



# A survey of ficolin-3 activity in Systemic Lupus Erythematosus reveals a link to hematological disease manifestations and autoantibody profile

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## ARTICLE INFO

### Keywords:

Systemic lupus erythematosus  
Complement system  
Lectin pathway  
Ficolin-3  
Autoantibodies

## ABSTRACT

The complement system plays a central role in the pathogenesis of Systemic Lupus Erythematosus (SLE), but most studies have focused on the classical pathway. Ficolin-3 is the main initiator of the lectin pathway of complement in humans, but its role in systemic autoimmune disease has not been conclusively determined. Here, we combined biochemical and genetic approaches to assess the contribution of ficolin-3 to SLE risk and disease manifestations. Ficolin-3 activity was measured by a functional assay in serum or plasma samples from Swedish SLE patients (n = 786) and controls matched for age and sex (n = 566). Genetic variants in an extended 300 kb genomic region spanning the *FCN3* locus were analyzed for their association with ficolin-3 activity and SLE manifestations in a Swedish multicenter cohort (n = 985). Patients with ficolin-3 activity in the highest tertile showed a strong enrichment in an SLE cluster defined by *anti*-Sm/DNA/nucleosome antibodies (OR 3.0, p < 0.001) and had increased rates of hematological disease (OR 1.4, p = 0.078) and lymphopenia (OR = 1.6, p = 0.039). Genetic variants associated with low ficolin-3 activity mapped to an extended haplotype in high linkage disequilibrium upstream of the *FCN3* gene. Patients carrying the lead genetic variant associated with low ficolin-3 activity had a lower frequency of hematological disease (OR 0.67, p = 0.018) and lymphopenia (OR 0.63, p = 0.031) and fewer autoantibodies (p = 0.0019). Loss-of-function variants in the *FCN3* gene were not associated with SLE, but four (0.5 %) SLE patients developed acquired ficolin-3 deficiency where ficolin-3 activity in serum was depleted following diagnosis of SLE.

Taken together, our results provide genetic and biochemical evidence that implicate the lectin pathway in hematological SLE manifestations. We also identify lectin pathway activation through ficolin-3 as a factor that contributes to the autoantibody response in SLE.

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## 1. Introduction

The complement system plays a central role in the pathogenesis of Systemic Lupus Erythematosus (SLE), a systemic autoimmune disorder characterized by the formation of autoantibodies against nuclear antigens [1]. Deficiencies in early components of the classical pathway of complement activation constitute strong risk factors for SLE, due to impaired clearance of immune complexes and apoptotic cell debris [2, 3]. The lectin pathway is structurally and functionally related to the classical pathway, but no consistent association with SLE phenotype has been established [4]. The lectin pathway is initiated by a series of pattern-recognition molecules (PRMs) that sense molecular patterns on the surface of pathogens or altered self. Target recognition and clustering of PRMs leads to activation of associated masp proteases, which activate complement C2 and C4 to form a C3 convertase, a process similar to the initiation of the classical pathway [5,6]. In humans, the main initiator of the lectin pathway is the PRM ficolin-3, which has >10-fold higher circulating concentration than other lectin pathway components including mannose-binding lectin (MBL) [7]. The physiological role of ficolin-3 remains poorly understood, but a role in the clearance of apoptotic and necrotic cells has been suggested [8,9]. A rare frameshift variant in the *FCN3* gene that encodes ficolin-3 in humans (rs532781899, chr1:27699670\_AG\_A) causes complete ficolin-3 deficiency in the homozygous state [10]. A recent publication summarized the known cases of ficolin-3 deficiency and suggested an enrichment of severe infections and SLE in deficient individuals [11], similar to the phenotype observed in classical pathway deficiencies.

In the current study, we performed a biochemical and genetic survey of the lectin pathway initiator ficolin-3 to systematically interrogate its contribution to SLE risk and disease manifestations. While an acquired ficolin-3 deficient state was observed in a small subset of patients, our results do not support that genetic ficolin-3 deficiency is a risk factor for SLE in patients with White European ancestry. Instead, we identified novel genetic variants associated with ficolin-3 activity, and present biochemical and genetic evidence that ficolin-3 is linked to hematological disease manifestations and specific autoantibody patterns in SLE patients.

## 2. Materials and methods

### 2.1. Study participants

Patients with SLE fulfilled the classification criteria for SLE as defined by the American College of Rheumatology (ACR) and/or the Systemic Lupus International Collaborating Clinics (SLICC) [12–14]. The Karolinska Institute (Stockholm) cohort was recruited between 2004 and 2014 and included SLE patients and population controls, individually matched to the first SLE patients for age, sex, and region of residence. The Umeå University Hospital SLE cohort was recruited in 2000 and included SLE patients and population controls individually matched for age and sex. Serum samples were available for 531 Karolinska SLE patients and 322 controls, and 255 Umeå SLE patients, and were collected at study inclusion. EDTA plasma samples from 244 Umeå controls were obtained from the Biobank of Northern Sweden. Clinical information was extracted from medical records. Disease activity at the time of sampling was measured by the SLE disease activity score (SLE-DAI-2K or SLEDAI). Ethical approval was granted from the regional ethics review boards in Umeå and Stockholm (Dnr 07–066M, and Dnr 03–556, respectively). Genetic data was extracted from the DISSECT study of SLE patients and population and blood donor controls from five Swedish tertiary hospitals [15]. The quality-controlled dataset comprised 958 patients with SLE and 1026 control individuals. 235 of the Karolinska patients, 228 of the Umeå patients, and 83 of the Umeå controls both had serum/plasma samples available and were represented in the genetic data set. The DISSECT study was approved by the local ethics review board in Uppsala (Dnr 2015-450, Dnr 2016-155, and

Dnr 2020–05065).

### 2.2. Complement and autoantibody analyses

Ficolin-3 activity in serum/plasma was measured by a functional ELISA where acetylated BSA (acBSA) was used as an affinity matrix to capture ficolin-3 from serum as described previously [16]. Complement C4 and C3 deposition was measured by the same setup. Ficolin-3 autoantibodies were analyzed by a modified ELISA setup. A detailed description of the assays is available in [Supplementary Fig. 1](#) and [Supplementary Methods](#). Analysis of complement C1q, C4, and C3 in the Karolinska cohort was described previously [17]. Autoantibodies were analyzed using multiplex assays or ELISA as previously described [18, 19]. For the DISSECT cohort, autoantibody analyses were performed at the immunological laboratories at the contributing hospitals.

