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Distinct gene dysregulation patterns herald precision medicine potentiality in systemic lupus erythematosus

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ABSTRACT

Objectives: We aimed at investigating the whole-blood transcriptome, expression quantitative trait loci (eQTLs), and levels of selected serological markers in patients with SLE versus healthy controls (HC) to gain insight into pathogenesis and identify drug targets.
Methods: We analyzed differentially expressed genes (DEGs) and dysregulated gene modules in a cohort of 350 SLE patients and 497 HC from the European PRECISESADS project (NTC02890121), split into a discovery (60%) and a replication (40%) set. Replicated DEGs qualified for eQTL, pathway enrichment, regulatory network, and druggability analysis. For validation purposes, a separate gene module analysis was performed in an independent cohort (GSE88887).
Results: Analysis of 521 replicated DEGs identified multiple enriched interferon signaling pathways through Reactome. Gene module analysis yielded 18 replicated gene modules in SLE patients, including 11 gene modules that were validated in GSE88887. Three distinct gene module clusters were defined i.e., "interferon/plasma cells", "inflammation", and "lymphocyte signaling". Predominant downregulation of the lymphocyte signaling cluster denoted renal activity. By contrast, upregulation of interferon-related genes indicated hematological activity and vasculitis. Druggability analysis revealed several potential drugs interfering with dysregulated genes within the "interferon" and "PLK1 signaling events" modules. *STAT1* was identified as the chief regulator in the most enriched signaling molecule network. Drugs annotated to 15 DEGs associated with *cis*-eQTLs included bortezomib for its ability to modulate *CTSL* activity. Belimumab was annotated to *TNFSF13B* (BAFF) and daratumumab was annotated to *CD38* among the remaining replicated DEGs.
Conclusions: Modulation of interferon, *STAT1*, PLK1, B and plasma cell signatures showed promise as viable approaches to treat SLE, pointing to their importance in SLE pathogenesis.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease marked by diverse clinical features as the result of an underlying maladaptive immune-mediated process involving leukocytes, antibodies, the complement system, and cytokines [1]. Reliable biomarkers for diagnosis and monitoring of patients with SLE constitute an unmet need [2,3]. Advances in the omics era include genetic profiling with next-generation sequencing (NGS) techniques, coupled with sophisticated bioinformatics. These methods have not only provided insights

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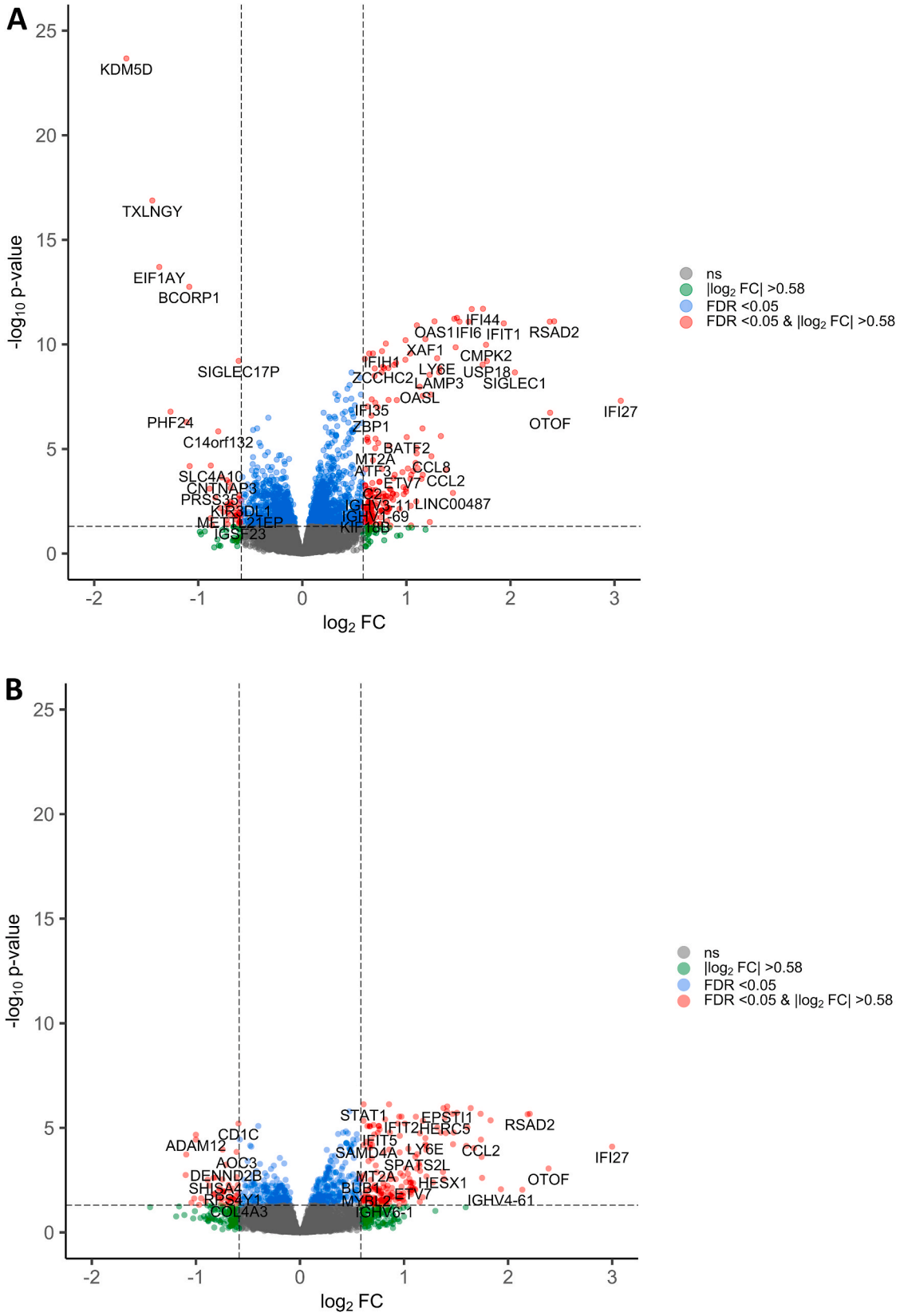


Fig. 1. Volcano plots of DEGs in SLE patients versus HC. The volcano plots show differentially expressed genes (DEGs) in the **(A)** discovery and **(B)** replication sets. DEGs which both passed the FDR <0.05 and the $|\log_2 FC|$ >0.58 are highlighted in red. DEGs which only passed the FDR threshold are denoted in blue. DEGs which only passed the FC threshold are highlighted in green. Non-significant DEGs are in grey. DEGs: differentially expressed genes; FC: fold change; FDR: false discovery rate; ns: non-significant; HC: healthy controls; SLE: systemic lupus erythematosus.

into the pathogenesis of SLE, but they have also addressed the clinical heterogeneity by identification of distinct molecular signatures with implications towards precision medicine. The characteristic interferon (IFN) gene signature [4] marks the starting point of such efforts, with the anti-type I IFN receptor monoclonal anifrolumab [5] having recently been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of SLE, but several other subgroups have also been identified, including the recently proposed patient stratification by neutrophil- and lymphocyte-driven clusters [4,6]. Furthermore, studies of single nucleotide polymorphisms (SNPs) in relation to expression of target genes integrating genotype and RNA-sequencing data through expression quantitative trait loci (eQTL) analysis have enhanced the study of genetic causality in SLE pathogenesis [7,8]. Given the complexity of the molecular mechanisms involved in SLE [1], each biological level provides an important source of information towards unravelling the complete picture of the dysregulated immune system underlying the disease at large, as well as specific manifestations. Integration of multiple levels of omics could contribute to a more granular understanding of the pathophysiology of SLE and provide implications for new drug targets or repurposing of existing drugs, towards personalized therapeutic approaches.

