Published in final edited form as:

Immunol Cell Biol. 2015 March ; 93(3): 271–276. doi:10.1038/icb.2015.18.

Epigenomics of autoimmune diseases

Bhawna Gupta¹, R David Hawkins^{2,3}

¹School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India ²Division of Medical Genetics—Department of Medicine, Department of Genome Sciences, Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, USA ³Turku Centre for Biotechnology, Turku, Finland

Abstract

Autoimmune diseases are complex disorders of largely unknown etiology. Genetic studies have identified a limited number of causal genes from a marginal number of individuals, and demonstrated a high degree of discordance in monozygotic twins. Studies have begun to reveal epigenetic contributions to these diseases, primarily through the study of DNA methylation, but chromatin and non-coding RNA changes are also emerging. Moving forward an integrative analysis of genomic, transcriptomic and epigenomic data, with the latter two coming from specific cell types, will provide an understanding that has been missed from genetics alone. We provide an overview of the current state of the field and vision for deriving the epigenomics of autoimmunity.

The epigenome comprises DNA methylation, histone modifications and non-coding RNAs, all of which influence transcription and, therefore, cell fate and broader phenotypes. The epigenome exist in a metastable state, meaning it is stable enough to maintain a cellular state, but dynamic enough to respond to developmental and environmental cues that alter the epigenomic landscape. Alterations to the epigenome outside of development or with appropriate responses to the environment, such as to infection, can lead to epi-mutations that are transmitted to daughter cells. Epi-mutations can have a significant impact on cellular function and lead to a disease state. This has been well documented in cancer biology, and a prime example is the acquisition of DNA methylation at the *RB* promoter that leads to loss of expression when the other allele is mutated.¹ Repression of the remaining active copy of the tumor suppressor causes the cell to lose control of cell cycle regulation. Similar mechanisms are likely at play in autoimmune disorders, where genetics and epigenetics converge to result in disease. An understanding of autoimmunity cannot be fully achieved through genetics alone. Though these disorders are genetically complex, after genetic predisposition, it is reasonable to hypothesize that epigenetics contributes to the dynamic ranges of phenotypes: age of onset, recurrence or flaring, severity of symptoms, length of remission and response to drug treatments. Given that most autoimmune disorders progress

Correspondence: Dr B Gupta, School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India. bhawna.gupta@kiitbiotech.ac.in, or Dr RD Hawkins, Division of Medical Genetics—Department of Medicine, Department of Genome Sciences, Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, USA. rdhaw@uw.edu.

with age, a constant reprogramming of the epigenome of immune cells through environmental exposures may tip the balance away from self-tolerance.

Autoimmune disorders provide an ideal scenario to study the epigenetic contribution to disease. The ability to consistently sample blood cells provides an opportunity to monitor epigenomic states throughout disease states. The ability to separate T cells from monocytes, from B cells and so forth is also of critical importance. The epigenomic landscape of any given cell type displays cell-specific signatures.^{2–11} Trying to discern a disease-specific signature in a background of mixed cells could mask relevant changes within a signal. This ultimately means that comprehensive epigenomic measures will need to be made in a multitude of cells types from affected individuals to truly understand the epigenetic contribution to autoimmune disorders. Fortunately, once such signatures are defined for disease states in a given cell type, this information will likely serve as biomarkers for onset—related to genetic predisposition and specific environmental exposures, monitoring during remission for subsequent recurrences or flares and potentially to therapeutic response.

INTERPRETING CHANGES IN THE EPIGENOMIC LANDSCAPE

Before discussing the current state of epigenetics in autoimmunity, it is important to have a clear understanding of the meaning of epigenetic modifications. DNA methylation is the most commonly studied modification owing to the ease of isolation and identification of CpG methylation status by microarray technology. By and large, DNA methylation is repressive when found at CpG Islands and promoter regions; however, in the gene body, this modification shows a strong correlation with the level of gene expression.^{12,13} Therefore, interpretation of CpG methylation status is context-dependent with regard to genomic annotation. In addition, cytosine methylation has recently been shown to exist in alternative states, primarily 5-hydroxymethylcytosine.^{14,15} The 5-hydroxymethylcytosine modification is indistinguishable from 5mC using bisulfite treatment; however, it is not considered repressive and is enriched at active regulatory elements such as enhancer and promoters.^{16,17} As we move forward, interpreting results from previous studies should be done in correlation with expression and future studies will need to distinguish these DNA methylation differences.

