VIEWING SLE THROUGH THE OMICS LENS

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Bibliography

Dr. Chandra Mohan is a Professor in the Division of Rheumatology. His research is focused on "genetic dissection" of lupus and lupus nephritis, and the identification of better biomarkers for SLE tapping upon various "OMICs" platforms and imaging modalities.

Purpose & Overview

The goal of this session is understand how various global molecular profiling technologies using "OMICs" platforms are augmenting our understanding of one of the most complex and systemic of all autoimmune disease, SLE.

Educational Objectives

1. As opposed to hypothesis driven research, "OMICs" platforms allow researchers to interrogate all genes (Genomics), RNAs (Transcriptomics), proteins (Proteomcs) and metabolites (Metabolomics) comprehensively in parallel.
2. Large scale genomic analysis of SLE reveals that a large number of genetic polymorphisms that impact the functioning of the adaptive immune system or the innate immune system can confer susceptibility to SLE.
3. Large scale transcriptomic analyses of PBMCs from SLE patients point to prominent contributions from Type I interferons as well as granulocytes.
4. Ongoing large scale proteomic and metabolomic analysis of SLE are helping researchers identify novel biomarkers of SLE and lupus nephritis, many of which await systematic evaluation in longitudinal studies.
5. Since SLE is very heterogenous (given that the diagnosis of SLE is based on fulfilling 4 out of 11 criteria involving multiple organ systems), novel biomarkers arising from these global "OMICs" platforms may help better diagnose, subset and monitor this systemic autoimmune disease.

Introduction

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, the diagnosis of SLE relies upon meeting at least four out of eleven clinical and laboratory criteria outlined by the American College of Rheumatology (4; Table 1). As one could imagine, this leads to a wide spectrum of potential disease manisfestations that could vary substantially from patient to patient; hence, SLE is by no means a homogenous disease. In addition, the complexity of the disease, relative non-specificity of the diagnostic criteria and the requirement of having to meet four ACR criteria often lead to a delay in diagnosis, or even misdiagnosis. A study from 2003 found that the interval between the first symptom and diagnosis was on the average 21.82 months [5].

Criteria	Description
Malar rash	Butterfly rash or flat/raised erythema sparing nasolabial fold
Discoid rash	Raised erythematous patches with adeherent keratotic scaling
Photosensitivity	Photosensitivity lasting for several weeks
Oral ulcers	Oral or nasopharyngeal ulceration
Arthritis	Nonerosive arthritis involving 2 or more peripheral joints
Serositis	Pleuritis or pericarditis
Renal disorder	Persistent proteinuria
Neurological disorder	Seizures or psychosis
Hematological disorder	Lymphopenia, hemolytic anemia, thrombocytopenia
Immunological disorder	Anti-DNA, anti-Sm, false-positive serology for syphilis
Antinuclear antibody	Abnormal titer of ANA at any point in time

Table 1. The ACR criteria for diagnosis of SLE. Currently, the diagnosis of SLE relies upon meeting at least four out of eleven clinical and laboratory criteria, as outlined by the American College of Rheumatology (4).

Among the different diagnostic criteria, the presence of antinuclear antibodies (ANA) is relatively more specific for SLE. Though ANA is positive in 98% of SLE patients, they can also be positive in other autoimmune diseases and healthy controls, making ANA positivity insufficient for the diagnosis of SLE [6]. Hence, the scientific community actively pursues the discovery of novel diagnostics in the hope of better identifying susceptible individuals in early stages of the disease, and discerning subgroups with specific organ involvement. In this respect, the "omics" platforms hold great promise.

Hypothesis-driven research versus OMICs-driven research

The traditional way of doing research is to begin with a well-defined hypothesis and to test that hypothesis. By design, such research is highly focused on a small, finite set of molecules (genes, proteins, etc) or on a single pathway, in order to pin-point a pathogenic process or validate a given therapeutic target. In contrast, OMICs-driven research is exploratory in nature, and seeks to interrogate the entire molecular landscape, with the idea that key pathways or nodes that are aberrant in a disease could be uncovered through a brute force approach. Thus, instead of studying one gene, OMICs researchers would interrogate the entire genome. Instead of an mRNA message, the entire transcriptome would be examined. Similarly, global approaches can be used to scan the entire proteome, metabolome, etc. Though OMICs approaches have the potential to highlight all molecules that are malfunctioning in a given disease, they are expensive, technology-intensive, and may end up generating more data than information. Nevertheless, 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 (http://www.nature.com/omics/index.html). The challenge ahead is to tap upon both hypothesis-driven research as well as the more exploratory global OMICs platforms in order to better understand, diagnose and manage SLE. In this review, we will survey some recent developments in our understanding of SLE, based on various OMICs studies.

1. GENOMICS OF SLE

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 [7]. The $\lambda_{sibling}$ rate of SLE has been reported to be 5.8 - 29, indicating the presence of familial aggregation [8]. 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 to SLE.

