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IN SILICO APPROACH TO GENETICS AND EPIGENETIC MECHANISMS IN SYSTEMIC LUPUS ERYTHEMATOSUS: FOCUS ON IMMUNE RESPONSE GENES

IN SİLİKO YAKLAŞIMLA SİSTEMİK LUPUS ERİTEMATOZUS'TA GENETİK VE EPİGENETİK MEKANİZMALAR: BAĞIŞIKLIK YANITI GENLERİNE ODAKLANMA

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ABSTRACT

Objective: Systemic lupus erythematosus (SLE) is a multifaceted autoimmune condition characterised by irregular immune reactions and genetic susceptibility. The aim of this in silico study was to investigate immune response genes during SLE pathogenesis, focusing on genetic and epigenetic regulation.

Materials and Methods: This study involving 1255 patients with SLE and 453 control subjects aimed to identify genes associated with the immune response and examine their expression and DNA methylation levels in both patients with SLE and controls. The study design utilized the ADEX open-access database.

Results: The study identified 10 differentially expressed immune response-related genes (*FAM117B, ZNF395, PGAP3, PIK3IP1, HLA-DMB, HLA-DPA1, HLA-DRB3, HLA-DQA1, HLA-DPB1,* and *CCL5*) in SLE pathogenesis, with 78 corresponding methylation sites. Upregulation of *HLA-DQA1, HLA-DMB,* and *CCL5* was observed in patients with SLE, whereas the remaining genes exhibited decreased expression. *HLA-DPA1, HLA-DPB1, HLA-DMB, HLA-DQA1, CCL5,* and *ZNF395* were found to be *the most hypermethylated genes in SLE. Methylation of the CpG islands of HLA-DPB1, CCL5, FAM117B, and ZNF395* is correlated with their expression levels in SLE.

Conclusion: Our findings shed light on the genetic and epigenetic mechanisms underlying SLE and underscore the importance of these genes in immune dysregulation and disease progression. Further research on the functional significance of these genes could provide valuable insights into the pathogenesis of SLE and potential therapeutic targets.

Keywords: Systemic lupus erythematosus, Immune response-related genes, HLA-DPB1, epigenetic regulation, FAM117B, ZNF395

ÖZ

Amaç: Sistemik lupus eritematozus (SLE), düzensiz bağışıklık tepkileri ve genetik yatkınlık ile karakterize edilen çok yönlü otoimmün bir durumdur. Bu in siliko çalışmanın amacı, SLE patogenezinde bağışıklık yanıtı genlerini incelemek ve genetik ile epigenetik düzenlemelere odaklanmaktır.

Gereç ve Yöntemler: 1255 SLE hastası ve 453 kontrol grubunu içeren bu çalışma, bağışıklık yanıtı ile ilişkilendirilen genleri belirlemeyi ve hem SLE hastalarında hem de kontrol grubunda bunların ekspresyon ve DNA metilasyon düzeylerini incelemeyi amaçlamaktadır. Çalışmada, açık erişimli bir veri tabanı olan ADEX veritabanını kullanmıştır.

Bulgular: Çalışma, SLE patogenezinde on farklı şekilde ekspresyon gösteren bağışıklık yanıtı ile ilişkilendirilen genleri (*FAM117B, ZNF395, PGAP3, PIK3IP1, HLA-DMB, HLA-DPA1, HLA-DRB3, HLA-DQA1, HLA-DPB1* ve *CCL5*) ve bunlara karşılık gelen 78 metilasyon sitesini belirlemiştir. SLE hastalarında *HLA-DQA1, HLA-DMB* ve *CCL5*'in aşırı ekspresyonu gözlenirken, diğer genlerin ekspresyonunda azalma görülmüştür. *HLA-DPA1, HLA-DPB1, HLA-DMB, HLA-DQA1, CCL5* ve *ZNF395*'in SLE'de en fazla hipermetile edilen genler olduğu bulunmuştur. *HLA-DPB1, CCL5, FAM117B* ve *ZNF395*'in CpG adalarındaki metilasyonları, bunların SLE'deki ekspresyon düzeyleri ile ilişkili bulunmuştur.

Sonuç: Bu bulgular, SLE'nin genetik ve epigenetik mekanizmalarına ışık tutmakta ve bu genlerin bağışıklık düzensizliği ve hastalık ilerlemesi üzerindeki önemini vurgulamaktadır. Bu genlerin fonksiyonel önemine ilişkin daha fazla araştırma, SLE patogenezine ve potansiyel terapötik hedeflere yönelik değerli bilgiler sağlayabilir.

Anahtar Kelimeler: Sistemik lupus eritematozus, İmmün yanıtla ilişkili genler, HLA-DPB1, Epigenetik düzenleme, FAM117B, ZNF395

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a persistent autoimmune condition that primarily affects women. The global prevalence of SLE varies between 43.7 cases per 100,000 people and is estimated to be 3.41 million people worldwide (1). SLE affects many organs, including the skin, joints, kidneys, heart, and brain. The pathogenesis of SLE involves dysregulated innate and adaptive immune responses regulated by diverse immune cell populations, inflammatory mediators, and cytokines. This dysregulation leads to tissue and organ damage through the formation of autoantibodies and immune complexes (2).