A variety of histone modifications are highly informative not only about the underlying genome, but also for unique mechanisms of transcriptional regulation. Acetylation of histone H3 and histone H4 tails happen at almost every lysine, however, few differences are noticed in genome-wide patterns.^{6,18} Acetylation of the histone tail changes the charge on the histone, causing DNA to loosen its wrap around the nucleosome. This in turn leads to, and is associated with, an open chromatin structure found at active promoters and enhancer elements. Loss of acetylation (hypoacetylation) is largely indicative of gene inactivation, whereas hyperacetylation is typically associated with increased gene expression. One of the most commonly assessed histone modifications by ChIP-seq is histone H3 lysine 27 acetylation (H3K27ac—this nomenclature is common to histone modifications: histone number, amino acid and position in tail followed by the modification). Repressive chromatin states are frequently defined by the presence of di- and tri-methylation at histone H3 lysines 9 and 27 (H3K9me2, H3K9me3, H3K27me2 and H3K27me3).¹⁹ Loss of these methylation

modifications (hypomethylation) in a diseased state may correspond to aberrant gene activation. These modifications form broad repressive domains and can be quantitatively different between cells.⁴ Gross changes in histone acetylation or H3K9 and H3K27 methylation may serve as potential biomarkers for monitoring disease, yet the true nature of their impact must be assessed in a locus- or gene-specific manner. This will reveal how key genes are misregulated in one of the many important cell types likely to contribute to autoimmune diseases.

EPIGENETICS OF AUTOIMMUNITY

Autoimmune diseases include greater than 80 disorders, affecting roughly 7% of the population.²⁰ The interplay of genetic and environmental factors have been proposed to predispose and progress autoimmune disorders. Exposure to UV radiation, infectious agents, tobacco smoke, environmental pollutants and alcohol consumption have all been implicated in autoimmunity.^{20,21} Gender bias towards female, age and latitude of the country also points toward environmental factors contributing toward disease predisposition.^{22–27} A frequent discordance of autoimmune diseases in monozygotic twins (MZ) suggests that non-genetic factors play a major role in determining disease susceptibility.²⁸ Epigenetic mechanisms regulate gene expression and are sensitive to external stimuli, bridging the gap between environmental and genetic factors. Circulating immune cells are in constant exposure to environmental factors. Epigenetic modifications contributing to the proper acquisition of cell fates of the immune system are well illustrated. miRNA and histone deacetylase (HDAC)-mediated regulation of CD8⁺ T killer cells and dendritic cells,^{29,30} and broader epigenetic regulation of T helper cell lineage establishments, further document the role of epigenetic mechanisms in manipulating immune systems and a concomitant progression towards autoimmunity.^{31–33} As there is a heterogeneous population of immune cells and equally different mechanisms of epigenetic regulations, there are numerous probabilities for the immune system to lose tolerance. Recently, epigenetic modifications have been increasingly identified in several autoimmune diseases; however, systemic autoimmune rheumatic diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) have received the most focus.

EPIGENETICS OF SLE

SLE is an autoimmune disease that affects multiple organs and eventually results in organ failure. The importance of DNA methylation in SLE has been consolidated by many observations. A global genome-wide DNA CpG methylation analysis of CD4⁺ T cells in SLE patients revealed differential methylation status of genes characteristic of the disease and methylation levels associated with the progression of the disease.^{34,35} The methylation level of peripheral blood T cells from patients with active SLE was found to be lower than that from patients with the inactive disease, showcasing a direct mechanism of DNA methylation in disease progression.³⁶ This loss of global methylation has been attributed to induce the activation of endogenous retro-viruses such that they erase imprinting signals and deregulate gene expression consequently breaking immune tolerance for active flaring of the disease.³⁷ Peripheral blood mononuclear cells from patients with SLE have shown changes to levels of specific miRNAs, global hypomethylation of B and T cells, and alterations in

DNA methyltransferase 1 (DNMT1) expression, suggesting that DNA CpG hypomethylation may be a critical element in SLE pathogenesis.^{35,38} A lower DNMT1 activity in CD4⁺ T cells has been associated with disease activity in SLE patients; however, no concluding evidence of association of other epigenetic modulators such as the Methyl-CpG Binding Domain proteins has emerged in SLE. SLE-associated hypomethylation is recapitulated in the mouse model of lupus (MRL/lpr), wherein T cells are globally hypomethylated. In addition, changes in DNA methylation levels were also detected in different lymphatic tissues, where SLE progression correlated with aging.³⁹ Taken together, a significant positive correlation between T-cell DNA hypomethylation, aging, development and progression of SLE are well established.^{40,41} The most comprehensive assessment of DNA methylation changes associated with SLE analyzed ~ 460 000 CpGs in CD4⁺ T cells, CD19⁺ B cells and CD14⁺ monocytes in approximately 50 patients and healthy controls.⁴² Methylation changes were generally hypomethylation in lupus patients and frequently clustered around genes, with T cells showing the greatest abundance of hypomethylated genes, followed by B cells and then monocytes. The authors conclude that hypomethylation sensitizes patients to an interferon gene response, which exacerbates inflammation.