### 2.3. Genotyping and imputation of genetic variants in the *FCN3* locus

Subjects in the DISSECT study were subjected to targeted DNA sequencing of immune-related genes as previously described [15]. In brief, DNA was extracted from blood samples, and target capturing for sequencing was performed using a custom NimbleGen array, including coding and regulatory regions of 1853 genes selected based on their involvement in immunological pathways, and sequenced on an Illumina HiSeq 2500. The design and implementation of the capturing array, as well as subsequent sequencing experiments and quality control, is described in detail in Sandling et al. [15]. Study subjects falling outside of the European subpopulation of the Human Genome Diversity Project (HGDP) reference set were excluded [20]. The quality-controlled dataset contained 287 354 single-nucleotide variants (SNVs). Imputation of additional variants was performed employing the Sanger imputation service with the Haplotype reference consortium r1.1 reference panel [21] and the ‘pre-phase with EAGLE2 and impute’ pipeline [22]. 200 genotyped SNVs were extracted from an extended genomic region encompassing 150 kb upstream and 150 kb downstream of the *FCN3* gene (GRCh37/hg19 coordinates chr1:27545601–27851343) ([Supplementary Fig. 4](#)). The *FCN3* gene was not included in the original gene list targeted in DISSECT, but most of the gene region was covered due to conservation and proximity to the *MAP3K6* gene. To increase coverage, 243 imputed SNVs from the same region were also included. The original DISSECT data set included only SNVs. Subsequent work by our group has extended the analysis to insertion-deletion variants [2,23], and we performed a focused analysis to call the rs532781899 frameshift variant (chr1:27699670\_AG\_A) in DISSECT participants. Further, we screened for other potential loss-of-function variants (frameshift, splice, or missense variants), and called genotypes for seven additional rare variants with potential impact on ficolin-3 activity.

### 2.4. Statistics

Patient and control characteristics are presented as medians with interquartile range (IQR) or percentages depending on data types. Groups were compared by Chi-square tests or non-parametric statistics (Mann-Whitney test or Kruskal-Wallis test). Correlations between continuous or ordinal data were assessed by Spearman’s rank correlation coefficient. Odds ratios (ORs) were calculated by logistic regression models adjusted for age and sex. A detailed description of the protein quantitative trait locus (pQTL) analysis for ficolin-3 activity and SLE association study can be found in [Supplementary Methods](#). All p-values were two-sided, and  $p < 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Screening of two SLE cohorts reveals four patients with ficolin-3 deficiency

Since previous studies have indicated an enrichment of ficolin-3 deficient individuals among patients with SLE, we sought to replicate these findings and analyzed ficolin-3 activity in SLE patient sera from two Swedish cohorts. In agreement with previous observations [24,25], we observed significantly higher ficolin-3 activity in SLE patients compared to controls in both the Karolinska and Umeå cohorts, albeit with a wide range (Fig. 1A–C). In the Karolinska cohort, three patients with very low or undetectable levels of ficolin-3 activity were identified, indicating a deficient state. In the Umeå cohort, one additional patient with an apparent ficolin-3 deficient state was found (Fig. 2A). There were no controls with ficolin-3 deficiency, and the lowest value for ficolin-3 activity recorded in controls was 18.9 %. Serum samples from deficient patients were also analyzed by capillary immunoelectrophoresis, where the results were in agreement with the activity assay and showed very low amounts of ficolin-3 protein in serum (Fig. 2B and C). In support of a ficolin-3 deficient state, sera from these patients failed to activate complement on acetylated BSA in a C4 and C3 deposition assay previously shown to be ficolin-3 dependent [16] (Fig. 2D and E). As ficolin-3 was originally identified as an autoantigen in SLE [26] we tested sera from deficient patients for the presence of ficolin-3 autoantibodies. Serum from the deficient patient in the Umeå cohort reacted strongly with recombinant ficolin-3, while sera from the other deficient patients showed similar reactivity as randomly selected SLE patients and controls representing cases with medium or high ficolin-3 activity (Supplementary Fig. 2A–C). Hence, ficolin-3 autoantibodies may explain one but not the other three cases of deficiency.

Serum samples collected at different time points were available for two of the deficient patients in the Karolinska cohort. Interestingly, one patient had normal ficolin-3 activity at the time of SLE diagnosis in the early 1990s, which was then depleted followed by a rebound at a later time point (Fig. 2F). Ficolin-3 activity appeared to follow complement C4 levels, where the ficolin-3 rebound was followed by a rise in C4 levels, implicating complement consumption as a possible cause of ficolin-3 depletion (Fig. 2F). Serial samples from a second patient also displayed fluctuating levels over time (Fig. 2G), indicating an acquired rather than constitutional deficiency in these patients. Clinical

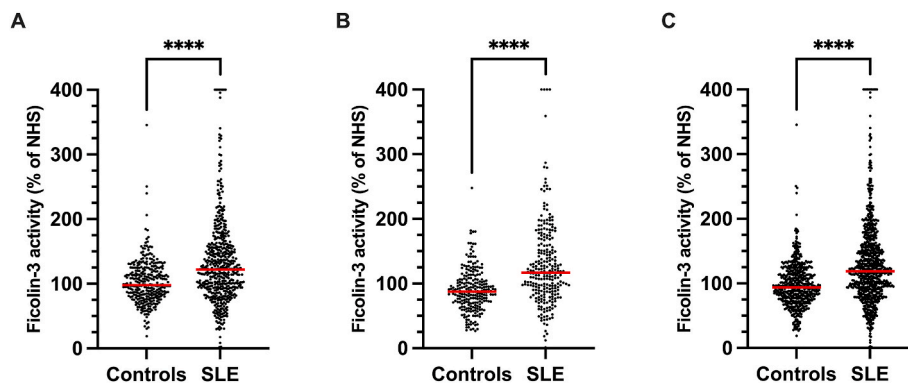
characteristics of the four ficolin-3 deficient patients are shown in Supplementary Table 1. All four patients fulfilled the criteria for photosensitivity, arthritis, immunological disorder, and ANA. The three Karolinska patients also had hematological disease with leukopenia and/or lymphopenia and C4 levels below the reference interval, while the patient with ficolin-3 autoantibodies exhibited a different phenotype with SSA/SSB antibodies, discoid lupus, and no hematological disease.

#### 3.2. Loss-of-function variants in the FCN3 gene are not enriched in SLE patients

To further investigate the association of ficolin-3 deficiency with SLE, genotypes for the FCN3 frameshift variant rs532781899 were called in the DISSECT cohort. Genotype frequencies for rs532781899 were similar in patients and controls (minor allele frequency (MAF) patients 0.008, MAF controls 0.009,  $p = 0.58$ ), and no minor allele homozygous individuals were found (Supplementary Fig. 3A). As expected, heterozygous rs532781899 minor allele carriers ( $n = 10$ ) had significantly lower ficolin-3 activity in serum ( $p < 0.001$ ) (Supplementary Fig. 3B). Additionally, when interrogating other potential FCN3 loss-of-function variants, there was no enrichment in patients vs. controls, and no homozygous or compound heterozygous individuals were found (Supplementary Table 2). Two of the patients with apparent ficolin-3 deficiency were represented in the genetic data set. None of these had the rs532781899 minor allele or any other coding variants that were predicted to be deleterious, in agreement with an acquired deficiency. Taken together, these results indicated an acquired rather than genetic cause of ficolin-3 deficiency in SLE.