Genetic susceptibility plays a crucial role in this process, with multiple susceptibility loci identified through genome-wide association (GWAS) and the study of candidate genes. Many of these susceptibility loci are located within or near immune response genes; this underlines their importance in SLE susceptibility and disease progression (3, 4). In recent decades, a thorough investigation has revealed many genetic and immunological elements influencing the development of SLE, notably focusing on immune response genes. The interplay between these genes and SLE pathology is intricate and multifaceted, reflecting the delicate equilibrium between host defence mechanisms and autoimmune responses (5, 6). Genes responsible for immune responses encode various proteins vital for immune control, modulation, and regulation of effector functions, thus playing key roles in shaping adaptive immune responses (7). The immune response genes most extensively studied in the context of SLE include those encoding components of the MHC, particularly the human leukocyte antigen (HLA) region. HLA molecules play critical roles in antigen presentation and immune regulation, and some HLA alleles are strongly associated with an increased risk of SLE and altered disease phenotypes (8). Additionally, genes encoding cytokines and chemokine such as tumor necrosis factor (TNF), C-C motif chemokine ligands (CCLs), and interleukins (Ils), have been shown to play a role in SLE susceptibility and disease pathogenesis, influencing immune cell activation and inflammation (9). Although certain HLA alleles are correlated with an increased risk of SLE, the exact mechanisms underlying their contribution to the disease remain unclear.

Furthermore, advances in high-throughput genomic and transcriptomic technologies have facilitated the comprehensive profiling of gene expression patterns and epigenetic modifications in patients with SLE, providing deeper insights into the molecular mechanisms underlying disease pathogenesis. Epigenetic regulation, which encompasses non-coding RNA, histone modifications, and methylation, has emerged as a critical factor affecting the expression and function of immune response genes in SLE (10-12). Dysregulated epigenetic marks contribute to altered gene expression profiles and abnormal immune responses (13). In light of these advances, understanding the complex relationships between immune response genes and SLE disease holds great promise for elucidating the mechanisms driving autoimmunity, identifying new biomarkers for diagnosis and prognosis, and developing targeted therapeutic strategies to restore immune homeostasis and improve the immune system. However, the ongoing investigation of immune response genes in the context of SLE represents a crucial frontier in autoimmune research, with profound implications for improving patient outcomes and advancing personalized medicine approaches for treating this complex and heterogeneous disorder (14).

Given the role of immune dysregulation in SLE pathology, new therapeutic approaches and genetic and epigenetic biomarkers. This study provided valuable evidence that immune response genes involved in the development of SLE are under epigenetic regulation. These findings offer new insights into the regulatory role of epigenetic factors in the immune response implicated in SLE pathogenesis, thereby enhancing our understanding of the molecular mechanisms involved.

MATERIALS AND METHODS

Datasets

Data sets for SLE were obtained from the GEO database. (http://www.ncbi.nlm.nih.gov/), (15). In total, 11 datasets were downloaded, namely GSE45291, GSE65391, GSE110169, GSE108497, GSE72509, GSE61635, GSE38351, GSE10325, GSE110174, GSE50772, and GSE82221. Among these, GSE45291, GSE65391, GSE110169, GSE108497, GSE72509, GSE61635, and GSE110174 involved whole blood (WB) samples, and high-throughput sequencing was employed for the experimental analysis. On the other hand, GSE38351, GSE10325, GSE50772, and GSE82221 utilized peripheral blood mononuclear cells (PBMC), with the experimental type being array-based.

Data analysis and visualization

In this study, the expression and methylation status of CpGs in relation to their known functions in disease progression were examined in SLE. The list of immune response genes with fully hypermethylated or hypomethylated CpGs associated with promoter regions and their expression were examined for enrichment in blood transcription modules (BTMs) using the predefined module DC.M8.83 (16). To ensure the integrity of methylation level assessment in both normal and SLE samples, two distinct peaks were identified, one near 0 and the other near 1. Methylation levels tended to be minimal at approximately 0 and maximal at approximately 1. Subsequently, the beta values are normalized using the ADEX tool to mitigate potential biases. Methylation sites were annotated using the GSE82221 platform file. Subsequently, genes associated with these distinct methylation sites were extracted. Data analysis and visualization were exclusively conducted within the ADEX programming environment (17).

Statistical analysis

The analyses utilized several R/Bioconductor packages, such as limma, to perform differential expression analysis between disease and control samples. The typical visualization approach includes a heatmap that displays the expression levels of the top differentially expressed genes (DEGs), which are arran-

ged based on their adjusted p values using the False Discovery Rate (FDR) method. Gene expression profiles from RNA-Seq platforms were analyzed using the standard pipeline of the DESeq2 package (18). In both cases, the differential expression analysis provided p values, adjusted p values by False Discovery Rate (FDR), and log2 fold change (FC) values. Group mean comparisons were performed using the Wilcoxon test when data did not conform to a normal distribution. To evaluate the normality of the expression data, the Shapiro test function was used in R (19).

RESULTS

Potential link between 10 immune response genes and the development of SLE

With the DC.M8.83 transcriptional module previously designed by Chaussabel et al., 10 potentially relevant genes were identified that were significantly implicated in the progression of SLE disease sites: *FAM117B, ZNF395, PGAP3, PIK3IP1, HLA-DMB, HLA-DPA1, HLA-DRB3, HLA-DQA1, HLA-DPB1,* and *CCL5*. Subsequently, the expression levels of 10 immune response-related genes were examined in a group of 1708 individuals, comprising 1255 patients with SLE and 453 case controls. SLE disease samples were designated cases in each dataset, whereas normal samples served as controls. The specifications of the 11 datasets are summarized in Table 1. The expression changes of these 10 genes were compared in GSE124939 (keratinocytes), GSE13887 (CD3-positive T cells), GSE11907 (blood), GSE30153 (B cells), GSE24706 (PBMC), GSE80183 (Blood), GSE10325 (Blood), and GSE11907 (Blood) as well as in 11 selected SLE datasets (Figure 1).