1A. HLA and complement genes: The first noted genetic association with lupus, the human leukocyte antigen (HLA) region, was reported in the 1970s. Selected HLA Class II DR and DQ alleles were noted to be associated with SLE [9]. *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 [9]. 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)* [10]. 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 anti-nuclear antibodies [11]. Murine complement gene knock-out models have also been created that develop SLE-like disease [12-15]. MutS protein homolog 5 (*MSH5*), a gene that is involved in immunoglobulin class switching, is also located in the HLA Class III locus and is highly associated with SLE [16]. To date, some of the strongest genes for SLE are the ones described about four decades ago - *DR2, DR3, C2, C4,* and *C1q*.

1B. GWAS studies: Besides the HLA and complement genes, multiple single nucleotide polymorphisms (SNPs) within several additional genes have been identified in

association with SLE [17-24]. These genes represent common polymorphisms with relatively small effect sizes, as summarized in Fig. 1. Some of the uncovered SNPs have also been noted to have racial associations to populations of African, European, Hispanic, Asian, and Amerindian origin [17-19, 21-23, 25-29]. The discovery of these associated gene polymorphisms has been made possible by genome-wide associations scans (GWAS). As tracked at genome.gov/gwastudies, an NIH database of published genome-wide association studies, there have been eight large-scale GWAS pertaining to SLE, which have collectively uncovered greater than two dozen genetic loci for SLE [30].

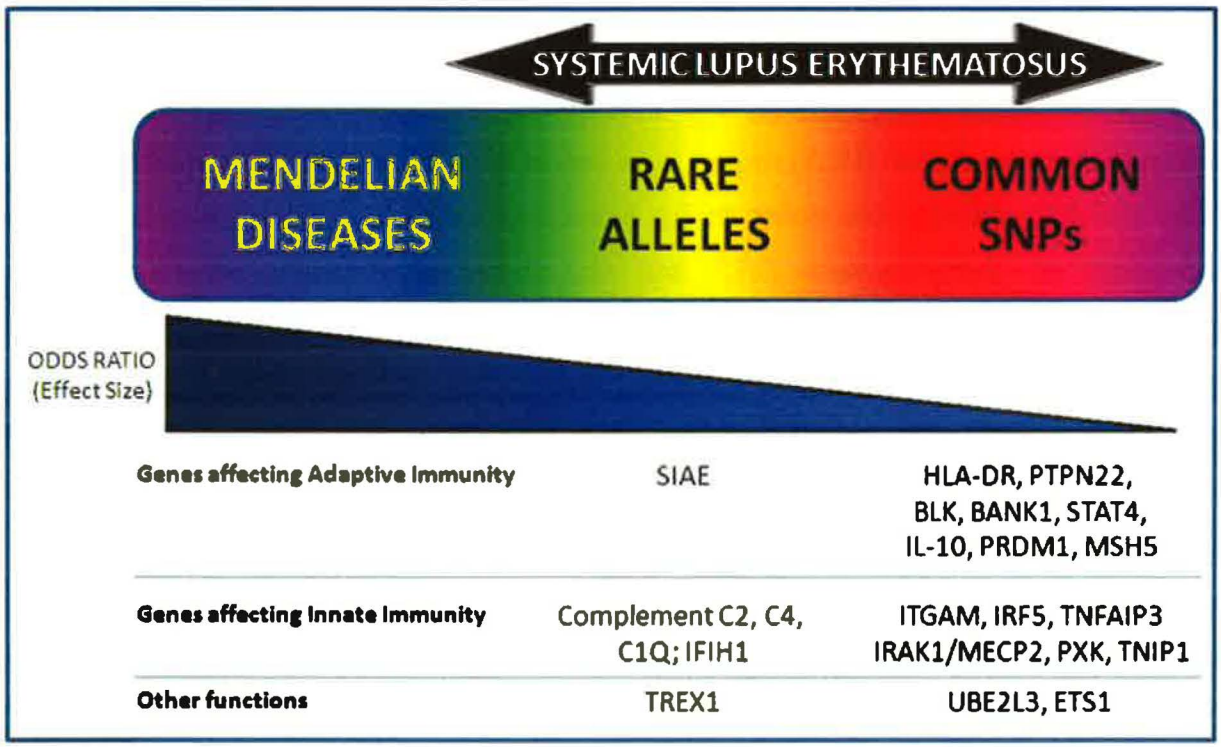

Fig. 1. The polygenic origins of SLE.

Most of the genes for SLE constitute common polymorphisms with small effect sizes, though a few rare genes with larger effect sizes have also been reported. As indicated, most of the SLE associated genes appear to impact either the adaptive or the innate immune system, as previously demonstrated in mouse models of lupus [32].