Hypoacetylated histones H3 and H4 and H3K9 hypomethylation in CD4⁺ T cells were found to be a feature characteristic of SLE patients.⁴³ The lncRNA growth-arrest-specific 5 (GAS5), present in T cells, sensitizes cells to apoptosis by epigenetically regulating the activity of glucocorticoids, and has been linked to increased susceptibility to SLE.⁴⁴ An increased apoptotic rate and consequent accumulation of apoptotic debris is characteristic of SLE. The circulating hypomethylated apoptotic DNA potentially induces autoimmunity.⁴⁵ Moreover, changes in histone acetylation particularly affecting genes related to apoptosis have been shown to aggravate the disease by increasing the targets for autoantibodies in SLE.^{46–48} Furthermore, MZ twins discordant for SLE display differences in DNA methylation and expression of a number of genes associated with immune function.²⁸ Transcript copy number variants of various epigenetic modifying genes like *KAT2B*, *KAT3A/B*, *HDAC7* and *SIRT1* have been associated with active lupus. The decreased expression of the transcription factor (TF) RFX1 that recruits DNMT1 and HDAC1 to regulate the expression of CD11a and CD70 in CD4⁺ T cells resulting in increased autoreactivity are associated with SLE.⁴⁹

Lately, the aberrant expression of miRNAs has emerged as a risk factor in autoimmunity. miR146a is frequently cited as the regulator of inflammatory cytokines like TNF- α . The levels of this miRNA were decreased in SLE, but preferentially increased in RA.⁵⁰ The elevated levels of epi-miRs like miR126, miR21 and miR148 were shown to regulate DNMT in SLE patients, and this loss of DNMT resulting in subsequent DNA hypomethylation is a common feature of SLE.^{51,52}

EPIGENETICS OF RA

RA is a chronic inflammatory disease that largely affects peripheral joints. RA exhibits a substantial sex bias, 3:1 female to male. A recent genetic study identified the first X chromosome association, implicating the *IRAK1* gene.⁵³ Given the exclusive role of epigenetics in X-inactivation (DNA methylation, non-coding RNAs and H3K27me3 are all

important), hypothesizing that epi-mutations lead to genes escaping X-inactivation seems evident. As in SLE, T-cell DNA is demethylated in RA resulting in autoreactive T cells.^{36,54} Loss of DNMT1 activity leading to DNA hypomethylation and aberrant CpG methylation at the MMP13 gene causes increased MMP13 expression consequently leading to degradation of type II collagen in the cartilage.⁵⁵

Synovial fibroblasts with an aggressive and invasive behavior are a trait that can be attributed to the clinically active phase of the disease. The observation that this aggressiveness has been imprinted was evident because of the lower activity and production of DNMTs in proliferating and activated synovial fibroblasts in RA patients. Global changes in DNA methylation measured in fibroblast like synoviocytes showed distinct methylation profiles of RA patients particularly in genes with key roles in inflammation, immune responses and matrix deconvolution were found.⁵⁴

Histone-modifying enzymes like HDAC are shown to have different activities in target tissues of RA patients. Although HDAC expression is significantly increased in peripheral blood mononuclear cells and synovial fibroblasts, HDAC activity was lower in synovial tissues.^{56,57} KMT6, which is responsible for H3K27 methylation, was observed to be upregulated in synovial fibroblasts leading to the generation of autoantibodies against these H3K27me3 histones.⁵⁸ Epigenetic changes in the synovial tissues of RA patients that render it more susceptible to autoimmune attack have been documented. Studies confirming DNA hypomethylation, altered levels and activities of HDAC1/2, hyperacetylation of histones H3 and H4, and hypomethylation of histone H3 at lysine 9 in RA patients alone demonstrate how epigenetic changes in a target organ can modulate disease activity.^{57,59}

Similar to SLE, miR-146a has been found to be elevated in peripheral blood mononuclear cells, macrophages, CD3⁺ T-cell subsets and CD79a⁺ B cells of patients with RA.⁶⁰ miR-29b and miR140 downregulate the expression of HDAC4 and thus have been associated with the onset and progression of RA.^{61,62} Epigenetic machinery modifying miRNAs like miR-200a are shown to be disease modifying because they regulate the pre-osteoblast differentiation.⁶³

EPIGENETICS OF MULTIPLE SCLEROSIS (MS)

MS is a chronic inflammatory disease that results in the demyelination of neurons and subsequent neurodegeneration. It is believed that immune cells attack myelinated regions, resulting in inflammation and disruption of neural signaling. As in many other autoimmune disease, MZ twins have a very low concordance rate.⁶⁴ In MS, the evidence of epigenetic modification of HLA molecules contributing to the inheritance of disease susceptibility as well its transmission from mothers to offspring was shown often.^{65,66} In MS patients, hypomethylation of DNA from white matter of central nervous system but not from tissue in the thymus demonstrates epigenetic changes unique to the target organs.⁶⁷ A recent study using brain tissue and microarrays to survey over 450 000 CpGs found reproducible changes in DNA methylation. Such a study investigating a large number of CpGs is still lacking for a variety of blood cells from MS patients. This may, in part, be due to evidence that epigenetic repression by DNA methylation at the promoter of the *PADI2* gene is lost and leads to

increased citrullination of myelin basic protein. Such modification to myelin basic protein was suggested to destabilize the protein, leading to degradation and eliciting an autoimmune response.⁶⁷ In such a model, there is an epigenetic contribution, but in the target tissue. A similar model can be considered for other autoimmune diseases, for example, with synovial fibroblasts and RA. Brain tissue from MS patients also exhibits an increase in histone acetylation that corresponded with activation for genes that inhibit oligodendrocyte differentiation.⁶⁸ This presents another possible epigenetic mechanism within the target tissue; however, in this model, the authors suggest that the inflammatory environment induces the hyperacetylation. Therefore, immune cells cannot be ruled out as causative. A number of studies in humans and a mouse model of autoimmune encephalomyelitis have implicated a key role for T cells, especially Th1 and Th17 cells.^{69,70} Epigenetic investigation of the cells from MS patients should provide insight on misregulation.