Heatmap showing the fold change of each relevant immune response gene selected for systemic lupus erythematosus (SLE) shows changes in expression in T cells and myeloid cell lines in the GSE10325 dataset. Upon analysing these genes across various cell and tissue types, it was discerned that *HLA-DRB3, CCL5, HLA-DQA1, FAM117B, HLA-DPA1,* and *HLA-DPB1* exhibited the strongest associations with SLE, contingent upon the specific tissue and cell type under investigation (Figure 1).

Differential expression of immune response genes in patients with SLE

The results demonstrated a notable increase in the expression of *HLA-DQA1* and *HLA-DMB* compared with the control group, as depicted in Figure 2A-2H. Additionally, a decrease in CCL5 expression was observed in all samples, except for those obtained from the GSE61635 dataset (Figure 2I-2L). The expression of the HLA-*DPA1, HLA-DRB3,* and *HLA-DPB1* genes from the HLA family, which are believed to be implicated in the immune response in SLE, were also assessed. A notable decrease in their expression levels was observed compared with the control (Figure 3). The expression of *FAM117B, ZNF395, PGAP3,* and *PIK3IP1*, among other genes potentially linked to the development of SLE, was also investigated. A similar trend was observed in the expression of these genes (Figure 4). These findings highlight the potential significance of these genes in the pathogenesis of SLE and highlight the necessity for further investigation into the roles of these genes in immune dysregulation and disease progression.

Table 1: Sample Characteristics in the SLE Datasets

	GSE45291	GSE65391	GSE110169	GSE108497	GSE72509	GSE61635	GSE38351	GSE10325	GSE110174	GSE50772	GSE82221
Series type	Array	Array	Array	Array	HTS	Array	Array	Array	Array	Array	Array
Case	292	116	82	325	99	79	14	13	144	61	30
Control	20	45	77	187	18	30	12	9	10	20	25
Total	312	161	159	512	117	109	26	22	154	81	55
Platform	GPL13158	GPL10558	GPL13667	GPL10558	GPL16791	GPL570	GPL96 GPL570	GPL96	GPL13158	GPL570	GPL10558 GPL13534
Tissue	WB	WB	WB	WB	WB	PBMC	PBMC	PBMC	WB	PBMC	PBMC
Clinical features	SLE	Pediatric SLE	SLE	Lupus pregnancy	SLE	SLE	SLE	SLE	SLE	SLE	SLE

WB: Whole Blood, HTS: High-throughput sequencing, SLE: Systemic Lupus Erythematosus

The hypermethylated genes most associated with SLE

Epigenetic phenomena have gained significant attention in understanding the molecular mechanisms of SLE. Although aberrant DNA methylation and histone modification have long been recognized as contributing factors to SLE pathogenesis, DNA hydroxymethylation has emerged as a relatively novel focus

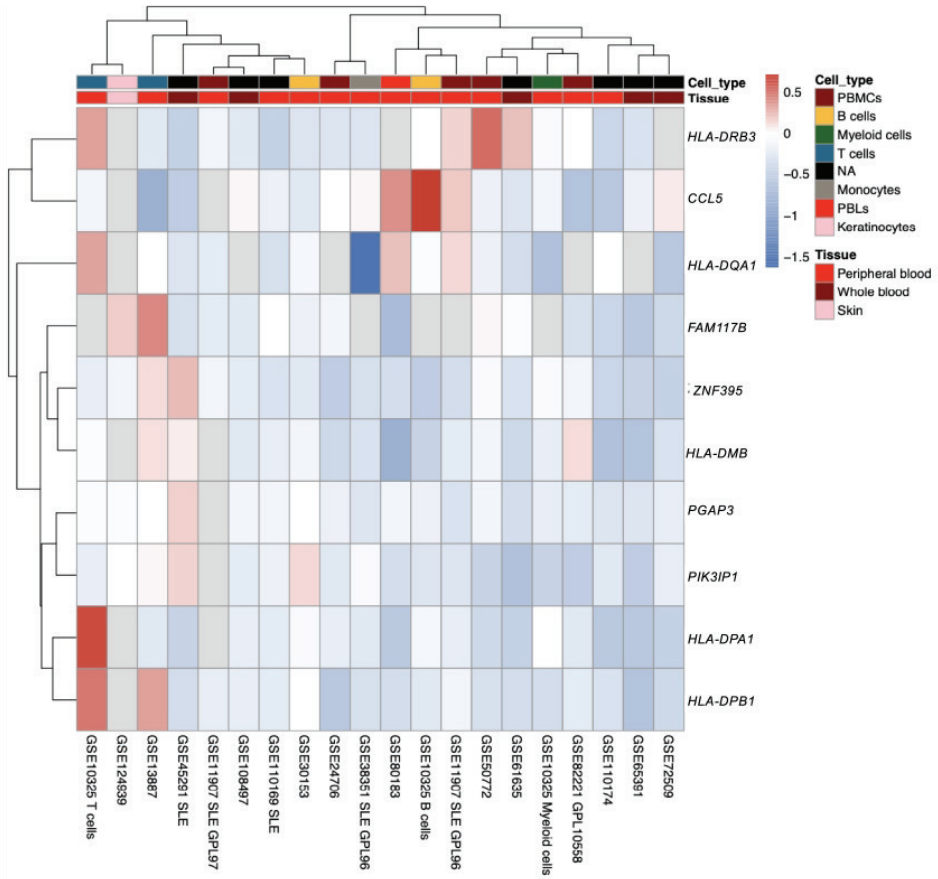

Figure 1: Heatmap with the fold change of each selected related immune response gene in systemic lupus erythematosus (SLE) disease

