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The clinical significance of plasma CFHR 1–5 in lupus nephropathy

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ABSTRACT

A deficiency of complement factor H may lead to excessive consumption of C3 and an increase in C3b deposition, which are important pathological characteristics of lupus nephritis. Complement factor H-related proteins (CFHRs), comprising CFHR1 to CFHR5 (CFHR1–5), are members of the wider factor H/CFHR family. Their role in lupus nephritis remains unclear. In this study, we compared circulating levels of CFHR1–5 in 152 patients diagnosed with lupus nephritis and 20 unrelated healthy individuals to explore the relationship between the expression of CFHR1–5 and development of the disease. We found that plasma levels of CFHR3 and CFHR5 were higher in patients with lupus nephritis than in healthy individuals; also, CFHR3 and CFHR5 concentrations increased with increasing systemic lupus erythematosus disease activity index (SLEDAI) values ($P < 0.05$). Pearson's and Spearman's correlation test results confirmed that plasma CFHR3 and CFHR5 levels in lupus nephritis patients were positively correlated with proteinuria and levels of creatinine (Cr) and anti-dsDNA (correlation coefficients = 0.491–0.717, $P < 0.05$), while they were negatively correlated with plasma C3 levels and eGFR [correlation coefficients = –(0.706–0.788), $P < 0.05$]. Receiver operating characteristic (ROC) curve analysis results confirmed that plasma CFHR3 and CFHR5 levels were predictive of SLEDAI values and disease end points (area under the curve = 0.664–0.884, $P < 0.05$), with patients with both high CFHR3 and high CFHR5 exhibiting the shortest progression-free survival. Thus, both CFHR3 and CFHR5 are of prognostic value in lupus nephritis status.

1. Introduction

Lupus nephritis is among the most common complications of systemic lupus erythematosus (SLE), which is characterized by immunocomplex deposition on the glomerular basement membranes (Bomback, 2018). The pathways that mediate the complement system, including recognition events characterized by the deposition of C3b (alternative pathway), the binding of antibodies or surfactant proteins (classical pathway), and mannose-binding lectins (lectin pathway) converge on the generation of a C3 convertase, which cleaves the central complement component C3 into the activation product C3b (Skerka et al., 2013). Complement factor H has been established as a key factor that regulates the deposition of C3b by inhibiting C3 convertase generation, thus suppressing the alternative pathway (Cabezas et al., 2018; Geerlings et al., 2018). In lupus nephritis, it has been reported that complement factor H deficiency leads to excessive consumption of C3 and an increase in C3b deposition (Bao et al., 2015), which may contribute to renal injury.

Complement factor H-related proteins (CFHRs), comprising CFHR1, CFHR2, CFHR3, CFHR4, and CFHR5 (CFHR1–5) are members of the wider factor H/CFHR family. Genes encoding the members of this family are located on a distinct segment on human chromosome 1q32 within the regulation of complement activation (RCA) cluster (Togarsimalemath et al., 2017). The C-terminal regions of the CFHR proteins exhibit a high degree of sequence identity with complement factor H. Functionally, CFHR1–5 and complement factor H co-participate in the regulation of the classical pathway, with both complementary and antagonistic interactions possible between each pair of

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proteins (Jiang et al., 2016). Previous research has confirmed that mutations, genetic deletions, duplications, or rearrangements in the individual CFHR genes are associated with numerous glomerular lesions, including C3 glomerulonephritis, IgA nephropathy, and SLE (Tan and Zhao, 2018, Zhu et al., 2018), but whether CFHR1–5 levels are related to the occurrence and development of lupus nephritis remains unclear. The aim of this study was to elucidate the contributions of CFHR1–5 to lupus nephritis by measuring circulating levels of CFHR1–5 in lupus nephritis patients during planned follow-up visits and to analyze their relationship with patient clinical characteristics and prognosis.

2. Material and methods

2.1. Study population

A total of 152 patients with lupus nephritis registered by the Hunan Provincial People's Hospital and The Affiliated Zhu Zhou Hospital Xiang-ya Medical College from July 2012 to November 2014 for whom a sufficient volume of plasma sample was available were selected for this study. All patients had been diagnosed with the disease in accordance with the American College of Rheumatology Guidelines (Hahn et al., 2012) and followed-up for at least 2 years. Patients were divided into one of three groups on the basis of their SLE disease activity index (SLEDAI) test result (Bombardier et al., 1992): Group 1: SLEDAI ≤ 9, 95 patients; Group 2: 9 < SLEDAI ≤ 14, 35 patients; Group 3: SLEDAI ≥ 15, 22 patients. In addition, 20 healthy volunteers (male:female ratio = 9:1) were recruited as controls. Patients with Henoch–Schonlein purpura, rheumatic disease, liver cirrhosis, diabetes, or renal damage caused by other diseases were excluded after detailed clinical and laboratory examination. The demographic data of the patients and healthy volunteers are shown in Table 1.

2.2. Clinical data

Clinical parameters were collected from patient medical records, including age; sex; serum creatinine (Cr); plasma C3, C4, IgA, IgM, and IgG levels; 24-h urine protein excretion (to detect proteinuria); neutrophil count (N); lymphocyte count (L); estimated glomerular filtration rate (eGFR); and levels of anti-double-stranded DNA (anti-dsDNA) and antinuclear antibodies (ANA). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Kong et al., 2013), and both anti-dsDNA and ANA were determined by antibody titer. Activity index (AI) and chronicity index (CI) values were evaluated in accordance with the measures of disease activity reported for lupus nephritis by Balow and Austin (1988). Seventy-six patients had renal biopsies, the slides of which were stained with hematoxylin and eosin (HE), periodic acid–Schiff (PAS), Masson's stain, and immunofluorescence for IgG, and the pathological type and scores of which were reviewed and graded by an independent pathologist who was blinded to all other clinical data. Patient renal biopsies were categorized into five types in accordance with current International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification (Seo et al., 2010), with 23 categorized as class III, 30 as class IV, 7 as class V, 7 as class III/V, and 9 as class IV/V.

