# Systemic lupus erythematosus diagnostics in the 'omics' era

Systemic lupus erythematosus is a complex autoimmune disease affecting multiple organ systems. Currently, diagnosis relies upon meeting at least four out of eleven criteria outlined by the ACR. The scientific community actively pursues discovery of novel diagnostics in the hope of better identifying susceptible individuals in early stages of disease. Comprehensive studies have been conducted at multiple biological levels including: DNA (or genomics), mRNA (or transcriptomics), protein (or proteomics) and metabolites (or metabolomics). The 'omics' platforms allow us to re-examine systemic lupus erythematosus at a greater degree of molecular resolution. More importantly, one is hopeful that these 'omics' platforms may yield newer biomarkers for systemic lupus erythematosus that can help clinicians track the disease course with greater sensitivity and specificity.

**KEYWORDS: epigenomics ■ genomics ■ metabolomics ■ proteomics ■ systemic lupus erythematosus ■ transcriptomics**

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**Learning objectives**

Upon completion of this activity, participants should be able to:

- Evaluate the standard diagnostic process for systemic lupus erythematosus (SLE)
- Assess the use of genomics to diagnose SLE
- Distinguish gene expression pathways most commonly found to be upregulated in cases of SLE
- Analyze the use of proteomics and metabolomics in SLE

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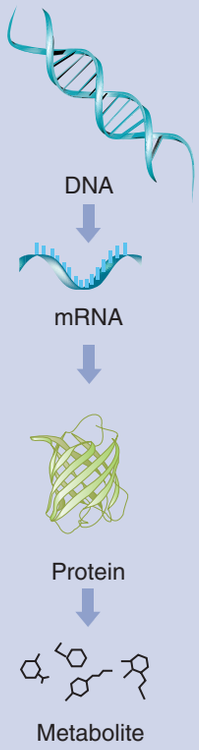
Systemic lupus erythematosus (SLE) is a complex autoimmune disease affecting multiple organ systems. The lifetime risk of diagnosis with SLE is 0.9% for females and 0.2% for males [1]. In the 1950s a 50% survival rate at 5 years was reported [2]. A more recent study in 2003 noted great improvements with a 10-year survival rate of 92% [3]. Currently, diagnosis relies upon meeting at least four out of 11 clinical and laboratory criteria outlined by the ACR. A 0.96 sensitivity and 0.96 specificity and a calculated area under the receiver operating curve (AUC) of 0.96 has been noted when patients were classified based on the 1971 criteria for diagnosis [4]. The complexity of the disease and requirement of meeting four ACR criteria, often leads to a delay in diagnosis. A study from 2003 found that the interval between first symptom and diagnosis was on the average 21.82 months [5]. Antinuclear antibodies (ANAs) are antibodies that target proteins within the nucleus of cells. Although ANAs are positive in 0.98% of SLE patients, they can also be positive in other autoimmune diseases and healthy controls, making ANA-positivity insufficient for diagnosis of SLE [6]. In fact, approximately a quarter of the general population has a measurable ANA [7]. The scientific community actively pursues discovery of novel diagnostics in the hope of better identifying susceptible individuals in early stages of disease, and subgroups with specific organ involvement predisposition. Research aims to improve diagnosis, surveillance, and treatment of SLE. Comprehensive studies of SLE have been conducted at multiple biological levels including: DNA (or genomics), mRNA (or transcriptomics), protein (or proteomics) and metabolites (or metabolomics). Indeed, comprehensive profiling using multiple 'omics' platforms has yielded novel insights on a wide spectrum of diseases, as summarized through the Nature Publishing Group's Omics Gateway [201]. These global technologies are proving to be equally informative in the study of SLE (Figure 1).

## Genomics

Genomics is the comprehensive analysis of the DNA of living beings. The observation of familial aggregation of SLE in the 1970s sparked interest in the study of the DNA of SLE patients. Monozygotic twins have been noted to have a 24% concordance of SLE [8]. The sibling recurrence-risk ratio ($\lambda_s$) is the ratio of risk of being affected by a disease given that one's sibling is affected and the risk of the disease in the general population. The SLE $\lambda_s$ has been reported to be 5.8–29, alluding to the presence of familial aggregation [9]. Also, it is well established that the incidence of SLE is greater in females and in particular racial groups. These observations have led to great efforts to identify regions in the genome that confer susceptibility for SLE.

The first noted genetic association with lupus, the HLA locus, was discovered in the 1970s. HLA class II alleles *DR* and *DQ* are associated with SLE [10]. *HLA-DRB1*15* and *HLA-DRB1*16* (jointly formerly *DR2*) and *HLA-DRB1*03* (formerly *DR3*) alleles were noted to be present in two-thirds of SLE patients, with those heterozygous for *DR2* and *DR3* or homozygous for *DR3* having the highest risk [10]. Anti-Ro and anti-La antibodies have been noted to be associated with *HLA-B*08* (*B8*), *HLA-DRB1*03* (*DR3*), *HLA-DQB1*02* (*DQ2*), and *HLA-C4AQ0* (*C4AQ0*) [11]. In the decades following, associations with HLA class III, more specifically the complement system, including C2, C4 and C1q, were discovered. Patients with each of these three complement deficiencies were noted to have clinically diagnosed SLE with ANAs [12]. Murine complement gene knockout models have also been created that develop SLE-like disease [13–16]. In addition to the above genes, several newer candidates




