Journal of Clinical Immunology (2019) 39:421–429
https://doi.org/10.1007/s10875-019-00627-2

ORIGINAL ARTICLE

# Ficolin-3 Deficiency Is Associated with Disease and an Increased Risk of Systemic Lupus Erythematosus

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Received: 5 March 2019 / Accepted: 7 April 2019 / Published online: 1 May 2019


## Abstract

**Purpose** Ficolin-3 deficiency is caused by a mutation *(+1637delC)* in the *FCN3* gene. It is a rare condition and has been associated with both infection and autoimmune disease including systemic lupus erythematosus (SLE). Here we investigated if ficolin-3 deficiency is more frequent in patients than in controls and tried to identify a common phenotype among ficolin-3 deficient individuals. Since a significant part of patients identified with ficolin-3 deficiency was diagnosed with SLE, we explored whether the heterozygous state of the *FCN3+1637delC* variant represents a risk factor in the development of SLE. Further, we examined other possible causes of ficolin-3 deficiency when the *FCN3+1637delC* is not present.

**Methods** A systematic literature search for studies measuring ficolin-3 was carried out. We examined 362 SLE patients and 596 controls for the presence of the variant *FCN3+1637delC*. We established assays for measurements of ficolin-3 and of autoantibodies against ficolin-3. We sequenced the coding and non-coding regions of the *FCN3* gene in an SLE patient with ficolin-3 deficiency not carrying the *+1637delC*.

**Results** Ficolin-3 deficiency leads to an 8-time increased odds of having a disease ($p < 0.05$). Three out of nine patients with deficiency had SLE. The heterozygous state of the deficiency variant is not associated with increased risk of developing SLE ($p = 0.18$).

**Conclusion** By systematically reviewing the literature for the described cases of ficolin-3 deficiency, an autoimmune phenotype is emerging. Thirty-three percent of the ficolin-3 deficient patients had SLE. Heterozygosity for the *FCN3* gene deletion causing the deficiency does not seem to be associated with the development of SLE.

**Keywords** SLE · ficolin-3 deficiency · complement · complement deficiency · autoimmunity

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10875-019-00627-2) contains supplementary material, which is available to authorized users.

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## Introduction

The plasma protein ficolin-3 (also called H-ficolin and originally named Hakata antigen) was discovered in 1990 as an autoantigen when antibodies against the protein were identified in patients with systemic lupus erythematosus (SLE) [1]. Ficolin-3 is a soluble pattern recognition molecule (PRM) of the innate immune system. It binds to acetylated molecules, e.g., as found in acetylated carbohydrate structures or on proteins via its fibrinogen-like recognition domain [2]. The fitting patterns recognized by ficolin-3 may be present on apoptotic and necrotic cells, and this gives ficolin-3 the possibility of acting as an initiator of scavenging actions [3]. When bound to a surface, ficolin-3 can activate the complement system via the lectin pathway (LP), giving rise to both anti-microbial defense and homeostatic balance. To enable this activation, ficolin-3 relies on activation of attached enzymes, i.e., so-called Mannose-binding lectin (MBL)-associated serine proteases

(MASPs) [4]. Ficolin-3 is the most abundant of the five PRMs of the LP (as compared with ficolin-2, ficolin-1, MBL, and collectin-LK).

In our previous investigations, we have consistently observed high levels of ficolin-3 in patients with SLE [5, 6]. This is in line with investigations by other research groups [1, 7, 8]. However, when examining a large SLE cohort of 424 patients, we also identified two ficolin-3 deficient individuals [6]. One of the patients carried the only known mutation-causing ficolin-3 deficiency; the other patient (patient-X) did not.

Ficolin-3 is encoded by the *FCN3* gene. A functional gene is only present in primates whereas, e.g., the rodent *FCN3* orthologue is a pseudogene [9, 10]. Among the known polymorphisms of *FCN3*, only one leads to complete deficiency in variant homozygotes. A frameshift mutation causing ficolin-3 deficiency is located on exon 5 of the *FCN3* gene (*+1637delC*, rs532781899) [11]. It has a gene frequency of 0.01, and thus, homozygosity is expected in 1 out of 10,000 individuals [12, 13]. Before our findings in a cohort of SLE patients [6], only males with ficolin-3 deficiency had been identified [14].

The PRMs of the LP provide defense against a large array of pathogens and are primarily mediated by recognition of highly conserved pathogen-associated molecular patterns, i.e., repetitive sugar arrays on the surface of microorganisms that are rare or non-existent on mammalian cells [15]. Deficiencies of the PRMs are therefore hypothesized to be associated with serious infections by several research groups [16, 17]. Ficolin-3 deficiency has only been described in a limited number of humans, and the alleged infectious phenotype is controversial [14]. Secondary ficolin-3 deficiency caused by autoantibodies against the protein has been described in SLE patients and has been suggested as a biomarker for disease activity and glomerulonephritis [1, 18, 19].