A meta-analysis of all GWAS studies requiring at least two reports with a $P \leq 1X10^{-5}$ for significance has identified HLA-DRB1*0301 (*HLA-DR3*), HLA-DRB1*1501 (*HLA-DR2*),

protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), interferon regulatory factor 5 (*IRF5*), signal transducer and activator of transcription 4 (*STAT4*), B lymphoid tyrosine kinase (*BLK*), integrin alpha M (*ITGAM*), and tumor necrosis factor, alpha-induced protein 3 (*TNFAIP3*) as genes confirmed to be associated with SLE [31], as listed in Fig. 1. These can be further categorized according to the immune function they impact. *IRF5*, *IRAK1*, *TNFAIP3* and ITGAM play key roles in the innate immune response, while *HLA-DR3, HLA-DR2, PTPN22, BLK*, and *STAT4* impact lymphocyte activation and/or function in the adaptive immune system. 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 genetic dissection studies in murine lupus, which point to 3 genetically determined steps in lupus development - breach in immune tolerance in the adaptive immune system, hyperactivation of the innate immune system, and chronic end-organ inflammation [32].

Individually, these genetic associations have small effect sizes, and are not strongly predictive of SLE. However, the development of SNP panels for potential diagnostics appears promising. A panel of 11 SNPs (found within the following genes - *HLA, ITGAM, IRF5/TNPO3, KIAA1542, PXK)* is noted to have an area under the receiver operating curve (ROC) of 0.67 with a reasonable degree of sensitivity and specificity for diagnosing SLE [33]. 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, BANK1* has been reported to have an AUC of 0.679, that improves to 0.689 when weighted based on odds ratios [34]. Researchers continue refining these SNP panels in order to improve their utility as diagnostic tools for the future. More recent studies have discerned specific sets of genes being associated with specific manifestations of SLE [34], and it is possible that we may one day be able to subset SLE into several overlapping syndromes driven by different gene sets and pathogenic pathways.

1C. Rare genes for SLE: The common genetic variants identified using GWAS confer relatively small effect sizes, as measured by their odds ratios, and collectively account for only a modest fraction of total disease heritability. In contrast to these common genetic polymorphisms 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. These include *SIAE*, *TREX1*, and *IFIH1,* as reviewed elsewhere [35], and summarized in Fig. 1. These rare genetic variants, particularly if highly penetrant, could be more predictive of SLE than the commonly occurring gene variants uncovered using GWAS. Continued discovery and characterization of common and rare genetic polymorphisms will likely lead to improved diagnostic gene panels for SLE. Ongoing efforts in the field employ next generation sequencing approaches aimed at targeted regions in the genome or the whole exome with the hope of uncovering additional genes underlying SLE.

2. TRANSCRIPTOMICS OF SLE

Transcriptomics evaluates comprehensive gene expression, or the RNA message that is being transcribed from the DNA sequence in a global fashion. Compared to genomics, transcriptomics allows researchers to focus just on the expressed fraction of the genome. Microarray technology has allowed rapid and comprehensive evaluation of gene expression differences between SLE patients and healthy controls. Unlike genomics, transcriptomic analyses focus on particular cells or tissues. Given the known immunologic effects of SLE, the focus of transcriptomic analyses in SLE have mostly concentrated on white blood cell populations. Nearly ten such studies have thus far been completed [36-45].

2A. Type 1 Interferon Signature: One of the earliest of these studies compared the transcriptomes of peripheral blood mononuclear cells (PBMCs) of various autoimmune diseases including SLE and healthy controls, and documented differences in gene

expression levels related to apoptosis, cell migration, cell differentiation, and cell cycle progression in SLE [36]. Another study undertook a comparison of PBMCs of SLE patients to controls using a focused cDNA array of cytokines, chemokines, growth factors, apoptosis, and immunomodulatory genes, and highlighted the up-regulation of genes related to TNF/death receptor, IL-1, and IL-8 [37]. Similarly, one more PBMC-based microarray study noted the up-regulation in death receptors and IL-1, Fc receptors, cell adhesion molecules, and multiple Type I interferon pathway genes [38]. Indeed, upregulation of genes in the Type 1 interferon (IFN-I) pathway has emerged as the dominant gene signature in SLE, virtually dwarfing all other observed pathway changes [39-45]. A second signature that has been noted in some of the studies encompasses granulocyte-specific genes [39]. A caveat should be considered in interpreting all of the above studies. The study of peripheral blood mononuclear cells' expression profiles can yield biased results due to the heterogeneity of the cell populations in patients versus healthy controls; hence, future studies should examine isolated leukocyte populations from SLE patients for their gene expression signatures.

These studies suggest a central role for IFN-I in SLE pathogenesis. Indeed interferon alpha treatment can induce a lupus-like syndrome in 0.1-2.2% of patients [46]. Although there is an association with SLE, the type I interferon signature has been noted in other diseases as well. Dermatomyositis patients have also been reported to over express interferon induced genes [47]. The type I interferon pathway has also been noted to be active in primary Sjögren's syndrome [48]. Treatment with IFN-alpha has additionally been linked to the development of diabetes, thyroid disease, Raynaud's phenomenon, rheumatoid arthritis, vasculitis, sarcoidosis, pernicious anemia, mixed connective tissue disease, psoriasis, and psoriatic arthritis [46]. Thus an upegulated IFN-I pathway has high sensitivity but low specificity for SLE.