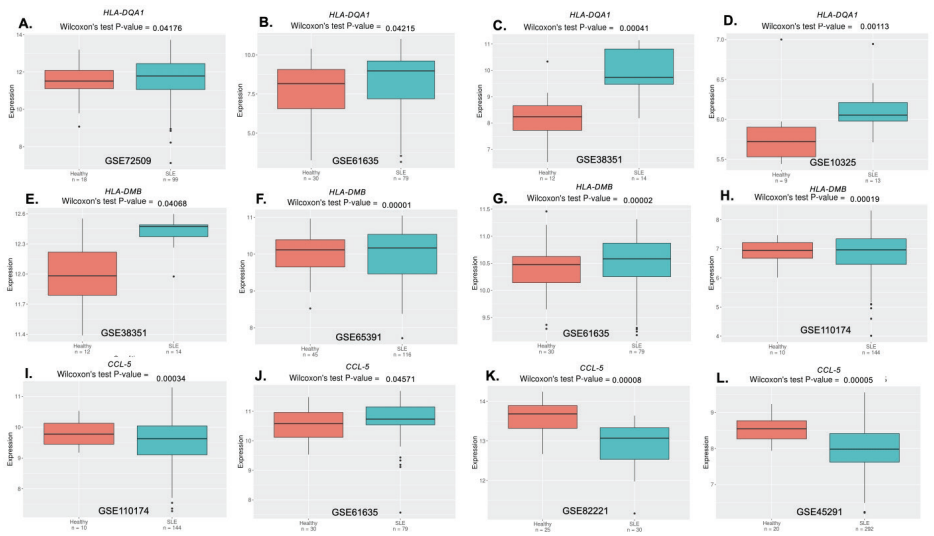

Figure 2: Expression profile of various immune response genes in systemic lupus erythematosus (SLE) disease A. Expression of *HLA-DQA1* in GSE72509 dataset B. Expression of *HLA-DQA1* in GSE61635 dataset C. Expression of *HLA-DQA1* in GSE38351 dataset D. Expression of *HLA-DQA1* in GSE10325 dataset E. Expression of *HLA-DMB* in GSE38351 dataset F. Expression of *HLA-DMB* in GSE65391 dataset G. Expression of *HLA-DMB* in GSE61635 dataset H. Expression of *HLA-DMB* in GSE110174 dataset I. Expression of *CCL5* in GSE110174 dataset J. Expression of *CCL5* in GSE61635 dataset K. Expression of *CCL5* in GSE82221 dataset L. Expression of *CCL5* in GSE45291 dataset

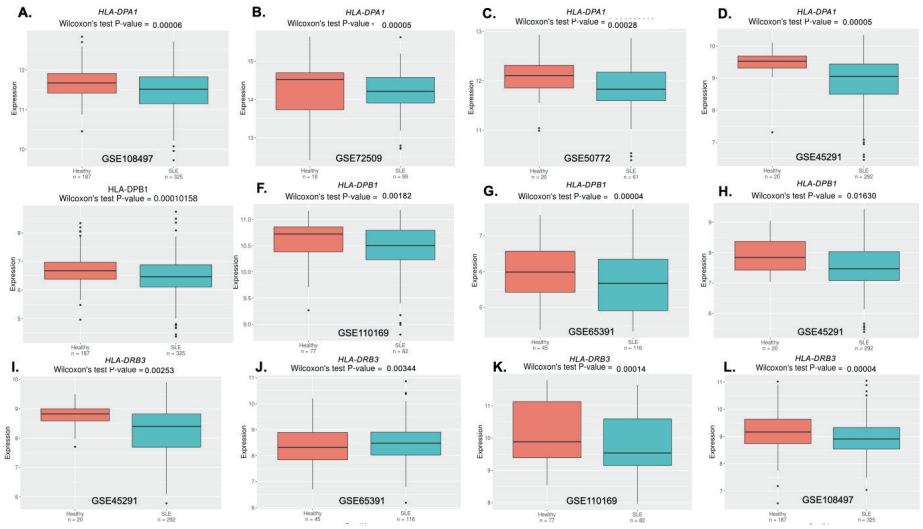

Figure 3: Expression profile of immune response family members in systemic lupus erythematosus (SLE) disease A. Expression of *HLA-DPA1* in GSE108497 dataset B. Expression of *HLA-DPA1* in GSE72509 dataset C. Expression of *HLA-DPA1* in GSE50772 dataset D. Expression of *HLA-DPA1* in GSE45291 dataset E. Expression of *HLA-DPB1* in GSE108497 dataset F. Expression of *HLA-DPB1* in GSE110169 dataset G. Expression of *HLA-DPB1* in GSE65391 dataset H. Expression of *HLA-DPB1* in GSE45291 dataset I. Expression of *HLA-DRB3* in GSE45291 dataset J. Expression of *HLA-DRB3* in GSE65391 dataset K. Expression of *HLA-DRB3* in GSE110169 dataset L. Expression of *HLA-DRB3* in GSE108497 dataset