#### 3.3. Ficolin-3 activity is associated with hematological SLE manifestations and a broad autoantibody response

Elevated ficolin-3 activity in SLE patients indicated that an increase in lectin pathway activity is a feature of the immunological activation in SLE. Yet, we did not observe a significant correlation with disease activity measured by SLEDAI score at the time of sampling ( $r = 0.06$ ,  $p = 0.10$ ), nor was ficolin-3 activity associated with ongoing immunosuppressive treatment (Supplementary Table 3). We therefore continued by exploring associations between ficolin-3 activity categorized into tertiles and specific SLE disease manifestations and autoantibodies in the combined Karolinska and Umeå cohorts. Ficolin-3 activity in the highest



**Fig. 1.** Ficolin-3 activity in SLE patients.

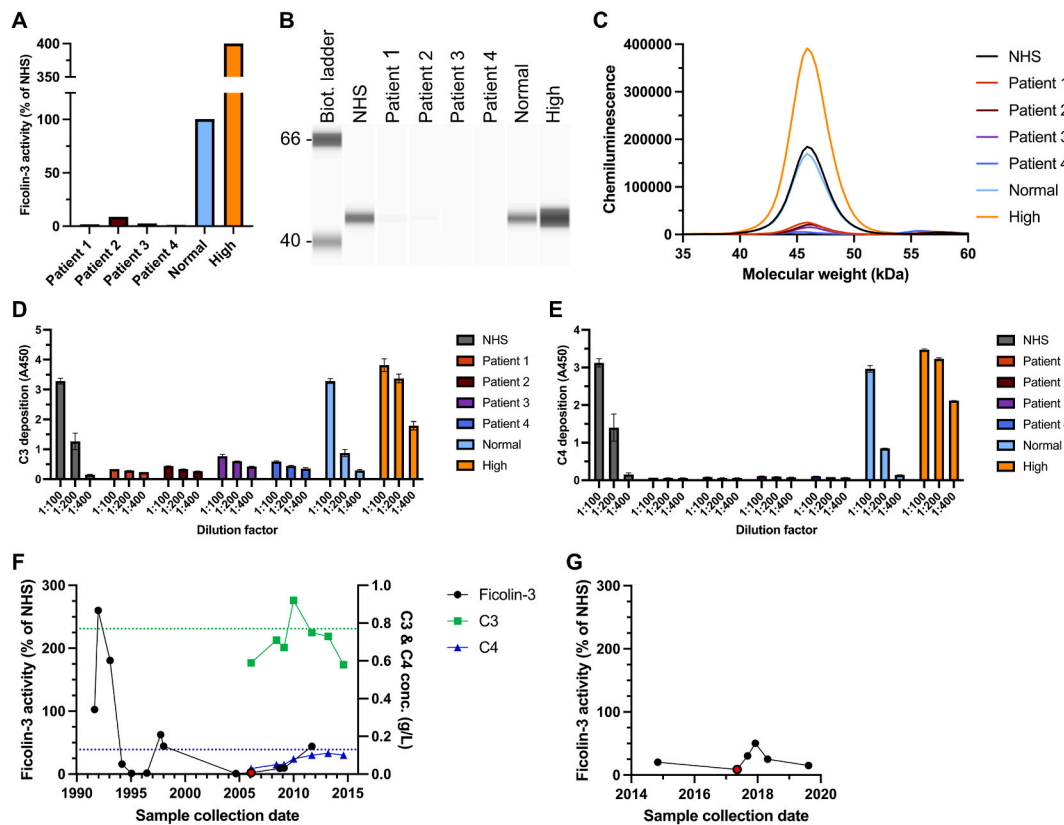
Ficolin-3 activity was measured in serum/plasma samples from patients with SLE and population controls from two Swedish cohorts using an in-house developed functional ELISA. Ficolin-3 activity was measured in relation to a normal human serum (NHS) pool.

A. Ficolin-3 activity in the Karolinska cohort was 121.8 % of NHS (92.2–157.0) for SLE patients ( $n = 531$ ), and 97.7 % (80.6–122.3) for controls ( $n = 322$ ),  $p < 0.0001$ .

B. Ficolin-3 activity in the Umeå cohort was 116.8 % of NHS (87.9–159.2) for SLE patients ( $n = 255$ ), and 84.47 % (70.4–105.9) for controls ( $n = 244$ ),  $p < 0.0001$ .

C. Ficolin-3 activity in the Karolinska and Umeå cohorts combined was 119.0 % of NHS (91.0–157.4) for SLE patients ( $n = 786$ ), and 93.9 % (75.8–115.2) for controls ( $n = 566$ ),  $p < 0.0001$ .

Data are presented as medians with interquartile range. Red horizontal lines indicate the median values. Statistical analysis was performed with Mann-Whitney  $U$  test. \*\*\*\* =  $p < 0.0001$ .



