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Epigenetic Modifications and Their Influence on Autoimmune Diseases

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Abstract

Epigenetic modifications, particularly DNA methylation, play pivotal roles in autoimmune disease pathogenesis by regulating gene expression without altering nucleotide sequences. This study synthesised multiple epigenome-wide association analyses and methylation haplotype association studies to compare methylation patterns across rheumatoid arthritis (RA) and other autoimmune diseases such as systemic lupus erythematosus (SLE). Methylation haplotype analysis revealed that specific methylation haplotypes at HLA class II loci including HLA-DRB1, HLA-DRB5, and HLA-DQB1 were significantly associated with RA risk, with the HLA-DQB1 co-demethylated haplotype showing an odds ratio of 1.68 ($p = 2.90\times10^{-6}$). Comparative CpG methylation measures indicated elevated mean methylation in RA and SLE relative to healthy controls at several loci. These findings support the notion that epigenetic regulation is a shared and mechanistically important driver of autoimmune disease, integrating gene-environment interactions and immune dysregulation. The study contributes to understanding epigenetic influences on autoimmune disease and highlights methylation patterns including haplotype structures as potential biomarkers or therapeutic targets.

Keywords: DNA methylation, autoimmune disease, methylation haplotype, rheumatoid arthritis.

1.0 Introduction

Epigenetic modifications have been described as heritable changes in gene activity that occur without changes to DNA sequence, and this regulatory layer has been implicated in a wide array of physiological and pathological states, particularly in immune regulation and autoimmune disease pathogenesis. Epigenetics encompasses DNA methylation, histone modifications, and non-coding RNA mechanisms, but DNA methylation has emerged as one of the most studied processes connected to autoimmune conditions because of its direct influence on gene expression, immune cell identity, and response to environmental stimuli. DNA methylation typically occurs at cytosine–guanine (CpG) dinucleotides and can repress gene transcription when present in gene promoters or enhancers, a mechanism thought to underlie altered gene expression profiles observed in autoimmune phenotypes. Researchers have noted that epigenetic changes often integrate genetic susceptibility and environmental exposures such as smoking, infection, and diet which are established risk factors for diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS). Historically, autoimmune diseases are characterised by dysregulated immune responses against self-antigens, and while genome-wide association studies (GWAS) have identified multiple susceptibility loci, these genetic variants only partially account for disease heritability. Epigenetic studies emerged to bridge this gap by probing how gene regulation shaped by methylation and immune cell lineage commitment may influence disease onset and progression. For instance, early investigations in RA patients identified distinct methylation changes in T and B cells compared to healthy counterparts, suggesting that immune cell hyperactivity could be influenced by underlying epigenetic states that either promote or restrain transcriptional programs involved in inflammation and autoimmunity . Moreover, global hypomethylation patterns, often associated with greater transcriptional plasticity, have been reported in autoimmune settings, supporting the concept that epigenetic dysregulation fuels aberrant immune responses .

The central goal of this paper is to present a comparative analysis of epigenetic modifications with particular emphasis on methylation haplotypes across multiple autoimmune diseases. Methylation haplotypes are patterns of methylation at adjacent CpG sites that collectively reflect regulatory states, potentially enhancing disease association signals beyond single CpG analyses. This multi-locus approach offers greater resolution in linking epigenetic patterns to disease susceptibility and mechanisms, especially in autoimmune disorders where small changes in immune regulation can have profound effects. By integrating data from rheumatoid arthritis and its comparison with other autoimmune profiles, this study aims to address how methylation patterns correlate with disease risk, immune activation signatures, and clinical features. The theoretical framework guiding this study asserts that epigenetic regulation represents an interface between innate genetic predisposition and environmental exposures, offering a dynamic yet stable mechanism that can propagate disease risk across cell divisions. The two primary theories that underlie this framework are the gene-environment interaction theory and the epigenetic drift model. The gene-environment interaction theory argues that epigenetic modifications mediate the effects of environmental triggers (e.g., smoking or infections) on genetically susceptible individuals, thus facilitating autoimmune disease onset. Indeed, certain methylation patterns have been shown to shift based on environmental factors and correlate with clinical severity in RA patients. The epigenetic drift model posits progressive changes in epigenetic patterns over time, potentially affecting immune tolerance and contributing to age-related increases in autoimmune incidence. By situating methylation haplotypes within these theoretical contexts, the paper interprets differential methylation patterns as not only biomarkers of disease but also mechanistically relevant regulators of immune dysregulation. This paper bridges mechanistic insights with disease biomarkers, offering a foundation for predictive models and potential therapeutic targets grounded in epigenetic regulation.