| Technology | Potential markers of SLE |
|---|---|
| Genomics | *AFF1, APOBEC4, ARID5B, ATF65, ATGS, BACH2, BANK1, BLK, C1q, C2, C4, C6orf10, C10orf64, CD44, CD80, CD247, CDKN1B, CLEC16A, COL25A1, CREBL2, DDA1, DGUOK, DRAM1, ELF1, ETS1, FCλR2A, FCλR3A/B, GHR, GPR19, HIC2, HIP1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRB1, IFIH1, IKZF1, IL10, IRAK1/MECP2, 1RF5, IRF7, ITGAM, ITGAX, JAZF1, KIAA1542, LAMC2, LEF1, LRRC18, LYN, MICB, MSH5, NAALADL2, NEGR1, NOTCH4, NTNG2, OR4A15, PRDM1, PRKCB, PTPN22, PTTG1, PXK, RASGRP3, RPL7AP59, RTKN2, SCN10A, SEZ6L2, SIAE, SLC15A4, SLC1A7, SLC29A3, SOCS6, STAT4, TET3, TMC2, TNFAIP3, TNFSF4, TNIP1, TNPO3, TNXB, TREX1, UBE2L3, UHRF1BP1, WDFY4* [10–12,17–46,60–66,70] |
| Transcriptomics | *APOBEC3B, DEFA1, EIF2AK2/PRKR, FCAR, FCGR1A/CD64, IFI44, IFI44L, IFIT1, IFIT3, IFIT4, IL1B, IL1R2, IL1RN, IRF7, LGALS3BP, LY6E, MAP3K8, MX1, OAS1, OAS2, OASL3, OASL, PLSCR, SERPING1, TRAIL, XAF1* [89–98] |
| Proteomics | MALDI-TOF MS (m/z 3376.02, 4070.09, 7770.45, 28045.1), iTRAQ (e.g., PBMC: brain acid soluble protein 1, cDNA FLJ61039, histone H2A type 1, isoform 2 of zinc finger protein 549, myeloblastin, neutrophil defensin 1, protein S100-P, kidney: aldolase, annexins, arginosuccinate synthetase, heterogeneous nuclear ribonucleoprotein), 2D-gel and MALIDI-TOF MS (e.g., SA100A9), targeted protein microarray (e.g., CCL3/MIP-1A, CCL7/MCP-3, CCL8/MCP-2, CLIC2, CXCL8/IL-8, GDTP1, IL-2SRA, IL-6, MORF4L1, PBOV1), autoantigen microarray (e.g., antibody to: chromatin, dsDNA, EBV, hyaluronic acid, laminin, myosin, ssDNA, vimenfin), B-C3d, B-C4d, IFN-λ. IL-1, IL-2, IL-10, IL-12, IL-16, NGAL, Siglec-1/CD169, T-C3d,T-C4d, TGF-β, TNF-α, urinary ICAM-1, urinary MCP-1, urinary OPG, urinary TWEAK, urinary VCAM-1 [111–130,135–138] |
| Metabolomics | 5-HETE, 9-HODE, 13-HODE, α-ketoglutarate, DHA, EPA, γ-glutamylalanine, glutamine, GSH, lactate, LDL, laukotriene B4, MDA, pyruvate [141–143] |

**Figure 1. Markers of systemic lupus erythematosus emerging from 'omics' studies.**
DHA: Docosahexaenoic acid; EBV: Epstein–Barr virus; EPA: Eicosapentaenoic acid; GSH: Glutathione; iTRAQ: Isobaric tags for relative and absolute quantitation; LDL: Low-density lipoprotein; MALDI-TOF: Matrix-assisted laser desorption/ionization time-of-flight; MDA: 3,4-methylenedioxyamphetamine; MS: Mass spectrometry; PBMC: Peripheral blood mononuclear cell; SLE: Systemic lupus erythematosus.

in this genetic interval have also been linked to SLE susceptibility. *MSH5*, a gene that is involved in immunoglobulin class switching, is located in the HLA class III region and is highly associated with SLE [17]. A focused evaluation of this region identified *MICB*, *ATF6B/BREBL1*, *C6orf10* and *NOTCH4* in addition to *HLA-DQB2, HLA-DQA1* and *HLA-DRB1* as being associated with SLE [18,19]. To date, some of the strongest genes for SLE are the ones described approximately four decades ago – DR2, DR3, C2, C4 and C1q. Similarly, receptors for the Fc region of IgG (FcγR) have been associated with SLE for decades [20]. Thus, the HLA region on chromosome 6 and the FcγR region on chromosome 1 remain important genetic loci in SLE.

Besides the HLA and complement genes, multiple single nucleotide polymorphisms (SNPs) within several additional genes have been identified to be associated with SLE. Some of these SNPs are noted to have racial associations to populations of African, European, Hispanic, Asian and Amerindian origin [18,21–32]. Technological advances have facilitated the discovery of more candidate genes using genome-wide association studies (GWAS). As tracked at genome.gov (a NIH database of published GWAS) there have been 14 studies reported pertaining to SLE, however some have overlap of patients [202]. Greater than fifty genes for SLE have been uncovered by these GWAS [33–46]. *STAT4*, *IRF5*, *BLK*, *WDFY4*, *ETS1*, *ITGAM* and *UBE2L3* are noted to have association with SLE in multiple studies in the NIH database; interestingly *STAT4*, *IRF5*, *BLK*, and *ETS1* are also reported in rheumatoid arthritis GWAS (Table 1) [47–56]. *STAT4* and *IRF5* have also been reported with systemic sclerosis [57–59]. Several additional studies have reported significant genetic associations to yet other genes [60–66].