**Fig. 2.** Characterization of SLE patients with ficolin-3 deficiency.

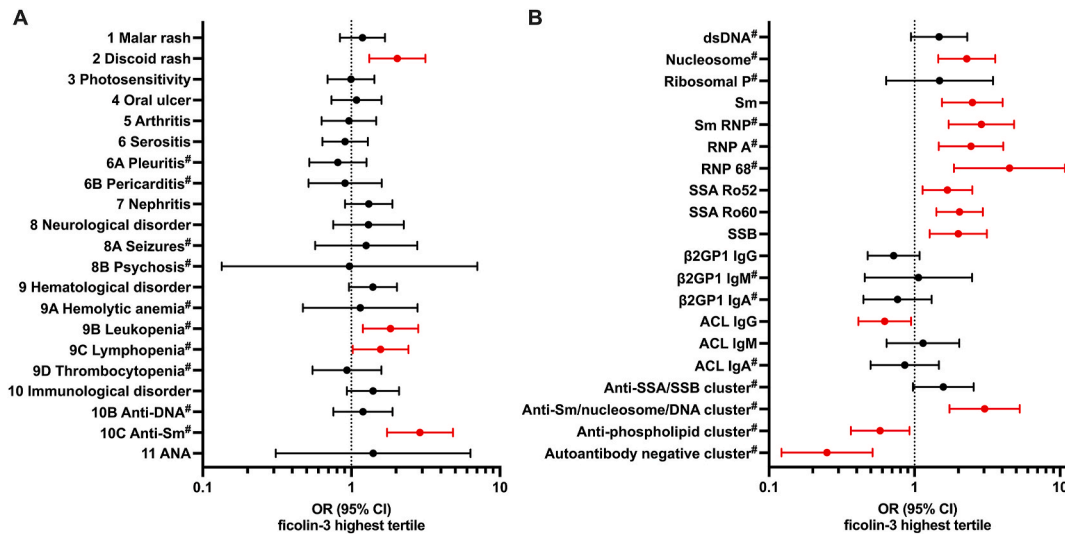
**A.** Four patients with low or undetectable levels of ficolin-3 activity indicating a deficient state were identified in the Karolinska and Umeå cohorts. Ficolin-3 activity was 1.8 %, 8.7 %, 2.6 %, and 1.2 % for patients 1 to 4 respectively, displayed in comparison to patients with normal (100.3 %) and high (400 %) ficolin-3 activity. **B–C.** Capillary immunoelectrophoresis analysis of ficolin-3 in diluted serum visualized by virtual plot view (**B**) and electropherogram (**C**) of chemiluminescence. **D–E.** Lectin pathway mediated C3 (**D**) and C4 (**E**) deposition for ficolin-3 deficient patients 1 to 4 compared to NHS and patients with normal and high levels of ficolin-3 activity. Deposition measured by in-house functional ELISA with diluted serum pre-incubated with Polyanetholesulfonic acid sodium salt (SPS) to block binding of non-lectin pathway complement proteins. Error bars indicate the standard deviation (SD) of replicates. **F–G.** Ficolin-3 activity over time in ficolin-3 deficient patients 1 (**F**) and 2 (**G**) for 20 and 5 years respectively. The red dots indicate the time points analyzed in the original screening. Patient 1 displayed a maximum ficolin-3 activity of 260 % and a minimum of 0.9 %, whereas patient 2 had a max of 50.4 % and a min of 8.8 %. For patient 1 (**F**), longitudinal C3 (green) and C4 (blue) concentrations were available. Green and blue dotted lines indicate the minimum value of the reference interval for C3 (0.77–1.62 g/L) and C4 (0.13–0.32 g/L), respectively.

tertile was significantly associated with discoid rash (OR 2.0,  $p = 0.0013$ ), leukopenia (OR 1.8,  $p = 0.0053$ ), lymphopenia (OR 1.6,  $p = 0.039$ ), and the *anti*-Sm criterion (OR 2.9,  $p < 0.001$ ) (Fig. 3A, Supplementary Table 3). These results indicated an association between ficolin-3 activity and cytopenias in the myeloid and lymphoid lineages. Hematological indices at the time of sampling were available for controls and patients in the Karolinska cohort. Interestingly, while ficolin-3 activity in controls was positively correlated with white blood cell counts and lymphocyte counts ( $r = 0.22$ ,  $p < 0.001$ , and  $r = 0.16$ ,  $p = 0.0047$ , respectively), the trend was reversed in SLE patients where a negative correlation with ficolin-3 activity was observed ( $r = -0.18$ ,  $p < 0.001$  for white blood cell count, and  $r = -0.16$ ,  $p < 0.001$  for lymphocyte count). Furthermore, elevated ficolin-3 activity displayed a strong association with the presence of Sm, RNP, and SSA/SSB antibodies, but not with anti-phospholipid antibodies (Fig. 3B, Supplementary Table 3). A recent publication by our group described four serologically defined SLE clusters [18]. Ficolin-3 activity strongly associated with a cluster characterized by *anti*-Sm/DNA/nucleosome antibodies (OR 3.0,  $p < 0.001$ ) and showed an inverse association with the seronegative cluster negative for all tested antibodies (OR 0.25,  $p < 0.001$ ). In agreement with these results, the number of positive autoantibodies in the Karolinska cohort was strongly linked to ficolin-3 activity (4 autoantibodies (2–6) in tertile 3 vs. 3 (1–5) in tertile 1,  $p < 0.001$ ).

#### 3.4. pQTL analysis identifies genetic variants associated with ficolin-3 activity

These results demonstrated a positive association between elevated lectin pathway activity and clinical and serological SLE manifestations. To address if increased ficolin-3 activity in SLE may be causally involved in disease pathogenesis or, alternatively, secondary to increased inflammation, we undertook a systematic survey of the genetic contribution to ficolin-3 activity in SLE. We matched serum/plasma samples from the Karolinska and Umeå cohorts with the DISSECT data set, and using ficolin-3 activity as the quantitative variable we performed a locus-wide pQTL analysis of an extended 300 kb genomic region encompassing the *FCN3* gene. The analysis revealed 132 SNVs to be significantly associated with ficolin-3 activity in SLE patients after multiple testing corrections (Fig. 4A). In contrast, no association was found in controls (Fig. 4B). Statistically significant SNVs mapped to a 146 kb region upstream of the *FCN3* gene, ranging from 3.2 kb upstream of the transcription start site (TSS) of the *FCN3* gene to the most distal significant SNV located 149 kb upstream of the TSS. All significant variants showed the same direction of association and were associated with lower ficolin-3 activity in SLE patients. The same set of genetic variants were tested for their association with SLE in the complete DISSECT cohort. In agreement with the lack of association for loss-of-function variants, no SNVs in this data set showed an association with





**Fig. 3.** Associations between ficolin-3 activity and SLE manifestations and autoantibodies.

The graphs show forest plots with odds ratios (OR) and 95 % confidence intervals (CI) for patients with ficolin-3 activity in the highest tertile compared to the lowest tertile.

**A.** ORs for SLE criteria according to the 1982 ACR classification.  $n = 778$ –780 (combined cohort) or 520–524 (Karolinska cohort only<sup>#</sup>).

**B.** ORs for autoantibody specificities.  $n = 765$ –767 (combined cohort) or 504–527 (Karolinska cohort only<sup>#</sup>).

All analyses were adjusted for sex and age at follow-up. Significant ORs ( $p < 0.05$ , unadjusted for multiple comparisons) are shown in red.

SLE after multiple testing corrections (Fig. 4C).

### 3.5. Lead SNV selection for further analyses

As significant SNVs were distributed over an extended genomic region, we analyzed linkage disequilibrium (LD) in the *FCN3* locus. All significant pQTLs were in high LD with the top SNV in the DISSECT data set (Fig. 4D), and analysis using LDlink [27] confirmed a high degree of LD in European populations (data not shown). As expected from the high degree of LD between significant pQTL variants, stepwise multiple regression failed to identify pQTLs independent from the top SNV. While most SNVs in the data set were imputed, 43 SNVs with a MAF  $> 0.01$  were also genotyped by direct sequencing. As sequenced genotypes are considered more robust than imputed ones, significant pQTLs were therefore prioritized based on the degree of LD with the top SNV and call rate in the genotyped data set. rs2474288 (chr1:27742308\_G\_A, adjusted  $p = 0.0021$ ) located approximately 40 kb from the *FCN3* TSS showed a complete call rate among SLE patients in DISSECT, with genotypes missing only from three DISSECT controls (Fig. 4E and F). Using the DISSECT data set, rs2474288 was in high LD with the top pQTL variant rs61551751 ( $R^2 = 0.90$ ), and the most significant genotyped pQTL rs1005413 ( $R^2 = 0.99$ ), and was therefore selected as a tag SNV for the region for further analysis.