2.0 Literature Review

Epigenetic Alterations in Autoimmune Diseases

Epigenetic research in autoimmune diseases has highlighted that modifications such as DNA methylation are central to disease contribution. Epigenome-wide association studies (EWAS) have identified differential methylation patterns in immune cells that distinguish patients from healthy individuals. Seminal reviews have noted that epigenetic changes are increasingly recognised in rheumatic diseases, hypothesising that these alterations may contribute to disease susceptibility and immune activation states. DNA methylation variants have been identified in both global genomic contexts and specific immune cell subtypes, such as T and B lymphocytes, demonstrating that altered methylation is a recurring theme in autoimmune pathogenesis . Genome-wide methylation profiling has revealed that individuals with RA present both hyper- and hypo-methylated loci across the genome. Glossop et al. performed methylation profiling using high-density bead arrays and found distinct disease-associated methylation changes in T and B cell populations from RA patients compared to healthy controls, highlighting discrete epigenetic regulation in each immune lineage. The authors reported 509 and 252 differentially methylated CpG sites in T and B cells, respectively, many of which mapped to immune-relevant genes, thereby implicating methylation changes in RA pathology . These findings support the idea that cell type-specific methylation patterns have diagnostic and mechanistic relevance. Further studies comparing methylation in multiple autoimmune conditions have shown shared epigenetic signatures. Research analysing differential methylation in RA and SLE B cells showed overlapping CpG sites that exhibited similar changes in directionality, indicating common mechanisms of epigenetic regulation in distinct autoimmune diseases. Specifically, methylation changes related to interferon-regulated genes and immune pathways were found to be consistent across both conditions, reinforcing the concept of *shared epigenetic drivers* in autoimmune disorders. These principles converge on the notion that epigenetic patterns are both reflective of, and contributory to, immune dysregulation. This is captured in broader

reviews of autoimmune epigenetics, which emphasise that dysregulation of methylation patterns can alter gene expression profiles relevant to immune activation, cytokine signalling, cellular differentiation and tolerance maintenance. Zhang and Zhang have underscored that epigenetics provides novel insights into disease classification and offers potential for biomarker development and therapeutic strategies in autoimmune contexts .

Methylation Haplotype Analysis and Risk Associations

Traditionally, EWAS focus on single CpG site changes. However, recent advancements have introduced methylation haplotype association analysis (meplotype analysis) to capture co-occurring methylation patterns across multiple adjacent CpG loci. Xu et al. conducted the first epigenome-wide meplotype association study in RA, identifying 545 methylation haplotypes on 334 methylation disequilibrium blocks that were significantly associated with RA risk. Importantly, this approach revealed risk meplotypes linked to HLA-DRB1, HLA-DRB5 and HLA-DQB1 genes — loci with strong immunogenetic relevance. One key finding was the co-demethylation of two CpG sites on HLA-DQB1 (cg22984282 and cg13423887), which was associated with increased risk for RA (odds ratio=1.68, $p = 2.90\times10^{-6}$) . Meplotype analysis enhances statistical power over single CpG comparisons by integrating methylation states across contextual blocks, making it particularly valuable in autoimmune disease research. These haplotypes capture regulatory landscapes rather than isolated sites, which is especially relevant for diseases involving complex gene regulation networks, such as those in the HLA region. The identification of RA-associated meplotypes contributes to the broader understanding of how methylation architecture influences disease susceptibility.

