

Identification of Fatty Acid-Binding Protein 4 as a Potential Biomarker and Therapeutic Target for Antineutrophil Cytoplasmic Antibody-Associated Glomerulonephritis

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Keywords

Antineutrophil cytoplasmic antibody-associated glomerulonephritis · Acute kidney injury · Fatty acid-binding protein 4 · Renal function · Urinary biomarker

Abstract

Introduction: Fatty acid-binding protein 4 (FABP4) is a novel adipokine that is critically involved in many inflammatory and immune diseases. However, the role of FABP4 in antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (ANCA-GN) remains unclear. The current study aimed to investigate the role of FABP4 in patients with ANCA-GN. **Methods:** Plasma and urine samples from 37 patients with active ANCA-GN and kidney biopsy specimens from another group of 56 patients with ANCA-GN were collected. The plasma and urinary levels of FABP4 were measured by enzyme-linked immunosorbent assay and the kidney FABP4 expression was determined by immunohistochemistry and immunofluorescence staining. Associations between FABP4 levels with clinical and pathologic parameters were analyzed. To further elucidate the role of FABP4 in ANCA-GN, a novel FABP4 inhibitor, BMS309403, was employed in a recognized rat model of experimental autoimmune vasculitis (EAV). **Results:** Plasma and urinary levels of

FABP4 in active ANCA-GN patients were significantly higher than those in normal controls (52.8 ± 23.6 ng/mL vs. 16.9 ± 8.8 ng/mL, $p < 0.01$; median 126.6 (interquartile range [IQR] 28.4–311.2) ng/g Cr vs. median 0.0 (IQR 0.0–0.0) ng/g Cr, $p < 0.01$, respectively). Immunohistochemical analysis revealed higher glomerular and tubular expression of FABP4 in the kidneys of ANCA-GN patients than those in normal controls (0.015 ± 0.012 vs. 0.004 ± 0.003 , $p < 0.001$; 0.053 ± 0.026 vs. 0.011 ± 0.010 , $p < 0.001$, respectively). Moreover, for ANCA-GN patients, urinary FABP4 levels were significantly higher in active ANCA than those in remission (184.3 ± 187.0 ng/g Cr vs. 9.4 ± 23.9 ng/g Cr, $p < 0.01$). Correlation analysis showed that urinary levels of FABP4 correlated with serum creatinine ($r = 0.596$, $p < 0.0001$), urinary albumin/Cr ($r = 0.523$, $p = 0.001$), blood neutrophil ratio ($r = 0.386$, $p = 0.018$), PT ($r = 0.583$, $p = 0.001$), APTT ($r = 0.364$, $p = 0.034$), hemoglobin level ($r = -0.398$, $p = 0.015$), estimated glomerular filtration rate ($r = -0.680$, $p < 0.0001$), crescent proportion ($r = 0.661$, $p = 0.032$), and all-cause death of ANCA-GN patients (HR 2.93, 95% CI [1.05–8.19]). Furthermore, FABP4 inhibition by BMS309403 ameliorated renal injury in a rat model of ANCA-GN. **Conclusions:** Urinary FABP4 levels might reflect the

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disease activity and renal involvement of ANCA-associated vasculitis, and FABP4 might act as a promising therapeutic target against ANCA-GN.

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Introductions

Antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) is a group of systemic autoimmune diseases characterized by necrotizing inflammation of small vessels with a predilection for the respiratory tract and the kidneys [1]. AAV that affects the kidney as glomerulonephritis is termed as ANCA-associated glomerulonephritis (ANCA-GN) and is histologically characterized by fibrinoid necrosis of the capillary loops and crescent formation without apparent deposition of immune complex [2]. Despite recent advances in treatment including immunosuppressive therapy, patients with ANCA-GN are still at high risk of death and progression to end-stage kidney disease (ESKD) [3–5]. Timely treatment could largely improve patient outcome while there still lacks efficient and noninvasive biomarkers for the early recognition of disease till now [6, 7]. Moreover, as a chronic and progressive autoimmune disease, ANCA-GN is often characterized by a relapsing disease course and patients with relapsing ANCA-GN are at higher risk for poor outcomes [8–10]. Unfortunately, traditional laboratory tests (i.e., urinalysis and serum creatinine) could often not reliably discriminate between active disease and chronic damage, which leads to the urgent need for novel markers, especially those with noninvasive measurements, to monitor the activity, severity as well as relapse of diseases [6, 11].