In the GTEx database of expression QTLs [28], carriers of the rs2474288 minor allele associated with low ficolin-3 activity had lower *FCN3* mRNA levels in liver tissue ( $p = 1.3 \times 10^{-6}$ ). The liver is the main site of synthesis of circulating ficolin-3, indicating that the functional variant tagged by rs2474288 acts by lowering mRNA transcription from the *FCN3* gene.

### 3.6. Replication of clinical associations in the DISSECT cohort

Next, rs2474288 was used as a genetic marker of low ficolin-3 activity to replicate the associations between ficolin-3 and SLE manifestations in the DISSECT cohort (Fig. 5A, Supplementary Table 4). Genotype frequencies for rs2474288 were similar in patients and controls (MAF patients 0.097, MAF controls 0.10,  $p = 1.0$ ). 168 SLE patients heterozygous and nine SLE patients homozygous for the rs2474288 minor allele were present in the DISSECT data set. When these patients

were categorized together as minor allele carriers, significantly lower rates of hematological (OR 0.67,  $p = 0.018$ ) and immunological disease (OR 0.68,  $p = 0.031$ ) compared to patients with two major alleles were observed. Of hematological subcriteria, lymphopenia showed a significant association (OR 0.63,  $p = 0.031$ ). The nine SLE patients homozygous for the rs2474288 minor allele were also analyzed separately. Interestingly, compared to heterozygous patients these had even lower rates of hematological disease (44 % vs 57 %,  $p$  for trend = 0.013) and lymphopenia (17 % vs 31 %,  $p$  for trend = 0.024), and none fulfilled the *anti*-Sm criterion or were positive for *anti*-Sm antibodies.

Next, we explored associations between rs2474288 genotype and autoantibodies (Fig. 5B, Supplementary Table 4). In the Karolinska cohort, rs2474288 minor allele carriers displayed the inverse serological profile compared to patients in the highest ficolin-3 tertile with the exception of anti-phospholipid antibodies. The lowest ORs were observed for Sm or RNP antibodies, specificities that also displayed a strong association with ficolin-3 activity. No individual antibody in the Karolinska cohort reached statistical significance, but a strong association with the number of positive antibodies was observed, mirroring the results for ficolin-3 activity (2 antibodies (1–4) for rs2474288 minor allele carriers vs. 3 (2–5) for major allele homozygotes,  $p = 0.0019$ ). The clustering analysis was concordant with the associations with ficolin-3 activity. rs2474288 minor allele carriers were enriched in the autoantibody negative cluster while the lowest frequency was observed for the *anti*-Sm/DNA/nucleosome cluster, although the associations were not statistically significant. Data on *anti*-SSA/SSB, *anti*-Sm, and anti-phospholipid antibodies were available for DISSECT participants, and all except *anti*-β2GP1 IgG antibodies showed lower ORs in rs2474288 minor allele carriers.

### 3.7. Complement factor levels in relation to ficolin-3 activity

Lastly, we analyzed complement factor levels in relation to ficolin-3 activity and rs2474288 genotypes in the Karolinska cohort. Ficolin-3 activity correlated with complement C4 and C3 levels in controls, but the correlation was absent in SLE patients (Fig. 6A and B) indicating that the lectin pathway is uncoupled from classical pathway consumption in SLE. rs2474288 minor allele carriers had significantly higher C1q and C4 levels compared to patients homozygous for the major allele while C3