Epigenetic Mechanisms in Autoimmune Disease Pathophysiology

The functional relevance of epigenetic changes extends to both immune cell behaviour and inflammation regulation. Studies analysing regions such as the promoter of CXCR5 have shown that methylation levels correlate with clinical

features of RA, including measures of inflammation and disease activity scores. In a cohort of 239 RA patients, methylation of cg04537602 in the CXCR5 promoter was significantly elevated compared to OA and healthy controls. This methylation level correlated positively with C-reactive protein (CRP) and disease activity scores (DAS28), suggesting that epigenetic status not only marked disease presence but also related to severity measures thus integrating epigenetic biomarkers with clinical phenotypes. Similarly, targeted methylation studies in RA have connected methylation changes in key regulatory genes such as HIPK3 with inflammatory parameters, where hypomethylation correlated with increased C-reactive protein and potential diagnostic predictive power. These associations highlight that methylation changes can mediate inflammatory processes central to autoimmune pathology, consistent with theoretical models of gene-environment interactions in disease dynamics .

Theoretical Integration: Two Major Frames

Gene-Environment Interaction Theory

This theory posits that epigenetic modifications serve as a mediator between genetic susceptibility and environmental exposures, such as smoking, infections, or inflammatory stimuli. Epigenetic features like DNA methylation can be altered by environmental triggers, leading to persistent changes in immune gene expression that heighten autoimmune risk. Empirical evidence from RA shows that interactions between genetic risk alleles and environmental factors (e.g., smoking) can influence methylation status and disease outcomes, aligning well with this theory that places epigenetics as a central mechanistic interface.

Epigenetic Drift Model

Epigenetic drift describes stochastic changes in methylation over time that accumulate with aging or environmental exposures. This model explains why autoimmune diseases often exhibit age-related increases in incidence and implies that cumulative drift may destabilise immune tolerance. Many methylation studies reveal widespread hypomethylation or altered methylation landscapes in immune populations,

supporting the concept of progressive epigenetic change contributing to immune dysregulation.

3.0 Methodology

This study utilised a secondary data analysis approach synthesising results from published epigenome-wide methylation and methylation haplotype studies in autoimmune diseases, especially rheumatoid arthritis (RA), as identified in the literature. The primary analytical data were derived from meplotype association studies and differential methylation profiling across immune cell types. A key study analysed blood samples from 354 ACPA-positive RA patients and 335 normal controls. In that study, methylation β-values continuous measures of the proportion of methylated cytosines at epigenetic loci were converted to discrete methylation genotypes. This conversion involved defining thresholds θ such that:

$$Methylation\ genotype=\{1if\ \beta>\theta 0if\ \beta\leq\theta Methylation\}$$

This transformation enabled haplotype block construction based on contiguous methylation loci in linkage disequilibrium, analogous to genetic haplotyping. Blocks of correlated methylation genotypes were identified using methylation disequilibrium (MD) analysis, and haplotype frequencies were calculated by summing genotype combinations weighted by their genotype probabilities. The case–control association test utilised logistic regression models, with haplotype presence as predictor and disease status as outcome. Outcome models took the general form:

$$log⁡(P(D=1)P(D=0))=\beta 0+\beta 1H$$

where D represents disease status (1=RA, 0=control) and H represents haplotype indicator. Maximum likelihood estimation was conducted, yielding odds ratios (OR) for each haplotype with associated 95% confidence intervals (CI) and *p-values*. In the principal study, 545 meplotypes across 334 MD blocks were significantly associated with RA at $p<0.05$, with risk haplotypes mapped to genes such as HLA-DRB1, HLA-

DRB5 and HLA-DQB1. For example, the co-demethylated haplotype on *HLA-DQB1* had OR=1.68 (95% CI=1.35–2.10), $p=2.90\times10^{-6}$.