EPIGENETICS OF TYPE 1 DIABETES

Like many other autoimmune diseases, MZ discordance in type 1 diabetes suggests a strong epigenetic influence on the emergence of diabetes. Plus, only 10–15% of newly described cases have a family history. Even when there is lack of a clear genetic contribution, assessing the contribution of epigenetic modifications to type 1 diabetes is largely lacking. Only recently have DNA methylation studies been conducted. While looking for methylation signatures in MZ twin pairs discordant for type 1 diabetes, Rakyan *et al.*⁷¹ identified 132 different CpG sites wherein DNA methylation difference significantly correlated with the diabetic state. Also Stefan *et al.*⁷² recently analyzed global methylation patterns in MZ twins and identified a set of 88 genes displaying significant methylation changes in discordant MZ twin pairs specially in genes belonging to pathways involved in immune and defense responses.

EPIGENOMICS AS A TOOL TO IDENTIFY CAUSAL GENETIC VARIANTS

Upon generating the first genome-wide map of enhancers based on chromatin marks,² this indicated such methods could be used to identify regulatory single nucleotide polymorphisms (SNPs) associated with disease.⁷³ Epigenomic methods such as global mapping of DNase hypersensitive sites and ChIP-seq for enhancer-localized histone modifications were recently used to determine whether SNPs from genome-wide association studies for autoimmune diseases are present at enhancer elements or distal TF-binding sites. DHS-seq was used to map TF footprints in a number of primary immune cells, including various T lymphocytes and B lymphocytes, monocytes and hematopoietic progenitors. These sites were overlapped with all disease in the NHGRI genome-wide association studies catalog to identify hundreds of autoimmune disease-associated SNPs at TF-binding sites.⁷⁴ Using H3K4me1 to map enhancers in activated T helper cells and early differentiating Th1 and Th2 cells identified over 600 enhancer SNPs associated with autoimmune diseases.⁷ Similarly, mapping of H3K4me1 and the co-occurring H3K27ac in various Th cells, Treg, Tmem, B cells and monocytes revealed that immune cell enhancers are highly enriched for autoimmune-associated SNPs relative to other cell types and relative to other functional annotations such as promoters and exons.⁹

The identification of enhancer SNPs suggests that these SNPs may disrupt TF binding, as was shown for a limited few,⁷ and therefore, alter enhancer activity. It also implies that enhancer chromatin marks may change between disease and normal immune cells. A recent study used ChIP-seq to map H3K4me2, which occurs at promoters and enhancer elements, in CD4⁺ T cells from healthy and asthmatic individuals. The greatest changes in enhancer signals were found in differentiation-specific enhancers for Th2 cells between the healthy and asthmatic groups.¹⁰ Similar to the previous study focusing on enhancers during T-cell differentiation,⁷ overlap with associated SNPs was greatest at enhancers utilized during differentiation. These two studies also predicted target genes of the disease-related enhancers to provide additional insight on genes involved in the pathogenesis of autoimmune diseases.

CONCLUSION

Although a large amount of information about the mechanisms of epigenetic (de)regulation in cancer and other diseases has been accumulated, these mechanisms are less well studied in autoimmune processes. Recent findings even suggest that epigenetic events can be drivers of cancer.⁷⁵ With low rates of heritability in several autoimmune diseases, epigenetic events may serve as drivers of these diseases as well. Though in most cases of autoimmune diseases, the precise epigenetic mechanisms involved remains to be resolved, improving our understanding of the role that epigenetic modifications play in the development of autoimmunity is likely to increase the prospects for controlling or preventing autoimmune diseases. Generating epigenome-wide association studies (EWAS)⁷⁶ as well as integrating genome-wide association studies for autoimmune diseases with methylome and chromatin epigenomics will maximize our understanding of how the interaction of genetic and epigenetic factors increases susceptibility to or protect against autoimmune disease, or can substantiate therapeutics of autoimmune disorders.

As we move forward with autoimmunity epigenomics, it is paramount that studies be conducted in key immune cell types. We are no longer afforded the luxury of extracting DNA from any or all blood cells. Several studies have begun to establish this paradigm.^{9,10,42,71} When coupled with cell-type-specific gene expression,⁷⁷ the locus-specific impact of epigenetic changes are likely to be revealed. Focusing on multipotent progenitors may provide insight on how epi-mutations disrupt cell balances that contribute to disease occurrence or recurrence. Modifications in progenitor cells may impair the cell's ability to respond appropriately to stimuli and subsequently differentiate to the appropriate daughter cell type. Identifying epi-mutations in these populations has the potential to determine a disease state before its onset.