SLE is a chronic autoimmune disease. The disease course is unpredictable and characterized by periods of remission followed by periods of ongoing disease activity (flares). Defects in the clearance of apoptotic cells leading to the release of intracellular autoantigens resulting in the induction of autoantibody production have been proposed as a key pathogenic mechanism of SLE [20, 21]. In agreement with this, proteins that are implicated in the stringently orchestrated clearance of dying cells are likely to play a role in host protection against SLE.

The objectives of the current study were to investigate if ficolin-3 deficiency would be more common in patients than in controls by reviewing published literature on ficolin-3 and identifying the number of patients and controls that had been investigated. We aimed to identify a common phenotype among ficolin-3 deficient individuals. Further, since a significant part of patients identified with ficolin-3 deficiency were diagnosed with SLE, we explored whether the heterozygous state (C/del) of rs532781899 would represent a risk factor in the development of SLE. Lastly, we examined an SLE patient, who is ficolin-3 deficient, but not carrying the *+1637delC* mutation for other potential causes leading to ficolin-3 deficiency. Further, we describe the first female patient with ficolin-3 deficiency.

## Methods

### Study Populations

SLE patients followed at the out-patient clinic of the Department of Rheumatology,

Aarhus University Hospital, Denmark ($n = 169$), the Rheumatology out-patient clinic of Odense University Hospital, Denmark ($n = 203$), and SLE patients from the rheumatology out-patient clinic of Basel, Switzerland, ($n = 52$) have previously been described [6]. The Danish cohort is a cross-sectional cohort and represents patients in full remission as well as patients with active disease. All patients fulfilled the 1997 revised ACR classification criteria for SLE [22]. Controls ($n = 596$) used in the study were included with informed consent at the blood bank at Aalborg University Hospital, Denmark.

### Patient-X

The patient is a male SLE patient (from our Danish SLE cohort) diagnosed at the age of 27 with classical SLE symptoms (arthritis, malar rash, lupus nephritis, positive anti-dsDNA, anti-nuclear antibodies, low complement). In the 8-year course of disease, he has had three severe nephritis flares. He currently has ongoing proteinuria of more than 5 g/day and is treated with prednisolone (10 mg/day), mycophenolate-mofetil (3 g/day), and hydroxychloroquine (400 mg/day). The patient was admitted with biopsy-verified vasculitis of the colon in 2015 treated with high dose intravenous prednisolone. He has not had documented recurrent infectious episodes.

### First Female Patient with Ficolin-3 Deficiency

The patient is a Swiss SLE patient (from our own SLE cohort) diagnosed at the age of 18 with classical SLE symptoms (arthritis, pleuritis, malar rash, lupus nephritis, positive anti-dsDNA, anti-nuclear antibodies, low complement, lupus headache, Coombs-positive anemia, lymphopenia, high anti-C1q). One year after diagnosis, she presented with meningococcal meningitis (type non-B/C) in cerebrospinal fluid, and in peripheral blood (culture), consecutively, she developed septic shock with septic cardiomyopathy, acute renal failure, disseminated intravascular coagulation, and respiratory failure. At the time, she was under mild immunosuppression with

prednisone (10 mg/day) and azathioprine (50 mg/day). She was successfully treated with ceftriaxone. Four years after her SLE diagnosis, she presented with severe pneumonia (without identification of a pathogen), and 5 years after diagnosis with acute *Escherichia coli* cystitis with pyelonephritis. In the same year, she had a renal flare and was started on mycophenolate-mofetil. The mother of this patient was also diagnosed with SLE and was found to be heterozygous for the *FCN3* gene deletion [6].

## Literature Review Search

Articles were identified by systematic literature searches in MEDLINE, Embase, Web of science, and Scopus covering the period from 1980 to January 1, 2018. The following search terms were used and adjusted based on the search engine used: "H-ficolin", "Ficolin 3", "FCN3 gene", "Ficolin", "Complement Pathway, Mannose-Binding Lectin".

Inclusion criteria were clinical studies on humans where ficolin-3 concentrations had been measured in a patient group and/or control group, or studies where genetic testing for ficolin-3 deficiency had been performed.

Exclusion criteria were studies in which methods were not described sufficiently, data were not available for evaluation, studies in which neither ficolin-3 plasma or serum concentrations nor genetic data were available, or review articles not containing original data (Supplementary Fig. 1 outlines the screening and selection process of the literature search).

After removing duplicates, the remaining articles were screened by title and abstract based on the exclusion criteria, which left 64 articles for full-text assessment. Sixty-four articles were screened independently and evaluated for inclusion and exclusion criteria. Ten of these articles were excluded due to missing information on measured plasma concentrations. In the remaining 54 articles, the number of patients and controls examined for ficolin-3 were then added up. Results from the current article and the article in which our two ficolin-3 deficient SLE patients were identified [6] were added to the group of articles ending up with a total of 56 articles.