2B. Simplifying the dimensionality of OMICs data: Microarray studies result in a deluge of transcriptomic data, the meaning of which is often difficult to fathom. The challenge in data analysis is to reduce dimensionality so that the key differences become readily apparent. Commercially available pathway analysis programs are commonly used for

this purpose. Recently, Pascual and colleagues have reported a modular data mining approach for reducing dimensionality and identifying transcriptomic fingerprints in a given disease. In this study, PBMC transcriptomic data comprised of 4742 transcripts harnessed from 239 patients with SLE, juvenile idiopathic arthritis, type I diabetes, metastatic melanoma, liver-transplant recipients undergoing immunosuppressive therapy, and acute infections were collapsed into 28 module sets (based on the known properties of the differentially expressed transcripts). The expression patterns of 11 of these 28 modules were noted to be statistically significant in SLE patients versus healthy controls or other diseases [49]. By tracking the activation of these 11 gene modules, the authors demonstrated that disease subtypes and activity can be readily monitored. Though the results of this study will need further evaluation and validation, the approach adopted clearly illustrates how the dimensionality of OMICs data can be readily simplified, so that clinically useful information can be gleaned.

3. PROTEOMICS OF SLE

All biological processes encoded in the genome are expressed through their respective RNA transcripts. Nevertheless, all bodily functions and disease phenotypes are ultimately mediated by proteins, not DNA or RNA. Hence, defining the cellular proteome may yield a better snapshot of the pathological processes leading up to a disease. The goal of global proteomics is to provide a comprehensive evaluation of all proteins within individual cells or tissues; however, the current stage of the technology allows only a fraction of the entire human proteome to be interrogated at reasonable costs, though our capabilities are rapidly evolving. This contrasts with global transcriptomics and genomics, both of which can be carried out far more comprehensively, encompassing all genes in the body. Regardless, two preliminary proteomic studies have already been executed, focusing on SLE using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and isobaric Tagging for Relative and Absolute protein Quantification (iTRAQ).

3A. Global Proteomic Studies in SLE: MALDI-TOF MS combined with weak cationic exchange (WCX) magnetic beads was used by Huang et al to analyze the serum proteomes of SLE patients, compared to healthy controls [50]. Utilizing four differentially expressed protein peaks with m/z ratios of 4070.1, 7770.5, 28045.1, and 3376.0, an AUC of 0.955 was obtained, differentiating SLE patients from controls; however the identities of the proteins underlying these peaks remain unknown [50]. The confirmatory results in a second patient set were as follows: 25/32 SLE patients were correctly identified with 7 falsely classified as diseased controls, 36/42 diseased controls (Rheumatoid Arthritis, Sjögren's syndrome, and Systemic Sclerosis) were correctly identified with 3 falsely classified as SLE and 3 as healthy controls, and 36/42 healthy controls were correctly identified with 4 falsely classified as diseased controls [50]. A second study adopted another large-scale platform for analyzing complete proteomes, named iTRAQ, an approach that allows for quantitative proteomics. Total protein extracted from PBMCs isolated from 6 active SLE, 6 stable SLE, 6 RA, and 6 healthy controls was blocked, digested, and labeled [51]. A total of 452 proteins were identified, with 67 of these showing significant expression differences between the groups, including 9 differentially-expressed proteins between stable SLE and healthy controls, and 35 differentially-expressed proteins between active SLE and controls. Though both the above approaches are being expanded and validated by multiple groups working in this field, they represent the very first attempts to unravel the SLE proteome. As is true in SLE, the full potential of global proteomics is yet to be realized in clinical medicine, including various rheumatic diseases.

3B. Targeted Proteomic Studies in SLE: In contrast to global proteomic scans, focused or targeted proteomics biased towards finite sets of target proteins is far easier to execute. One well established platform for targeted proteomic analysis is the planar array. Planar arrays have been useful in uncovering novel protein biomarkers as well as novel autoantibodies. Planar arrays pre-coated with antibodies to potential biomarker proteins can be used to interrogate the blood levels of various proteins such as cytokines, chemokines, shed receptors, growth factors, etc, as illustrated in Fig. 2.

Conversely, planar arrays coated with a battery of autoantigens can be used to assay the levels of multiple autoantibodies in parallel (Fig. 2). Both these approaches are beginning to pave the way towards the identification of novel biomarkers in SLE.