The aim of this study was to investigate the transcriptome, eQTLs, and levels of selected cytokines and autoantibodies in patients with SLE compared with healthy controls (HC) from the PRECISESADS project [9] to gain insight into underlying biological mechanisms and dysregulated signaling pathways, and suggest targets that hold promise for drug development or repurposing to treat SLE.

2. Material and methods

2.1. Study population and data

Clinical data and blood samples from two cross-sectional cohorts of patients with SLE (n = 463), all fulfilling the revised American College of Rheumatology (ACR) criteria for SLE [10], and 497 HC were obtained within the frame of the 5-year European PRECISESADS project (NTC02890121; see Supplementary Material, page 5 for complete inclusion and exclusion criteria). All patients and HC provided written informed consent prior to recruitment, and the study was approved by local ethics review boards at the 18 participating centers (see Supplementary Material, page 6 for a list of local investigators). Genome-wide DNA-genotyping and peripheral whole-blood RNA-sequencing were performed using Illumina assays (Illumina Inc., San Diego, CA, USA), as previously described [9]. Analysis of serum levels of a broad panel of cytokines and autoantibodies was performed, as described elsewhere [9]. In brief, 88 cytokines were initially measured in a subset of patients with SLE and HC to select a panel of 14 cytokines that were subsequently measured with a customized assay, both analyses using Luminex technology (Luminex xMAP™ Technology and R&D Systems Luminex assay, Luminex Corporation, Austin, TX, USA). Additionally, matrix metalloproteinase-2 (MMP-2), C-reactive protein (CRP), Tumor Necrosis Factor alpha (TNF-α), interleukin (IL)-6, B cell activating factor belonging to the TNF ligand family (BAFF), and transforming growth factor beta (TGF-β) were analyzed using a quantitative sandwich enzyme immunoassay (Biorad Laboratories Inc., Hercules, CA, USA). Autoantibody levels were analyzed with an automated chemiluminescent immunoanalyser (IDS-iSYS, Immunodiagnostic Systems Holdings Ltd., East Boldon, United Kingdom), a turbidimetric immunoassay (SPAPLUS analyzer, The Binding Site Group Ltd., Birmingham, United Kingdom), and an anti-dsDNA-NcX enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany).

For validation purposes, publicly available genome-wide transcriptomic data from the ILLUMINATE-1 (NCT01205438) and ILLUMINATE-2 (NCT01196091) trials [11] of the anti-BAFF human IgG4 monoclonal antibody tabalumab in patients with SLE were utilized. These data were generated through analysis of peripheral whole-blood mRNA using the Affymetrix Human Transcriptome Array 2.0 (Thermo Fisher Scientific, Santa Clara, CA, USA) and NanoString (NanoString, Seattle, WA, USA), as previously described [11]. The dataset comprised data from 1760 patients with SLE, all fulfilling the revised ACR criteria for SLE (14), and 60 HC, and was retrieved from the Gene Expression Omnibus (GEO) database repository (ID: GSE88887) [11,12]. Samples from SLE patients were obtained prior to the trial intervention (at the trial baseline).

2.2. Statistical analysis

The 350 patients with SLE and 497 HC with available transcriptome data were randomly split into a discovery (60%) and a replication (40%) set. Differentially expressed gene (DEG) and weighted gene co-expression network analysis (WGCNA) was performed in the discovery and replication sets separately. Replicated gene modules were functionally annotated based on gene expression data from Chaussabel et al. [13] and Li et al. [14]. Dysregulation of replicated gene modules and DEGs was assessed by mean z-scores of genes for each SLE patient compared with all sex-matched HC, adopting a similar methodology as that recently reported by Toro-Domínguez et al. [15]. A separate DEG analysis and gene module analysis was performed in GSE88887 to validate the DEGs and gene modules that were replicated in the discovery and replication sets.

The dysregulation of replicated gene modules was analyzed in relation to serological markers. Pathway enrichment analysis was performed by means of over-representation analysis (ORA) and gene set enrichment analysis (GSEA). In order to investigate genetic markers with a potential to guide choice of treatment, eQTL analysis of the replicated DEGs was performed. The most upregulated and downregulated replicated DEGs from the discovery set were imputed in iRegulon [16] through Cytoscape [17] to yield signaling molecule networks and their chief regulators. Replicated DEGs were run through the Drug Gene Interaction database (DGIdb) for druggability analysis. Comparisons of unrelated continuous data were made using the Mann-Whitney *U* test, associations between unrelated binomial variables were investigated using Pearson's chi squared (χ^2) or Fisher's exact tests, and correlations were assessed using Pearson or Spearman's rank correlation coefficients, as appropriate. All p values < 0.05 and a false discovery rate (FDR) < 0.05 (Benjamini-Hochberg) were considered statistically significant. Analyses were performed using the R software version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). A detailed description of all methods can be found in the Supplementary Material, page 7–11.

2.3. Patient and public involvement

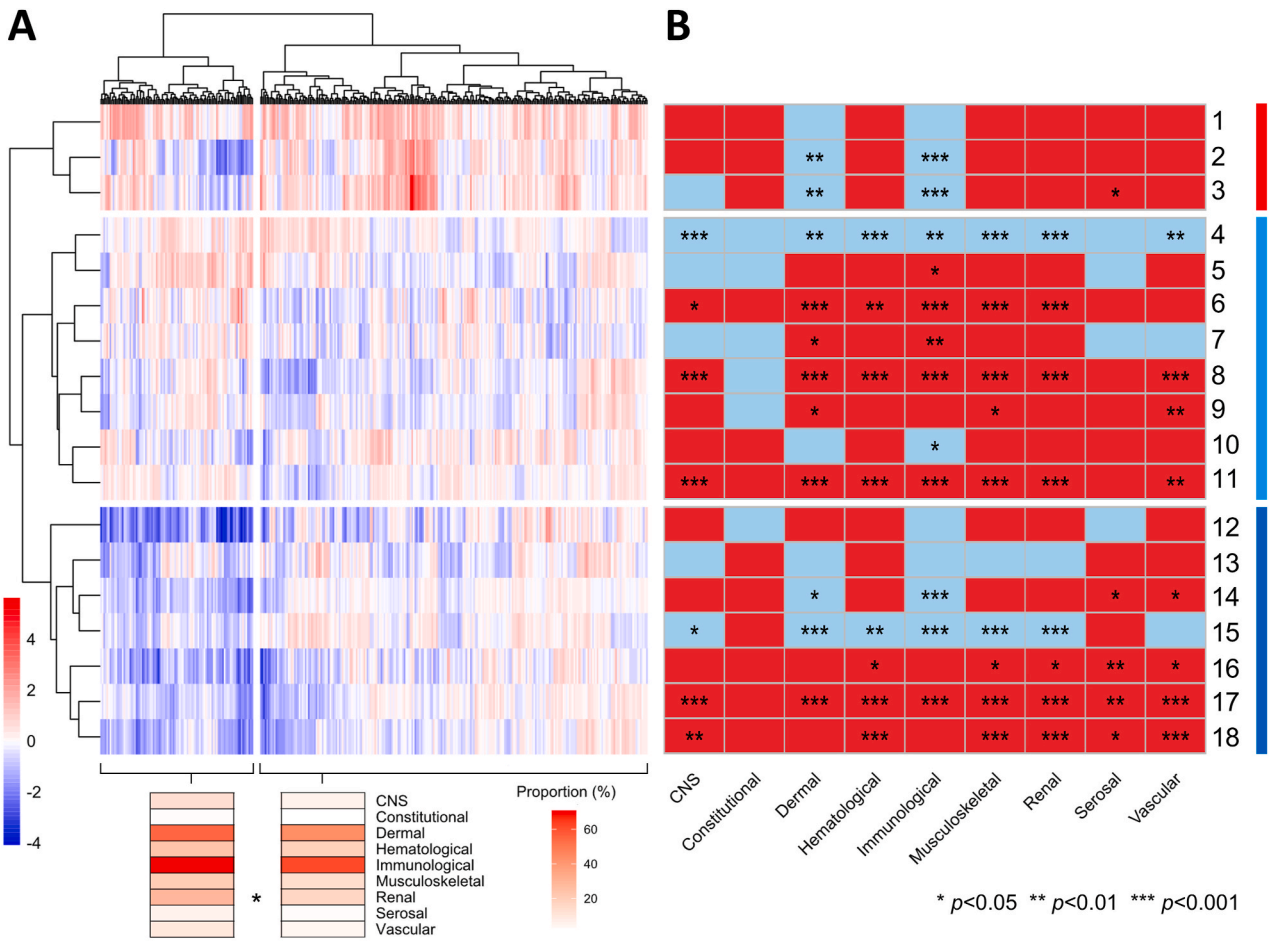
A patient research partner (YE) was involved in the design and reporting of this research. The public was not involved in the design, analysis, or reporting or dissemination plans of this research.