## PCR Analysis for FCN3+1637delC Mutation

DNA from SLE patients and controls were extracted from peripheral blood mononuclear cells (PBMCs) using the Maxwell16 Blood DNA kit on the Maxwell16 Instrument (Promega).

To cover the frameshift mutation *FCN3+1637delC* (rs532781899) [12], a fragment of 366 bp of *FCN3* exon 5 was amplified by PCR using the following primers: 5′-ggccaagatcctccccaca-3′ and 5′-tctggtgggttctggctcc-3′. PCR amplifications were carried out in 50 μl volumes containing ~ 50 ng genomic DNA, 0.5 mM of each primer, 1× PCR buffer II, 2.5 mM MgCl2, 0.2 mM dNTP, and 0.75 units of AmpliTaq DNA polymerase (Invitrogen Life Technologies). The PCR reactions were performed at; 5 m94 °C, 35 cycles (30 s94 °C, 30 s62 °C, 30 s72 °C), 5 m72 °C.

After a cleaning step (FlashGel Recovery System, Lonza, Inc.), the fragment was sequenced in both directions using the ABI BigDye cycle sequencing terminator kit, V 1.1 (Applied Biosystems). PCR amplifications were performed in 20 μl volumes at: 1 m96 °C, 25 cycles (10 s96 °C, 5 s50 °C, 4 m60 °C). PCR products were purified (BigDye XTerminator, Applied Biosystems) and sequences analyzed (ABI Prism 3500 Genetic Analyzer, Applied Biosystems). CLC main workbench software was used for alignment of resulting DNA sequence.

## FCN3 Gene Sequencing

A total of 6510 bases of the *FCN3* gene (covering all 8 exons of the gene) were sequenced covering 1350 base pairs (bp) upstream of exon 1, exon 1, intron 1, exon 2, intron 2, exon 3, intron 3, exon 4, intron 4, exon 5, and 300 bp upstream of intron 5, 482 bp downstream of exon 6, exon 6, intron 6, exon 7, and 271 bp upstream of intron 7, 308 bp downstream of exon 8, besides 1485 bp upstream of the 3' UTR part. The sequence was aligned to the NG_016279 sequence from the NCBI nucleotide database. The sequencing was performed as a resequencing service at Eurofins Genomics GmbH Freiburg, Germany.

## Ficolin-3 Assay

Microtitre plates (Nunc, #437958 or # 43791) were incubated with acetylated bovine serum albumin (Ac-BSA) (Sigma #B2518) 10 μg/ml coating buffer (0.1 M sodium bicarbonate, pH 9.6 with 0.09% (*w*/*v*) sodium azide, and Ampliqon #AMPQ44048.1000) and incubated overnight at room temperature (RT). Residual binding sites were blocked by incubation for 1 hour (h) with 200 μl of 1 mg human serum albumin (HSA)/ml TBS (10 mM Tris, 145 mM NaCl, pH 7.4) per well, and plates were washed three times with TBS/TW (TBS with 0.05% Tween 20). Standard, quality controls, and samples were then added to the plate in duplicates. The standard curve was obtained by diluting the standard (pool of human plasma with a known concentration of 20 μg/ml ficolin-3) 1/10 and further seven times twofold dilutions with TBS/Tween/$Ca^{2+}$/HSA (TBS/TW, 5 mM $CaCl_2$, and 1 mg/ml HSA). The concentration of ficolin-3 in the standard is based on comparisons of the signal obtained in dilutions of standard with that obtained in dilutions of purified plasma ficolin-3. Samples and quality controls were diluted 1/50 in TBS/Tween/$Ca^{2+}$/HSA. After overnight incubation at 4 °C and three times wash in TBS/Tween/$Ca^{2+}$ biotinylated mouse anti-human-ficolin-3, clone 4H5 (conc. 0.34 μg/ml, Hycult biotechnology #HM2089b) diluted 1/1000 in TBS/Tween/$Ca^{2+}$ were added

to each well at 100 μl/well and incubated for 2 h at RT. The subsequent steps followed our standard procedures for time-resolved immunofluorometric assay (TRIFMA) as previously described [6], i.e., incubation with europium-labeled streptavidin followed by wash and detection of the signal by time-resolved fluorometry.