2.3. Treatment and follow-up

All patients received a therapy regimen defined by the Kidney Disease Improving Global Outcomes (KDIGO) foundation consistent with the individual condition of each patient, with metacortandracin or methylprednisolone used as the principal drugs, combined with immunosuppressive agents for patients with persistent proteinuria. All patients had a follow-up consultation after 2 years, with the end point defined as a decline in eGFR of 30%, end-stage renal disease (ESRD), or death, whichever occurred first. This research was conducted in compliance with the principles of the Declaration of Helsinki and approved by the ethics committee of the Hunan Provincial People's Hospital and

Table 1
Demographic data of patients and healthy volunteers.

Data	Control(n = 20)	Group 1(n = 95)	Group 2(n = 35)	Group 3(n = 22)	P value
Age (yr)	37 (25.49)	38 (26.52)	37 (28.53)	39 (28.52)	0.729[b]
Sex (female)	2 (10%)	11 (11.6%)	4 (11.4%)	3 (13.6%)	0.987[a]
Education [n (%)]					0.122[b]
Graduate from primary school	3 (15.0%)	11 (11.6%)	7 (20.0%)	5 (22.7%)	
Graduate from Middle school	9 (45.0%)	47 (49.5%)	14 (40.0%)	8 (36.4%)	
Undergraduate	8 (40.0%)	32 (33.7%)	11 (31.4%)	5 (22.7)	
Postgraduate	0	5 (5.3%)	3 (85.7%)	4 (18.2%)	
Occupation [n (%)]					0.190[a]
Farmer	4 (20.0%)	11 (11.6%)	7 (2.0%)	5 (22.7%)	
Worker	7 (35.0%)	32 (33.7%)	17 (48.6%)	12 (54.5%)	
Office clerk	7 (35.0%)	42 (44.2%)	10 (28.6%)	5 (22.7%)	
Others	2 (10.0%)	10 (10.5%)	1 (2.9%)	0	
Family history [n(%)]	0	6 (6.3%)	2 (5.7%)	3 (13.6%)	0.345[a]
Nation [n (%)]					0.242[a]
Ethnic Han	20 (100.0%)	91 (95.8%)	35 (100.0%)	20 (90.9%)	
Ethnic minorities	0	4 (4.2%)	0	2 (9.1%)	
Residences [n(%)]					0.730[a]
Village	6 (30.0%)	26 (27.4%)	11 (31.4%)	4 (18.2%)	
Cities and towns	14 (70.0%)	69 (72.6%)	24 (68.6%)	18 (81.8%)	
Comorbidity [n(%)]					
Hypertension	0	7 (7.4%)	2 (5.7%)	1 (4.5%)	0.633[a]
Infectious diseases	0	10 (10.5%)	5 (14.3%)	5 (22.7%)	0.126[a]

Group 1: SLEDAI ≤ 9; Group 2: SLEDAI = 9 < SLEDAI ≤ 14; group 3; Group 3: SLEDAI ≥ 15.
SLEDAI: systemic lupus erythematosus disease activity index.
Family history: SLE had been diagnosed in third degree relatives of the patients.
[a] Indicates Chi-squared test.
[b] Indicates Kruskal–Wallis test.

Table 2
Clinical examination data of patients and healthy volunteers.

Data	Control (n = 20)	Group 1 (n = 95)	Group 2 (n = 35)	Group 3 (n = 22)	P_1 value	P_2 value	P_3 value
Age (yr)	37 (25.49)	38 (26.52)	37 (28.53)	39 (28.52)	0.884[c]	0.805[c]	0.663[c]
Sex (male)	2 (10%)	11 (11.6%)	4 (11.4%)	3 (13.6%)	0.967[a]	0.775[a]	0.867[a]
Plasma C3 (g/L)	1.02 ± 0.24	0.68 ± 0.12	0.52 ± 0.13	0.46 ± 0.09	< 0.001[b]	< 0.001[b]	< 0.001[b]
Plasma C4 (g/L)	0.51 ± 0.14	0.48 ± 0.09	0.46 ± 0.14	0.44 ± 0.13	0.066[b]	0.114[b]	0.105[b]
Plasma Ig A (g/L)	3.82 (3.12, 5.63)	3.52 (3.03, 5.45)	3.72 (3.14, 5.55)	3.84 (3.27, 5.15)	0.624[c]	0.553[c]	0.633[c]
Plasma Ig M (g/L)	1.62 (1.33, 2.22)	1.71 (1.19, 2.54)	1.64 (1.26, 2.37)	1.57 (1.38, 2.42)	0.252[c]	0.196[c]	0.337[c]
Plasma Ig G (g/L)	12.2 (5.1, 13.2)	13.5 (6.6, 14.5)	13.1 (6.0, 15.0)	12.5 (6.0, 14.7)	0.224[c]	0.335[c]	0.106[c]
N (10^9/L)	2.14 (1.51, 3.88)	3.85 (1.42, 8.32)	5.02 (2.88, 11.49)	5.41 (3.05, 13.26)	< 0.001[c]	< 0.001[c]	< 0.001[c]
L (10^9/L)	1.54 (1.05, 3.22)	1.28 (1.02, 2.64)	1.25 (0.83, 1.85)	1.05 (0.55, 1.46)	0.007[c]	0.003[c]	< 0.001[c]
NLR	1.52 ± 0.48	3.11 ± 0.81	4.22 ± 1.02	6.01 ± 1.46	< 0.001[b]	< 0.001[b]	< 0.001[b]
Cr (μmol/L)	67.5 ± 13.2	98.6 ± 13.2	132.5 ± 18.2	164.5 ± 22.3	< 0.001[b]	< 0.001[b]	< 0.001[b]
Proteinuria (g/d)	0.13 (0.08, 0.15)	1.26 (0.61, 2.24)	1.33 (0.66, 2.33)	1.51 (1.11, 2.84)	< 0.001[c]	< 0.001[c]	< 0.001[c]
eGFR (ml/min/1.73 m^2)	115.2 ± 18.3	82.3 ± 12.3	77.2 ± 8.6	73.3 ± 7.6	< 0.001[b]	< 0.001[b]	< 0.001[b]
Anti-dsDNA positive	0	48 (50.2%)	25 (71.4%)	17 (77.3%)	–	0.033[a]	0.626[a]
1:10		21	5	3	–	0.032[c]	0.029[c]
1:100		20	13	3			
1:320		7	6	5			
1:1000		0	1	6			
ANA positive	0	87 (91.6%)	31 (88.6%)	19 (86.4%)	–	0.599[a]	0.805[a]
1:100		26	7	3	–	0.620[c]	0.481[c]
1:320		21	7	5			
1:1000		19	11	5			
1:3200		21	6	6			