3. Results

Patient characteristics and clinical data are presented in Supplementary Material, page 12.

3.1. Differentially expressed genes (DEGs) associated with SLE

DEG analysis in the discovery set resulted in 1726 significant DEGs after FDR correction, and subsequent analysis in the replication set resulted in 675 DEGs, with 521 DEGs overlapping between the two sets, which were considered replicated (Fig. 1; detailed in Supplementary Material, sheets 1 and 2). Among the replicated DEGs in the PRECISESADS cohort, a total of 361 (69.3%) DEGs overlapped with DEGs that reached statistical significance in the GSE88887 dataset (detailed in



- **1** Interferon
- **2** Plasma cells, immunoglobulins
- **3** PLK1 signaling events

Interferon/plasma cells

- **4** Inflammation
- **5** Lipid metabolism, endoplasmic reticulum
- **6** Erythrocytes
- **7** Platelets
- **8** CORO1A-DEF6 network
- **9** Olfactory receptors
- **10** Monocytes
- **11** Platelet activation

Inflammation

- **12** B cell
- **13** Regulation of transcription, transcription factors
- **14** Cell cycle
- **15** Enriched for ubiquitination
- **16** Cytotoxic/NK cell
- **17** Enriched in cell cycle
- **18** T cell signaling and co-stimulation

Lymphocyte signaling

- Validated in an independent cohort

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Fig. 2. Dysregulated gene modules in patients with SLE. (A) The heatmap shows replicated gene modules and their dysregulation in relation to the gene expression of HC, as measured by the z score, in patients with SLE. Columns denote SLE patients, and rows denote gene modules, clustered using hierarchical clustering with the Ward method. Clusters of gene modules are termed based on the predominant gene modules included in each cluster and are indicated by colored bars. Gene modules that were validated in GSE88887 are indicated by green dots. Proportions of SLE patients with specific clinical features, as determined by SLEDAI-2K, within the two main patient subgroups are indicated below the heatmap. All *p* values are derived from Pearson's chi squared (χ^2) or Fisher's exact tests. **(B)** Dysregulation of gene modules in relation to organ-specific activity based on the SLEDAI-2K. The group without organ-specific activity for each comparison was considered the reference group. Red and blue colors denote higher and lower z-scores compared with the reference group, respectively. All *p* values are derived from Mann-Whitney *U* tests. CORO1A-DEF6: coronin 1 A-differentially expressed in FDCP 6 homolog; NK: natural killer; HC: healthy controls; PLK1: polo like kinase 1; SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.

Supplementary Material, page 13 and sheet 3).

3.2. Dysregulated gene modules and molecular subgroups in SLE

WCGNA analysis yielded 18 replicated gene modules (detailed in Supplementary Material, sheets 4–6). As shown in the heatmap in Fig. 2A, the dysregulated gene modules were grouped into three main clusters, which were termed "interferon/plasma cells", "inflammation", and "lymphocyte signaling" based on the predominant gene modules included in each cluster. Furthermore, the SLE patients were clustered into two main subgroups, one characterized by varying dysregulation patterns in gene modules of the interferon/plasma cells and inflammation clusters but prominently downregulated gene modules within the lymphocyte signaling cluster, and one subgroup characterized by an upregulated interferon/plasma cells cluster and varying dysregulation patterns in gene modules within the inflammation and lymphocyte signaling clusters. The former patient subgroup displayed higher SLE Disease Activity Index 2000 (SLEDAI-2K) [18] scores (mean ± standard deviation: 8.3 ± 7.0 versus 5.4 ± 5.3; $p < 0.001$) and a higher proportion of patients with active renal disease (28.3% versus 16.3%; $p = 0.017$) compared with the other patient subgroup (detailed in Supplementary Material, page 14). Among the 18 replicated gene modules, 11 gene modules were validated in an independent cohort (GSE88887), including the "interferon", "plasma cells, immunoglobulins", "inflammation", "erythrocytes", "platelets", "monocytes", "platelet activation", "B cell", "regulation of transcription, transcription factors", "cytotoxic/NK cell", and "T cell signaling and co-stimulation" gene modules (Fig. 2; detailed in Supplementary Material, page 15).

Overall, dysregulation of the "inflammation", "erythrocytes", "CORO1A-DEF6 network", "platelet activation", "enriched for ubiquitination", "enriched in cell cycle", and "T cell signaling and co-stimulation" genes modules was similar across SLEDAI-2K organ domains (Fig. 2B; detailed in Supplementary Material, page 16–24).

As shown in the heatmap in Fig. 3, the 139 replicated DEGs with a $|\log_2$ fold change (FC)$|$ >0.58 i.e., FC < 0.66 for downregulation and >1.5 for upregulation, were grouped into four main gene clusters based on their degree of dysregulation. One gene cluster included genes within the "B cell", "cell cycle", and "cytotoxic/NK" gene modules (cluster 1), one cluster included genes within the "plasma cells, immunoglobulins", and "PLK1 signaling events" gene modules (cluster 2), one cluster exclusively included genes within the "interferon" gene module (cluster 3), and one gene cluster included genes within the "cell cycle", "enriched for ubiquitination", "enriched in cell cycle", "interferon", "platelet activation", and "T cell signaling and co-stimulation" gene modules (cluster 4).

Furthermore, the SLE patients were clustered into two main subgroups, one of which was characterized by predominant upregulation in gene clusters 2, 3, and 4, yet downregulation in cluster 1, and one subgroup characterized by varying dysregulation patterns across all four gene. The former patient subgroup was characterized by higher proportions of hematological activity (26.3% versus 13.4%; $p = 0.004$) and vasculitis (8.1% versus 2.4%; $p = 0.037$) as per SLEDAI-2K (detailed in Supplementary Material, page 25).

3.3. Pathway enrichment analysis

The most enriched pathways from the ORA and GSEA are shown in Supplementary Material, page 26 (detailed in Supplementary Material, sheets 7–10). Several pathways, such as virus disease and the necroptosis pathways, were enriched based on the KEGG library. Multiple interferon signaling pathways were enriched based on the Reactome database.

3.4. eQTL analysis

The eQTL analysis in SLE patients yielded a total of 4385 significant *cis*-eQTLs which involved groups of SNPs in blocks of high linkage of disequilibrium (LD) associated with expression levels of a total of 66 DEGs (detailed in Supplementary Material, sheet 11). No significant *trans*-eQTLs were found after FDR correction. As seen in Fig. 4A, most significant *cis*-eQTLs clustered close to the transcription start site of the respective genes. *Cis*-eQTLs included eight SNPs that were strongly associated with complement C3a receptor 1 (*C3AR1*), as well as rs9410942 G > T that was associated with cathepsin L (CTSL), and rs7918733 T > C that was associated with caspase 7 (*CASP7*; Fig. 4B–D). The expression levels of *C3AR1* and *CTSL* decreased whereas gene expression of *CASP7* increased with the number of alternative alleles for the most statistically significant SNPs (Fig. 4E–N).