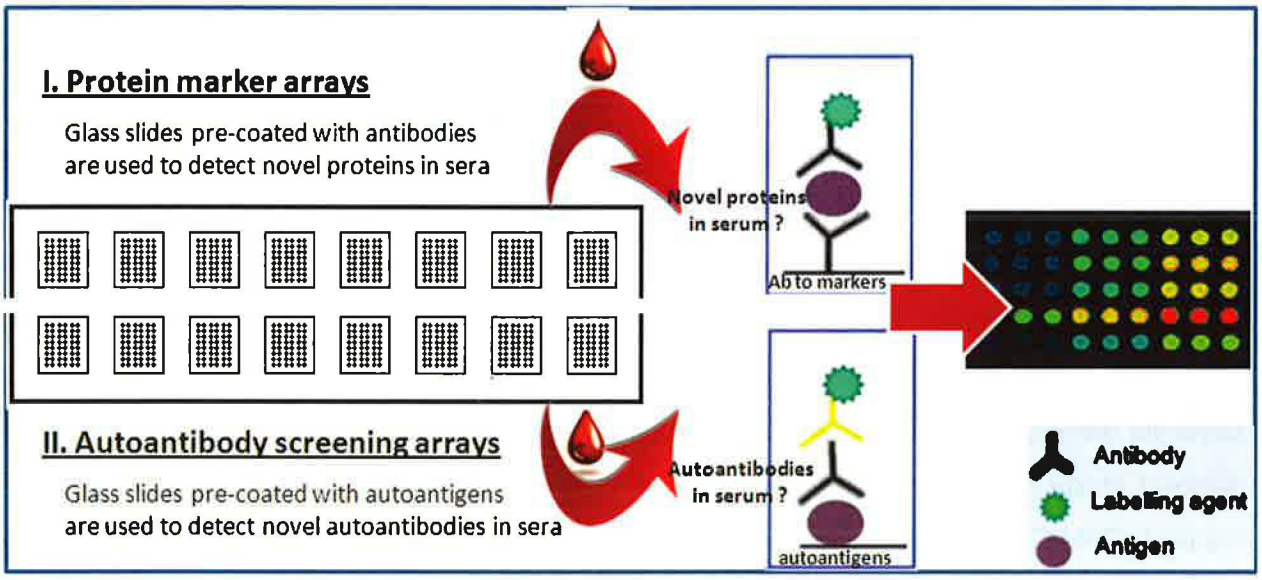

Fig. 2. Planar arrays allow targeted or focused proteomic analysis in 2 different ways. On the one hand, planar arrays pre-coated with antibodies to potential biomarker proteins can be used to interrogate the blood levels of various proteins such as cytokines, chemokines, shed receptors, growth factors, etc (I, above). Alternatively, planar arrays coated with a battery of autoantigens can be used to assay the levels of multiple autoantibodies in sera (II, below). Both these approaches are beginning to pave the way towards the identification of novel biomarkers in SLE.

3C. Array-based discovery of new biomarkers. Carlsson et al have recently reported the use of a planar array to interrogate the serum levels of 60 different moldcules (52). When sera from SLE patients, patients with systemic sclerosis, and healthy controls were compared, the serum protein profile had the capacity to distinguish between these diseases. Notably, SLE patients had significantly higher levels of IL-2, IL-5, IL-10, IL-12, IFN-γ, and CD40, and reduced levels of the complement proteins C1q, C3, C4, C5 and factor B, compared to the controls, with several of these differences correlating with disease activity. Though these differences are yet to be independently validated, they represent the first report using planar arrays to uncover novel lupus biomarkers.

A recent study employed a more extensive planar array to interrogate the serum levels of ~300 proteins in SLE (53). Thirty of the molecules that were upregulated in SLE sera on the arrays were subsequently validated using independent patient cohorts and orthogonal assay platforms. In addition to confirming several previously reported increases (including increased serum leptin, osteopontin, OPG, TGF-β, TNFR-II, and VCAM-1 in SLE), this new study also uncovered several additional proteins to be elevated in SLE serum, including angiostatin, EFG-R, Furin, IGFBP-4, Siglecs, TREM1 and VEGF-C (53). These newly identified biomakers warrant rigorous testing in longitudinal patient samples, in order to ascertain if these proteins could be used to subset SLE patients, predict disease severity and course, formulate targeted therapy, and/or to monitor treatment response. In addition, similar platforms are being applied to mine urine from patients for novel biomarkers of lupus nephritis. Examples of potential urinary markers of lupus nephritis that have arisen from initial array-based screens include VCAM-1, sTNFRI, p-selectin, CXCL16, total urinary protease, prostaglandin D synthase, serum amyloid P, and superoxide dismutase (54, 55). Altogether, this is an exploding field of research that promises to yield novel disease markers in the near future.

3D. Array-based autoantibody screens in SLE: The immune system drives many of the manifestations of SLE through autoantibody production. Anti-nuclear antibody (ANA) positivity is one of the 11 ACR criteria for diagnosis; additionally more specific antinuclear antibodies (e.g., anti-dsDNA and anti-Sm) can lead to fulfillment of a second diagnostic criterion (4; Table 1). In a study of 130 military personnel diagnosed with SLE, the presence of autoantibodies in serum often preceded their diagnosis. Eighty-eight percent were noted to have the presence of one lupus-related autoantibody occurring on average 3.3 years prior to fulfilling 4/11 ACR diagnostic criteria [56]. In addition to ANA and anti-DNA, targeted proteomic studies have expanded the serach for additional autoantibodies in SLE, as summarized below.