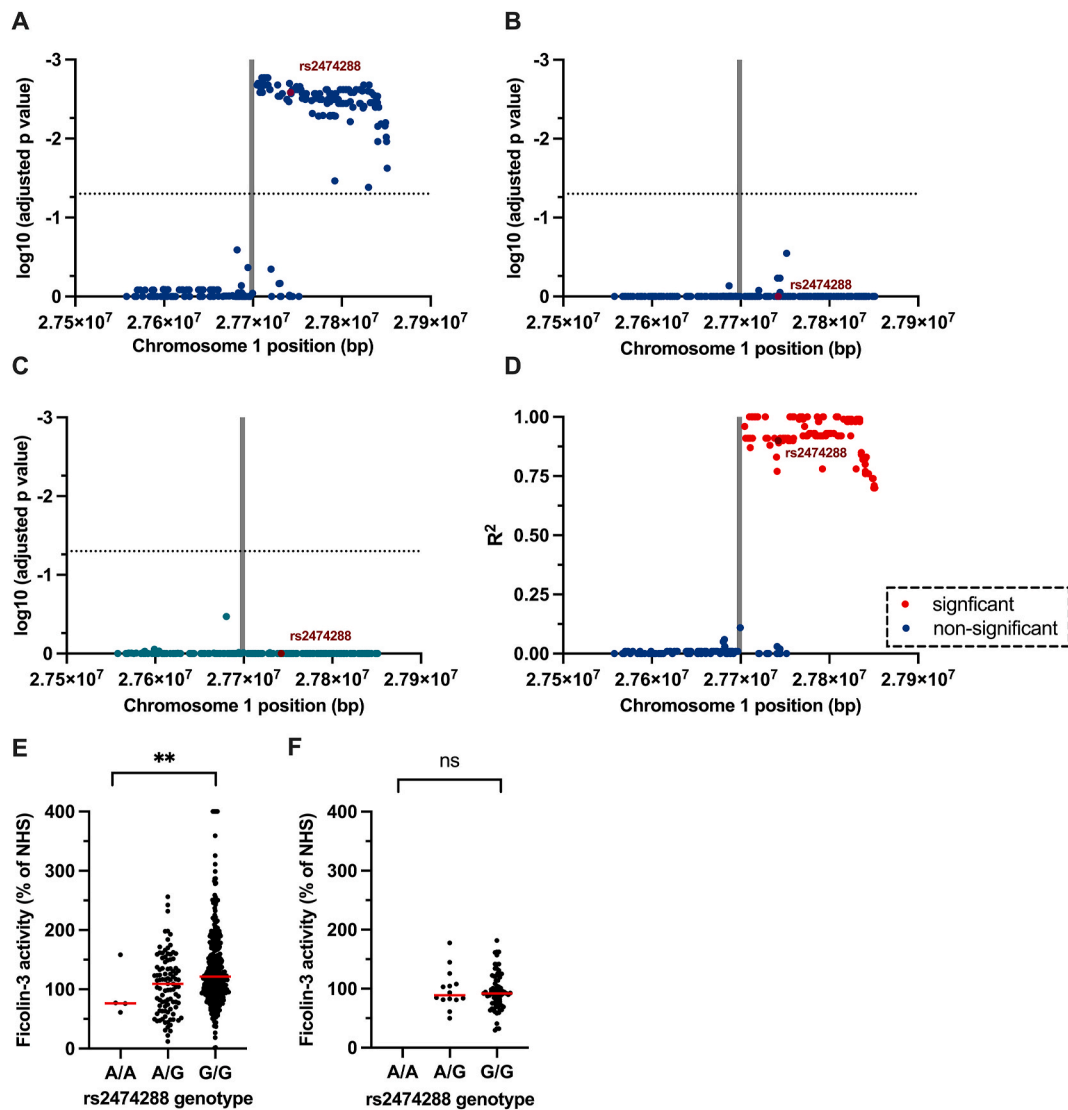


Fig. 4. Genetic associations with ficolin-3 activity and SLE in the *FCN3* gene region.

A-B. SNVs in the *FCN3* gene region were tested for their association with ficolin-3 activity in SLE patients (n = 463) (A) and controls (n = 83) (B). The graphs show adjusted p-values for individual SNVs. As the *FCN3* gene is transcribed from the reverse strand, significant pQTLs are located upstream of the TSS. Grey bar indicates the location of the *FCN3* gene. Dotted lines indicate significance threshold (adjusted p < 0.05).

C. Associations between individual SNVs and SLE disease status in the DISSECT cohort. n (SLE patients) = 958, n (controls) = 1026.

D. LD analysis of the *FCN3* gene region. The graph shows  $R^2$  values calculated from the DISSECT data set (n = 1984). SNVs that showed a significant association with ficolin-3 activity are indicated in red.

E-F. Ficolin-3 activity stratified by rs2474288 genotype. SLE patients: 76.5 % (65.0–138) for rs2474288-A/A (n = 4), 109 % (68.3–142) for rs2474288-A/G (n = 97), and 122 % (94.3–168) for rs2474288-G/G (n = 362), adjusted p = 0.0021 (E); Controls: 89.0 % (81.2–112) for rs2474288-A/G (n = 14), and 91.9 % (72.3–117) for rs2474288-G/G (n = 69), adjusted p = 1.0 (F). Data are presented as medians (red horizontal lines) with interquartile range.

levels did not differ (Fig. 6C–E), implying reduced complement turnover up to the C4 level in carriers of the ficolin-3 pQTL variant.

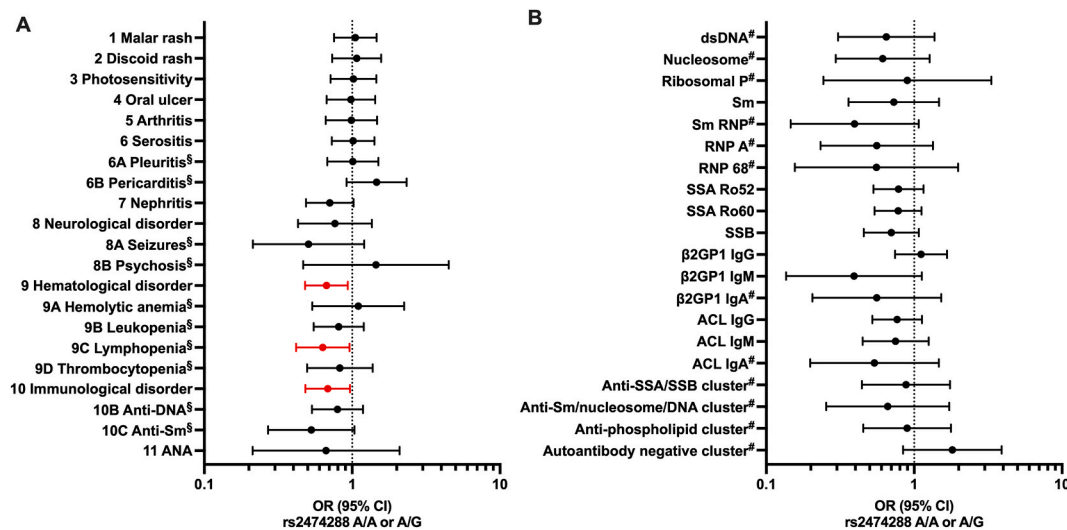
#### 4. Discussion

In the current study, we performed a comprehensive genetic and biochemical characterization of the lectin pathway initiator ficolin-3 in SLE. We demonstrate that high ficolin-3 activity associates with a broad autoantibody response, and a phenotype characterized by *anti*-Sm antibodies and the development of hematological disease manifestations.

SLE patients carrying the rs247288 minor allele associated with low ficolin-3 activity showed fewer clinical manifestations and autoantibody specificities compared with non-carriers, indicating that ficolin-3 may be causally involved in SLE pathogenesis. In further support of this notion, a pattern emerged where rs2474288 minor allele carriers

exhibited the inverse clinical and serological phenotype of patients with elevated ficolin-3 activity. Regarding SLE criteria, biochemical and genetic data were concordant for the association with immunological and hematological manifestations and, in particular, lymphopenia, indicating that ficolin-3 and the lectin pathway are involved in the development of these features of SLE. Interestingly, an association between ficolin-3 levels and lymphopenia was also observed by Andersen et al. [25]. Lymphopenia in SLE is often observed in active disease with a more severe phenotype and may be a predictor of damage accrual [29, 30]. The exact underlying mechanism is unclear, but lymphocyte depletion by lymphotoxic autoantibodies has been suggested [31]. Given the strong link we observed between ficolin-3 and autoantibody profiles, a plausible mechanism is that activation of the ficolin-3 pathway promotes the formation of such lymphotoxic autoantibodies.

Discoid rash showed a strong association with ficolin-3 activity but



**Fig. 5.** Associations between rs2474288 genotype and SLE manifestations and autoantibodies.

The graphs show forest plots with odds ratios (OR) and 95 % confidence intervals (CI) for the rs2474288 A/A or A/G genotypes (minor allele) (n = 177) compared to rs2474288 G/G genotype (major allele) (n = 781).

**A.** ORs for SLE criteria according to the 1982 ACR classification in the DISSECT cohort (<sup>§</sup>n = 727, otherwise n = 958).

**B.** ORs for autoantibody specificities. <sup>#</sup>Karolinska cohort only (n = 235), others DISSECT cohort (n = 442–943).