A trend towards higher expression of *C3AR1* was seen in serologically active SLE patients according to the SLEDAI-2K (anti-dsDNA positivity, low complement levels) than in serologically inactive patients (median [interquartile range]: 9.5 [8.9–10.0] versus 9.3 [8.8–9.9]; $p = 0.053$). This prompted further comparisons for the immunological descriptors of the SLEDAI-2K. This analysis revealed a higher expression of *C3AR1* in anti-dsDNA positive versus negative SLE patients (9.5 [9.0–10.1] versus 9.3 [8.8–9.9]; $p = 0.034$) but no statistically significant difference between patients with low versus normal/high complement levels (detailed in Supplementary Material, page 27–28).

3.5. Cytokine and autoantibody profiles in relation to dysregulated gene modules in SLE

As shown in Fig. 5A (detailed in Supplementary Material, page 29–64), dysregulation of the interferon gene module, as measured by z-score, was positively correlated with serum BAFF levels in SLE patients. Furthermore, the z-score of the interferon gene module was higher in patients with anti-dsDNA positivity versus negativity, anti-Sm positivity versus negativity, and low versus normal/high levels of C3c and C4, among several serological markers (Fig. 5B; detailed in Supplementary Material, page 65–80).

3.6. Druggable dysregulated genes in SLE

A total of 744 drugs from the DGIdb were annotated to 96 replicated DEGs (detailed in Supplementary Material, sheet 12). Most drugs were annotated to the "cell cycle" gene module (n = 283), followed by the interferon (n = 164), "inflammation" (n = 162), and "PLK1 signaling events" (n = 164) modules. Drugs annotated to 15 DEGs that were associated with *cis*-eQTLs included CHEMBL389348 for its ability to interfere with *C3AR1* activity, bortezomib for its ability to interfere with

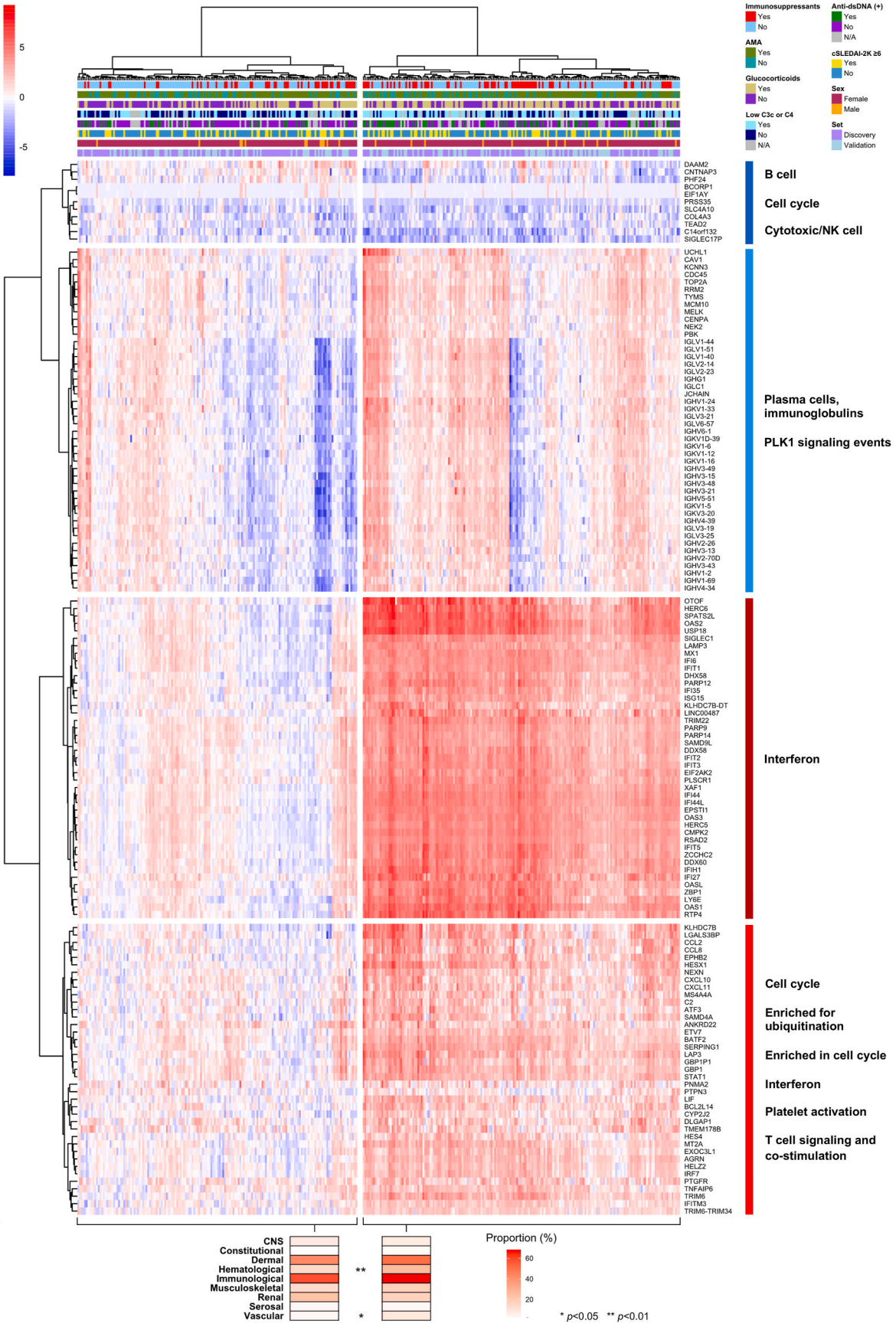

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Fig. 3. Heatmap of DEGs in SLE patients versus HC. The heatmap shows replicated DEGs and their dysregulation in relation to the gene expression of HC, as measured by the z score, in the discovery (in purple) and replication (in light blue) sets. Only DEGs that exceeded the $|\log_2 FC| > 0.58$ threshold in the discovery set are included in the heatmap. Columns denote SLE patients, and rows denote replicated DEGs, clustered using hierarchical clustering with the Ward method. Clusters of DEGs are annotated by their gene modules and are indicated by colored bars. Proportions of SLE patients with specific clinical features, as determined by SLEDAI-2K, within the two patient subgroups are indicated below the heatmap. Asterisks denote statistically significant differences. The annotations on the top of the heatmap annotate for immunosuppressant use (in red/light blue), AMA use (in olive/sea green), glucocorticoid use (in khaki/violet), low levels of C3 or C4 (in cadet blue/dark blue), anti-dsDNA positivity (in forest green/purple), cSLEDAI-2K ≥ 6 (in yellow/steel blue), and sex (in maroon/orange) for each patient with SLE. Grey color indicates missing data. All *p* values are derived from Pearson's chi squared (χ^2) or Fisher's exact tests. AMA: antimalarial agents; anti-dsDNA: anti-double stranded DNA; C3c: complement component 3c; C4: complement component 4; CPM: counts per million; cSLEDAI-2K: clinical Systemic Lupus Erythematosus Disease Activity Index 2000; DEGs: differentially expressed genes; FC: fold change; HC: healthy controls; SLE: systemic lupus erythematosus.

CTSL activity, and several drugs with ability to interfere with *CASP7*. Lastly, belimumab was annotated to *TNFSF13B* (BAFF) and daratumumab was annotated to *CD38*, two of the replicated DEGs.

3.7. Druggability potentiality through signaling molecule networks in SLE

The five top chief regulators and enriched motifs belonging to the most enriched signaling molecule networks, based on iRegulon, are displayed in the Supplementary Material, page 81. Signal transducer and activator of transcription 1 (STAT1) was associated with the factorbook-signal transducer and activator of transcription 2 (STAT2) motif, and enriched with a normalized enrichment score (NES) of 23.32 in the top signaling molecule network (Fig. 6). Drugs annotated to these DEGs included irinotecan for its ability to inhibit the expression of IFN-stimulated gene 15 (*ISG15*), which was upregulated in the DEG analysis.