A meta-analysis of all GWAS studies requiring at least two reports with a $p \leq 1 \times 10^{-5}$ for significance has identified *HLA-DRB1*0301* (*HLA-DR3*), *HLA-DRB1*1501* (*HLA-DR2*), *PTPN22*, *IRF5*, *STAT4*, *BLK*, *TGAM* and *TNFAIP3* as genes confirmed to be associated with SLE [67]. These can be further categorized according to the immune function they impact.

**Table 1. Candidate genes for systemic lupus erythematosus identified in genome-wide association studies listed by chromosomal location.**

| Region | Candidate gene(s) | SLE ref. | RA ref. | Systemic sclerosis ref. |
|---|---|---|---|---|
| 1p31.1 | *NEGR1* | [35] | | |
| 1p32.3 | *SLC1A7* | [43] | | |
| 1q24.2 | *CD247* | [44] | [47,48] | [57–59] |
| 1q25 | *TNFSF4, LAMC2, APOBEC4* | [38,46] | | |
| 1q31 | Intergenic | [43] | | |
| 2p13 | *DGUOK, TET3* | [46] | | |
| 2p16 | *REL* | [37] | [47,49] | |
| 2p22 | *RASGRP3, QPCT* | [38,46] | [50] | |
| 2q31 | Intergenic | [43] | | |
| 2q32.3 | *STAT4* | [35,37,38,41,43,45,46] | [47,48,51] | [57–59] |
| 3p14.3 | *PXK, ARHGEF3* | [36] | [47,52] | |
| 3p22 | *SCN10A* | [46] | | |
| 3q13.3 | *CD80* | [46] | | |
| 3q26.3 | *NAALADL2* | [43] | | |
| 4q21.3 | *AFF1* | [41] | | |
| 4q24 | *BANK1* | [34,46] | | |
| 4q25 | *COL25A1, LEF1* | [41,43] | | |
| 4q28 | Intergenic | [37] | | |
| 5p13.1 | *GHR* | [35] | | |
| 5q33.1 | *TNIP1* | [38,46] | | [58] |
| 5q33.3 | *PTTG1* | [43] | | |
| 7p12 | *IKZF1, GRB10* | [38,46] | | [59] |
| 7q11.23 | *HIP1* | [38,41] | | |
| 7q32 | *IRF5, TNPO3* | [35,36,38,43,45,46] | [47,53] | [57–59] |
| 8p23.1 | *BLK, C8orf13* | [35,37,38,41,43,45,46] | [49,52] | |
| 9q34.1 | *NTNG2* | [35] | | |
| 10q11.2 | *WDFY4, LRRC18, C10orf64* | [37–39] | | |
| 10q21.2 | *ARID5B, RTKN2* | [46] | [54,55] | |
| 10q22.1 | *SLC29A3* | [46] | | |
| 11p13 | *CD44* | [46] | | |
| 11p15.5 | *KIAA1542* | [36,43] | | |
| 11q11 | *OR4A15* | [43] | | |
| 11q23.3 | *DDX6* | [38] | [48] | |
| 11q24 | *ETS1, FLI1* | [38,39,41] | [55,56] | |
| 12p13.1 | *GPR19, CDKN1B* | [46] | | |
| 12p13.2 | *CREBL2, RPL41, ESYT1* | [46] | | [59] |
| 12q21.2 | *RPL7AP59* | [43] | | |
| 12q23 | *DRAM1* | [46] | | |
| 12q24.33 | *SLC15A4* | [38,46] | | |

*This table summarizes the 14 SLE genome-wide association studies included on Genome.gov in order of location with chromosome 6 removed due to variable reporting of the HLA region* [202]. *Overlap of SLE candidate genes with RA and systemic sclerosis genome-wide association studies on Genome.gov are included in the final columns.*
*RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus.*

**Table 1. Candidate genes for systemic lupus erythematosus identified in genome-wide association studies listed by chromosomal location (cont.).**

| Region | Candidate gene(s) | SLE ref. | RA ref. | Systemic sclerosis ref. |
|---|---|---|---|---|
| 13q14.1 | *ELF1, ENOX1* | [42,46] | [56] | |
| 16p11.2 | *ITGAM, ITGAX, SEZ6L2* | [35–38,43,46] | | |
| 16p13.1 | *CLEC16A* | [46] | | |
| 17p12 | Intergenic | [37] | | |
| 18q22 | *SOCS6, CBLN2* | [35] | [50] | |
| 19p13.1 | *DDA1* | [46] | [56] | |
| 20p13 | *TMC2* | [43] | | |
| 22q11.2 | *UBE2L3, HIC2* | [38,43,46] | | |

*This table summarizes the 14 SLE genome-wide association studies included on Genome.gov in order of location with chromosome 6 removed due to variable reporting of the HLA region* [202]. *Overlap of SLE candidate genes with RA and systemic sclerosis genome-wide association studies on Genome.gov are included in the final columns.*
*RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus.*

*IRF5*, *STAT4* and *TNFAIP3* have roles in the innate immune response, whereas *HLA-DR3, HLA-DR2, PTPN22, BLK* and *STAT4* have roles in lymphocyte activation and/or function, and *ITGAM* is involved in phagocytosis, cell adhesion and inflammation [17]. Collectively, these findings suggest that genetic aberrations that impact the adaptive immune system, as well as those that shape innate immunity, are both important in lupus pathogenesis, as suggested by previous studies in murine lupus [68].