Group 1: SLEDAI ≤ 9; Group 2: 9 < SLEDAI ≤ 14; Group 3: SLEDAI ≥ 15.
SLEDAI: systemic lupus erythematosus disease activity index; N: neutrophil count; L: lymphocyte count; NLR: ratio of neutrophil count to lymphocyte count; Cr: serum creatinine; eGFR: estimated glomerular filtration rate; anti-dsDNA: anti-double stranded DNA antibodies; ANA: antinuclear antibodies.
P_1 value indicated the difference between the control and group 1; P_2 value indicated the difference between groups 1 and 2; P_3 value indicated the difference between groups 2 and 3.
[a] Indicates Chi-squared test.
[b] Indicates LSD-*t* Test.
[c] Indicates Mann–Whitney *U* Test.

The Affiliated Zhu Zhou Hospital Xiang-ya Medical College. Written informed consent was provided by all enrolled individuals.

2.4. Quantification of plasma levels of CFHR1–5

The plasma levels of complement factor H in all patients and healthy volunteers were quantified by a DAS-ELISA kit (RAB1362-1KT) purchased from Sigma-Aldrich (St. Louis, MO, USA), while CFHR1–5 levels were quantified using DAS-ELISA kits from Shanghai Meilian Biotechnology Co. Ltd (Shanghai, China). For all ELISAs, the main steps were as follows: (1) All reagents and plasma samples were warmed to 25 °C prior to testing. All standards and samples were tested at least in duplicate. (2) A 100-μL aliquot of each standard or sample was placed into the appropriate wells of 96-well plates, which were then covered and incubated overnight at 4 °C with gentle shaking. (3) Each plate was emptied and washed four times with wash buffer, which was discarded after each rinse. The plates were inverted and blotted against a clean paper towel following the final wash. (4) Then, 100 μL of biotinylated detection antibody was added and incubated for 1 h at room temperature with gentle shaking. (4) After discarding the contents, each plate was washed as described above, and then 100 μL of HRP-streptavidin solution was added to each well and incubated for 45 min at room temperature with gentle shaking. (5) Each plate was again inverted and washed prior to the addition of 100 μL of ELISA Colorimetric TMB Reagent to each well. The plates were incubated for 30 min at room temperature in the dark with gentle shaking. (6) Stop solution was added (50 μL), and the absorbance of each well was measured using a microplate reader (SimpliAmp, Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm.

2.5. Statistical analysis

Statistical analyses were performed using SPSS software (version 22.0). All normally distributed quantitative variables are expressed as means ± SD, while the remainder are expressed as median (IQR) values. ANOVA, LSD-t, and Student–Newman–Keuls (SNK) tests were performed to compare differences among groups for normally distributed variables, with Mann–Whitney *U* and Kruskal–Wallis tests conducted for variables that were not normally distributed. Categorical data are expressed as frequencies and percentages, and chi-square tests were performed to compare differences between groups where data were dichotomous. A Kruskal–Wallis *H* test was performed for comparisons between groups with ranked data. In addition, Pearson's correlation and Spearman's correlation tests were used to correlate plasma CFHR3 and CFHR5 levels with other clinical data for each patient. ROC curve analysis was used to ascertain the predicted plasma concentrations of CFHR3 and CFHR5 for the manifestation of lupus nephropathy activity and poor prognosis. Kaplan–Meier and log-rank tests were used to compare the cumulative incidence of the end points in patients with different levels of CFHR3 and CFHR5. Logistic regression was used to ascertain whether CFHR3 and CFHR5 independently influence prognosis. Differences with a two-tailed P value less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics of patients with lupus nephritis

A comparison of clinical data (Table 2) demonstrated that significant differences in the values of plasma C3, N, L, the ratio of neutrophil to lymphocyte count (NLR), Cr, protein excretion, eGFR, and anti-dsDNA existed between patients and controls ($P < 0.05$). Compared with control subjects, L, eGFR, and plasma C3 levels in patients with lupus nephritis were lower. The values of these parameters decreased with increasing SLEDAI (groups 1–3). The values of N, NLR, Cr, protein excretion, and anti-dsDNA in patients with lupus nephritis were higher than those in controls and increased with increasing SLEDAI values (groups 1–3). Therefore, plasma C3, eGFR, NLR, Cr, protein