Autoantigen microarrays containing 196 biomolecules designed to detect antibodies to proteins, peptides, enzyme complexes, ribonucleoprotein complexes, DNA and post-

translationally modified antigens have helped document differing spectrums of 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 4-8 fold greater sensitivity than conventional ELISA [57]. Another planar microarray bearing nuclear antigens as well as autoantigens known to be expressed in the glomerular milieu has been used to study serum from murine lupus models as well as SLE patients, compared against RA and healthy controls (58). Several distinct clusters of autoantibodies were noted in SLE, with two of these clusters being particularly ominous. Autoantibody reactivity to laminin, myosin, Matrigel, heparan sulphate, and vimentin clustered together whereas reactivity to ssDNA, dsDNA, chromatin, and total glomerular lysate clustered separately; importantly, both these autoantibody clusters were significantly higher in the serum of patients with active disease and renal involvement [58]. A later study added forty new autoantigens to this array, and evaluated sera from SLE, incomplete lupus erythematosus (ILE), first degree relatives of SLE or ILE patients (FDR), and non-autoimmune control patients (either healthy controls or osteoarthritis). Serum from ILE and SLE patients exhibited several novel clusters of IgG and IgM autoantibodies compared to the controls, and some of these clusters correlated well with disease activity, number of ACR lupus criteria, and renal disease [59].

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, with improved specificity and sensitivity profiles. Clearly, all newly identified autoantibodies have to be carefully assessed for their disease predictive potential in longitudinal studies.

4. METABOLOMICS OF SLE

The word "metabolome" refers to the complete set of small-molecule metabolites in a biological sample, or organism. The first draft of the human metabolome was reported in 2007 [60]. Since then, comprehensive metabolomic scans have been applied to the study of only a handful of diseases. The very first metabolomic study in SLE has recently been completed, comparing the serum metabolome of 20 SLE patients against that of healthy controls, using LC/MS and GC/MS based platforms (61). Validation of key differences was performed using an independent cohort of 38 SLE patients and orthogonal assays. Interestingly, SLE sera showed evidence of profoundly dampened glycolysis, Krebs cycle, fatty acid β oxidation and amino acid metabolism, alluding to reduced energy biogenesis from all sources. Whereas long-chain fatty acids, including the n3 and n6 essential fatty acids were significantly reduced, medium chain fatty acids and serum free fatty acids were elevated. The SLE metabolome exhibited a profound degree of lipid peroxidation, reflective of oxidative damage. Deficiencies were noted in the cellular anti-oxidant, glutathione, and all methyl group donors, including cysteine, methionine, and choline, as well as phosphocholines. These differences did not appear to be related to the medications the patients were on. Whether the patients' nutritional status and the underlying disease process could potentially have contributed to the observed metabolic differences remain to be dissected out.

Table 2. Specificity and sensitivity profiles of 4 metabolic markers in SLE (61)

Metabolic Marker	AUC (SLE vs RA)	AUC (SLE vs Healthy)
Leukotriene B4	0.82	0.99
GGT1	0.67	0.87
MDA (lipid peroxidation)	0.97	0.95
Glutathione	0.78	0.83

Though these studies need to be extended to additional patient populations, they have yielded several novel propects for disease biomarkers. The best metabolite discriminators of SLE included elevated lipid peroxidation products, MDA, gamma-glutamyl peptides, GGT1, leukotriene B4 and 5-HETE; these novel serum markers had the capacity to distinguish SLE from healthy controls and RA disease controls with a good degree of specificity and sensitivity (Table 2). The utility of these markers in predicting disease in longitudinal studies remains to be established.

Conclusion

Systemic Lupus Erythematosus is a complex multi-system disease requiring 4/11 American College of Rheumatology criteria to be satisfied in order to diagnose the disease. This complex system of classification results in the inadvertent clustering of a wide spectrum of different disease manifestations under the seemingly homogenous diagnosis of SLE. Though evidence indicates that early diagnosis and treatment can improve long term disease outcome, the currently available yardsticks to detect early disease are far from being optimal. The "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. This is an exploding field of research, and one is hopeful that these "omics" platforms will yield better biomarkers for SLE, with superior sensitivity and specificity.

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References Cited

1. Crowson, C.S., et al., *The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases.* Arthritis Rheum, 2011. **63**(3): p. 633-9.
2. Merrell, M. and L.E. Shulman, *Determination of prognosis in chronic disease, illustrated by systemic lupus erythematosus.* J Chronic Dis, 1955. **1**(1): p. 12-32.
3. Cervera, R., et al., *Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients.* Medicine (Baltimore), 2003. **82**(5): p. 299-308.
4. Tan, E.M., et al., *The 1982 revised criteria for the classification of systemic lupus erythematosus.* Arthritis Rheum, 1982. **25**(11): p. 1271-7.
5. Ozbek, S., et al., *Delay in the diagnosis of SLE: the importance of arthritis/arthralgia as the initial symptom.* Acta Med Okayama, 2003. **57**(4): p. 187-90.
6. Maddison, P.J., *Is it SLE?* Best Pract Res Clin Rheumatol, 2002. **16**(2): p. 167-80.
7. Deapen, D., et al., *A revised estimate of twin concordance in systemic lupus erythematosus.* Arthritis Rheum, 1992. **35**(3): p. 311-8.
8. Alarcon-Segovia, D., et al., *Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort.* Arthritis Rheum, 2005. **52**(4): p. 1138-47.
9. Graham, R.R., et al., *Specific combinations of HLA-DR2 and DR3 class II haplotypes contribute graded risk for disease susceptibility and autoantibodies in human SLE.* Eur J Hum Genet, 2007. **15**(8): p. 823-30.