Individually, these genetic associations are not strongly predictive of SLE disease; however, the development of SNP panels for potential future diagnostics appears promising. A panel of 11 SNPs found within the following genes: *HLA, ITGAM, IRF5/TNPO3, KIAA1542* and *PXK* is noted to have an AUC of 0.67 with a reasonable degree of sensitivity and specificity for SLE [36]. Recently, a more extensive panel comprised of 22 SNPs within the following genes: *HLA-DRB1 (DR3* allele tag*), IRF5, ITGAM, STAT4, PTPN22, UHRF1BP1, IL10, TNIP1, TNFSF4, KIAA1542, FCλR2A, BLK, UBE2L3, HLA-DRB1 (DR2* allele tag*), IRAK1/MECP2, PTTG1, TNFAIP3, PRDM1, PXK, JAZF1, ATGS* and *BANK1* was reported to have an AUC of 0.679, that improved to 0.689 when weighted, based on odds ratios [69]. Researchers continue refining these SNP panels in order to improve their utility as a diagnostic tool for the future. In contrast to these common genetic polymorphisms identified using GWAS, which are relatively frequent in both the disease-affected and unaffected populations, rarer genes also exist that have larger effect sizes and a greater impact on SLE development including *SIAE*, *TREX1* and *IFIH1* [70]. Collectively, the common polymorphisms and rare variants that have thus far been identified as being SLE-associated, account for less than half of the estimated genetic contribution to SLE. Efforts are underway in multiple laboratories using high throughput next generation sequencing in order to get at this 'missed heritability'. Continued discovery and characterization of common and rare genetic polymorphisms will likely lead to improved gene-based diagnostic panels for this genetic disease.

Genetic relationships in responses to therapeutics are the focus of pharmacogenomics. For example, individuals possessing allelic variants in the CYP450 complex or the glutathione S-transferase enzyme system have higher risk of cyclophosphamide toxicity [71]. TPMT genetic polymorphism testing is often utilized in clinical practice prior to initiation of azathioprine or 6-mercaptopurine owing to the association with bone marrow toxicity. Beyond predicting toxicity, pharmacogenomics is being utilized to predict beneficial therapeutic response, though this emerging branch of science has not yet been applied to the study of SLE [71,72].

Closely linked to DNA sequences are alterations that directly influence the expression of genes. Epigenomics is the comprehensive evaluation of modifications to DNA including methylation, chromatin remodeling/histone modification, miRNAs and transcription factors, all of which ultimately impact gene expression. The contribution of epigenomics to the development of SLE is currently undergoing extensive evaluation. Genome-wide studies of $CD4^+$ T cells noted a majority of genes to be hypomethylated in lupus patients versus healthy controls [73,74]. Global hypomethylation was observed in samples of heterogeneous peripheral white blood cells,

with significant reduction of IL-10 and IL-1R2 methylation [75]. Histones are also noted to alter gene expression as a result of methylation and acetylation. $CD4^+$ T cells from lupus patients were noted to have hypomethylation and hypoacetylation of H3 and H4 histones [76]. Additional studies reported functional changes as a result of histone modifications [77–79]. Further studies are warranted to explore if these epigenetic changes are the consequence of the disease, or whether they may actually impact pathogenic pathways leading to lupus.

Another category of epigenetic contributors are small noncoding segments of RNA, known as miRNAs. miRNA arrays have been used to identify multiple upregulated and downregulated miRNAs in peripheral blood mononuclear cells (PBMCs) from patients with SLE. Three such studies were evaluated for overlaps and noted the downregulation of hsa-let7a, hsa-miR-196a, hsa-miR-17-5p and hsa-miR-383; and upregulation of hsa-miR-21, hsa-miR-342 and hsa-miR-198 in lupus [80–82]. Cell-free plasma has also been evaluated for circulating miRNAs leading to the identification of target genes involved in apoptosis, T-cell development, cytokine receptors and other signaling pathways [83,84]. The involvement of miRNAs in Toll-like receptor pathways is an additional area of investigation [85,86]. Transcription factors (TFs) are key drivers of gene expression through direct interaction with DNA. An array containing 345 TFs detected 78 upregulated and 14 downregulated TFs in PBMCs from SLE patients compared with controls [87]. The disease potential of TFs in SLE is well illustrated by a recent study evaluating cAMP-responsive element modulator α and its impact on IL-17 expression in SLE [88]. Our understanding of how miRNAs and various TFs regulate the expressed transcriptome in various immune cell types in the context of SLE is just beginning to blossom.