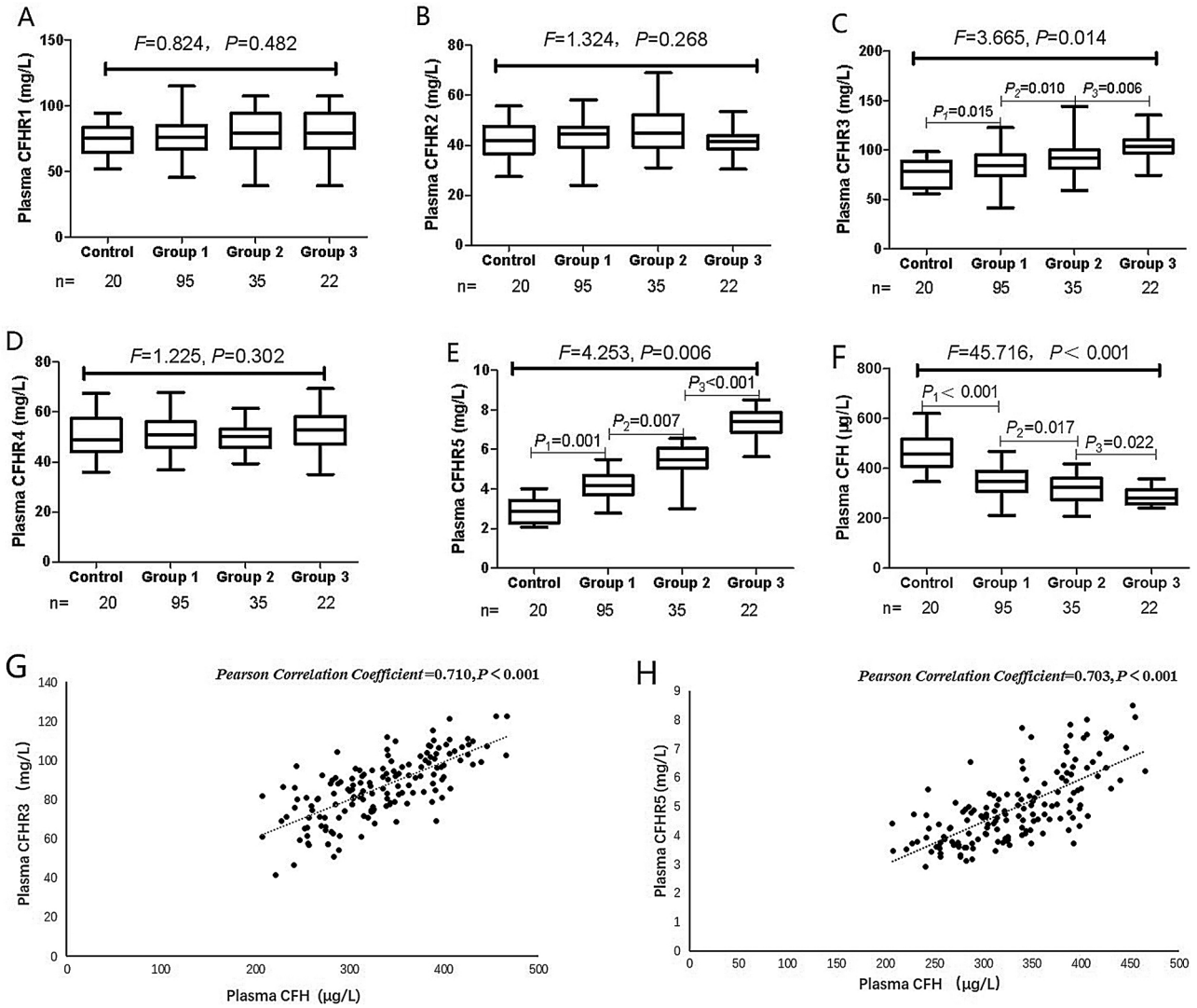

Fig. 1. Plasma levels of CFHR1–5 in patients and controls. (A) CHFR1; (B) CHFR2; (C) CHFR3; (D) CHFR4; (E) CHFR5; (F) CFH; (G) correlation between CFHR3 and CFH; (H) correlation between CFHR5 and CFH. Control: healthy volunteers; Group 1: SLEDAI ≤ 9; Group 2: 9 < SLEDAI ≤ 14; Group 3: SLEDAI ≥ 15.

excretion, and anti-dsDNA were parameters that were chosen for further analysis.

3.2. Plasma CFHR3 and CFHR5 levels are elevated in lupus nephritis and are associated with patient clinical characteristics

As demonstrated in Fig. 1, no significant differences in plasma levels of CFHR1, CFHR2, or CFHR4 were observed between patients and controls ($P > 0.05$). In contrast, plasma CFHR3 and CFHR5 levels in patients with lupus nephritis were higher than those in controls and were elevated in patients with higher SLEDAI values (groups 1 to 3), with significant differences between each group ($P < 0.05$). Plasma CFH levels in patients with lupus nephritis were lower than those in controls and decreased with increasing SLEDAI, with significant differences between each group ($P < 0.05$). Furthermore, there was a positive correlation between plasma CFHR3 and CFH levels and between CFHR5 and CFH levels (Pearson's correlation coefficients = 0.710, 0.703, respectively; $P < 0.05$). Moreover, as shown in Fig. 2A–D, plasma CFHR3 levels were positively correlated with Cr (Pearson's correlation coefficient = 0.685; $P < 0.05$) and negatively correlated with plasma C3 levels and eGFR (Pearson's correlation coefficients = −0.742, −0.698, respectively; $P < 0.05$). Plasma CFHR3 levels were also positively correlated with protein excretion, anti-dsDNA, AI, and CI (Spearman's correlation coefficients = 0.621, 0.585, 0.352, 0.700, respectively; $P < 0.05$; Fig. 2E–H). As shown in Fig. 3A–D, plasma CFHR5 levels were positively correlated with Cr (Pearson's correlation coefficient = 0.717; $P < 0.05$) and negatively correlated with plasma C3 levels and eGFR (Pearson's correlation coefficients = −0.753, −0.745, respectively; $P < 0.05$). Plasma CFHR3 levels also positively correlated with proteinuria, anti-dsDNA, AI, and CI (Spearman's correlation coefficients = 0.551, 0.491, 0.351, 0.669, respectively; $P < 0.05$; Fig. 3E–H).