10. Hartung, K., et al., *The genetic basis of Ro and La antibody formation in systemic lupus erythematosus. Results of a multicenter study. The SLE Study Group.* Rheumatol Int, 1992. **11**(6): p. 243-9.
11. Meyer, O., et al., *Genetic deficiency of C4, C2 or C1q and lupus syndromes. Association with anti-Ro (SS-A) antibodies.* Clin Exp Immunol, 1985. **62**(3): p. 678-84.
12. Paul, E., et al., *Anti-DNA autoreactivity in C4-deficient mice.* Eur J Immunol, 2002. **32**(9): p. 2672-9.
13. Mitchell, D.A., et al., *C1q deficiency and autoimmunity: the effects of genetic background on disease expression.* J Immunol, 2002. **168**(5): p. 2538-43.
14. Botto, M., et al., *Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies.* Nat Genet, 1998. **19**(1): p. 56-9.
15. Einav, S., et al., *Complement C4 is protective for lupus disease independent of C3.* J Immunol, 2002. **168**(3): p. 1036-41.
16. Moser, K.L., et al., *Recent insights into the genetic basis of systemic lupus erythematosus.* Genes Immun, 2009. **10**(5): p. 373-9.
17. Barcellos, L.F., et al., *High-density SNP screening of the major histocompatibility complex in systemic lupus erythematosus demonstrates strong evidence for independent susceptibility regions.* PLoS Genet, 2009. **5**(10): p. e1000696.
18. Douglas, K.B., et al., *Complement receptor 2 polymorphisms associated with systemic lupus erythematosus modulate alternative splicing.* Genes Immun, 2009. **10**(5): p. 457-69.
19. Furnrohr, B.G., et al., *Polymorphisms in the Hsp70 gene locus are genetically associated with systemic lupus erythematosus.* Ann Rheum Dis, 2010. **69**(11): p. 1983-9.
20. Kim-Howard, X., et al., *ITGAM coding variant (rs1143679) influences the risk of renal disease, discoid rash and immunological manifestations in patients with systemic lupus erythematosus with European ancestry.* Ann Rheum Dis, 2010. **69**(7): p. 1329-32.
21. Lu, R., et al., *Genetic associations of LYN with systemic lupus erythematosus.* Genes Immun, 2009. **10**(5): p. 397-403.

22. Namjou, B., et al., *Evaluation of C1q genomic region in minority racial groups of lupus.* Genes Immun, 2009. **10**(5): p. 517-24.
23. Webb, R., et al., *A polymorphism within IL21R confers risk for systemic lupus erythematosus.* Arthritis Rheum, 2009. **60**(8): p. 2402-7.
24. Armstrong, D.L., et al., *Identification of new SLE-associated genes with a two-step Bayesian study design.* Genes Immun, 2009. **10**(5): p. 446-56.
25. Bouali, H., et al., *Association of the G-463A myeloperoxidase gene polymorphism with renal disease in African Americans with systemic lupus erythematosus.* J Rheumatol, 2007. **34**(10): p. 2028-34.
26. Chung, S.A., et al., *European population substructure is associated with mucocutaneous manifestations and autoantibody production in systemic lupus erythematosus.* Arthritis Rheum, 2009. **60**(8): p. 2448-56.
27. Kamen, D.L., et al., *Autoantibody prevalence and lupus characteristics in a unique African American population.* Arthritis Rheum, 2008. **58**(5): p. 1237-47.
28. Moser, K.L., et al., *Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees.* Proc Natl Acad Sci U S A, 1998. **95**(25): p. 14869-74.
29. Sanchez, E., et al., *Genetically determined amerindian ancestry correlates with increased frequency of risk alleles for systemic lupus erythematosus.* Arthritis Rheum, 2010. **62**(12): p. 3722-9.
30. Hindorff LA, J.H., Hall PN, Mehta JP, and Manolio TA. *A Catalog of Published Genome-Wide Association Studies.* 2011; Available from: www.genome.gov/gwastudies.
31. Graham, R.R., et al., *Review of recent genome-wide association scans in lupus.* J Intern Med, 2009. **265**(6): p. 680-8.
32. Kanta, H. and C. Mohan, *Three checkpoints in lupus development: central tolerance in adaptive immunity, peripheral amplification by innate immunity and end-organ inflammation.* Genes Immun, 2009. **10**(5): p. 390-6.
33. Harley, J.B., et al., *Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci.* Nat Genet, 2008. **40**(2): p. 204-10.