## Purification of Ficolin-3 from Serum

Ficolin-3 was purified from human serum following a previously described procedure [23]. Serum was precipitated with polyethylene glycol (PEG) in two steps each followed by centrifugation, and at 4 °C, the supernatant was passed through a 10 ml HSA-sepharose (HSA coupled to CNBr-activated sepharose beads) column (this removes most L-ficolin), and the effluent was loaded onto a 20 ml-acetylated HSA-sepharose column to bind ficolin-3. The column was washed with 80 ml of TBS/tw/$Ca^{2+}$. Ficolin-3 was subsequently eluted from the beads with 1 M sodium acetate, pH 7.5, 1 ml/fraction. Fractions with ficolin-3 were pooled and dialyzed against 50 mM sodium acetate, 0.01% Tween-20, and pH 8.3. After dialysis, the preparation was centrifuged 9000*g* for 10 min. The ficolin-3 in the supernatant was purified further on an ion exchange column MonoQ 5/50 GL (GE Healthcare), using a gradient from 50 mM NaCl to 350 mM NaCl over 30 ml. The purity of the protein was tested by SDS-PAGE protein stain (Supplementary Fig. 2).

## Assay Measuring Auto-antibodies Against Ficolin-3

Microtitre plates (Nunc, Roskilde, Denmark; # 437958 or # 43791) were incubated with purified ficolin-3 at 0.5 μg/ml phosphate buffered saline (PBS) overnight at RT. Residual binding sites were blocked with 1 mg of HSA per ml of TBS. After washing with TBS/TW, 100 μl of test samples and three quality controls (plasma chosen to reflect a wide distribution of concentrations) were added to the plate diluted 1/10 in sample buffer (10 mM Tris/base, 145 mM NaCl, 10 mM EDTA, 0.05% Tween-20, 1 mg HSA/ml, heat-aggregated human immunoglobulin (Ig)G 100 mg/ml, pH 7.4). All samples were added in duplicates using a 1/10 dilution. A standard curve was constructed from a pool of four SLE EDTA plasma samples, initially diluted tenfold followed by serial threefold dilutions. After incubation overnight at 4 °C, the wells were washed with TBS/TW and incubated with biotin-rabbit-anti-human-kappa/lambda (Dako #A0194 and A0191) at 1/5000 in TBS/TW for 2 h at RT. After washing with TBS/TW, the wells were incubated with europium-labeled streptavidin (Perkin Elmer, USA; #1244-360) 1/1000 in TBS/TW, 25 μM EDTA for 1 h at RT. After washing, quantification of europium was performed by adding 200 ml of enhancement solution (Ampliqon laboratory reagents #Q99800; Ampliqon, Denmark) per well releasing and encapsulating the bound europium, and the fluorescence was read as time-resolved fluorometry on a Victor 5 from Perkin Elmer.

As a control, plates were also coated with human IgG to be able to adjust for the difference in background signal between SLE patients and controls. No significant binding was observed for neither SLE patient sera nor for controls, and no difference in background signal was observed between patients and controls.

## Western Blot

We tested whether ficolin-3 protein from patient-X could be detected by Western blot and compared with the signals seen in other SLE patient samples. Serum samples from six SLE patients with known ficolin-3 serum concentrations (one deficient, two with high concentrations, one with medium concentrations and two with low concentration), based on measurements in TRIFMA assays as described above, were added to SDS PAGE sample buffer (30 mM Tris-HCL, 10% (*v*/*V*) glycerol, 8 M urea, 3% (*w*/*v*) SDS, 0.1%(*W*/*v*) bromophenol blue, pH 8.9). TBS was added to reach the desired sample volume (30–45 μl). Dithiothreitol (DDT) was added to reach 60 mM in the samples to be reduced, followed by heating and addition of iodoacetic acid. Proteins were separated using an 18 well 4–15% gradient gel (Bio-Rad, Criterion TGX gels # 567-1084). Following electrophoresis, the proteins were blotted onto nitrocellulose membranes (Bio-Rad #170-4159). The membranes were then blocked by incubation for 30 min at RT in TBS with 0.1% Tween (*v*/v), washed, and developed with goat-anti-human-ficolin-3 (RD#F2367) at 0.5 mg/ml) in primary buffer (TBS, 1 mM EDTA, pH 7.4, with 1 mg HSA (CSL Behring #109697) and 100 μg human IgG (CSL Behring #007815) per ml). The membrane was subsequently washed and incubated with HRP-conjugated rabbit-anti-goat IgG antibody (DAKO #P0449) diluted 1/3000 in secondary buffer (TBS/Tween, 100 μg human IgG/ml, 1 mM EDTA, pH 7.4). After washing, the blot was developed with SuperSignal West Dura extended duration substrate (Pierce), and emission recorded by a charge-coupled device camera.

## Ethics

The project was performed according to the Helsinki Declaration. The Danish Data Protection Agency and the Central Denmark Region Committees on Health Research.

Ethics approved the study conducted in Aarhus (#1-10-72-214-13). The Southern Denmark Region Committees on Health Research Ethics approved the inclusion of patients in Odense (#2010 0015). For inclusion of the Swiss SLE population, the Ethical committee of Northwest and Central Switzerland approved the project (EKNZ.Ref. no. EK 262/06).