4. Discussion

In the present study, analysis of the whole-blood transcriptome of a large cohort of patients with SLE and HC resulted in 521 replicated DEGs that were subsequently used in analysis of pathway enrichment and regulatory networks. Moreover, we identified 18 replicated gene modules in SLE patients, including 11 gene modules that were validated in an independent cohort, and assessed their dysregulation patterns in relation to HC. Through gene module analysis performed to reduce dimensionality, three distinct gene module clusters were defined i.e., interferon/plasma cells, inflammation, and lymphocyte signaling, whose dysregulation patterns grouped the SLE patients into subgroups of distinct clinical phenotypes. In concrete, predominant upregulation of interferon-related genes indicated hematological activity and vasculitis while downregulation within the lymphocyte signaling gene module cluster denoted renal activity. We also investigated DEGs that were coupled to gene polymorphisms in eQTL analysis. Among DEGs assessed for druggability, *CTSL* and *CD38* were of particular interest, as was the implication for the prospect of caspase inhibition.

Following a workflow developed to integrate multiple layers of omics data, we identified several mediators and pathways of interest, both of well-known importance in SLE and less studied ones warranting further investigation. Among the former, we found high gene expression of BAFF in SLE patients versus HC in line with previous literature [19]. Besides its central role in the pathogenesis of SLE [20], BAFF has also been proven to be a relevant drug target, with the anti-BAFF belimumab being the first biological agent in history to be licensed for the treatment of SLE [5], and recently also for lupus nephritis [21,22]. To aid interpretation, it is worth noting that none of the SLE patients included in the present study had received belimumab therapy within 6 months prior to enrolment. In agreement with the well-known importance of IFN signaling in SLE [1,4], multiple IFN signaling pathways were enriched in the present investigation. Moreover, we found that dysregulation of the "interferon" gene module correlated with serum BAFF levels in SLE patients, in line with a previously reported association between BAFF levels and an interferon cluster based on gene and DNA methylation modules across several autoimmune diseases [9], as well as *in vitro* experiments showing that IFN-α accelerates BAFF secretion by SLE monocytes [23]. The interplay between interferons and B cell activation has also been highlighted in studies demonstrating that B cell-intrinsic deletion of the IFN gamma (γ) receptor decreases B cell activation and ameliorates disease in murine lupus [24,25]. Notably, *STAT1* was herein found to be a chief regulator of the most enriched signaling molecule network; STAT1 is an important transcription factor [26] that regulates the expression of interferon-stimulated among other genes [27] and has in previous research been suggested as a potential therapeutic target for SLE [27]. Moreover, our results suggested drug repurposing potentiality with the use of the topoisomerase inhibitor I irinotecan, for its ability to inhibit the expression of *ISG15*, a gene among the replicated DEGs of the present study that was upregulated in the enriched STAT1 network. In this respect, it is worth noting that irinotecan has shown promising results in studies of murine lupus [28] and a case report of human SLE [29], and has been canvassed as a potential drug for SLE [30].

In the present study, we identified 18 replicated gene modules in SLE patients, including 11 gene modules that were validated in an independent cohort, of which several modules have previously been reported in the context of SLE [15] and have been linked to known mechanisms involved in SLE pathogenesis [1]. Moreover, hierarchical clustering grouped these gene modules into three distinct clusters based on their dysregulation patterns, which we termed "interferon/plasma cells", "inflammation", and "lymphocyte signaling" gene module clusters based on gene module predominance. These clusters showed similarity to the "inflammatory", "lymphoid", and "interferon" patterns previously reported by Barturen et al. to be common across several autoimmune diseases [9]. Both DEG and gene module dysregulation patterns clustered SLE patients into distinct subgroups, differing in disease activity and/or clinical manifestations; while predominant upregulation of interferon-related genes indicated hematological activity and vasculitis, downregulation within the lymphocyte signaling gene module cluster denoted renal activity. Furthermore, druggability analysis revealed several potential drugs interfering with the dysregulation of genes included in the interferon module, in conformity with the demonstrated efficacy of type 1 interferon inhibition in SLE [31], as well as multiple potential drugs interfering with the dysregulation of genes included in the PLK1 signaling events module. Interestingly, PLK1 blockade was recently reported to be efficacious in murine lupus [32], further supporting the role of PLK1 signaling in SLE pathogenesis and its inhibition to ameliorate disease.

SLE is characterized by the production of autoantibodies by antibody-producing cells from autoreactive B cell lineages, which are considered important drivers of autoimmunity [33]. However, long-lived plasma cells resistant to standard immunosuppressive drugs and B cell depleting agents such as rituximab constitute a therapeutic challenge [33,34]. Moreover, failure to prominently reduce long-lived plasma cells measured in the periphery shortly after therapy commencement was recently shown to be associated with the development of severe SLE flares [35] and renal flares in particular [36], whereas clinical responders showed more prominent decreases [37]. In the present study, *CTSL* and *CD38* were upregulated in SLE compared with HC. While higher expression of cathepsin L has been documented in skin from patients with SLE as compared with skin from HC [38], plasma levels of cathepsin L did not differ between SLE patients and HC in a study by Zhang et al. [39]. The proteasome inhibitor bortezomib interferes with *CTSL* activity and was implicated in the present study