All analyses were adjusted for sex and age at follow-up. Significant ORs (p < 0.05, unadjusted for multiple comparisons) are shown in red.

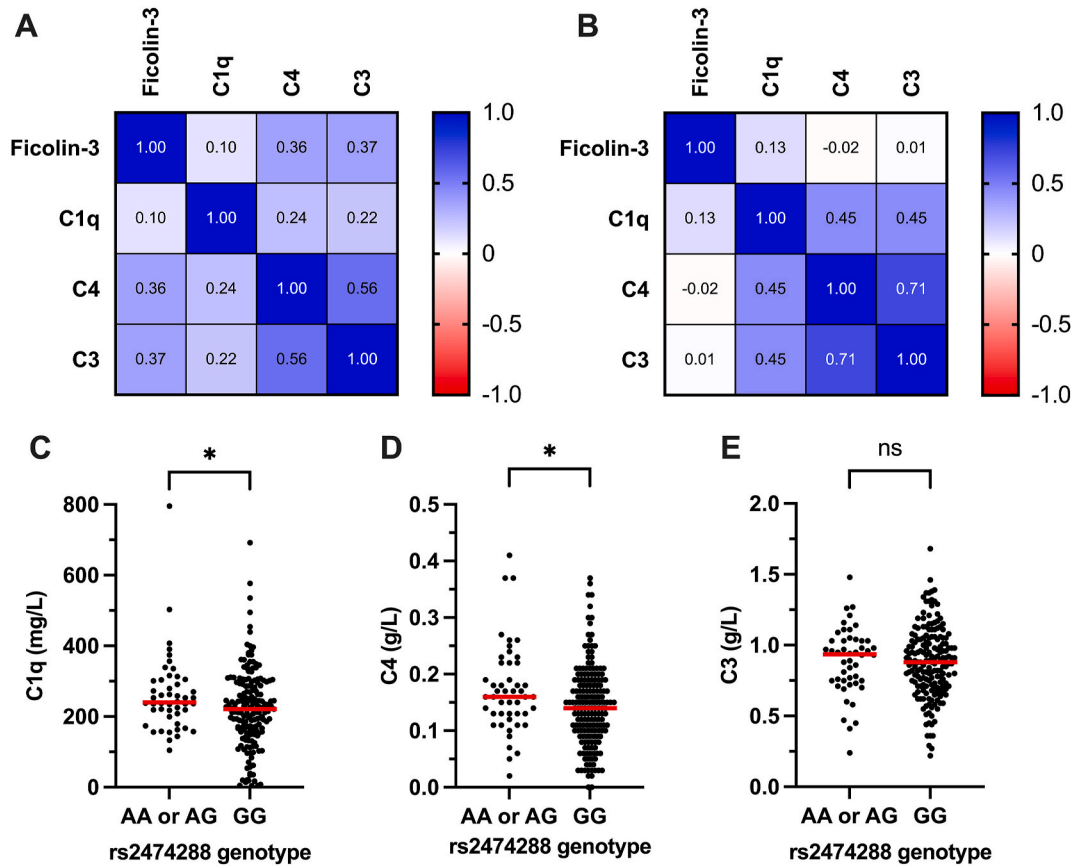
not with the rs2474288 genotype. This association therefore likely reflects increased inflammation and may be secondary to elevated ficolin-3 activity rather than causally related.

Elevated ficolin-3 activity was associated with several autoantibodies and showed a strong enrichment in the *anti*-Sm/DNA/nucleosome cluster, which defines a core SLE phenotype [18]. *Anti*-Sm antibodies are highly specific for SLE and are part of the serological ACR classification criteria, although the antibody's pathogenic role in the disease remains unclear [32]. Yet, a link between *anti*-Sm seropositivity and lymphopenia has been reported [29,33] suggesting that these SLE features associated with ficolin-3 may share a common underlying immunological process. Results from the genetic analysis in the DISSECT cohort were consistent with these observations. The lowest OR for rs2474288 minor allele carriers among ACR criteria was recorded for the *anti*-Sm criterion, further underlining a potential causal link to this SLE subtype. Additionally, rs2474288 minor allele carriers had fewer autoantibody specificities and were enriched in the autoantibody negative cluster. The seronegative cluster was characterized by a lower frequency of hematological manifestations in the original study [18], in concordance with lower rates of hematological disease in rs2474288 minor allele carriers in the present investigation. As the number of autoantibodies was strongly associated with both ficolin-3 activity and rs2474288 genotype, our data demonstrate a link between ficolin-3 and the breadth of the autoantibody response. The molecular mechanism that underlies these clinical associations remains to be determined. We propose a model where a sustained increase in ficolin-3 activity promotes the formation of *anti*-Sm antibodies and epitope spreading by an as yet unidentified mechanism. In this context, it is interesting to note that the role of complement in augmenting antibody responses is well recognized [34]. In mice, a moderate enhancing effect of lectin pathway activation through MBL on antibody responses has been reported, although the classical pathway appears to be more important [35]. However, ficolin-3 does not exist in rodents [36], and our results raise the possibility that ficolin-3 might act as a regulator of antibody responses in humans.

SNVs significantly associated with ficolin-3 activity tagged a large LD block upstream of the *FCN3* gene, covering >146 kb of DNA and encompassing 3 additional protein-coding genes (*CD164L2*, *GPR3*, and *WASF2*) (Supplementary Fig. 4). Currently, we cannot say where in this

region the functional SNV is located. GTEx revealed an association between the lead SNV rs2474288 and *FCN3* mRNA levels, but also with expression levels of *CD164L2* and *WASF2*. *CD164L2* encodes a gene product with a poorly characterized function that shows high expression in the digestive tract and female reproductive system [37], while *WASF2* encodes a ubiquitously expressed component of the actin cytoskeleton machinery that regulates cytoskeletal dynamics and cellular behavior in a range of cell types [38]. However, regarding the association with SLE phenotype, several lines of evidence indicate that *FCN3* is the functional gene. The rs2474288 SNV showed a robust association with both ficolin-3 activity in patient sera and with SLE phenotype. The clinical and serological phenotype in rs2474288 minor allele carriers was the inverse of what was observed in patients with high ficolin-3 activity, an observation which is in accordance with a functional role of ficolin-3 and the lectin pathway. Furthermore, carriers of the rs2474288 minor allele had increased serum levels of C1q and C4, directly linking a genetic variant associated with low ficolin-3 activity to a low-activating complement phenotype. Presumably, a lower degree of ficolin-3-driven complement activation leads to higher complement factor levels as these are not consumed at the normal rate. Interestingly, a similar phenomenon is observed in subjects with low C1q, where the absence of classical pathway activation leads to decreased turnover of downstream complement components such as C4 [39].