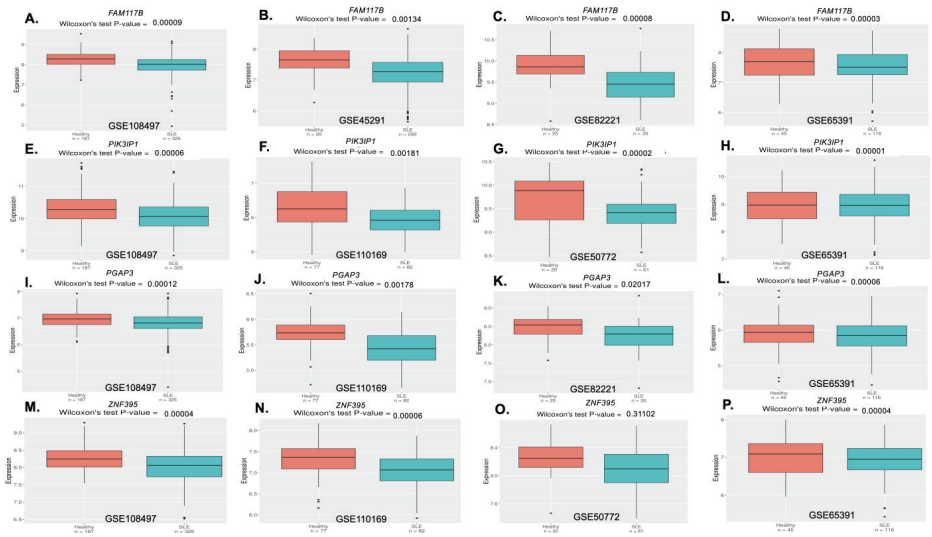

Figure 4: Expression profile of immune response family members in systemic lupus erythematosus (SLE) disease A. Expression of *FAM117B* in GSE108497 dataset B. Expression of *FAM117B* in GSE45291 dataset C. Expression of *FAM117B* in GSE82221 dataset D. Expression of *FAM117B* in GSE65391 dataset E. Expression of *PIK3IP1* in GSE108497 dataset F. Expression of *PIK3IP1* in GSE110169 dataset G. Expression of *PIK3IP1* in GSE50772 dataset H. Expression of *PIK3IP1* in GSE65391 dataset I. Expression of *PGAP3* in GSE108497 dataset J. Expression of *PGAP3* in GSE110169 dataset K. Expression of *PGAP3* in GSE82221 dataset L. Expression of *PGAP3* in GSE65391 dataset M. Expression of *ZNF395* in GSE108497 dataset N. Expression of *ZNF395* in GSE110169 dataset O. Expression of *ZNF395* in GSE50772 dataset P. Expression of *ZNF395* in GSE65391 dataset.