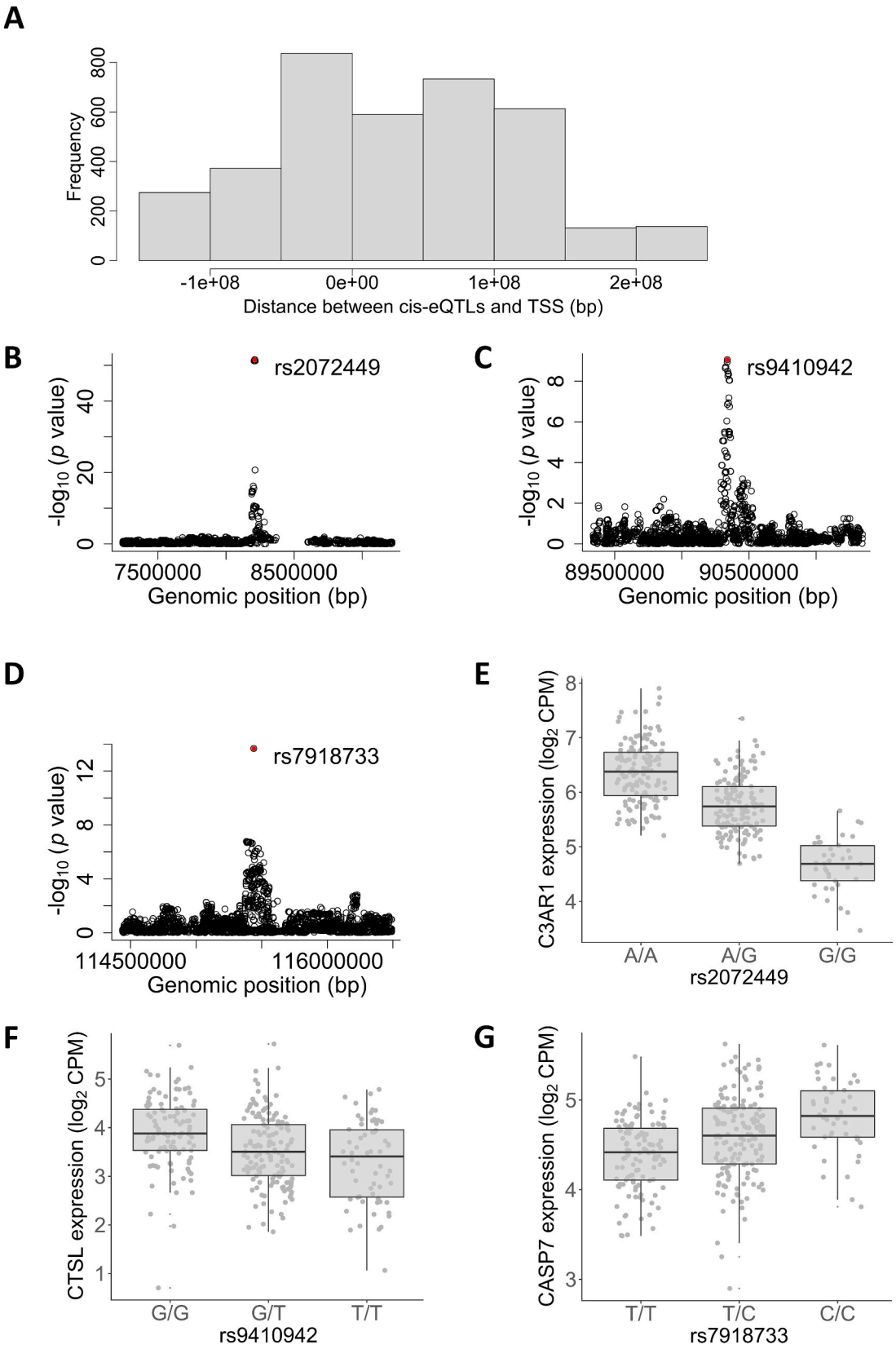
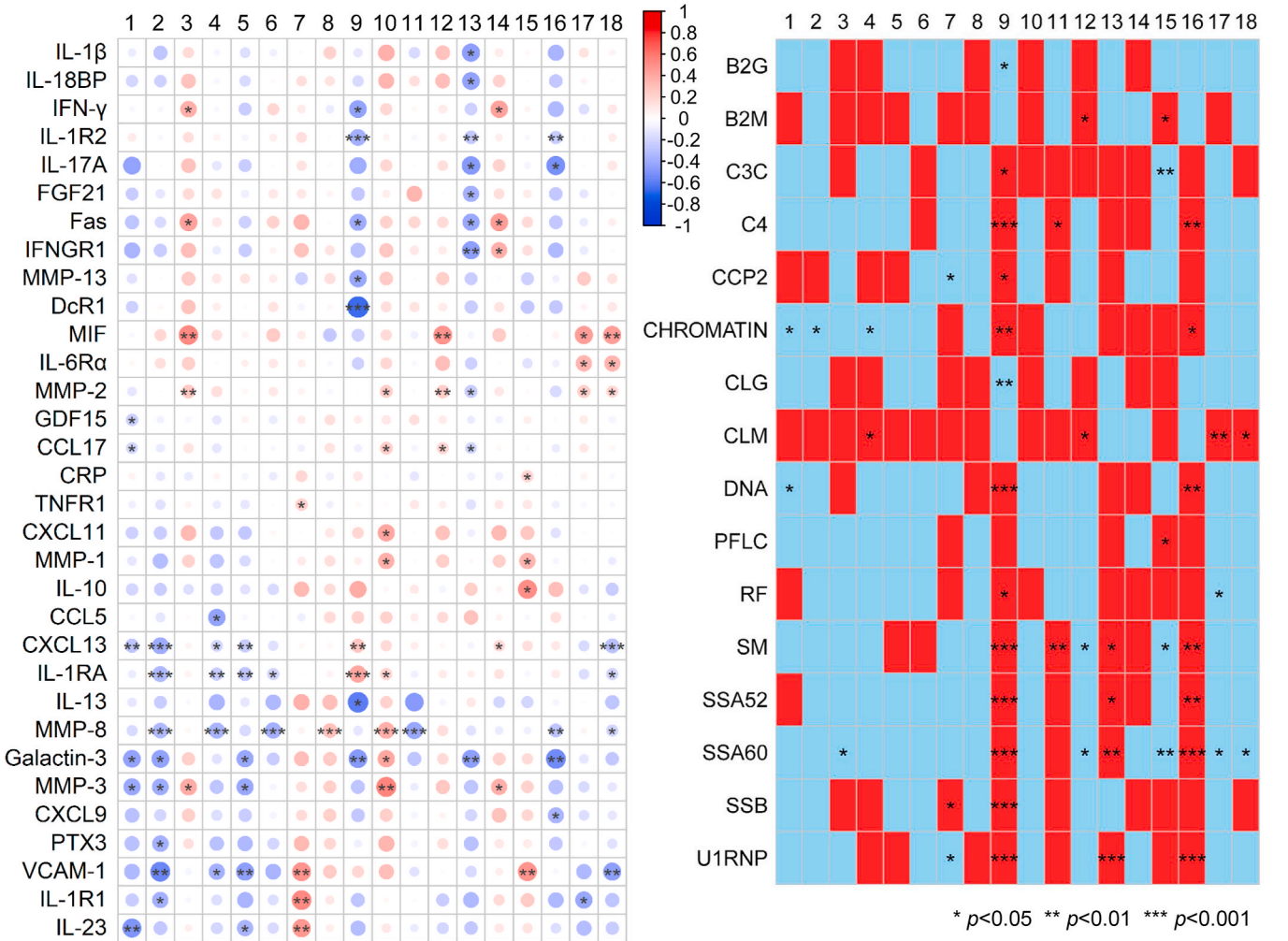


Fig. 4. ***Cis*-expression quantitative trait loci (eQTLs) in patients with SLE.** **(A)** Histogram of the distance between the significant *cis*-eQTLs and the transcription start site (TSS) of the respective genes. Locus plots for *cis*-eQTLs involved in gene expression of **(B)** *C3AR1*, **(C)** *CTSL*, and **(D)** *CASP7* genes in patients with SLE, with the most significant eQTL for each gene denoted in red. Expression levels of **(E)** *C3AR1*, **(F)** *CTSL*, and **(G)** *CASP7* in relation to the number of alternative and reference alleles for the most significant *cis*-eQTLs.bp: base pair; *C3AR1*: complement C3a receptor 1; *CASP7*; caspase 7: *CTSL*: cathepsin L; eQTL: expression quantitative trait loci; SLE: systemic lupus erythematosus; TSS: transcription start site.



1 B cell
2 Cell cycle
3 CORO1A-DEF6 network
4 Cytotoxic/NK cell
5 Enriched for ubiquitination
6 Enriched in cell cycle
7 Erythrocytes
8 Inflammation
9 Interferon
10 Lipid metabolism, endoplasmic reticulum
11 Monocytes
12 Olfactory receptors
13 Plasma cells, immunoglobulins
14 Platelet activation
15 Platelets
16 PLK1 signaling events
17 Regulation of transcription, transcription factors
18 T cell signaling and co-stimulation