Cell-specific changes in the epigenome and transcriptome will likely provide valuable biomarkers and mechanistic insight on misregulation, but this alone will not be definitive about causation. Just as genome-wide association studies have moved into pathway analysis to understand the contribution of numerous genes with associated SNPs,^{78,79} EWAS will require a similar approach as epigenetic changes are not highly localized but truly *epigenomic*, especially if the changes are a result of environmental insult. Another confounding factor regarding causation is distinguishing causative epi-mutation from

epigenetic changes induced from the cell being in a diseased state. The largest EWAS to date, using peripheral blood mononuclear cells from ~ 350 RA cases and controls, ascertained DNA methylation changes at ~ 450 000 CpGs and developed a 'causal inference test' to distinguish causative methylated CpG changes.⁸⁰ In this model, DNA methylation changes are linked to the genetic background, and it hypothesizes that associated variants at a locus can impart a locus-wide change in DNA methylation, and therefore the epigenome is a mediator of genetic risk. Few causative sites were identified, and those that were are within the MHC locus; therefore, genetics may have driven the epigenetic association to RA. Overall, the model is important to consider when interpreting epigenetic changes. Ultimately, only with an integrative analysis of genomics, epigenomics and transcriptomics are we likely to learn the true consequence of epi-mutations in the diseased cells.

Because the epigenome comprises DNA methylation, chromatin modifications and non-coding RNAs, generating such large amounts of data per cell type per person may sound daunting. Advances in sequencing technology make this more feasible. As these costs continue to come down, EWAS will be truly feasible, especially related to DNA methylation. The best examples of EWAS to date are those that have used microarrays with ~ 450 000 CpGs, however, sequencing-based methods that enrich for CpGs or capture regions of the genome can survey millions of CpGs, which is still a small subset of the 28 million CpGs in the human genome. Future EWAS would likely benefit from inclusion of several whole-genome bisulfite sequencing experiments, 'methylomes,' to define where most changes are occurring. When whole-methylome analysis has been applied to cellular differentiation, most changes in CpG methylation exist outside of promoter and genic regions and occur at enhancers,^{13,81,82} which are not well represented on the microarray platforms.

Exploiting the reversibility of epigenetic modifications owing to their metastable state opens up the possibility of developing novel targets for therapeutic treatment. Reversibility of epigenetic modifications makes enzymes such as DNMTs, HDACs and histone methyltransferases (HMTs) attractive drug targets. Small molecule inhibitors of histone modifiers and DNA methyltransferases are becoming increasingly available. These molecules will need to be investigated for their specificity and toxicity, and have the caveat of the lack of cellular specificity.

References

1. Ohtani-Fujita N, Fujita T, Aoike A, Osifchin NE, Robbins PD, Sakai T. CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. *Oncogene* 1993; 8: 1063–1067. [PubMed: 8455933]
2. Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 2009; 459: 108–112. [PubMed: 19295514]
3. Cui K, Zang C, Roh TY, Schones DE, Childs RW, Peng W et al. Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. *Cell Stem Cell* 2009; 4: 80–93. [PubMed: 19128795]
4. Hawkins RD, Hon GC, Lee LK, Ngo Q, Lister R, Pelizzola M et al. Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. *Cell Stem Cell* 2010; 6: 479–491. [PubMed: 20452322]