We did not find any evidence for an association between genetic ficolin-3 deficiency and SLE susceptibility. Instead, we observed four SLE patients with an apparent depletion of ficolin-3 in serum, a phenomenon illustrated by the disappearance of circulating ficolin-3 over time in longitudinal samples. In support of these findings, two of the three ficolin-3 deficient SLE patients described in the literature have no confirmed coding mutations in the *FCN3* gene [11,25]. Serum from one of the four deficient patients in our study displayed strong autoreactivity against ficolin-3, implying autoantibodies as a possible cause of the deficient state. Yet, this did not appear to be the case in the remaining deficient patients. Instead, we speculate that a hitherto unknown component in blood or tissues strongly activates the lectin pathway in these patients, leading to complete consumption of ficolin-3. Low C4 levels supported that complement consumption occurred as a consequence of lectin pathway activation. As the binding specificity and function of ficolin-3 is poorly defined, this activating factor remains to



**Fig. 6.** Analysis of complement factor levels in relation to ficolin-3 activity in the Karolinska cohort.

**A-B.** Correlation matrices showing Spearman's rank correlation coefficients for ficolin-3 activity and C1q, C4, and C3 antigen levels in controls and SLE patients. Ficolin-3 activity is significantly correlated with C4 ( $r = 0.36$ ,  $p < 0.001$ ) and C3 ( $r = 0.37$ ,  $p < 0.001$ ), but not C1q ( $r = 0.10$ ,  $p = 0.07$ ) in controls (A); While other complement factors show strong associations in SLE patients, there is no correlation between ficolin-3 activity and C4 ( $r = -0.017$ ,  $p = 0.71$ ) or C3 ( $r = 0.013$ ,  $p = 0.77$ ). Ficolin-3 and C1q show a weak but statistically significant correlation ( $r = 0.13$ ,  $p = 0.0036$ ) (B). **C-E.** Complement factor levels in relation to rs2474288 genotype. C1q levels were 240 mg/L (202–303) for rs2474288 A/A or A/G, and 221 mg/L (163–292) for rs2474288 G/G,  $p = 0.048$  (C); C4 levels were 0.16 g/L (0.13–0.22) for rs2474288 A/A or A/G, and 0.14 g/L (0.10–0.19) for rs2474288 G/G,  $p = 0.029$  (D); C3 levels were 0.94 g/L (0.75–1.03) for rs2474288 A/A or A/G, and 0.88 g/L (0.71–1.05) for rs2474288 G/G,  $p = 0.67$  (E).

Data are presented as medians with interquartile range. Red horizontal lines indicate the median values.

be identified.

The apparent lack of association between genetic ficolin-3 deficiency and SLE risk *per se* is in stark contrast to the classical pathway, where deficiencies in early components are strong risk factors for SLE. Thus, despite their many structural and functional similarities, this fact indicates a fundamental difference between these two complement activation pathways. Furthermore, while depletion of classical pathway components is a common feature of SLE [40], it appears to be a rare phenomenon for ficolin-3. However, a large variation in ficolin-3 activity was observed, likely reflecting a balance between increased synthesis due to inflammation and increased consumption. The latter led to complete ficolin-3 deficiency in a small number of patients, but likely operates to a varying degree in SLE patients as a whole.

There are limitations to the current study. While our study included SLE patients from several Swedish cohorts, the patients were mainly of White European origin. An important future task will therefore be to replicate the findings in cohorts with SLE patients of other ancestries. Furthermore, it is well recognized that autoantibody specificities may vary depending on methods and choice of cut-offs [41,42]. In the Karolinska cohort, an extended autoantibody panel was analyzed in a cross-sectional design, while autoantibodies in the other cohorts were analyzed by different methods at the participating hospitals. In any case, data were consistent with a lower frequency of autoantibodies in patients carrying the pQTL variant associated with low ficolin-3 activity.

## 5. Conclusions

To conclude, we provide biochemical and genetic evidence that implicates lectin pathway activation through ficolin-3 in autoantibody formation and the development of hematological disease manifestations in SLE. Furthermore, we show that a state of acquired ficolin-3 deficiency develops in a small subset of patients with active disease, where ficolin-3 levels are depleted by a mechanism yet to be identified.

## Author contributions

Conceptualization: LL, SRD, LR, IG, ES, OE; Data curation: LL, SRD, CL, ES, IG, OE; Formal analysis: LL, CL, MGH, OE; Funding acquisition: DL, LR, CS, AJ, AAB, KLT, BN, IG, ES, OE; Investigation: LL, SRD, CL, AS, MGH, IG, ES, OE; Methodology: LL, CL, OE, MB, SVK, KLT; Project administration: OE; Resources: SRD, JKS, DL, LR, HE, CS, AJ, AAB, MB, SVK, KLT, KNE, BN, IG, ES; Supervision: OE; Visualization: LL, OE; Writing – original draft: LL, OE; Writing – review & editing: SRD, CL, JKS, DL, AS, LR, HE, CS, AJ, AAB, MGH, LMDG, MB, SVK, KLT, KNE, BN, IG, ES.

## Declaration of competing interest

The authors declare no competing financial interests.



## Acknowledgments

This work was supported by the Agnes and Mac Rudberg Foundation, the Göran Gustafsson Foundation, and the Jeansson Foundation to OE, the Swedish Rheumatism Association (to OE, SRD, LR, CS, ES), the King Gustaf V 80-year Anniversary Foundation (to OE, SRD, LR, CS, ES), the Swedish Society of Medicine (to OE, and the Ingegerd Johansson donation to LR and ES), the Swedish Research Council for Medicine and Health (grant number 2018-02551 to SRD, grant number 2018-02399 to LR, grant numbers 2020-05762, 2021-02252 to BN, grant numbers 2018-02535, 2022-00783 to ES), the Gustafsson Foundation (to CS), the Swedish Heart-Lung Foundation (to ES), and regional ALF grants (to SRD, CS, BN, ES). Dr. Lindblad-Toh's work was supported by a Wallenberg Scholarship. DNA sequencing and genotyping were performed at the SNP&SEQ Technology Platform in Uppsala, part of the National Genomics Infrastructure Sweden and Science for Life Laboratory. Computations were enabled by support from National Bioinformatics Infrastructure Sweden (NBIS) and by resources provided by the National Academic Infrastructure for Supercomputing in Sweden (NAISS) and the Swedish National Infrastructure for Computing (SNIC) at the Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) partially funded by the Swedish Research Council (grant numbers 2018-05973, 2022-06725). The Biobank Research Unit at Umeå University is acknowledged for providing blood samples. Professor Peter Garred, University of Copenhagen, Denmark, is acknowledged for providing recombinant ficolin-3. Members of the DISSECT consortium and the ImmunoArray development consortium are listed in the Supplementary Information.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaut.2023.103166>.

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