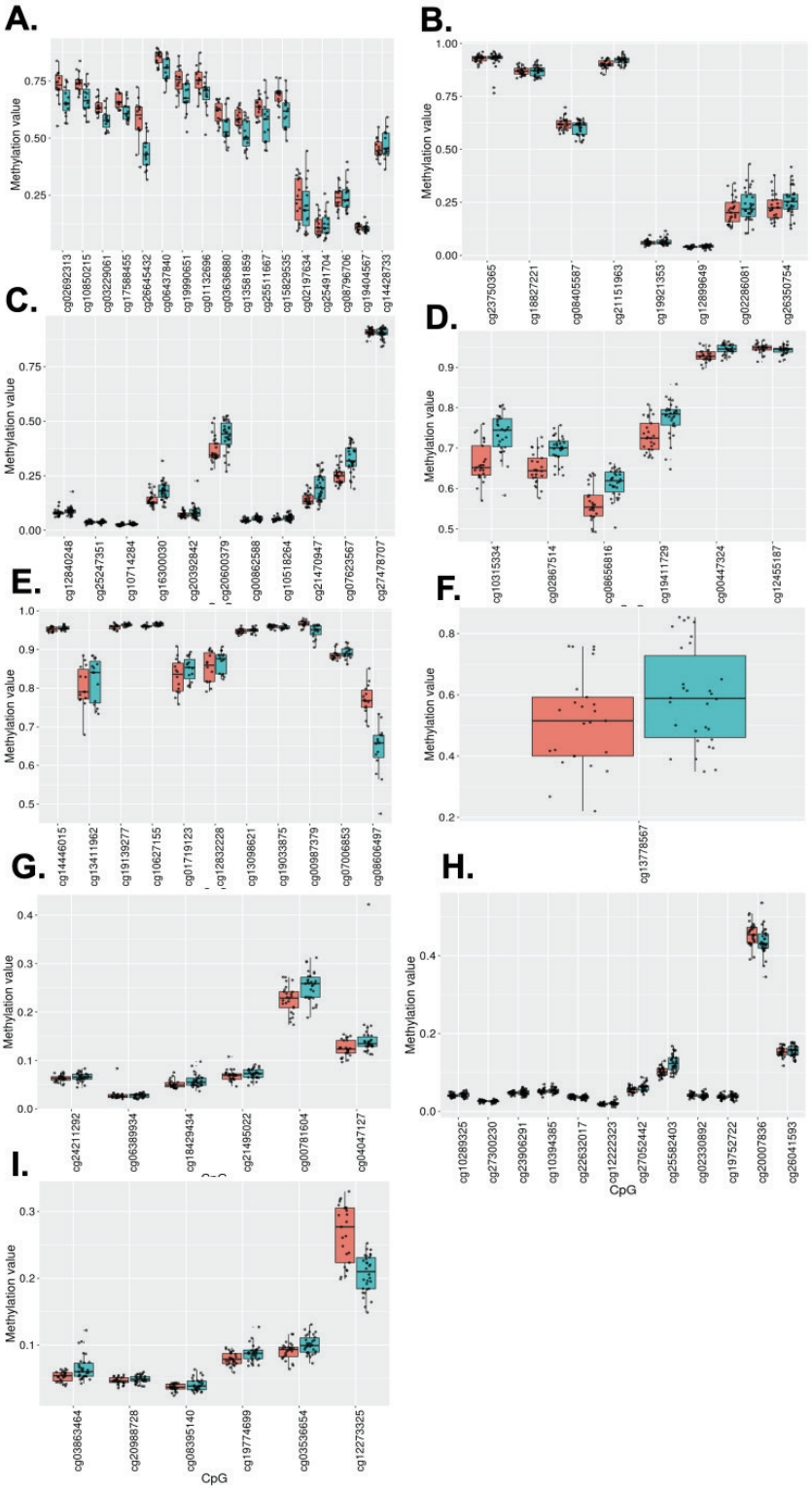

Figure 5: Overview of analyzed methylation sites and genomic organization of related genes. A. *HLA-DPA1* B. *HLA-DPB1* C. *HLA-DMB* D. *CCL5* E. *ZNF395* F. *HLA-DQA1* G. *FAM117B* H. *PGAP3* I. *PIK3IP1*. A boxplot illustrates the distribution of beta-values for individual CpG sites in both case and control samples. Each boxplot represents the beta values observed for a specific CpG site, with the middle line denoting the median value and the box encompassing the interquartile range. Red blots indicate healthy individuals and blue blots indicate systemic lupus erythematosus (SLE) patients

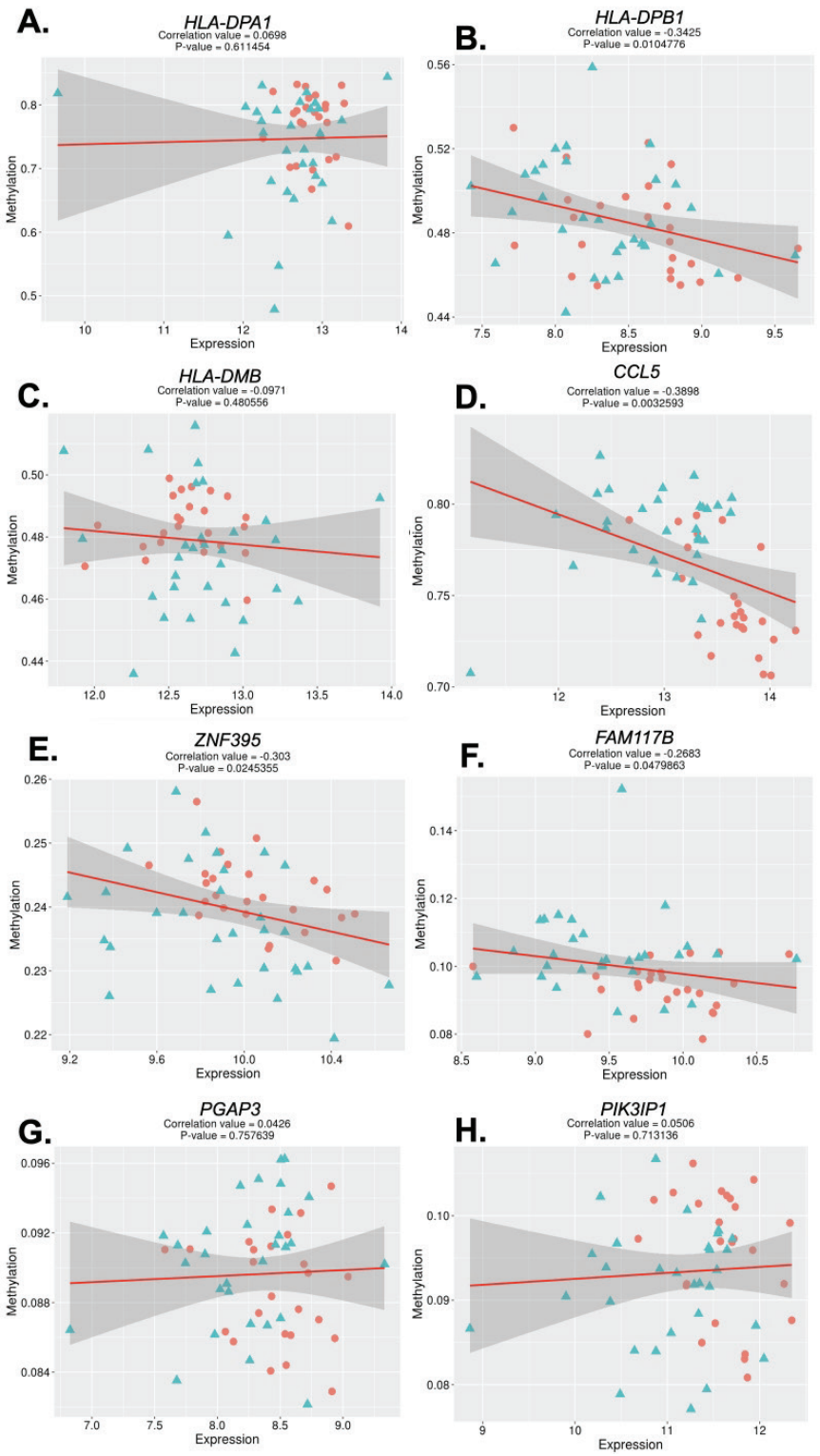

Figure 6: Correlation of methylation and expression of related genes. A. *HLA-DPA1* B. *HLA-DPB1* C. *HLA-DMB* D. *CCL5* E. *ZNF395* F. *FAM117B* G. *PGAP3* H. *PIK3IP1*. A scatter plot depicting the relationship between gene expression levels and mean methylation values of selected CpG sites is displayed. Each data point represents a sample, with the x-axis indicating the expression level of the gene and the y-axis representing the average methylation value across the specified CpG sites. Red blots indicate healthy individuals and blue blots indicate systemic lupus erythematosus (SLE) patients

in epigenetic investigations because of its activating potential. Against this backdrop, an inquiry was undertaken to assess the influence of ten genes implicated in immune response methylation on SLE pathology using data from the GSE82221 dataset.