3.3. Plasma CFHR3 and CFHR5 levels are associated with pathological classification as lupus nephritis

In all, 76 patients had renal biopsies, the slides of which were stained with hematoxylin and eosin (HE), periodic acid–Schiff (PAS), Masson's stain, and immunofluorescence for IgG. In a typical pathological report (Fig. 4), HE staining (Fig. 4A) indicated glomerulosclerosis, PAS staining (Fig. 4B) suggested the formation of fibrous crescents, Masson's staining indicated the formation of small cell crescents (Fig. 4C), and immunofluorescence staining (Fig. 4D) demonstrated IgG deposition in the glomerular capillary. Plasma levels of CFHR3 and CFHR5 in class IV, V, and IV/V patients were consistently higher than those in class III ($P < 0.05$; Fig. 4E–F).

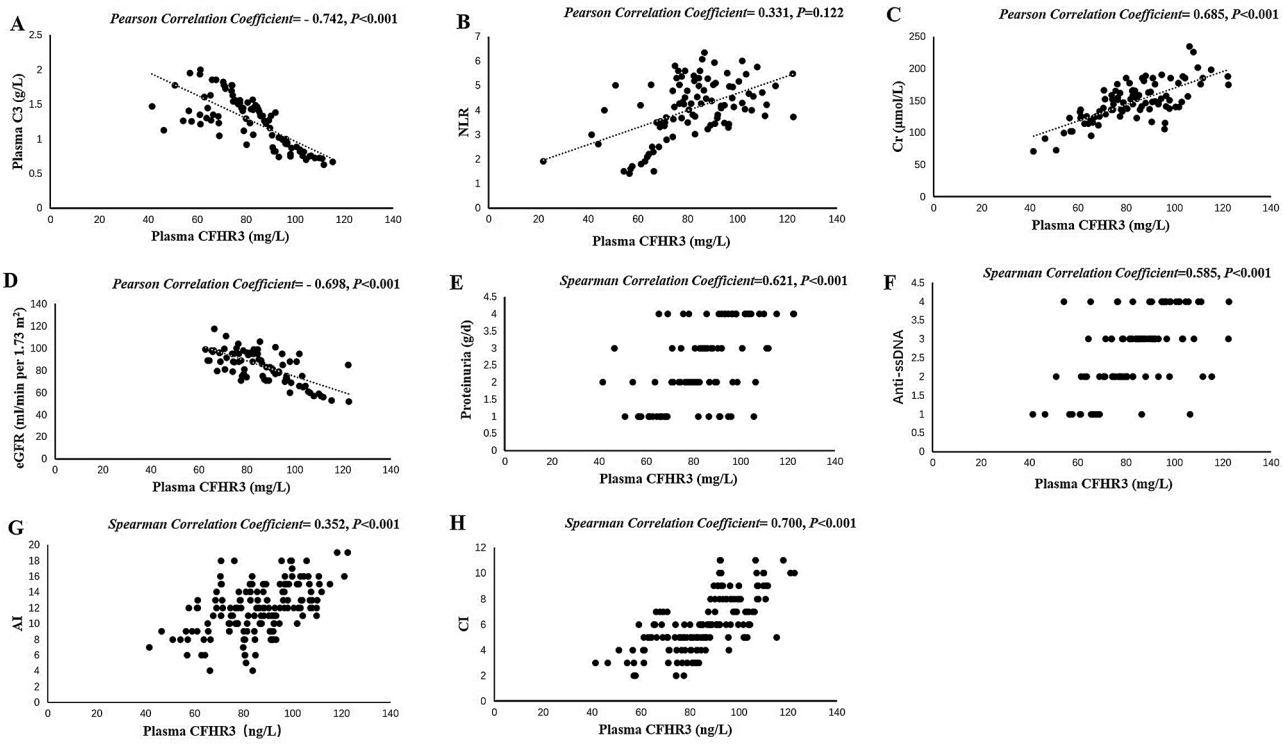

Fig. 2. Correlation between CFHR3 and patient characteristics. Correlations between (A) CFHR3 and C3, (B) CFHR3 and NLR, (C) CFHR3 and Cr, (D) CFHR3 and eGFR, (E) CFHR3 and protein excretion, (F) CFHR3 and anti-dsDNA antibodies, (G) CFHR3 and AI, and (H) CFHR3 and CI.