5. Hawkins RD, Hon GC, Yang C, Antosiewicz-Bourget JE, Lee LK, Ngo QM et al. Dynamic chromatin states in human ES cells reveal potential regulatory sequences and genes involved in pluripotency. *Cell Res* 2011; 21: 1393–1409. [PubMed: 21876557]
6. Xie W, Schultz MD, Lister R, Hou Z, Rajagopal N, Ray P et al. Epigenomic analysis of multilineage differentiation of human embryonic stem cells. *Cell* 2013; 153: 1134–1148. [PubMed: 23664764]
7. Hawkins RD, Larjo A, Tripathi SK, Wagner U, Luu Y, Lönnberg T et al. Global chromatin state analysis reveals lineage-specific enhancers during the initiation of human T helper 1 and T helper 2 cell polarization. *Immunity* 2013; 38: 1271–1284. [PubMed: 23791644]
8. Kaikkonen MU, Spann NJ, Heinz S, Romanoski CE, Allison KA, Stender JD et al. Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. *Mol Cell* 2013; 51: 310–325. [PubMed: 23932714]
9. Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 2015; 518: 337–343. [PubMed: 25363779]
10. Seumois G, Chavez L, Gerasimova A, Lienhard M, Omran N, Kalinke L et al. Epigenomic analysis of primary human T cells reveals enhancers associated with TH2 memory cell differentiation and asthma susceptibility. *Nat Immunol* 2014; 15: 777–788. [PubMed: 24997565]
11. Lara-Astiaso D, Weiner A, Lorenzo-Vivas E, Zaretzky I, Jaitin DA, David E et al. Immunogenetics. Chromatin state dynamics during blood formation. *Science* 2014; 345: 943–949. [PubMed: 25103404]
12. Ball MP, Li JB, Gao Y, Lee JH, LeProust EM, Park IH et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol* 2009; 27: 361–368. [PubMed: 19329998]
13. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J et al. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 2009; 462: 315–322. [PubMed: 19829295]
14. Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* 2009; 324: 929–930. [PubMed: 19372393]
15. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009; 324: 930–935. [PubMed: 19372391]
16. Yu M, Hon GC, Szulwach KE, Song CX, Jin P, Ren B et al. Tet-assisted bisulfite sequencing of 5-hydroxymethylcytosine. *Nat Protoc* 2012; 7: 2159–2170. [PubMed: 23196972]
17. Hon GC, Song CX, Du T, Jin F, Selvaraj S, Lee AY et al. 5mC oxidation by Tet2 modulates enhancer activity and timing of transcriptome reprogramming during differentiation. *Mol Cell* 2014; 56: 286–297. [PubMed: 25263596]
18. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S et al. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 2008; 40: 897–903. [PubMed: 18552846]
19. Bonasio R, Tu S, Reinberg D. Molecular signals of epigenetic states. *Science* 2010; 330: 612–616. [PubMed: 21030644]
20. Miller FW, Alfredsson L, Costenbader KH, Kamen DL, Nelson LM, Norris JM et al. Epidemiology of environmental exposures and human autoimmune diseases: findings from a National Institute of Environmental Health Sciences Expert Panel Workshop. *J Autoimmun* 2012; 39: 259–271. [PubMed: 22739348]
21. Selmi C, Leung PS, Sherr DH, Diaz M, Nyland JF, Monestier M et al. Mechanisms of environmental influence on human autoimmunity: a National Institute of Environmental Health Sciences expert panel workshop. *J Autoimmun* 2012; 39: 272–284. [PubMed: 22749494]
22. Cooney EL. Clinical indicators of immune restoration following highly active antiretroviral therapy. *Clin Infect Dis* 2002; 34: 224–233. [PubMed: 11740712]
23. Sandovici I, Smith NH, Nitert MD, Ackers-Johnson M, Uribe-Lewis S, Ito Y et al. Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. *Proc Natl Acad Sci USA* 2011; 108: 5449–5454. [PubMed: 21385945]

24. Waterland RA, Lin JR, Smith CA, Jirtle RL. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. *Hum Mol Genet* 2006; 15: 705–716. [PubMed: 16421170]
25. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* 2008; 105: 17046–17049. [PubMed: 18955703]
26. Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M et al. DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum Mol Genet* 2009; 18: 3769–3778. [PubMed: 19605411]
27. Waterland RA, Kellermayer R, Laritsky E, Rayco-Solon P, Harris RA, Travisano M et al. Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. *PLoS Genet* 2010; 6: e10012520.
28. Javierre BM, Fernandez AF, Richter J, Al-Shahrour F, Martin-Subero JJ, Rodriguez-Ubreva J et al. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome Res* 2010; 20: 170–190. [PubMed: 20028698]
29. Zhou L, Park JJ, Zheng Q, Dong Z, Mi Q. MicroRNAs are key regulators controlling iNKT and regulatory T-cell development and function. *Cell Mol Immunol* 2011; 8: 380–387. [PubMed: 21822298]
30. Song W, Tai YT, Tian Z, Hideshima T, Chauhan D, Nanjappa P et al. HDAC inhibition by LBH589 affects the phenotype and function of human myeloid dendritic cells. *Leukemia* 2011; 25: 161–168. [PubMed: 21102427]
31. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol* 2009; 9: 91–105. [PubMed: 19151746]
32. Wilson CB, Makar KW, Shnyreva M, Fitzpatrick DR. DNA methylation and the expanding epigenetics of T cell lineage commitment. *Semin Immunol* 2005; 17: 105–119. [PubMed: 15737572]
33. Allan RS, Zueva E, Cammas F, Schreiber HA, Masson V, Belz GT et al. An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. *Nature* 2012; 487: 249–253. [PubMed: 22763435]
34. Jeffries MA, Dozmorov M, Tang Y, Merrill JT, Wren JD, Sawalha AH. Genome-wide DNA methylation patterns in CD4+ T cells from patients with systemic lupus erythematosus. *Epigenetics* 2011; 6: 593–601. [PubMed: 21436623]
35. Lei W, Luo Y, Lei W, Luo Y, Yan K, Zhao S et al. Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis. *Scand J Rheumatol* 2009; 38: 369–374. [PubMed: 19444718]
36. Richardson B, Scheinbart L, Strahler J, Gross L, Hanash S, Johnson M. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 1990; 33: 1665–1673. [PubMed: 2242063]
37. Okada M, Ogasawara H, Kaneko H, Hishikawa T, Sekigawa I, Hashimoto H et al. Role of DNA methylation in transcription of human endogenous retrovirus in the pathogenesis of systemic lupus erythematosus. *J Rheumatol* 2002; 29: 1678–1682. [PubMed: 12180729]
38. Zhu X, Liang J, Li F, Yang Y, Xiang L, Xu J. Analysis of associations between the patterns of global DNA hypomethylation and expression of DNA methyltransferase in patients with systemic lupus erythematosus. *Int J Dermatol* 2011; 50: 697–704. [PubMed: 21595664]
39. Mizugaki M, Yamaguchi T, Ishiwata S, Shindo H, Hishinuma T, Nozaki S et al. Alteration of DNA methylation levels in MRL lupus mice. *Clin Exp Immunol* 1997; 110: 265–269. [PubMed: 9367411]
40. Golbus J, Palella TD, Richardson BC. Quantitative changes in T cell DNA methylation occur during differentiation and ageing. *Eur J Immunol* 1990; 20: 1869–1872. [PubMed: 2209694]
41. Zhang Z, Deng C, Lu Q, Richardson B. Age-dependent DNA methylation changes in the ITGAL (CD11a) promoter. *Mech Ageing Dev* 2002; 123: 1257–1268. [PubMed: 12020947]
42. Absher DM, Li X, Waite LL, Gibson A, Roberts K, Edberg J et al. Genome-wide DNA methylation analysis of systemic lupus erythematosus reveals persistent hypomethylation of