Fatty acid-binding protein 4 (FABP4) is a member of the cytoplasmic fatty acid-binding protein family with a molecular weight of approximately 15 kDa [12, 13]. It is predominately identified in adipocytes and macrophages, while recent studies also reported its expression in some vascular endothelial cells [14]. In the kidney, increased FABP4 expression was reported to be induced in glomerular endothelial cells and macrophages upon glomerular injury, the extent of which was closely associated with the severity of proteinuria and renal dysfunction [15]. As a matter of fact, accumulating evidence has suggested the pathogenic role of FABP4 in multiple kidney diseases such as diabetic kidney disease (DKD), IgA nephropathy, rhabdomyolysis-induced acute kidney injury and chronic kidney diseases by orchestrating mechanisms of inflammation, apoptosis, angiogenesis

and oxidative stress [15–18]. Meanwhile, FABP4 has recently also emerged as a critical player in several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and autoimmune diabetes [19–22]. However, in patients with ANCA-GN, the clinical correlation of FABP4 and its role in disease progression remains unknown. In this study, we detected the levels of FABP4 in the plasma, urine, and kidney biopsy samples of patients with ANCA-GN and analyzed their association with clinical parameters and outcomes of patients. To further demonstrate the role of FABP4, we also measured the renal effect of FABP4 inhibition by BMS309403 in a well-established rat model of ANCA-GN.

Methods

Study Design and Participants

This study included a total of 93 active ANCA-GN patients diagnosed at West China Hospital of Sichuan University from January 2014 to December 2018. Plasma and urine samples from 37 active ANCA-GN patients were collected 3 days or less before the day of the renal biopsy. These patients were followed up by telephone contact every 6 months or at their regular ambulatory visits, and the last follow-up visit was 31 September 2021. Blood samples of 10 patients who achieved remission after immunosuppressive therapy were collected at their regular ambulatory visits, of which 8 urine samples were collected. All 10 patients were among the 37 patients described above. Plasma and urine samples of 41 age- and gender-matched health donors were collected as the normal control. Plasma and urine samples of 15 patients with kidney biopsy-proven DKD at the same center were collected as the disease control. Kidney biopsy specimens from another group of 56 patients with active ANCA-GN were collected. All the patients met the Chapel Hill Consensus Conference definition of AAV and none of them had coexistence of other kidney diseases at the time of kidney biopsy [23]. Disease activity of ANCA-GN was determined using the Birmingham Vasculitis Activity Score (BVAS) version 3 and those with a score of zero were defined as reaching remission [24]. Kidney tissue from normal parts of the nephrectomized kidneys of 10 patients with renal carcinoma from the same center was obtained and considered normal control. This study was in compliance with the Declaration of Helsinki and approved by the Ethics Committee of West China Hospital of Sichuan University. Written informed consent was obtained from all participants.

Outcomes: Study Variables

ESKD was defined as the initiation of long-term kidney replacement therapy [25]. The kidney histology of patients with ANCA-GN was evaluated according to the previous standardized protocol [26, 27]. The estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Detection of the Expression of FABP4 in the Blood, Urine, and Kidneys

The plasma and urine levels of FABP4 were determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions for the Human FABP4 Quantikine ELISA Kit (DFBP40, R&D Systems). The expression of FABP4 in the kidney was detected by immunohistochemistry staining and immunofluorescence staining. The details were described in the online supplementary material (for all online suppl. material, see <https://doi.org/10.1159/000543940>).

Animal Study

The experimental autoimmune vasculitis (EAV) model was induced according to a previously reported protocol [28]. Briefly, female Wistar Kyoto rats (purchased from Charles River, Shanghai, China, 6 weeks, weighing 95–110 g) were first immunized intramuscularly with 400 µg/kg of human serum albumin (HSA, A5843, Sigma-Aldrich), and then intraperitoneally injected with 0.5 µg of pertussis toxin (P7208-50, Sigma-Aldrich) at day 0 and day 2 after immunization, glomerulonephritis was reported to be established 28 days after immunization with presentations of hematuria and albuminuria. Rats with sole HSA immunization were considered the control group (HSA group). To illustrate the effect of FABP4 inhibition on ANCA-GN, an FABP4 inhibitor, BMS309403, was purchased from Selleck (S6622, China) and was given at a dosage of 28 mg/kg/day by gavage starting from day 21. The drug was dissolved in Tween80 and PEG400, and further diluted with distilled H₂O. The animals were sacrificed on day 56, and the plasma, urine and kidney samples were collected for further analysis. The animal procedures and experimental protocols were approved by the Experimental Animal Ethics Committee of West China Hospital of Sichuan University.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), and categorical variables were shown as count with percentage. To assess differences between two

groups, *t* test or Mann-Whitney U test were performed for normally or skewedly distributed data, respectively. Pearson or spearman correlation was used to analyze the correlation between two continuous variables as appropriate. To examine the associations of urinary and plasma FABP4 levels with the occurrence of ESKD and death, time-to-event analysis was performed. We used complete case analysis as missing data were less than 3%. Cox proportional hazard models were only adjusted for eGFR due to limited sample size, and we confirmed no violation of proportional hazards assumption. A two-side *p* value <0.05 was considered statistically significant. All analysis were performed with SPSS version 22.0 and R software version 4.1.1.