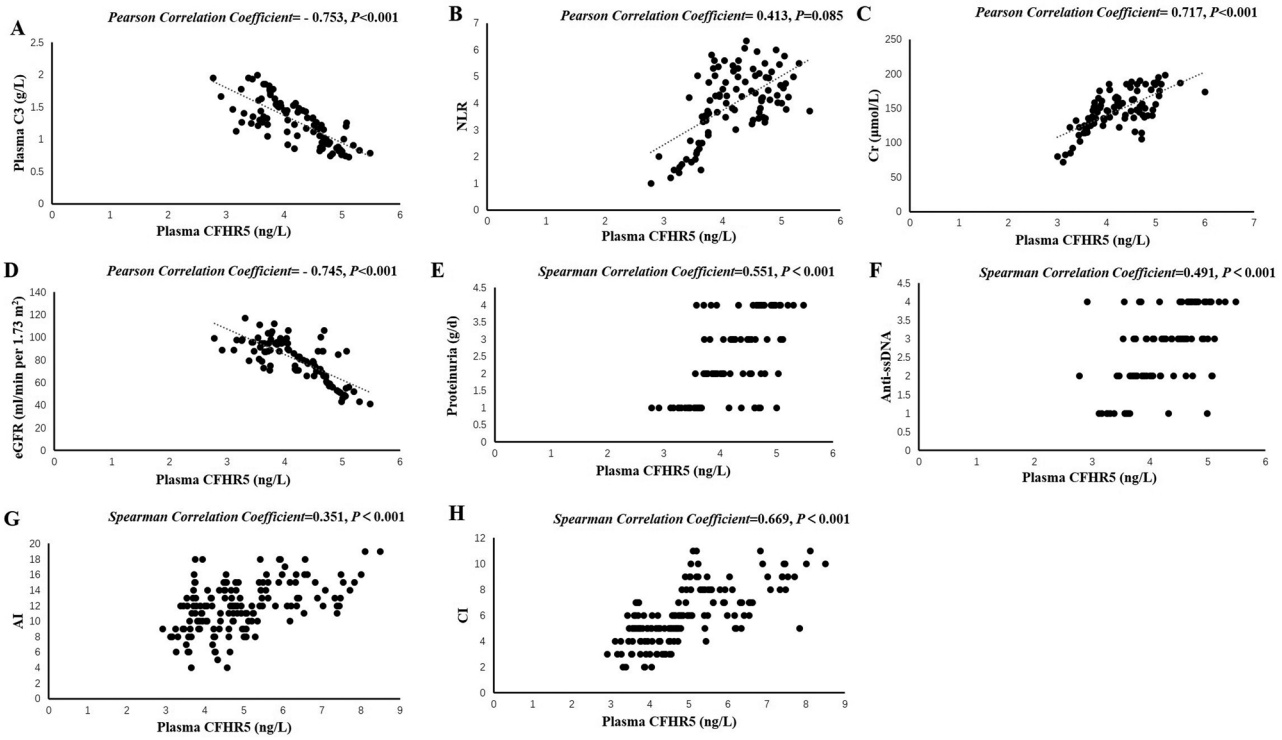

Fig. 3. Correlation between CFHR5 and patient characteristics. Correlations between (A) CFHR5 and C3, (B) CFHR5 and NLR, (C) CFHR5 and Cr, (D) CFHR5 and eGFR, (E) CFHR5 and protein excretion, (F) CFHR5 and anti-dsDNA antibodies, (G) CFHR5 and AI, (H) CFHR5 and CI.

3.4. Plasma CFHR3 and CFHR5 levels are predictive of lupus erythematosus disease activity and disease end point

Based on the results presented above, we speculated that plasma CFHR3 and CFHR5 levels might have value in the prognosis of lupus nephritis. ROC curve analysis confirmed this hypothesis (Fig. 5). The results demonstrated that both plasma CFHR3 and plasma CFHR5 levels predicted SLEDAI and disease end point. The optimal operating point (OOP) for plasma CFHR3 for predicting SLEDAI ≥ 15 was a concentration of 92.3 mg/L, with a sensitivity of 77.3% and a specificity of