These findings also revealed that 17 promoter-associated CpG islands were identified for *HLA-DPA1*, 8 for *HLA-DPB1*, 11 for *HLA-DMB*, six for *CCL5*, 11 for *ZNF395*, 1 for *HLA-DQA1*, six for *FAM117B*, 12 for *PGAP3*, and six for *PIK3IP1* in patients with SLE (Figure 5 A, B, C, D, E, F, G, H, I). Inconsistencies were observed among all 10 genes when comparing the methylation levels of genes across GSE82221. Specifically, high methylation levels were detected for *the HLA-DPA1, HLA-DPB1, HLA-DMB, CCL5, ZNF395*, and *HLA-DQA1* genes, whereas low methylation levels were observed for *FAM117B, PGAP3,* and *PIK3IP1*. Furthermore, the CpG islands exhibiting the highest levels of methylation were cg06437840, cg23750365, cg20600379, cg00447324, cg10627155, and cg13778567, respectively. The genes *PIK3IP1, FAM117B,* and *PGAP3* exhibited methylation levels well below 0.5 but did not demonstrate significant hypermethylation. In contrast, a specific subset of six genes, namely *HLA-DPA1, HLA-DPB1, HLA-DMB, CCL5, ZNF395,* and *HLA-DQA1,* maintained consistent methylation levels. The observed stability in methylation levels indicate persistent epigenetic alterations compared with the hypermethylation patterns noted across all samples within this particular subset.

Relationship between methylation status and HLA-DPB1, CCL5, FAM117B, and ZNF395 gene expression in SLE

The interaction between immune response gene expression and methylation status is complex and multifaceted. DNA methylation is typically related to reduced expression or gene silencing, especially in promoter regions. This relationship is influenced by several factors, including the specific genes involved, the methylation patterns of their regulatory regions, and the cellular and environmental contexts in which these genes are active.

This investigation aimed to explore the correlation between immune response gene expression and methylation status in SLE. The study revealed that alterations in the expression of four genes, namely *HLA-DPB1, CCL5, FAM117B*, and *PGAP3*, were linked to hypermethylation in the CpG islands of these genes. In particular, methylation of CpG sites in the promoter regions of these genes inhibited their expression levels (Figure 6). These results indicate that methylation plays a regulatory role in modulating the expression of immune response genes in SLE.

DISCUSSION

SLE is a complex inflammatory condition that affects various organs and has a known genetic component. Epigenetic mechanisms (DNA methylation, histone modification) and mutations (polymorphism) are crucial for the regulation of gene expression (10). Alterations in DNA methylation patterns contribute to the aberrant immune responses characteristic of SLE. Specifically, changes in DNA methylation levels of genes involved in the regulation of T cell function, activation of B cells, and clearance of immune complexes have been implicated in SLE pathogenesis (20). These alterations may disturb the equilibrium of immune response, generating autoantibodies and the inflammatory cascades characteristic of SLE (21). Despite the identification of genes exhibiting altered expression in SLE, the underlying causes of these changes remain unclear. Therefore, this study aimed to elucidate the relationship between gene expression related to immune response and the development of SLE. Additionally, specific CpG methylation sites associated with SLE in patients were identified through the analysis of the analysis of publicly available datasets, integrating clinical, laboratory, and biological factors.

HLA-DQA1 and *HLA-DMB* encode the alpha and beta chains of the HLA-D protein, a major histocompatibility complex (MHC) class II molecule. MHC class II molecules are pivotal in the immune system because they present antigens to T cells, initiating immune responses. Certain variants of these genes may be linked to an elevated risk of developing SLE (22-24). Studies have indicated that specific *HLA-DQ* and *HLA-DM* alleles are more common in individuals with SLE than in the general population (22, 25). In this study, the expression levels of *HLA-DQA1* and *HLA-DMB* were higher in patients with SLE than in controls. These alleles could contribute to the autoimmune response in SLE by presenting self-antigens to T cells, which in turn triggers the production of autoantibodies and inflammation.

HLA alleles can also stimulate an uncontrolled increase in the production of various cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), which play crucial roles in inflammation and immune responses (26). For instance, certain populations exhibit a heightened risk of SLE with *HLA-DR2, HLA-DR3*, and *HLA-DR4* alleles (23, 25). Shirakawa et al. demonstrated a notable decrease in the number of HLA-DR-positive monocytes and expression of HLA-DR antigens in active patients with SLE. These levels returned to normal in inactive SLE patients (25, 27). The diminished presence of HLA-DR-positive monocytes disrupts the regulation of immune response crucial for SLE development. The observed reduction in HLA-DR3 levels in this study might contribute to an aberrant immune response in patients with SLE, potentially intensifying disease progression (23, 25). Furthermore, Takona et al. noted a marked elevation in HLA-DP+ T-cell counts among patients with SLE compared with healthy individuals. Concurrently, another study indicated that elevated HLA-DP levels in patients with SLE were correlated with increased T cell frequency and insufficient IL-2 expression (28). Moreover, any alteration in carbon metabolism has been shown to downregulate genes involved in the hypermethylation of essential genes like *RFC1* and *MHC2TA* while also affecting other critical genes such as *HLA-DR* by altering promoter CpG island methylation (29). Considering these mutational and epigenetic influences, the decline in *HLA-DPA1, HLA-DPB1,* and *HLA-DRB3* gene expression noted in SLE patients in this investigation could be linked to post-transcriptional elements such as DNA hypermethylation and polymorphism.