## Transcriptomics

Transcriptomics evaluates comprehensive gene expression, or the RNA message that is being transcribed from the DNA sequence in a global fashion. Microarray technology has allowed rapid and comprehensive evaluation of gene expression differences between SLE patients and healthy controls. Unlike genomics, transcriptomics focuses on particular cells or tissues. Given the known immunologic effects of SLE, the focus of transcriptomic analyses in SLE has mostly been geared towards peripheral blood white blood cell populations. A study comparing PBMCs from patients with various autoimmune diseases, including SLE, and healthy controls found differences in gene expression levels related to apoptosis, cell migration, cell differentiation and cell cycle progression [89]. Another study involved a comparison of PBMCs of SLE patients to controls using a focused cDNA array of cytokines, chemokines, growth factors and apoptosis and immunomodulatory genes. It highlighted the upregulation of genes related to TNF/death receptors, IL-1 and IL-8 [90]. Similarly, one more PBMC-based microarray study noted the upregulation in death receptors and IL-1, Fc receptors, cell adhesion molecules and multiple type I interferon (IFN) pathway genes [91]. The importance of the IFN pathway was reinforced by another study of PBMCs from pediatric SLE patients; this report also highlighted a second upregulated pathway comprised of granulocyte-specific genes [92]. IFN pathway genes were also found to be upregulated in peripheral white cells of SLE patients, including: *IFN-ω, IFIT1, IFIT2, IFIT4, OAS1, OASL* and *LY6E* [93]. Similar profiles were noted in a few additional microarray studies in SLE [94–98]. Given that the study of peripheral blood mononuclear cells' expression profiles can yield biased results due to the heterogeneity of the cell populations (in patients versus controls), some cell-specific studies have been undertaken in T cells [99], B cells [100] and plasma cells from SLE patients [101].

Seven published microarray studies of PBMCs from SLE patients versus controls were evaluated for overlaps. Any gene transcript that was identified in two or more of the studies by Baechler *et al.*, Bennett *et al.*, Biesen *et al.*, Crow and Wohlgemuth, Han *et al.*, Lyons *et al.* and Rus *et al.* is included in the following list of 26 genes: *APOBEC3B, DEFA1, EIF2AK2/PRKR, FCAR, FCGR1A/CD64, IFI44, IFI44L, IFIT1, IFIT3, IFIT4, IL1B, IL1R2, IL1RN, IRF7, LGALS3BP, LY6E, MAP3K8, MX1, OAS1, OAS2, OASL3, OASL, PLSCR, SERPING1, TRAIL* and *XAF1* [90–93,96–98]. The vast majority of these genes are involved in the type I IFN pathway, as noted by others [91,102–105]. For instance, 29 out of 30 pediatric lupus patients were found to have an IFN signature [106]. Indeed IFN-α treatment can induce a lupus-like syndrome in 0.1–2.2% of patients [107]. Although there is an association with SLE, the type I IFN signature has been noted in other diseases as well. Dermatomyositis patients were also noted to have overexpression of IFN-induced genes [108]. The type I IFN pathway is also noted to be active in primary Sjögren's syndrome [109]. Treatment with IFN-α has additionally been linked to development of diabetes, thyroid disease,

Raynaud's phenomenon, rheumatoid arthritis, vasculitis, sarcoidosis, pernicious anemia, mixed connective tissue disease, psoriasis and psoriatic arthritis [107]. Hence, the prediction would be that this 26-gene panel may possess high sensitivity, but low specificity, for SLE.

False-positive results, and variation of results between facilities and platforms, are potential obstacle for comparing transcriptomics data. The high dimensionality of transcriptomic data also warrants careful processing in order to render it useful for clinical diagnostics and prognostics. Modular data mining is a possible solution for future transcriptomics analysis in order to reduce dimensionality and to discern transcriptomic fingerprints in order to identify biomarkers. An illustration of this approach is the recent report by the Pascual group [110]. Module construction was based on microarray analysis of PBMCs from 239 patients with: SLE, juvenile idiopathic arthritis, Type I diabetes, metastatic melanoma, liver transplant recipients undergoing immunosuppressive therapy and acute infections (*Escherichia coli, Staphylococcus aureus*, influenza A) that resulted in 4742 transcripts distributed into 28 module sets. Then, 11 of the 28 modules were determined to be significantly differentially expressed in SLE, comprised of a total of 628 transcripts. The differential expression profiles of these 11 modules were noted to be statistically significant ($p < 0.01$) for SLE patients versus healthy controls [110]. The results of this study will need further evaluation and validation, but appear promising. The ability of peripheral blood transcriptomic profiles to predict flares in SLE, and the transcriptomic profiles of the diseased end organs, are active areas of research in several laboratories.

## Proteomics

Proteomics focuses on the products of translation from RNA to proteins. Although transcriptomics indicates gene activity with 'intention' for protein synthesis, proteomics studies the product as a better indication of the underlying biologic processes. All bodily functions and disease phenotypes are ultimately mediated by proteins. Global proteomics provides a comprehensive evaluation of all proteins within a given sample, but is only in an infant stage at this time. This contrasts with transcriptomics and genomics, both of which can be carried out far more comprehensively, encompassing all genes in the body. The current stage of the technology allows only a fraction of the entire human proteome to be interrogated, but our capabilities are rapidly evolving. All proteomic studies can be classified into one of two broad categories – unbiased approaches, and biased or targeted approaches. Whereas the former attempts to scan the entire proteome, the latter platforms focus on interrogating limited predetermined subsets of the proteome. Owing to the technical difficulty of the former approach, and the fact that global proteomic technology is still evolving, very few such studies have been accomplished in SLE.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry combined with weak cationic exchange magnetic beads was used to analyze the serum proteomes of SLE patients [111]. Utilizing four differential protein peaks with m/z ratios of 4070.09, 7770.45, 28,045.1 and 3376.02 an AUC of 0.955 was obtained; however, the identities of the proteins underlying these peaks remain unknown. The validation results were as follows: 25 out of 32 SLE patients were correctly identified while seven were falsely classified as diseased controls; 36 out of 42 diseased controls (rheumatoid arthritis, Sjögren's syndrome and systemic sclerosis) were correctly identified, while three were falsely classified as SLE and three as healthy controls; 36 out of 42 healthy controls were correctly identified, while four were falsely classified as diseased controls [111]. Autoantibodies to brain proteins in the serum of CNS lupus patients have been identified utilizing two platforms referred to as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MS), in conjunction with liquid chromatography–tandem MS [112].