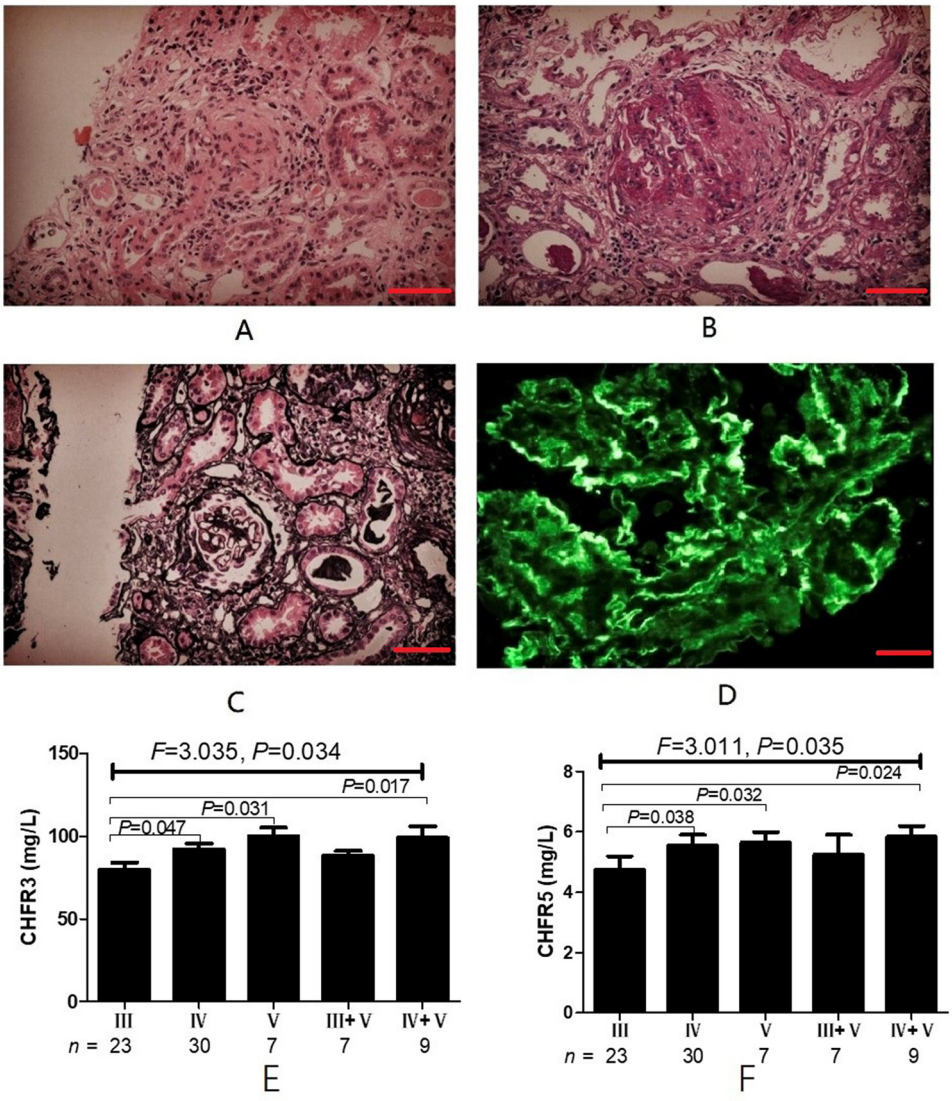

Fig. 4. Renal biopsy results and plasma CHFR3 and CHFR5 levels in patients from different classes. (A) HE staining demonstrated glomerulosclerosis (scale bar = 100 μm). (B) PAS staining indicated the formation of fibrous crescents (scale bar = 50 μm). (C) Masson's staining indicated the formation of small cell crescents (scale bar = 100 μm). (D) Immunofluorescence demonstrated IgG deposition in the glomerular capillary (scale bar = 25 μm). (E) Plasma CFHR3 levels in patients of different pathological classifications. (F) Plasma CFHR5 levels in patients of different pathological classifications.

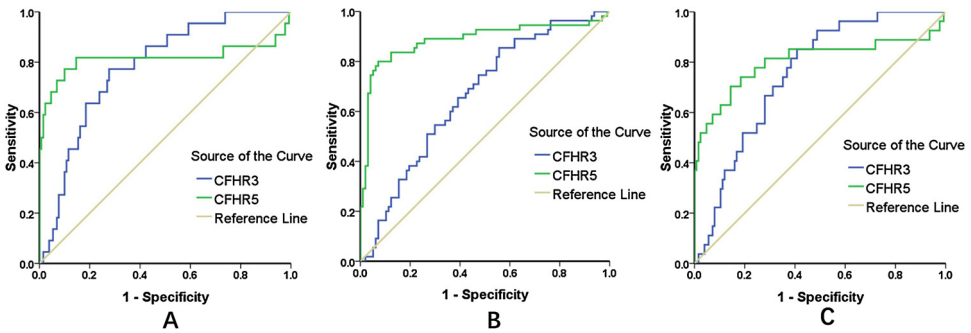

Fig. 5. ROC curve analysis results for plasma CFHR3 and CFHR5.

72.3% (AUC = 0.779, 95% CI: 0.687–0.871); to predict SLEDAI ≥ 9, the OOP was 91.3 mg/L, with a sensitivity of 54.5% and a specificity of 70.1% (AUC = 0.664, 95% CI: 0.577–0.751). A plasma CFHR3 level of 86.7 mg/L predicted disease end point with a sensitivity of 85.2% and a specificity of 60.0% (AUC = 0.753, 95% CI: 0.668–0.838). For CFHR5, the OOP to predict SLEDAI ≥ 15 was 5.60 mg/L, with a sensitivity of 81.8% and a specificity of 85.4% (AUC = 0.815, 95% CI: 0.670–0.961), and to predict SLEDAI ≥ 9, it was 5.20 mg/L, with a sensitivity of 80.0% and a specificity of 92.8% (AUC = 0.884, 95% CI: 0.818–0.952). The OOP of plasma CFHR5 for the prediction of disease end point was 4.82 mg/L, with a sensitivity of 85.2% and a specificity of 63.3% (AUC = 0.804, 95% CI: 0.684–0.925). In order to explore the

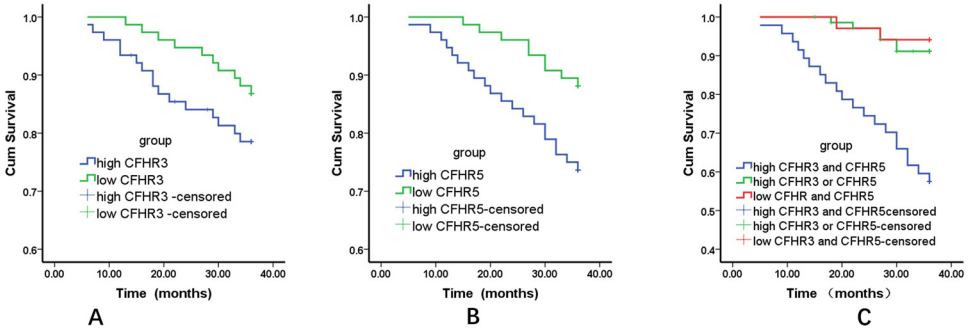

Fig. 6. Survival curves of patients with different levels of CFHR3 and CFHR5. (A) Cumulative survival curves of patients with low and high plasma CFHR3. Log-rank test, $\chi^2 = 3.416$, $P = 0.065$. (B) Cumulative survival curves of patients with high and low plasma CFHR5. Log-rank test, $\chi^2 = 5.484$, $P = 0.019$. (C) Cumulative survival curves of patients with various levels of CFHR3 and CFHR5. Log-rank test, $\chi^2 = 28.242$, $P < 0.001$.

Table 3
ROC curve analysis results for the predictive value of CFHR3 and CFHR5.