CCL5 gene expression was downregulated in all datasets except the GSE61635 dataset. This dataset investigated gene expression in the blood of patients with SLE positive for RNP autoantibodies. The presence of RNP autoantibodies can disrupt the clearance of apoptotic cells, resulting in the release of self-antigens and subsequent activation of the immune system. Ultimately, RNP autoantibodies present in the blood of patients with SLE can disrupt immune responses, sustain inflammation, and play a role in the development of the disease (30). *FAM117B*, or Family with sequence similarity 117 member B, is a protein-coding gene. Its exact role in SLE has yet to be fully understood. This study revealed that *FAM117B* is downregulated in patients with SLE and is implicated in the pathogenesis of this disease. Upon encountering pathogen infection, the interferon (IFN) pathway activation triggers the upregulation of IFN-stimulated genes, including proinflammatory cytokines, essential for robust antiviral immune reactions. Nevertheless, aberrant expression of these genes can pose risks to the host. *ZNF395* has been recognized as a gene that operates independently of IFN but boosts the IFN-mediated expression of chemokine (31). In this study, posttranscriptional modifications influenced the expression of *ZNF395*, and its irregular expression could play a role in the development of SLE. These results emphasize the intricate role of immune dysregulation in SLE and underscore the importance of these genes in driving the disease process.

In the present study, a subset of genes with consistent methylation levels, including *HLA-DPA1, HLA-DPB1, HLA-DMB, CCL5, ZNF395,* and *HLA-DQA1, were* identified. Consistent methylation levels across all samples indicate that these genes were hypermethylated. Unlike methylation levels, gene silencing is often associated with hypermethylation, although this relationship is not always direct and may be influenced by additional regulatory mechanisms (32). Interestingly, the upregulation of hypermethylated genes, such as *CCL5, HLA-DQA1,* and *HLA-DMB*, was observed. The regulation of gene expression is influenced by various mechanisms beyond methylation, such as specific transcription factors and histone modifications, which can override the repressive effects of DNA methylation (33). Although promoter hypermethylation generally results in gene silencing, methylation at different sites may have contrasting effects, including increased gene expression. Despite the observed consistent methylation patterns in our analysis, the presence of technical and biological variability in high-throughput datasets like GSE61635 might influence gene expression differences in individual samples. These findings highlight the intricate nature of epigenetic regulation, indicating that hypermethylation does not always lead to gene silencing. Further experimental investigations are necessary to elucidate the specific mechanisms driving gene expression patterns in patients with SLE (34). The present study also identified *HLA-DPB1, CCL5, FAM117B,* and *ZNF395* as genes potentially regulated by methylation in SLE, with CpG methylation within their promoter regions likely exerting a suppressive effect on their expression levels. Aberrant DNA methylation is implicated as a contributing factor to the dysregulated expression of these genes in SLE, and other mechanisms, such as RNA interference, may also play a role. Further studies are needed to validate these findings and elucidate the precise regulatory mechanisms.

This study highlighted the complex network of immune response genes and their regulatory elements, providing valuable insights into the molecular mechanisms underlying SLE pathogenesis. Second, the potential use of epigenetic modifications, particularly DNA methylation, as biomarkers for disease diagnosis and prognosis of SLE was highlighted. By elucidating the role of specific genes and their epigenetic regulation in the development of SLE, this study provides a growing body of knowledge to better understand and manage this complex autoimmune disorder.

Limitations of the study

This study has several limitations. Despite extensive analyses across various cell and tissue types, further studies using additional cohorts and experimental models are required to confirm the results and determine their broader significance. Moreover, the cross-sectional design of this study precludes the establishment of causal relationships. The findings highlight the importance of longitudinal studies to elucidate the temporal patterns of gene expression and epigenetic regulation during the progression of SLE. Despite these limitations, these findings provide a solid foundation for future research efforts to understand the intricate mechanisms of SLE pathogenesis and identify novel therapeutic targets for this complex autoimmune disease.

CONCLUSION

This study underscores the intricate regulatory mechanisms of systemic lupus erythematosus (SLE), focusing on the roles of genetic and epigenetic factors. The present study revealed that alterations in DNA methylation patterns, particularly those of genes involved in immune response regulation, play a significant role in SLE pathogenesis. Notably, genes such as HLA-DQA1, HLA-DMB, CCL5, ZNF395, and others were found to exhibit consistent methylation levels, indicating that hypermethylation as a regulatory mechanism. The present study also highlighted the potential of specific CpG methylation sites and gene expression profiles as biomarkers for SLE diagnosis and prognosis. The identification of differentially expressed genes, such as HLA-DQA1, HLA-DMB, CCL5, FAM117B, and ZNF395, linked to immune response dysregulation provides valuable insights into the molecular mechanisms driving SLE. These findings emphasize the importance of further experimental investigations to validate the observed methylation patterns and elucidate the regulatory mechanisms.

Informed Consent: Since this study utilizes data from a publicly available database, no direct contact with human subjects was made, and informed consent was not required. The data was accessed by the terms of use provided by the database.

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