34. Taylor, K.E., et al., *Risk alleles for systemic lupus erythematosus in a large case-control collection and associations with clinical subphenotypes.* PLoS Genet, 2011. **7**(2): p. e1001311.
35. Satterthwaite, A.B. and C. Mohan, *Genetics: Rare genes for autoimmunity-the new kids on the block.* Nat Rev Rheumatol, 2010. **6**(12): p. 678-9.
36. Maas, K., et al., *Cutting edge: molecular portrait of human autoimmune disease.* J Immunol, 2002. **169**(1): p. 5-9.
37. Rus, V., et al., *Expression of cytokine- and chemokine-related genes in peripheral blood mononuclear cells from lupus patients by cDNA array.* Clin Immunol, 2002. **102**(3): p. 283-90.
38. Baechler, E.C., et al., *Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus.* Proc Natl Acad Sci U S A, 2003. **100**(5): p. 2610-5.
39. Bennett, L., et al., *Interferon and granulopoiesis signatures in systemic lupus erythematosus blood.* J Exp Med, 2003. **197**(6): p. 711-23.
40. Han, G.M., et al., *Analysis of gene expression profiles in human systemic lupus erythematosus using oligonucleotide microarray.* Genes Immun, 2003. **4**(3): p. 177-86.
41. Mandel, M., et al., *Autoimmunity gene expression portrait: specific signature that intersects or differentiates between multiple sclerosis and systemic lupus erythematosus.* Clin Exp Immunol, 2004. **138**(1): p. 164-70.
42. Nakou, M., et al., *Gene expression in systemic lupus erythematosus: bone marrow analysis differentiates active from inactive disease and reveals apoptosis and granulopoiesis signatures.* Arthritis Rheum, 2008. **58**(11): p. 3541-9.
43. Crow, M.K. and J. Wohlgemuth, *Microarray analysis of gene expression in lupus.* Arthritis Res Ther, 2003. **5**(6): p. 279-87.
44. Biesen, R., et al., *Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus.* Arthritis Rheum, 2008. **58**(4): p. 1136-45.

45. Lyons, P.A., et al., *Novel expression signatures identified by transcriptional analysis of separated leucocyte subsets in systemic lupus erythematosus and vasculitis.* Ann Rheum Dis, 2010. **69**(6): p. 1208-13.
46. Gota, C. and L. Calabrese, *Induction of clinical autoimmune disease by therapeutic interferon-alpha.* Autoimmunity, 2003. **36**(8): p. 511-8.
47. Greenberg, S.A., et al., *Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis.* Ann Neurol, 2005. **57**(5): p. 664-78.
48. Bave, U., et al., *Activation of the type I interferon system in primary Sjogren's syndrome: a possible etiopathogenic mechanism.* Arthritis Rheum, 2005. **52**(4): p. 1185-95.
49. Chaussabel, D., et al., *A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus.* Immunity, 2008. **29**(1): p. 150-64.
50. Huang, Z., et al., *MALDI-TOF MS combined with magnetic beads for detecting serum protein biomarkers and establishment of boosting decision tree model for diagnosis of systemic lupus erythematosus.* Rheumatology (Oxford), 2009. **48**(6): p. 626-31.
51. Wang, L., et al., *Comparative proteome analysis of peripheral blood mononuclear cells in systemic lupus erythematosus with iTRAQ quantitative proteomics.* Rheumatol Int, 2010.
52. Carlsson A, Wuttge DM, Ingvarsson J, et al. Serum protein profiling of systemic lupus erythematosus and systemic sclerosis using recombinant antibody microarrays. Mol Cell Proteomics 10.1074, 2011
53. Wu T, Han J, and Mohan C. Identification of novel serum biomarkers of SLE using planar arrays. Manuscript in preparation.
54. T Wu, C Xie, HW Wang, XJ Zhou, N Schwartz, M Mackay, C Aranow, C Putterman, and C Mohan. 2007. Elevated urinary VCAM-1, P-selectin, sTNFR-1 and CXCL16 in multiple murine lupus strains and human lupus nephritis. J Immunol *179(10):7166-75*
55. Tianfu Wu, Yuyang Fu, Deirdre Brekken, Columbine Che, Noa Schwartz, Ho Bing Oei, Xin J Zhou, Chul Ahn, Chaim Putterman and Chandra Mohan. 2010.

Urine proteome scans uncover total urine protease as a potential marker of lupus nephritis. J Immunol 184(4):2183-93;

56. Arbuckle, M.R., et al., *Development of autoantibodies before the clinical onset of systemic lupus erythematosus.* N Engl J Med, 2003. **349**(16): p. 1526-33.
57. Robinson, W.H., et al., *Autoantigen microarrays for multiplex characterization of autoantibody responses.* Nat Med, 2002. **8**(3): p. 295-301.
58. Li, Q.Z., et al., *Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays.* J Clin Invest, 2005. **115**(12): p. 3428-39.
59. Li, Q.Z., et al., *Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes.* Clin Exp Immunol, 2007. **147**(1): p. 60-70.
60. Wishart DS, Tzur D, Knox C, *et al.* (January 2007). "HMDB: the Human Metabolome Database". *Nucleic Acids Research* 35 (Database issue): D521–6.
61. Tianfu Wu, Chun Xie, Jie Han, Jim Weiel, Quan Li, Irene Blanco, Chul Ahn, Nancy Olsen, Chaim Putterman, Ramesh Saxena, Chandra Mohan. Metabolic disturbances underlying systemic lupus erythematosus. Plos One (under revision); 2012.