Another comprehensive method of analyzing complete proteomes is isobaric tagging for relative and absolute protein quantification. Total protein extracted from subsets of pooled PBMCs isolated from six active SLE, six stable SLE, six RA and six healthy controls was blocked, digested and labeled. A total of 452 proteins were identified with 67 differentially expressed unique proteins, including nine differentially expressed proteins between stable SLE and healthy controls, and 35 between active SLE and controls [113]. The identities of all the proteins are known, although these findings have not yet been validated. Comparison of the proteomes from lupus nephritis and healthy control renal tissue using isobaric tagging for relative and absolute protein quantification identified significant differences in the levels of heterogeneous nuclear ribonucleoprotein, annexins, arginosuccinate synthetase and aldolase [114]. An additional study involved 2D-gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass

spectrometry to compare the proteome of SLE PBMCs to that of healthy controls and has identified 98 unique proteins. SA100A9, a protein involved in leukocyte recruitment, was upregulated in SLE and was related to higher levels of low-density granulocytes that produce higher levels of IFN-α [115]. Though all of the above global proteomic approaches hold great promise, these studies warrant careful validation with larger, well-defined patient cohorts.

Although the fruits of global proteomics have not yet been fully realized, several individual proteins or groups of proteins have been examined for their predictive potential in SLE. Using protein microarray-based targeted proteomics, sera from 15 healthy controls, 15 SLE patients with high gene expression of IFN-regulated transcripts, and 15 SLE patients with low gene expression of IFN-regulated transcripts were analyzed for the levels of 160 protein analytes. Of these, 30 were noted to have significant differences between SLE high and controls, 17 between SLE low and controls, 11 between SLE high and SLE low and 27 between pooling of both SLE groups versus controls. Most of the differentially expressed protein analytes were inducible by type I IFN and were noted to correlate with disease activity [116]. A much larger microarray containing 5011 human proteins identified PBOV1, MORF4L1, CLIC2 and GDTP1 in SLE sera [117].

As opposed to adopting a proteomic approach to uncovering novel disease biomarkers, others have pursued individual proteins based on their known biology. These include Siglec-1, complement activation products and various urinary proteins, as discussed below. As mentioned above, type I IFN signatures predominate SLE patients' whole blood and PBMCs, and further transcriptome analysis of SLE patient's monocytes also revealed an IFN signature, including the transcript for Siglec-1 cell surface protein. Validation studies using antibodies to Siglec-1 (CD169) have noted exclusive expression on monocytes as detected by flow cytometry with increased frequency of Siglec-1$^+$ monocytes in SLE versus healthy controls that correlated with disease activity [97]. Likewise, measurements of lymphocyte-bound complement activation products, specifically T-cell bound C4d and C3d (T-C4d and T-C3d) and B-cell bound C4d and C3d (B-C4d and B-C3d) by flow cytometry revealed increased expression in SLE patients, compared with other autoimmune or inflammatory diseases with an AUC exceeding 0.72 [118]. Another flow cytometry-based platform, phosphoflow, evaluates phosphorylation signaling at the cellular level. For example, phosphoflow analysis has uncovered differences in the MAPK and signal transducer and activator of transcription pathways in cells of SLE patients [119,120]. Additional cell-based technologies include the comprehensive assessment of cell surface markers through leukocyte-capture arrays, a novel technology that warrants independent validation [121]. Various interleukins (IL-1, IL-2, IL-6, IL-10, IL-12 and IL-16), IFN-γ, TGF-β and TNF-α have exhibited expression differences at the protein level in SLE. Cytokine profiles have also been evaluated in SLE as markers of disease activity [122,123]. However, some of these differences are limited to the sites of pathology rather than being elevated systemically, limiting their utility as potential diagnostic markers [124].

In addition, various proteins in the urine have also been examined for their biomarker potential. TNF-like weak inducer of apoptosis is a cytokine that is involved in cellular proliferation, migration, survival, differentiation, induction of apoptosis and induction of inflammation via chemokines, cytokines and adhesion molecules. Urinary TNF-like weak inducer of apoptosis distinguishes lupus nephritis (LN) SLE patients from non-LN SLE with an AUC of 0.724, sensitivity of 0.50 and specificity of 0.90 [125]. Lipocalin-2 or neutrophil gelatinase-associated lipocalin, a protein secreted by leukocytes and epithelial cells in inflammatory conditions, differentiated LN patients from non-LN patients yielding a sensitivity of 0.50, specificity of 0.91, and AUC of 0.71 [126], with similar findings noted in pediatric SLE [127]. In SLE patients, two additional markers, urinary MCP-1 and urinary OPG, differentiated those with high renal activity (renal activity score ≥4) from SLE patients with low renal activity (renal activity score <4) with AUCs of 0.66 and 0.73, respectively [128]. Similar findings have been reported for two adhesion molecules, urinary VCAM-1 and urinary ICAM-1 [129]. A very recent urinary marker of chronic lupus nephritis that joins this list is angiostatin [130]. However, it should be pointed out that this is just the tip of the iceberg, as several additional proteins with great promise are currently under scrutiny. Serum and urine proteomics is an exploding field with several additional biomarkers in the pipeline currently being evaluated in several laboratories.