**Table 1** Patients with ficolin-3 deficiency described in the literature

| Clinical presentation | Age | Gender (M/F) | Ficolin-3 concentration μg/ml (plasma or serum) | Homozygosity for the FCN3+1637delC | Autoantibodies against ficolin-3 | Reference | Dominant phenotype |
|---|---|---|---|---|---|---|---|
| Recurrent pulmonary infections, brain abscesses, and recurrent warts on fingers; bronchiectasis and pulmonary fibrosis | 32 | M | < 0.1 | Yes | NA | [12] | Autoimmune |
| SLE no other clinical data available | NA | NA | < 1.1 | No | No | [7] | Autoimmune |
| Invasive necrotizing enterocolitis and repeated skin infections with staphylococci; no other infectious diseases during 4-year follow-up | 29 weeks GA | M | < 0.1 | Yes | NA | [24] | Infectious |
| Fatal necrotizing enterocolitis | 28 weeks GA | M | 0.8 | NA | NA | [24] | Infectious |
| Perinatal *S. agalactiae* infection; microcephaly, growth and mental retardation; ADHD; no severe infections during 8-year follow-up | 35 weeks GA | M | < 0.1 | Yes | NA | [25] | Infectious |
| Membranous nephropathy; nephrotic syndrome; EBV infection; no other recurrent infections | 50 | M | < 0.1 | Yes | NA | [14] | Autoimmune |
| Congenital heart disease; pneumonia; no severe infections during follow-up after cardiac surgery | 11 months | M | < 0.1 | Yes | NA | [14] | Infectious |
| Healthy individual (control person) no clinical data available | NA | NA | < 0.1 | Yes | NA | [26] | NA |
| Healthy individual (control person) no clinical data available | NA | NA | < 0.1 | Yes | NA | [26] | NA |
| SLE (arthritis, positive ANA, anti-ds-DNA, glomerulonephritis, low C3 and C4). Meningococcal sepsis. 2nd severe lung infections. | 18 | F | < 0.1 | Yes | NA | [6] | Autoimmune |
| SLE (malar rash, arthritis, glomerulonephritis, ANA positive, anti-ds-DNA positive, low complement C3 and C4) | 27 | M | 0.3 | No | No | [6] | Autoimmune |

**Table 2** Cumulated number of patients and controls in the published literature where ficolin-3 has been measured based on a systematic literature review. An OR of having disease when being ficolin-3 deficient was calculated based on the number of ficolin-3 individuals in each group (patients and controls)

| Ficolin-3 measured | Patients (p) | Controls (c) | Deficient (p/c) |
|---|---|---|---|
| Total | 9.294 | 8.227 | 9/1 |

Odds for disease when being ficolin-3 deficient: OR 8.0 (95% CI 1.01–63.0, $p < 0.05$)

## Results

### Ficolin-3 Deficiency

Based on our literature search, a total of 10 individuals with ficolin-3 deficiency are now described in the literature, nine males and one female. All individuals with described ficolin-3 deficiency where evaluated for disease phenotype, age, gender, ficolin-3 concentration, genetic data, and autoantibody status against the ficolin-3 protein where this information was available (Table 1).

Based on all published data on ficolin-3, we observed that a total of 9294 patients and 8227 controls had been evaluated for either ficolin-3 concentration and/or ficolin-3 deficiency by genetic testing (Supplementary Table 1). These numbers are based on the addition of the total number of patients and the total number of controls reported in the included articles of the literature search. Among these two groups, nine patients and only one control were deficient for the *FCN3*del1637, yielding an odds ratio for having a disease phenotype when being ficolin-3 deficient of 8.0 (95% CI 1.01–63.0, $p < 0.05$) (Table 2).

### FCN3 Mutation in SLE

Since three of the nine identified patients with ficolin-3 deficiency were SLE patients, we examined (in our own cohorts) whether being a heterozygous carrier of the *FCN3 + del1637C* would be a risk factor for the development of SLE. SLE patients ($n = 362$) and controls ($n = 596$) were assessed for the mutation. In SLE patients, 2.8% carried the mutation, and in controls, this was true for 1.5%. This difference was not significant ($p = 0.18$) (Fig. 1).

### Investigations of the Cause of Ficolin-3 Deficiency in Patient-X

Sixteen randomly chosen SLE patients from our SLE cohort with different serum concentrations of ficolin-3 and our ficolin-3 deficient patient without the known mutation-causing ficolin-3 deficiency (patient-X), and 18 randomly chosen controls were examined for auto-antibodies against ficolin-3. No significant difference was observed between controls and SLE patients (Fig. 2a), and no correlation was observed between ficolin-3 plasma concentration and concentration of auto-antibodies against ficolin-3 (Fig. 2b). One patient had very high levels of antibodies against ficolin-3; however, the patient had normal plasma levels of ficolin-3 (38 μg/ml). Patient-X did not have higher concentrations of autoantibodies against ficolin-3 than the controls (open circle Fig. 2a).