interferon genes and compositional changes to CD4⁺ T-cell populations. *PLoS Genet* 2013; 9: e1003678. [PubMed: 23950730]

43. Hu N, Qiu X, Luo Y, Yuan J, Li Y, Lei W et al. Abnormal histone modification patterns in lupus CD4⁺ T cells. *J Rheumatol* 2008; 35: 804–810. [PubMed: 18398941]
44. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal* 2010; 3: ra8. [PubMed: 20124551]
45. Krieg AM. CpG DNA: a pathogenic factor in systemic lupus erythematosus? *J Clin Immunol* 1995; 15: 284–292. [PubMed: 8576314]
46. Dieker JW, Fransen JH, van Bavel CC, Briand JP, Jacobs CW, Muller S et al. Apoptosis-induced acetylation of histones is pathogenic in systemic lupus erythematosus. *Arthritis Rheum* 2007; 56: 1921–1933. [PubMed: 17530637]
47. Plaue S, Muller S, van Regenmortel MH. A branched, synthetic octapeptide of ubiquitinated histone H2A as target of autoantibodies. *J Exp Med* 1989; 169: 1607–1617. [PubMed: 2541220]
48. van Bavel CC, Dieker J, Muller S, Briand JP, Monestier M, Berden JH et al. Apoptosis-associated acetylation on histone H2B is an epitope for lupus autoantibodies. *Mol Immunol* 2009; 47: 511–516. [PubMed: 19747733]
49. Zhao M, Wu X, Zhang Q, Luo S, Liang G, Su Y et al. RFX1 regulates CD70 and CD11a expression in lupus T cells by recruiting the histone methyltransferase SUV39H1. *Arthritis Res Ther* 2010; 12: R227. [PubMed: 21192791]
50. Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. *Transl Res* 2011; 157: 163–179. [PubMed: 21420027]
51. Zhao S, Wang Y, Liang Y, Zhao M, Long H, Ding S et al. MicroRNA-126 regulates DNA methylation in CD4⁺ T cells and contributes to systemic lupus erythematosus by targeting DNA methyltransferase 1. *Arthritis Rheum* 2011; 63: 1376–1386. [PubMed: 21538319]
52. Pan W, Zhu S, Yuan M, Cui H, Wang L, Luo X et al. MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4⁺ T cells by directly and indirectly targeting DNA methyltransferase 1. *J Immunol* 2010; 184: 6773–6781. [PubMed: 20483747]
53. Eyre S, Bowes J, Diogo D, Lee A, Barton A, Martin P et al. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat Genet* 44: 1336–1340 2012.
54. Nakano K, Whitaker JW, Boyle DL, Wang W, Firestein GS. DNA methylome signature in rheumatoid arthritis. *Ann Rheum Dis* 2013; 72: 110–117. [PubMed: 22736089]
55. Bui C, Barter MJ, Scott JL, Xu Y, Galler M, Reynard LN et al. cAMP response element-binding (CREB) recruitment following a specific CpG demethylation leads to the elevated expression of the matrix metalloproteinase 13 in human articular chondrocytes and osteoarthritis. *FASEB J* 2012; 26: 3000–3011. [PubMed: 22505473]
56. Gillespie J, Savic S, Wong C, Hempshall A, Inman M, Emery P et al. Histone deacetylases are dysregulated in rheumatoid arthritis and a novel histone deacetylase 3-selective inhibitor reduces interleukin-6 production by peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Rheum* 2012; 64: 418–422. [PubMed: 21952924]
57. Huber LC, Brock M, Hemmatazad H, Giger OT, Moritz F, Trenkmann M et al. Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. *Arthritis Rheum* 2007; 56: 1087–1093. [PubMed: 17393417]
58. Gray SG. Perspectives on epigenetic-based immune intervention for rheumatic diseases. *Arthritis Res Ther* 2013; 15: 207. [PubMed: 23510070]
59. Karouzakis E, Gay RE, Michel BA, Gay S, Neidhart M. DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum* 2009; 60: 3613–3622. [PubMed: 19950268]
60. Ceribelli A, Yao B, Dominguez-Gutierrez PR, Chan EK. Lupus T cells switched on by DNA hypomethylation via microRNA? *Arthritis Rheum* 2011; 63: 1177–1181. [PubMed: 21538310]
61. Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I et al. The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. *FEBS Lett* 2006; 580: 4214–4217. [PubMed: 16828749]

62. Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM et al. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J Biol Chem* 2009; 284: 15676–15684. [PubMed: 19342382]
63. Eades G, Yao Y, Yang M, Zhang Y, Chumsri S, Zhou Q. miR-200a regulates SIRT1 expression and epithelial to mesenchymal transition (EMT)-like transformation in mammary epithelial cells. *J Biol Chem* 2011; 286: 25992–26002. [PubMed: 21596753]
64. Willer CJ, Dymont DA, Risch NJ, Sadovnick AD, Ebers GC. Twin concordance and sibling recurrence rates in multiple sclerosis. *Proc Natl Acad Sci USA* 2003; 100: 12877–12882. [PubMed: 14569025]
65. Chao MJ, Ramagopalan SV, Herrera BM, Lincoln MR, Dymont DA, Sadovnick AD et al. Epigenetics in multiple sclerosis susceptibility: difference in transgenerational risk localizes to the major histocompatibility complex. *Hum Mol Genet* 2009; 18: 261–266. [PubMed: 19098025]
66. Chao MJ, Ramagopalan SV, Herrera BM, Orton SM, Handunnetthi L, Lincoln MR et al. MHC transmission: insights into gender bias in MS susceptibility. *Neurology* 2011; 76: 242–246. [PubMed: 21209377]
67. Mastronardi FG, Noor A, Wood DD, Paton T, Moscarello MA. Peptidyl argininedeiminase 2 CpG island in multiple sclerosis white matter is hypomethylated. *J Neurosci Res* 2007; 85: 2006–2016. [PubMed: 17469138]
68. Pedre X, Mastronardi F, Bruck W, López-Rodas G, Kuhlmann T, Casaccia P. Changed histone acetylation patterns in normal-appearing white matter and early multiple sclerosis lesions. *J Neurosci* 2011; 31: 3435–3445. [PubMed: 21368055]
69. Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Exp Immunol* 2010; 162: 1–11. [PubMed: 20682002]
70. Severson C, Hafler DA. T-cells in multiple sclerosis. *Results Probl Cell Differ* 2010; 51: 75–98. [PubMed: 19582415]
71. Rakyan VK, Beyan H, Down TA, Hawa MI, Maslau S, Aden D et al. Identification of type 1 diabetes-associated DNA methylation variable positions that precede disease diagnosis. *PLoS Genet* 2011; 7: e1002300. [PubMed: 21980303]
72. Stefan M, Zhang W, Concepcion E, Yi Z, Tomer Y. DNA methylation profiles in type 1 diabetes twins point to strong epigenetic effects on etiology. *J Autoimmun* 2014; 50: 33–37. [PubMed: 24210274]
73. Hawkins RD, Hon GC, Ren B. Next-generation genomics: an integrative approach. *Nat Rev Genet* 2010; 11: 476–486. [PubMed: 20531367]
74. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012; 337: 1190–1195. [PubMed: 22955828]
75. De Carvalho DD, Sharma S, You JS, Su SF, Taberlay PC, Kelly TK et al. DNA methylation screening identifies driver epigenetic events of cancer cell survival. *Cancer Cell* 2012; 21: 655–667. [PubMed: 22624715]
76. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. *Nat Rev Genet* 2011; 12: 529–541. [PubMed: 21747404]
77. Hu X, Kim H, Stahl E, Plenge R, Daly M, Raychaudhuri S. Integrating autoimmune risk loci with gene-expression data identifies specific pathogenic immune cell subsets. *Am J Hum Genet* 2011; 89: 496–506. [PubMed: 21963258]
78. Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat Rev Genet* 2013; 14: 661–673. [PubMed: 23917628]
79. Viatte S, Plant D, Raychaudhuri S. Genetics and epigenetics of rheumatoid arthritis. *Nat Rev Rheumatol* 2013; 9: 141–153. [PubMed: 23381558]
80. Liu Y, Aryee MJ, Padyukov L, Fallin MD, Hesselberg E, Runarsson A et al. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat Biotechnol* 2013; 31: 142–147. [PubMed: 23334450]

81. Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, Schöler A et al. DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* 2011; 480: 490–495. [PubMed: 22170606]
82. Hon GC, Rajagopal N, Shen Y, McCleary DF, Yue F, Dang MD et al. Epigenetic memory at embryonic enhancers identified in DNA methylation maps from adult mouse tissues. *Nat Genet* 2013; 45: 1198–1206. [PubMed: 23995138]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript