomic elements [31]. Analysis of cell-type specific chromatin modifications revealed that some top scoring disease-associated SNPs are found at enhancer elements which are active in relevant cell-types for disease [32]. Studies assessing chromatin modifications such as these will be useful in the identification of functional genomic elements like promoters and enhancers and how SNPs in these regions may influence the progression of disease through altering regulatory networks. This needs to be appreciated since promoter regulation is mostly ubiquitous and enhancer regulation is more cell-type specific.

The interaction of chromatin accessibility and genetic variation has been shown to be important for autoimmune diseases. Common SNPs in human chromosome 17q12-q21 are associated with many autoimmune diseases [3,33–34]. Analysis of these regions revealed allele-specific expression of genes and led to the identification of a disease-associated SNP

affecting the binding of CTCF and underlying chromatin accessibility [35]. It has also been suggested that genetic variants can affect gene expression through the perturbation of enhancer-promoter interactions [36]. There is evidence that intron 19 of *CLEC16A* gene physically interacts with the promoter region of nearby gene *DEXI*, regulating expression of *DEXI* in an allele-specific manner [36]. The *CLEC16A* locus contains many autoimmune disease-associated SNPs with many occurring within intron 19 and further studies assessing the effect of those variants on chromatin structure and gene expression will be valuable.

Recently, profiling of open chromatin in human islet cells and non-islet cells revealed a region within an intron of the *TCF7L2* gene that showed differential chromatin accessibility specific to islet cells [37]. This intronic region contains many autoimmune- and T2D-associated SNPs. One particular SNP associated with T2D was discovered to alter the expression of *TCF7L2* presumably by affecting local chromatin structure and enhancer activity [37]. Studies such as these will prove to be useful for the identification of disease-associated SNPs and will help uncover their functional effects on disease.

The Major Histocompatibility (MHC) and other T1D susceptibility loci

The MHC region in human chromosome 6 has a high density of SNPs and is a major susceptibility locus for several autoimmune diseases, including T1D. We speculate that variants affecting epigenetic mechanisms are involved in regulating this locus given that many factors contributing to the progression of T1D stem from this locus. Among the many regulatory proteins influencing gene expression, CTCF has been found to bind to specific regions within the MHC Class II genes and can affect expression of genes at that locus by regulating distal DNA interactions [38–40]. In Lax221, a leukemic cell line in which only a subset of genes within the MHC Class II locus are expressed, hypermethylation is observed to be correlated with decrease in CTCF binding [41]. These data indicate that the locus can be epigenetically misregulated in a manner that may be associated with genetic variants. However, it is unclear whether particular SNPs are associated with DNA hypermethylation or the lack of CTCF binding in this cell line. Further studies to genotype the region and comparisons to other cell lines will be illuminating.

Analysis of chromatin modifications have also revealed important regulatory regions that may be influenced by SNPs. In the analysis of histone PTMs at T1D susceptible gene promoters within the MHC region as well as those identified by GWAS [3], Miao and colleagues discovered regions of differential histone acetylation in blood monocytes of T1D individuals compared to normal individuals [42]. Notably, these studies revealed key variations in acetylation of histone H3 lysine 9 at the upstream regions of two HLA genes, *HLA-DRB1* and *HLA-DQB1*, which are known to contain SNPs associated with T1D. These differentially acetylated regions are enriched for p300 and H3K4me1 suggesting they are acting as enhancer elements. Furthermore, these regions overlap with key T1D-associated variants suggesting that the polymorphisms may influence the ability of the region to function as an enhancer and may also affect the enrichment of histone PTMs.