Indexes	Area	Std. Error[a]	*p*	Asymptotic 95% Confidence Interval	OOP (mg/L)	Sensitivity (%)	Specificity (%)
SLEDAI ≥ 15							
CFHR3	0.779	0.047	< 0.001	0.687–0.871	92.3	77.3	72.3
CFHR5	0.815	0.074	< 0.001	0.670–0.961	5.60	81.8	85.4
SLEDAI ≥ 9							
CFHR3	0.664	0.044	0.001	0.577–0.751	91.3	54.5	70.1
CFHR5	0.884	0.034	< 0.001	0.818–0.952	5.20	80.0	92.8
The end point							
CFHR3	0.753	0.043	< 0.001	0.668–0.838	86.7	85.2	60.0
CFHR5	0.804	0.062	< 0.001	0.684–0.925	4.82	85.2	63.3

CFHR3: complement factor H-related protein 3; CFHR5: complement factor H-related protein 5; Definition of end point: 30% decline in eGFR, ESRD or death, whichever occurred first; OOP: optimal operating point.

association of plasma CFHR3 and CFHR5 with lupus erythematosus, we divided patients into two equal groups based on the median values of CFHR3 and CFHR5. Kaplan–Meier and log-rank tests (Fig. 6) clearly showed that patients with both high CFHR3 and high CFHR5 levels exhibited the shortest progression-free survival ($P < 0.05$) (Table 3).

4. Discussion

Previous studies have reported that lupus nephritis is the current leading cause of morbidity and mortality in SLE, developing in 50% to 75% of Asian SLE patients (Shaltout et al., 2016). Dysfunction of complement factor H has been observed in a umber of active lupus nephritis patients and is associated with clinical phenotype and certain clinical features, in addition to prognosis (Tan et al., 2017; Wang et al., 2016). Within the factor H/CFHR family, CFHR1–5 have a similar structure and exhibit functions closely related to those of complement factor H (Cantsilieris et al., 2018). These observations attracted our attention to the role of CFHR1–5 in lupus nephritis. Here, we measured circulating CFHR1–5 levels in 152 patients with lupus nephritis and 20 healthy volunteers and found that both CFHR3 and CFHR5 levels were elevated in lupus nephritis. A number of recent studies have suggested that CFHR1, CFHR3, and CFHR5 may compete with complement factor H for binding to C3b at physiological concentrations, thereby antagonizing its activity (Ding et al., 2017; Munch et al., 2017; Zhai et al., 2016). Thus, we speculated that circulating CFHR3 and CFHR5 may be important participants in the pathological mechanisms of lupus nephropathy development.

Following an analysis of clinical data, we found that plasma C3, NLR, Cr, protein excretion, eGFR, and anti-dsDNA values changed significantly in patients in whom lupus nephritis was detected and progressing, so we first analyzed the relationship between circulating CFHR3 and CFHR5 levels and these parameters. Pearson's and Spearman's correlation tests confirmed that plasma CFHR3 and CFHR5 levels in patients with lupus nephritis were positively correlated with Cr, protein excretion, and anti-dsDNA and negatively correlated with plasma C3 levels and eGFR. Cr and protein excretion (proteinuria) are both classical renal damage markers, with increased values due principally to damage to the glomerular filtration function (Ishii et al., 2015; Shaharir et al., 2015). The correlation between a decrease in serum complement components and an increase in SLE disease activity has been confirmed in many studies, with low levels of serum complement components C4 and C3, especially C3, serving clinically as reliable markers of active disease (Chimenti et al., 2014; Kim et al., 2018; Troldborg et al., 2018). ANA and anti-dsDNA are important autoantibodies that are elevated in SLE, and clinicians use them as a tool for assessing disease activity (Bhattacharya et al., 2018). Previous studies have reported that approximately 90% of SLE patients are positive for ANA, while 50–70% are positive for anti-dsDNA (Olson et al., 2013). Past studies have suggested that SLE patients with the anti-dsDNA-positive subtype exhibit increased risk for a more aggressive form of disease, particularly those with lupus nephritis (Barbhaiya et al., 2018; Zigon et al., 2011). Our results demonstrate that both plasma CFHR3 and plasma CFHR5 levels are positively correlated with the anti-dsDNA positive rate. Since a decrease in C3 changes the value of SLEDAI and since CFHR3 and CFHR5 are independently associated with decreased C3 levels, we also evaluated AI and CI, which are not influenced by C3 levels; the results showed that both CFHR3 and CFHR5 were positively correlated with AI and CI. Together, these results suggest that CFHR3 and CFHR5 may be closely related to the disease activity of lupus nephritis.

In total, 76 patients had renal biopsies. We found that plasma CFHR3 and CFHR5 levels in class IV, V, and IV/V patients were higher in all cases than those in class III patients. According to the ISN/RPS classification, class IV and V patients exhibit relatively serious symptoms, and studies have revealed that being class IV or V serves as a significant risk factor for renal outcome (Sada and Makino, 2009). Our study demonstrated that class IV and V patients had higher CFHR3 and CFHR5 levels, suggesting that CFHR3 and CFHR5 may be potential biomarkers for predicting the prognosis of lupus nephritis. Using ROC curve analysis, we found that both plasma CFHR3 and plasma CFHR5 predicted SLEDAI and disease end point. The OOPs for predicting SLEDAI ≥ 15, SLEDAI ≥ 9, and disease end point were 92.3 mg/L,

91.3 mg/L, and 86.7 mg/L for plasma CFHR3, respectively, and 5.60 mg/L, 5.20 mg/L, and 4.82 mg/L for plasma CFHR5, respectively. Moreover, our results demonstrated that patients with both high CFHR3 and high CFHR5 levels exhibited the shortest progression-free survival.

In conclusion, CFHR3 and CFHR5 may participate in the pathological mechanism of lupus nephritis, and plasma levels of these proteins appear to have prognostic value in determining disease status.

Conflict of interest

Each author approved the final version of this manuscript. They report no conflict of interest.

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