To examine whether ficolin-3 protein was present in patient-X in a form not detectable in our immunoassay, we investigated patient serum by Western blotting. Ficolin-3 is normally detected at 34 kDa under reducing conditions (Fig. 3a) and as several high oligomeric bands above 250 kDa under non-reducing conditions (Fig. 3b). No

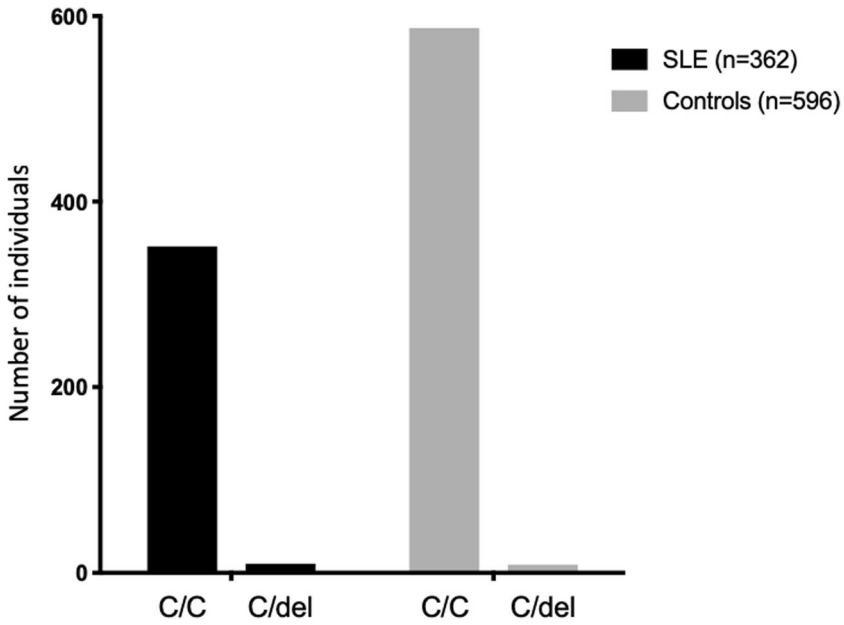


**Fig. 1** Number of carriers of the heterozygous mutation *FCN3+ 1637delC* in Danish SLE patients and controls. In the SLE patients, 2.8% (10 / 362) were heterozygous carriers of the *FCN3+ 1637delC*, the same was true for 1.5% (9/596) of the controls. *P* value reflects $\chi^2$ for the difference between the two groups

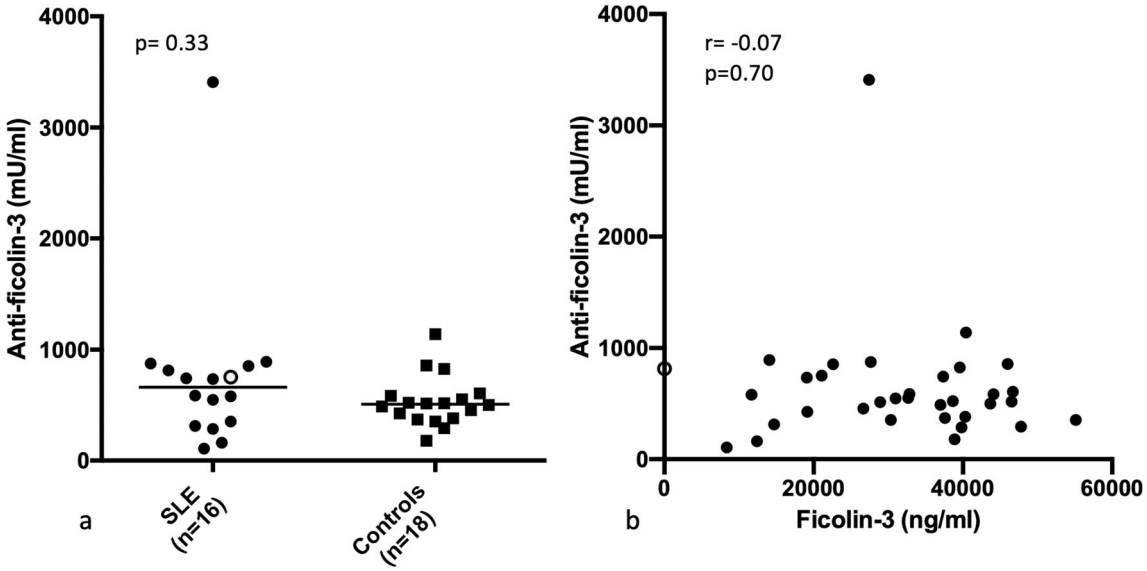


**Fig. 2** Auto-antibodies against ficolin-3. **a** Auto-antibodies against ficolin-3 in SLE patients and controls. Open circle represents patient-X with ficolin-3 deficiency not carrying the known mutation causing ficolin-3 deficiency (*FCN3 + 1637delC*). Auto-antibodies against ficolin-3 did not explain the observed deficiency. **b** Correlation between ficolin-3 plasma concentrations and auto-antibodies against ficolin-3

ficolin-3 was detectable in patient-X (lane 1 Fig. 3a (reducing) and lane 1 Fig. 3b (non-reducing)).

For patient-X, genetic sequencing of all exons [1–8] and flanking sequences of the *FCN3* gene was carried out to detect potential new mutations to account for the observed deficiency. No such mutations were discovered. Only a heterozygous mutation located upstream of exon1 in the promoter region was observed (Supplementary Fig. 3c).

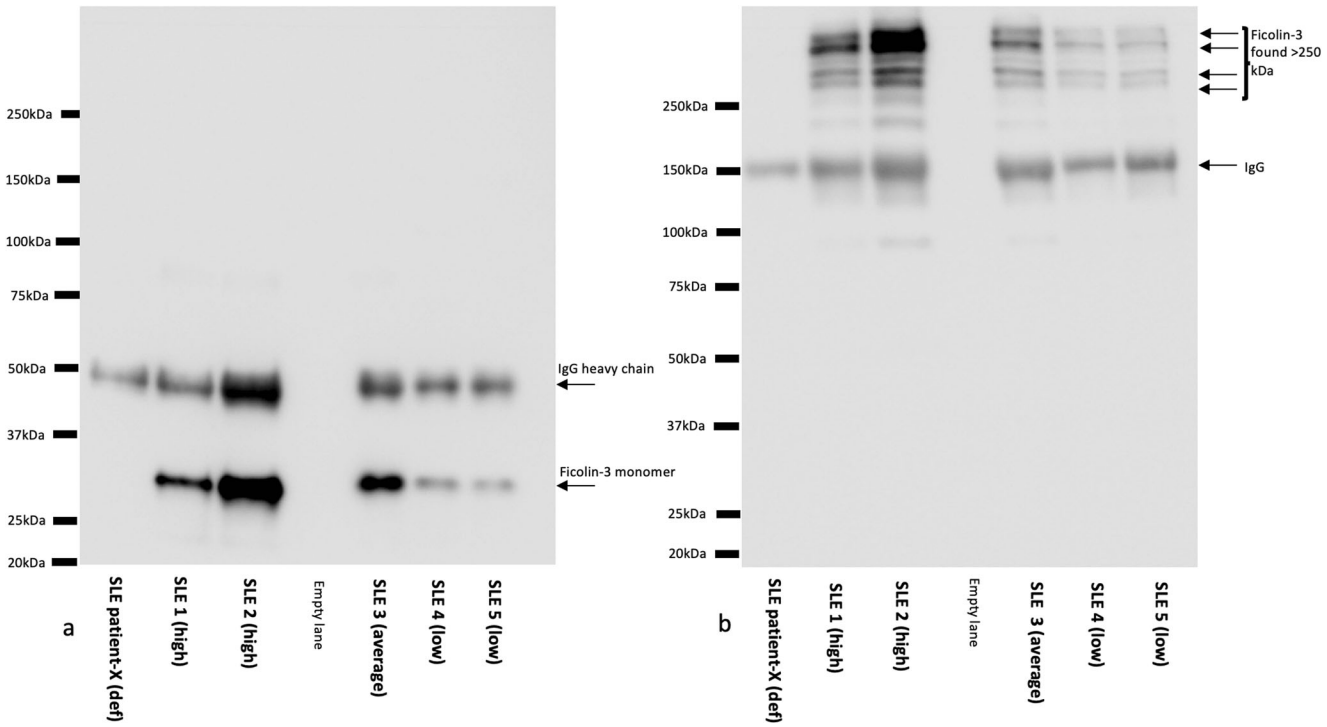


**Fig. 3** Test for ficolin-3 using Western blotting of samples from the SLE deficient patient not carrying *FCN3 1637delC* (patient-X). Proteins were separated on a 4–15% gel and blotted, and the blot developed with goat-anti-ficolin-3. **a** Ficolin-3 is detected at 34 k Da under reducing conditions (lanes 2, 3, 4–6). No ficolin-3 was present in patient-X (lane 1). **b** Ficolin-3 is detected as several oligomeric bands above 250 kDa under non-reducing conditions. No ficolin-3 was present in patient-X (lane 1, a + b). Lanes 2–3 and 5–7 on each blot represents serum from SLE patients with high (lane 2–3), medium (lane 5), and low [6, 7] concentrations in serum of ficolin-3

## Discussion

By systematically reviewing the literature, we identified 10 individuals with ficolin-3 deficiency. Eight carried the known *FCN3del1637* mutation. Three of the ten described cases had a diagnosis of SLE. The heterozygous state of the *FCN3del1637* mutation, which leads to a 50 % reduction in ficolin-3 plasma concentration was not associated with the development of SLE.