The immune system drives many of the manifestations of SLE through autoantibody

production. ANA positivity is one of the 11 ACR criteria for diagnosis; additionally the presence of a more specific antinuclear antibody (e.g., anti-dsDNA and anti-Sm) can fulfill a second diagnostic criterion. In a study of 130 military personnel diagnosed with SLE, the presence of autoantibodies in serum often preceded their diagnosis. In total, 88% of these subjects were noted to have the presence of one lupus-related autoantibody occurring on average 3.3 years prior to meeting four out of 11 ACR diagnostic criteria with particular specificities evolving in an ordered progression [131]. Established lupus autoantibody profiles have been interrogated utilizing bead-based automated multiplex assays, yielding comparable results to traditional clinical testing. However, ANA detection by BioPlex 2200 exhibited decreased sensitivity compared with indirect immunofluorescence [132–134].

Targeted proteomics is also revolutionizing new autoantibody discovery, based on the use of arrays coated with various putative autoantigens. The use of autoantigen microarrays containing 196 biomolecules including various proteins, peptides, enzyme complexes, ribonucleoprotein complexes, DNA and post-translationally modified antigens have helped detect autoantibodies in serum from patients with Sjögren's syndrome, SLE, polymyositis, mixed connective-tissue disease, primary biliary sclerosis, diffuse scleroderma, limited scleroderma, and rheumatoid arthritis with four- to eight-fold greater sensitivity than conventional ELISA [135]. A multiplexed proteome microarray containing 30 autoantigens known to be expressed in the glomerular milieu was used to interrogate the serum in SLE patients compared with RA and healthy controls. Serum antibody reactivity to laminin, myosin, Matrigel™ (BD Biosciences, CA, USA), heparan sulphate and vimentin clustered together whereas reactivity to ssDNA, dsDNA, chromatin and total glomerular lysate clustered separately and both clusters exhibited significantly higher reactivity in patients with elevated disease activity and more severe renal pathology [136]. A later study added 40 new autoantigens to these arrays (totaling 70 autoantigens and four controls) and evaluated the autoantibody profiles in SLE, incomplete lupus erythematosus, first degree relatives of SLE patients and nonautoimmune control patients (either healthy controls or osteoarthritis). Serum from incomplete lupus erythematosus and SLE patients exhibited higher levels of IgG antibodies to 50 autoantigens and IgM antibody to 12 autoantigens, with several of these autoantibody clusters correlating with disease activity, the number of ACR lupus criteria and renal disease status [137].

A study in 2010 utilized a larger chip containing 694 antigens to interrogate autoantibodies from three groups of SLE patients (in renal remission, with active lupus nephritis, and without renal involvement) and healthy controls. A global analysis of the antibody reactivities between all SLE patients and healthy controls demonstrated a sensitivity of 0.90 and a specificity of 0.81 for the diagnosis of SLE, yielding a calculated AUC of 0.855 that increased with various subset analyses. The antibodies that distinguished healthy control subjects from SLE included upregulation of IgG antibodies to dsDNA, ssDNA, hyaluronic acid and Epstein–Barr virus; and downregulation of IgM antibodies to myeloperoxidase, IGFBP-1, CD99 and cardiolipin [138]. Currently, arrays bearing >10,000 human proteins are being tested in multiple laboratories, and it would be interesting to see if these assays reveal any additional autoantibody specificities in SLE that may perhaps achieve even higher AUCs.

Technologies are under development that allow the detection of antigen–antibody and peptide–antibody binding in a comprehensive fashion. A plasmonic gold film peptide array using near infrared fluorescence-based detection reports improved sensitivity of autoantibody detection [139]. Nonfluorescence detection-based proteomic techniques are also being developed utilizing silicon-based peptide arrays [140]. This real-time technology is capable of evaluating peptide interactions and peptide modifications (e.g., methylation) and is likely to add a further dimension to the 'omics' analysis of SLE.

## Metabolomics

Metabolomics is the study of the complete set of smal molecule metabolites in a biological sample or organism. The first metabolomic study in SLE has recently been reported, utilizing liquid chromatography/MS and gas chromatography/MS platforms. When compared with healthy controls, sera from SLE patients exhibited reduction of energy substrates, elevation of markers of oxidative stress, elevation of markers of inflammation and altered lipid profiles [141]. Another metabolomic study utilizing nuclear magnetic resonance spectroscopy was performed on SLE sera and found similar reductions in energy substrates, increased low-density lipoproteins and increased lactate [142]. Urine nuclear magnetic resonance-derived

metabolomics profiles have been studied as potential diagnostic tools for differentiating proliferative and membranous lupus nephritis [143]. Glycomics is the study of complete saccharide profiles including sugars that are free and bound to proteins or lipids. Studying the potential contribution of carbohydrate changes to autoimmunity is a novel area of study that holds promise [144,145]. The potential of metabolomics is yet to be fully realized but may have potential utility in diagnostics and disease monitoring, extrapolating from the initial leads as described above.