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Fig. 5. Dysregulated gene modules in relation to serological markers in patients with SLE. (A) The correlation heatmap shows Spearman's rank correlation coefficients for correlations between levels of selected cytokines and dysregulation of gene modules, as measured by the z score. **(B)** Dysregulation of gene modules in relation to autoantibody positivity or low levels of C3C or C4. The group without autoantibody positivity or low levels of C3C or C4 for each comparison was considered the reference group. Red and blue colors denote higher and lower z-scores compared with the reference group, respectively. All *p* values are derived from Mann-Whitney *U* tests. Asterisks denote statistically significant correlations or differences. B2G: anti-β2 glycoprotein I IgG; B2M: anti-β2 glycoprotein I IgM; BAFF: B cell activating factor belonging to the tumor necrosis factor ligand family; C3C: complement component 3c; C4: complement component 4; CCL13: C–C Motif Chemokine Ligand 13; CCL17: C–C Motif Chemokine Ligand 17; CCL18; C–C Motif Chemokine Ligand 18; CCL2; C–C Motif Chemokine Ligand 2; CCL20; C–C Motif Chemokine Ligand 20; CCL3; C–C Motif Chemokine Ligand 3; CCL4; C–C Motif Chemokine Ligand 4; CCL5; C–C Motif Chemokine Ligand 5; CCL7; C–C Motif Chemokine Ligand 7; CCL8; C–C Motif Chemokine Ligand 8; CCP2: anti-cyclic citrullinated peptide (second generation); CLG: anti-cardiolipin IgG; CLM: anti-cardiolipin IgM; CRP: C-reactive protein: CXCL1: C-X-C motif ligand 1; CXCL10: C-X-C motif ligand 10; CXCL11: C-X-C motif ligand 11; CXCL13: C-X-C motif ligand 13: CXCL16: C-X-C motif ligand 16; CXCL5: C-X-C motif ligand 5; CXCL9: C-X-C motif ligand 9; DcR1: decoy receptor 1; DcR3: decoy receptor 3; DNA: anti-double stranded (ds)DNA; EGF: epidermal growth factor; FasL; Fas ligand; FGF2: fibroblast growth factor 2; FGF21: fibroblast growth factor 21; G-CSF: granulocyte colony-stimulating factor; GDF15: growth differentiation factor 15; GH: growth hormone; HGF: hepatocyte growth factor; ICAM-1: intercellular adhesion molecule 1; IFN-γ: interferon γ; IFNGR1: interferon γ receptor 1; IL-10: interleukin 10; IL-12: interleukin 12; IL-13: interleukin 13; IL-17 A: interleukin 17 A; IL-17 F: interleukin 17 F; IL-18BP: interleukin 18 binding protein; IL-1α: interleukin 1 α; IL-1R1: interleukin 1 receptor type 1; IL-1R2: interleukin 1 receptor type 2; IL-1RA: interleukin 1 receptor antagonist; IL-1β: interleukin 1β; IL-2: interleukin 2; IL-23: interleukin 23; IL-27: interleukin 27; IL-28 A: interleukin 28 A; IL-2Rα: interleukin 2 receptor α; IL-31: interleukin 31; IL-33: interleukin 33; IL-5: interleukin 5; IL-6: interleukin 6; IL-6Rα: interleukin 6 receptor α; IL-8: interleukin 8; M-CSF: macrophage colony-stimulating factor; MDC: macrophage-derived chemokine; MIF: macrophage migration inhibitory factor; MMP-1: matrix metalloproteinase 1; MMP-13: matrix metalloproteinase 13; MMP-2: matrix metalloproteinase 12; MMP-3: matrix metalloproteinase 3; MMP-7: matrix metalloproteinase 7; MMP-8: matrix metalloproteinase 8; MMP-9: matrix metalloproteinase 9; MPO: myeloperoxidase; PF4: platelet factor 4; PFLC: polyclonal free light chains of kappa and lambda type; PR3: anti-proteinase 3; PTX3: pentraxin 3; RF: rheumatoid factor; SSA52: anti-SSA/Ro52; SSA60: anti-SSA/Ro60; SSB: anti-SSB/La; TACI: transmembrane activator and calcium modulator and cyclophilin ligand interactor; TGF-β: transforming growth factor β; TNF-α: transforming growth factor α; TNFR1: tumor necrosis factor receptor 1; TNFR2: tumor necrosis factor receptor 2; VCAM-1: vascular cell adhesion protein 1; VEGF: vascular endothelial growth factor; VEGF-C: vascular endothelial growth factor C; VEGFR3: vascular endothelial growth factor receptor 3.

among drugs with potentiality to treat SLE. Bortezomib is a potent inhibitor of the 26 S proteasome and induces cell death via accumulation of unfolded proteins through inhibition of the ubiquitin-proteasome system and suppression of autophagy [33,40]. The high immunoglobulin production by plasma cells makes those cells particularly sensitive to proteasome inhibition, as shown by the depletion of short-lived and long-lived plasma cells upon bortezomib treatment in murine lupus [41]. Besides, inhibition of cathepsin L activity with bortezomib was shown to block autophagy and induce cell death in estrogen receptor $(ER)^{+}$ breast cancer cells [42]. Whether a part of the effect conferred from bortezomib is exerted by a similar mechanism in SLE plasma cells remains unknown. Nevertheless, our finding is of particular interest in light of recent reports of bortezomib ameliorating disease activity in refractory SLE cases, *inter alia* in a phase II clinical trial, although some patients experienced adverse reactions such as fever and severe hypersensitivity [5,43,44]. Furthermore, in a recent study by Garantziotis et al. bortezomib was shown to reverse gene dysregulation in a neutrophilic cluster of SLE patients [45]. The upregulation of *CD38* in the SLE population of the present study is in accordance with previous reports of higher expression in several peripheral blood immune cell subsets in patients with SLE compared with HC [46,47]. Interestingly, the plasma cell depleting monoclonal anti-CD38 daratumumab, a biological agent that is approved for the treatment of multiple myeloma [48], was demonstrated to induce improvement in clinical and laboratory parameters in two patients with severe and refractory SLE [49]. Taken together, our results along with current literature point to drug repurposing potentiality with modulation of *CTSL* and *CD38* activity in patients with SLE, corroborating the central role of plasma cells in SLE pathogenesis.

In the present study, eQTL analysis in SLE patients revealed multiple DEGs that were associated with *cis*-eQTLs, including *C3AR1*, *CTSL*, and *CASP7*. *C3AR1* has previously been reported to be upregulated in kidney tissue from patients with lupus nephritis, and increasing renal deposition of C3aR was seen in murine lupus models as the disease progressed [50]. Moreover, C3aR antagonists have been shown to reduce renal disease and neurodegeneration in murine lupus [51,52]. Our findings that *C3AR1* and *CTSL* were upregulated in patients with SLE compared with HC and that their gene expression in SLE patients was decreased in carriers of certain SNPs suggest that these genetic polymorphisms may be indicative of anticipated non-response to C3aR antagonists and bortezomib, with implications towards a more precise and individualized drug selection.

Defective clearance of apoptotic cell debris is considered a hallmark of the immune aberrancies that characterize SLE [1]. Caspase 3 and caspase 7 have been shown to cleave CD3ζ, which is involved in signal transduction during T cell activation, and loss of this chain has been associated with tumor-induced immune dysfunction and T cell apoptosis [53]. A similar role for caspase 3 and caspase 7 has been suggested in SLE upon observations of restoration of CD3ζ expression in SLE T cells upon caspase 3 and pan-caspase inhibition [54]. In the same direction, CASP7 was herein found to be upregulated in patients with SLE compared with HC. It is worth noting that CASP7 has been linked to susceptibility to other autoimmune diseases e.g., rheumatoid arthritis [55], type 1 diabetes mellitus [56], and vitiligo [57], thus indicating a generic rather than disease-specific role of this pathway in autoimmunity. Alongside, we corroborated the importance of *STAT1* in SLE pathogenesis, here identified as the chief regulator of the most enriched signaling molecule network in the present study. STAT1 has been shown to induce apoptosis in cancer cells, a process mediated by caspase 2, caspase 3, and caspase 7 [58]. However, apart from a role in apoptosis, which is of known significance in SLE, STAT1 has an established role in IFN-mediated responses, which is mechanistically linked to interactions between IFN receptors and STAT1 signaling, also of immense importance in the pathogenesis of SLE [26,27,59]. Supportive of this was also the recent demonstration of diminished expression of interferon-stimulated genes in SLE patients treated with tofacitinib, a small molecule that interferes with the Janus kinase (JAK)-STAT signaling pathway through inhibition of mainly JAK1 and JAK3 [60,61], justifying systematic current efforts to target this pathway [62–64].

Notably, expression of *CASP7* was increased in carriers of the gene variant rs7918733, suggesting that only some SLE patients may benefit from caspase inhibition, and that these patients could be identified by biomarkers such as this genetic polymorphism. In this regard, it is worth underscoring the herein suggested potential of gene polymorphisms identified through eQTL analysis as stable markers of anticipated response to therapies, contributing towards novel precision medicine concepts.