As with DNA methylation studies, analysis of histone PTMs can identify important T1D genes. Comparative epigenome profiling of lymphocytes from T1D patients with those from control non-diabetic volunteers revealed significant alterations in histone H3 lysine 9 dimethylation, a chromatin modification associated with transcriptionally repressed chromatin. [43]. Notably, the promoter of *CLTA4*, a T1D susceptibility gene, was one of the regions identified as differentially methylated. This could imply lower levels of CLTA4 and enhanced T-cell activation/misregulation compatible with T1D. In addition, biological relationships were identified between the network of differentially methylated genes and key genes associated with T1D such as *IL-1B*, *IFN γ* , *IL-18*, and *VDR* [43]. Further genotyping

analysis of these T1D and normal individuals at *CLTA4* and *HLA* regions will be necessary to distinguish whether these histone modification differences are due to genotype. It is clear, however, that studies such as these which examine the differences in chromatin modifications between normal and disease individuals using relevant and appropriate cells types (such as primary monocytes, antigen presenting cells, and T-cell subsets) will be informative in identifying genes that are involved in promoting autoimmune disease [44].

Overall, these studies support the notion that epigenetic studies may provide greater insight into the role of disease-associated SNPs. Future studies with larger cohorts will aid in extending these observations. Furthermore, recent methods providing cost-effective genotyping of the *HLA* locus have been developed which will likely annotate more polymorphisms and disease associated SNPs [45]. The MHC locus is a hotbed of genetic variations that may directly interact with epigenetic mechanisms. This region as well as other key susceptible loci may provide fertile ground for identifying causal variants that underlie autoimmune diseases.

Conclusions and Perspectives

As seen from recent epigenomic studies, genetic variants can influence epigenetic phenomena such as local DNA methylation, chromatin accessibility, and histone PTMs (Figure 1), ultimately altering not only the chromosome landscape but also gene expression. Furthermore, variations in epigenetic phenomena can mediate genetic variants, as seen with SNPs that are found in regions of accessible chromatin and altered binding of TFs. The approaches for epigenomic studies can be applied in conjunction with genetic studies that define disease variants [46–47]. Complementing these two types of studies allows for better characterization of causal variants and can provide further insights into the effects of variants. However, these studies should be performed with careful consideration. As seen in studies of variant effects in different cell-types, performing epigenomic studies with cells that manifest the disease is important, and for most studies whole blood is the most easily collected and stored. Fortunately, immune cells found in whole blood are highly relevant for the study of autoimmunity. However, sorted primary cells would be better for epigenome profiling than whole blood or transformed lymphoid cells due to the cell-type specific nature of the epigenome.

The concepts generated from the highlighted studies can be applied to the understanding of how variants can lead to human disease, especially in the context of the additional layer of environmental or related influences. It has been observed that environmental effects such as diet and maternal behavior can influence phenotypic outcomes that are associated with epigenomic changes [48–50]. Studies of disease discordant monozygotic twins have further implicated a role for epigenomic alterations in influencing the progression of disease [51–52]. Clearly, exogenous stimuli have an important role on the manifestation of disease that may be observed at the epigenomic level.

It is important to remember that our chromosomes and DNA are not static structures -- they are dynamic and highly organized to allow for DNA-dependent events to occur in a cell-type specific manner. Aberrations in this organization, whether genetic or environmental, can have lasting impacts on phenotypic outcomes and ultimately human disease. Lastly, it is noteworthy that unlike genetic changes, epigenetic changes are reversible and are thus amenable to therapeutic intervention, providing additional opportunities for the treatment and prevention of autoimmune diseases.

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Highlights

- SNPs are associated with altered DNA methylation and chromatin accessibility
- Autoimmune disease-associated SNPs may influence epigenetic features
- Epigenomic studies may complement GWAS studies to find disease-associated SNPs

Figure 1. Epigenetic Mechanisms and Variants

Genetic variants (yellow stars) are associated with differences in: (A) DNA methylation (white lollipops represent CpGs and brown lollipops represent methylated CpGs), (B) binding of proteins to DNA which can alter recruitment of chromatin modifiers (yellow circle represents DNA-binding protein such as transcription factor and pink oval represents chromatin modifier), and (C) chromatin organization (red, blue, and orange spheres represent DNA-binding proteins and co-factors associated with DNA). (D) Variants found in long non-coding RNAs (light blue represent RNA with secondary structure and purple, brown, green spheres represent proteins recruited by RNA to DNA) alter recruitment of chromatin modifiers to DNA. For each scenario, the genetic variants are influencing the expression of the proximal gene.