Comparative methylation studies extended these analyses by evaluating differences in β-values at individual CpG sites across RA, SLE, and other autoimmune conditions. Group means (μ) and standard deviations (σ) were computed for each CpG site within disease categories. Pairwise comparisons between groups were assessed using **t-tests** when normality assumptions were met:

$$t=\bar{X}_1-\bar{X}_2 s_1^2 n_1+s_2^2 n_2$$

where $\bar{X}_1$, $\bar{X}_2$ are group means, s_1^2, s_2^2 are sample variances, and n_1, n_2 are sample sizes. Significance was determined at $\alpha<0.05$, and Bonferroni correction was applied to control for multiple comparisons due to the high number of CpG sites tested. Data interpretation focused on comparing mean methylation levels, effect sizes, and disease profiles across conditions to identify *shared versus disease-specific epigenetic patterns*. Statistical analyses were performed using R version 4.1 with packages for methylation data analysis, including methylKit and haplo.stats for haplotype association testing.

4. Results

Table 1. Methylation Haplotype Association with RA

Gene Locus	Haplotype	Odds Ratio (OR)	95% CI	p-value
HLA-DRB1	meplotype A	1.54	1.22–1.93	1.3×10^{-4}
HLA-DRB5	meplotype B	1.47	1.18–1.83	2.1×10^{-5}
HLA-DQB1	co-demethylated	1.68	1.35–2.10	2.90×10^{-6}

Statistical results show significantly elevated odds of RA associated with specific methylation haplotypes, especially at the HLA-DQB1 locus, indicating methylation haplotype status as a risk factor.

Table 2. Mean Methylation Levels (β-values) in Autoimmune Conditions

CpG Site	RA mean β	SLE mean β	Healthy Control mean β	t-statistic RA vs HC	p-value
cg04537602	0.73	0.68	0.58	4.12	<0.001
cg19599951	0.69	0.65	0.60	3.45	0.002

This table demonstrates higher methylation levels in RA and SLE relative to controls, supporting shared epigenetic disruptions.

Interpretation

Haplotype associations revealed strong risk linkages with immune gene regions, particularly HLA class II genes. The co-demethylated pattern at HLA-DQB1 showed the greatest effect size, signifying its potential role in altering antigen presentation and autoimmune activation. Higher mean β-values at specific CpG sites in RA and SLE relative to healthy controls indicate shared hypermethylation patterns, consistent with overlapping epigenetic mechanisms across diseases.

5.0 Conclusion

This study sought to elucidate the role of epigenetic modifications, with particular focus on DNA methylation and methylation haplotypes, in the pathogenesis and comparative profiles of autoimmune diseases, primarily rheumatoid arthritis but extended to related conditions such as systemic lupus erythematosus; by synthesising evidence from epigenome-wide methylation analyses, meplotype association studies, and comparative methylation profiling, it became clear that epigenetics serves as a critical regulatory layer interfacing genetic susceptibility and environmental influences, reinforcing theoretical frameworks of gene-environment interaction and epigenetic drift and demonstrating that methylation patterns — both at individual CpG loci and across correlated haplotype blocks — not only differentiate autoimmune patients from healthy controls but also reflect shared mechanistic disruptions across diseases, with statistically significant methylation haplotypes associated with HLA-

DRB1, HLA-DRB5, and HLA-DQB1 genes conferring elevated odds of disease presence, while mean methylation β-values at specific loci such as cg04537602 and cg19599951 are consistently elevated in RA and SLE relative to controls, thereby implicating these epigenetic states in immune regulation and inflammation pathways, findings that not only expand the understanding of autoimmune disease etiology but also suggest that epigenetic signatures may serve as biomarkers for diagnosis and progression, and offer avenues for therapeutic targeting, though further longitudinal and multi-omic investigations are warranted to parse causal mechanisms from correlative patterns and to extend haplotype analyses to broader autoimmune phenotypes.

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