In the current omics era, we are witnessing a paradigm shift in several medical disciplines from clinical diagnoses to the establishment of novel taxonomies and therapeutic decision-making on the basis of molecular and cellular profiles. This paves the way towards precision medicine, tailored to the individual patient, or even the actual phase of a patient's disease course, being particularly appealing in SLE that is characterized by a prominent heterogeneity not only in terms of clinical

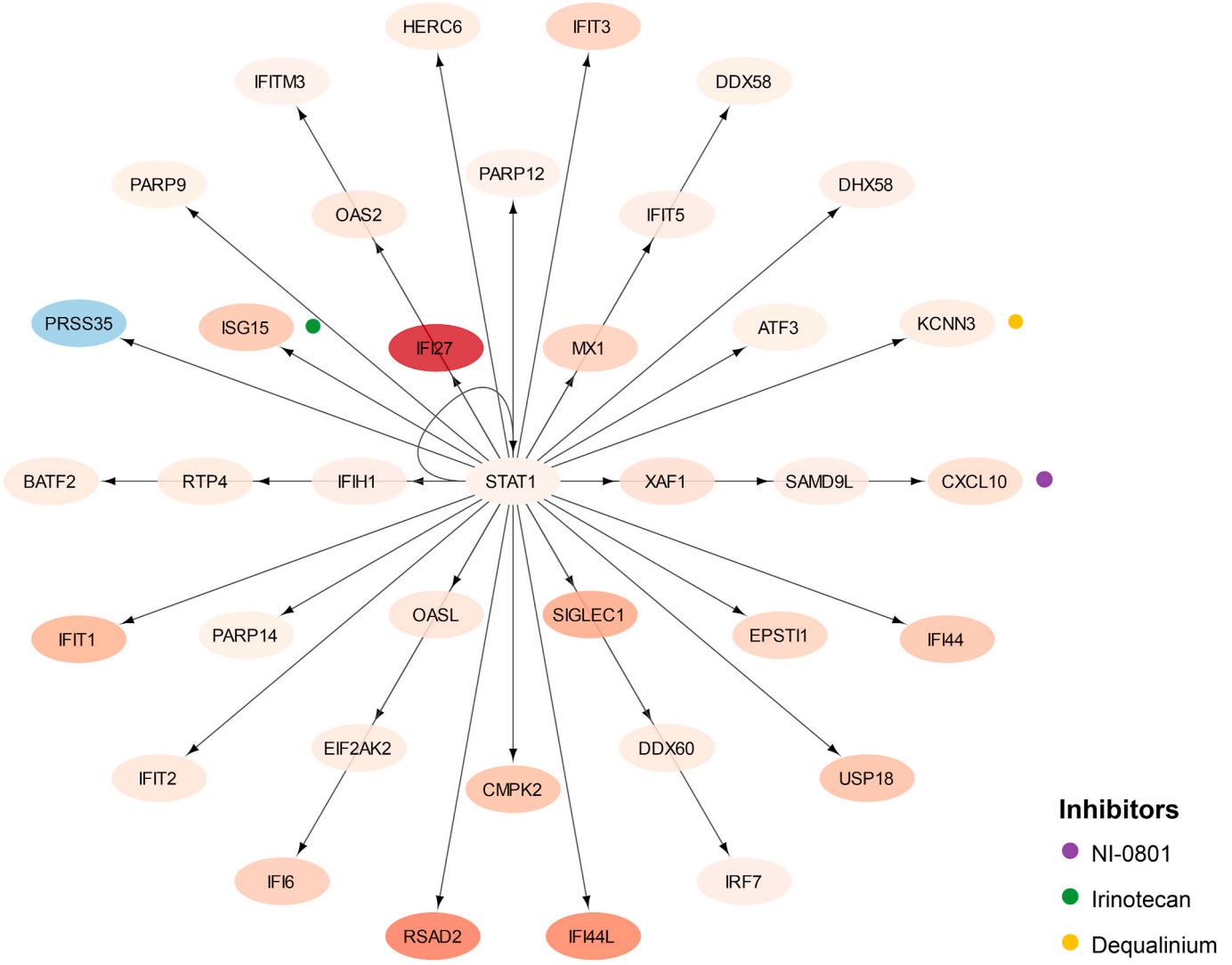

Fig. 6. The *STAT1* signaling molecule network and annotated drug targets. A total of 139 replicated SLE DEGs with an FDR-corrected p value < 0.05 and a $|\log_2 FC| > 0.58$ were imputed in iRegulon through Cytoscape to yield signaling molecule networks. The most enriched signaling molecule network is plotted, with the chief regulator *STAT1* in the central node. The color of the nodes ranges from light blue (downregulated genes) to increasing intensities of red (upregulated genes). Inhibiting drugs and their upregulated targets are indicated by colored dots. DEGs: differentially expressed genes; FC: fold change; FDR: false discovery rate.

features but also immunological aberrancies. The clinical management of SLE has improved considerably since the introduction of targeted therapies such as the anti-BAFF belimumab and the anti-type I IFN receptor anifrolumab [5]. The interferon gene signature has been proposed as a determinant of response to anifrolumab [31,59] and has also been implied to signify a favorable response to belimumab [65]. However, a comprehensive understanding of immune milieus that would signify a benefit from BAFF or type I IFN inhibition, B cell depletion, or other immunomodulation remains an urgent need. In light of this, the findings of the present study contribute to the development of a framework for informed selection of suitable treatments for each individual SLE patient and their current disease state. The overall lack of apparent correlates between disease manifestations and distinct patterns of gene dysregulation implies that molecular profiling may be superior to clinical features in predicting immune responses to different therapies. We found *CASP7*, *C3AR1*, and *CTSL* to be upregulated in patients with SLE compared with HC. *CASP7* expression increased whereas gene expression of *C3AR1* and *CTSL* decreased with the number of certain genetic variants, suggesting that carriers of those specific polymorphisms could be anticipated to show a favorable response to caspase inhibition or non-response to C3aR antagonists or bortezomib. This concept carries some novelty, as does the entire emerging field of pharmacogenetics, here rendered through patient characterization based on distinct gene polymorphism profiles that could provide guidance in drug selection, tailored to attributes in an individual SLE patient. This introduces the advantage of personalized medicine, circumventing the common issue of biomarker variability over time.

All study participants were of European origin, which limits the generalizability of the results. Moreover, while whole-blood RNA-sequencing formed the basis for the analyses, single-cell profiling would provide complemental information and while it was excluded from the initial analysis plan in the PRECISEADS project due to feasibility constraints, it is warranted in future survey. Furthermore, the cross-sectional nature of the study precluded analyses of biological changes over time and in relation to treatment responses, which nevertheless was beyond the scope of the present study. The large number of patients with SLE with available multilevel omics data constituted an important strength of the present work, which along with the usage of systems biology and an integrative workflow provided implications for drug development and repurposing for this complex autoimmune disease, as well as indicators for their expected efficacy.

5. Conclusions

Integrative multilevel omics analysis provided insights into key immune mechanisms underlying SLE. The large number of study participants allowed transcriptome analysis in a discovery and a replication set, and revealed a set of important dysregulated gene modules and distinct dysregulated gene module clusters, enriched immune pathways, and regulatory networks. Our findings support the prospect of *CTSL*, *CD38*, and *ISG15* inhibition for the treatment of SLE, warranting further investigation of the potentiality of e.g., bortezomib, daratumumab, and irinotecan, respectively. Moreover, the prospect of caspase inhibition was implicated.

Ethical approval

The study complied with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all study participants prior to enrolment in the PRECISESADS project. The PRECISESADS project was approved by regional ethics review boards for all participating centers, and the study protocol for the present analysis was reviewed and approved by the Swedish Ethical Review Authority (2022-03907-01).

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ioannis Parodis reports a relationship with Amgen, AstraZeneca, Aurinia Pharmaceuticals, Elli Lilly and Company, Gilead Sciences, GlaxoSmithKline, Janssen Pharmaceuticals, Novartis, Otsuka Pharmaceutical, and F. Hoffmann-La Roche AG that includes: consulting or advisory and funding grants.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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