Our immediate hypothesis was, that the discovery of ficolin-3 deficiency in diseased individuals, could be due to diseased populations being studied more rigorously than controls. However, when evaluating the literature, we found that approximately the same number of diseased individuals and healthy individual has been investigated since the discovery of ficolin-3. Based on the currently available literature, this implies that the deficient state is a risk factor for disease and not just a selection bias. The clinical consequences of ficolin-3 deficiency are still unclear and definite conclusions cannot be drawn based on 10 individuals. However, a pattern of infection in the young cases and an autoimmune phenotype in the adult cases, does seem to be emerging.

Michalski et al. reported, that heterozygosity for the *FCN3* gene deletion does not seem to have major clinical importance in neonates [25]. This also appears to be the case for SLE, i.e., we did not find a significantly higher number of heterozygous carriers among SLE patients. In concurrence with previous reports, ficolin-3 concentrations in the heterozygous patients were on average half of the expected normal concentration [6, 12].

In patient-X, we were not able to detect a genetic explanation for the observed deficiency. We did find a heterozygous mutation upstream of exon1. It is, however, unlikely to be the explanation of the deficiency in patient-X, since the mutation was heterozygous. Autoantibodies against ficolin-3 could be an explanation for a pseudo deficient state. This phenomenon is known from complement C1q, where autoantibodies against the protein are strongly associated with protein levels and disease activity [27, 28]. Autoantibodies against ficolin-3 were recently reported in as much as 37% of the SLE population investigated [18]. We did, however, not observe higher levels in patient-X, and although only a small number of patients and controls were examined in the present study (SLE $n = 16$, controls $n = 16$), we did not (on average) see any difference in antibodies against ficolin-3 between SLE patients and controls, nor did we see a correlation between ficolin-3 levels and antibodies against ficolin-3. The deficiency could be caused by epigenetic regulation. Epigenetic regulation of the *FCN3 gene* has been described in relation to high ficolin-3 levels in leprosy [29]. We did, however, not have liver tissue available from patient-X, which would be necessary to investigate this hypothesis further.

In the original studies on antibodies against ficolin-3 by Inaba et al, 283 SLE patients and 398 patients with other autoimmune diseases were examined [1]. In the SLE cohort, 15 patients with unmeasurable amounts of ficolin-3 were identified, 3 of who were also positive for antibodies against ficolin-3. In the group of other autoimmune diseases, two patients were identified as ficolin-3 deficient without detectable antibodies against ficolin-3, one with chronic glomerulonephritis and one with primary biliary cirrhosis. The study was carried out prior to the purification of ficolin-3, and the authors proposed the deficient state to be caused by autoantibodies against ficolin-3 although they did not detect antibodies against ficolin-3 in most of the patients.

Based on the DNA deposits gathered at the Broad Institute [13], 26 people homozygous for the *FCN3 del1637* mutation have been detected resulting in an allele frequency of 0.017. Clinical data on these individuals are not available.

The question still remains, whether ficolin-3 deficiency is the cause of disease or acts as a disease modifier. In relation to infections, it has been proposed that the crucial role of ficolin-3 may be to control normal (commensal) flora that can cause opportunistic infections rather than to protect from obligatory pathogens [14]. With regard to autoimmunity, it has been demonstrated, that ficolin-3 binds to apoptotic cells and activates the complement system [3, 30]. It is well established, that clearance defects are strongly associated with autoimmune diseases [20], and it is striking to see that among the diseased adults with ficolin-3 deficiency SLE, glomerulonephritis, and lung fibrosis are the clinical presentations.

We speculate that ficolin-3 deficiency, like C1q deficiency, could increase the risk of serious infections especially in early childhood; whereas, the deficient state in adulthood is a potential risk factor for autoimmune diseases like SLE, in which clearance defects seem to play an important role.

**Acknowledgements** We thank the Swiss Systemic Lupus Erythematosus Cohort Study (SSCS) for collaboration in the project. We are grateful to all patients participating in the project.

**Authors Contribution** AT, ST, and MT designed the study. AT performed the literature search. AT, AH, and KG performed the laboratory experiments. RS performed the genetic analysis. AT was in charge of collecting blood samples and handling the blood samples after they were drawn; AT, AV, and KS handled patient inclusion and clinical assessments. ST developed the assays used in the project and supervised laboratory procedures. AT, ST, and KS wrote the manuscript, and all authors participated in the editing of the article.

**Funding Information** The authors would like to acknowledge the Danish Rheumatism association, Aase and Ejnar Danielsens fond, Lundbeck Foundation, and the Danish National Research Foundation for financial support.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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