## Conclusion

SLE is a complex multisystem autoimmune disease requiring four out of 11 ACR criteria to be satisfied in order to diagnose the disease. Although this approach is fairly sensitive and specific, it often leads to delayed or missed diagnosis. This system of classification results in the inadvertent clustering of a wide spectrum of different disease manifestations under the seemingly homogenous diagnosis of SLE. More importantly, the currently available instruments to gauge disease activity or renal pathology are inadequate at predicting oncoming flares, or are invasive. There is an acute need in the field to identify better biomarkers that can not only help diagnose and subdivide SLE, but also predict oncoming flares and predict (and guide) treatment response. Emerging 'omics' platforms – genomics, transcriptomics, proteomics and metabolomics – allow us to re-examine SLE at a greater degree of molecular resolution so that disease subsets may be better defined. Pathway analysis and integration of 'omics' platforms in order to undertake a systems biology analysis is a growing field of research likely to yield additional insights in this field [146–148]. More importantly, one is hopeful that these 'omics' platforms may yield newer biomarkers for SLE that can help clinicians track the disease course with greater sensitivity and specificity.

## Future perspective

A wide spectrum of 'omics' platforms are being tested in an attempt to identify better biomarkers for the diagnosis and monitoring of SLE, and to shed light on the molecular cascades that lead to the pathogenesis of lupus. Advances in technology and bioinformatics will continue to expand the repertoire of platforms and biomarker dimensionality over the next several years. Defining the interactions between different molecular elements and integrating the various levels of 'omics' using a systems biology approach promise to yield novel perspectives on the pathogenic cascades leading to SLE. Defining the underlying science will also pave the way to better therapies for this dreaded autoimmune disease.

### Executive summary

***Genomics***

- HLA, complement, and Fc region of IgG-related genes identified decades ago remain significant in systemic lupus erythematosus (SLE) genomics.
- Genome-wide association studies (GWAS) in SLE have uncovered potentially pathogenic single nucleotide polymorphisms in and around several candidate genes.
- Combining GWAS-identified single nucleotide polymorphisms into panels is a promising diagnostic approach.
- Rare genes with SLE association are being identified.
- Epigenomics may bridge the gap between genetic information, environmental influences and phenotype.

***Transcriptomics***

- Gene microarrays of peripheral blood mononuclear cells have provided information regarding gene-expression changes in SLE.
- The interferon signature, though not specific for SLE, has repeatedly been demonstrated to be upregulated in SLE.
- A few cell type targeted transcription studies have also been completed in SLE.

***Proteomics***

- Global proteomic scans using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, isobaric tagging for relative and absolute protein quantification, and other platforms are becoming feasible, but have not been widely used to study SLE.
- Targeted proteomic studies focusing on selected proteins, antigens, or cytokines have been undertaken in the study of SLE with interesting leads.
- With both of the above approaches, independent validation of the hits is warranted.

***Metabolomics***

- Altered metabolites in SLE include reduced energy substrates; and increased lipids, oxidative stress and inflammation.
- The potential of metabolomics is yet to be fully realized but may have potential utility in diagnostics and disease monitoring.

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## Medscape EDUCATION Systemic lupus erythematosus diagnostics in the 'omics' era

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| **Activity evaluation: where 1 is strongly disagree and 5 is strongly agree.** | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| The activity supported the learning objectives. | | | | | |
| The material was organized clearly for learning to occur. | | | | | |
| The content learned from this activity will impact my practice. | | | | | |
| The activity was presented objectively and free of commercial bias. | | | | | |

1. You are seeing a 40-year-old woman with a history of joint pain and laboratory evidence of nephritis. What should you consider regarding the diagnosis of systemic lupus erythematosus (SLE)?

- ☐ **A** SLE is more than 4 times more common among women than men
- ☐ **B** At least 8 of 11 American College of Rheumatology (ACR) criteria are necessary to make the diagnosis of SLE
- ☐ **C** Most patients are diagnosed with SLE within 3 months of the onset of symptoms
- ☐ **D** Antinuclear antibodies (ANA) are highly specific in the diagnosis of SLE

2. What should you consider regarding the genomics of SLE?

- ☐ **A** The concordance rate of SLE diagnosis among monozygotic twins is 70%
- ☐ **B** Human leukocyte antigen (HLA)- and Fc region of IgG (FcγR)-related mutations are no longer considered relevant in the pathogenesis of SLE
- ☐ **C** Mutations in IRF5 alone are strongly predictive of SLE
- ☐ **D** Pharmacogenomics can identify risks of drug toxicity but not predict response to therapy for SLE

| 3 | Which is the most common finding using transcriptomics in cases of SLE? | |
|---|---|---|
| ☐ | **A** | Downregulation of interleukin (IL)-1 |
| ☐ | **B** | Upregulation of IL-3 |
| ☐ | **C** | Upregulation of interferon |
| ☐ | **D** | Downregulation of tumor necrosis factor (TNF) |

| 4 | Which of the following statements regarding proteomics and metabolomics in SLE is most accurate? | |
|---|---|---|
| ☐ | **A** | Proteomics is generally performed on a patient's entire spectrum of proteins |
| ☐ | **B** | Urinary TNF-like weak inducer of apoptosis (TWEAK) distinguishes patients with lupus nephritis from those without lupus nephritis |
| ☐ | **C** | Metabolomics focuses only on a limited number of metabolites in each patient |
| ☐ | **D** | Metabolomics in SLE is characterized by increased energy substrates and reduced lipid values |