Results

Patient Cohort

Among the 37 active ANCA-GN patients with plasma and urine samples, 19 (51.4%) were men, and 18 (48.6%) were female, with an average age of 52.1 ± 16.5 years at diagnosis. All 37 patients in our study presented renal involvement of vasculitis. Myeloperoxidase ANCA (MPO-ANCA) and proteinase-3 ANCA (PR3-ANCA) were detected in 25 (67.6%) and 4 (10.8%) patients; one (2.7%) patient had both MPO-ANCA and PR3-3 ANCA detected, whereas 7 patients had no ANCA serum reactivity. The level of initial eGFR and urinary protein was 22.6 ± 18.6 mL/min/1.73 m² and 2.6 ± 2.1 g/24 h, respectively. The level of BVAS in the 37 patients in the active stage was 17.9 ± 6.6 and in the 10 patients in remission it was 0. The detailed patient characteristics are listed in Table 1.

Kidney biopsy samples were obtained from another group of 56 ANCA-GN patients. The mean age was 51.8 ± 13.5 years and 60.7% were female. The levels of eGFR and serum creatinine at sampling were 16.6 (IQR 11.2–29.2) mL/min/1.73 m² and 321.5 ± 186.0 µmol/L, respectively. Among these 56 patients, 48 (85.7%) patients tested positive for MPO-ANCA, 5 patients tested positive for PR3-ANCA (8.9%), and 3 patients tested positive for both MPO-ANCA and PR3-ANCA (5.4%). The average BVAS score was 17.1 ± 4.3 . Patient characteristics at the time of kidney biopsy are depicted in Table 2.

Plasma and Urinary FABP4 Levels in Patients with ANCA-GN

Elevated levels of plasma FABP4 (P-FABP4) were found in patients with active ANCA-GN and ANCA-GN in remission as compared with normal controls (52.8 ± 23.6 ng/mL vs. 16.9 ± 8.8 ng/mL, 39.7 ± 15.2 ng/mL vs.

Table 1. General data of patients with ANCA-GN for measuring plasma and U-FABP4

Parameters	Active stage	Remission stage	<i>p</i> values
Number	37	10	–
Gender (male/female)	19/18	5/5	1.000
Age at diagnosis, years	52.1±16.5	46.0±15.2	0.286
Target antigen of ANCA	25 MPO/4 PR3/1 both	–	–
eGFR, mL/min/1.73 m ²	22.6±18.6	56.9±21.0	<0.001
Urinary protein, g/24 h	2.6±2.1	–	–
BVAS	17.9±6.6	0.0±0.0	<0.001

ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; PR3, proteinase 3; eGFR, estimated glomerular filtration rate. Statistics are shown as mean ± standard deviation.

Table 2. Clinical and histopathologic data of patients with AAV for detecting renal FABP4 expression

Parameters	Value
Number	56
Gender (male/female)	22/34
Age at diagnosis, years	51.8±13.5
eGFR, mL/min/1.73 m ²	16.6 (11.2–29.2)
Serum creatinine, μmol/L	321.5±186.0
Target antigen of ANCA	48 MPO/5 PR3/3 both
Urinary protein, g/24 h	2.1 (1.1–3.7)
Skin rash	2 (3.6%)
Arthralgia	5 (8.9%)
Muscle pain	2 (3.6%)
Pulmonary	37 (66.1%)
ENT	1 (1.8%)
Ophthalmic involvement	3 (5.4%)
Gastrointestinal involvement	2 (3.6%)
Nervous system	1 (1.8%)
BVAS	17.1±4.3
Average glomeruli per biopsy	17.6±8.6
Glomerular lesions, %	
Total crescents	20 (1–49)
Glomerulosclerosis	29 (10.5–56.5)
Tubulointerstitial lesions	
Interstitial infiltration (0/1/2/3)	2/28/18/8
Interstitial fibrosis (0/1/2)	15/32/9
Tubular atrophy (0/1/2)	6/42/8

AAV, antineutrophil cytoplasmic antibody; BVAS, Birmingham Vasculitis Activity Scores; ENT, ear, nose, and throat.

16.9 ± 8.8 ng/mL, respectively, $p < 0.01$) (Fig. 1a). However, no difference was detected between patients with active ANCA-GN and those in remission (Fig. 1a, $p > 0.05$). In the 15 patients with DKD, a similar increase in P-FABP4 level was also noticed (active ANCA-GN vs. DKD: 52.8 ± 23.6 ng/mL vs. 37.0 ± 30.9 ng/mL, $p > 0.05$) (Fig. 1a). For 10 patients with sequential blood samples, the P-FABP4 level remained unchanged between active ANCA-GN and ANCA-GN in remission (45.9 ± 22.1 ng/mL vs. 39.7 ± 15.2 ng/mL, $p > 0.05$) (Fig. 1b). The level of urinary FABP4 (U-FABP4) was normalized by the urinary creatinine to correct for variations in dilution. The levels of U-FABP4 were significantly higher in active ANCA-GN patients than those in remission or normal controls (184.3 ± 187.0 ng/g Cr vs. 9.4 ± 23.9 ng/g Cr, $p < 0.01$; 26.6 [28.4, 311.2] ng/g Cr vs. 0.0 [0.0, 0.0] ng/g Cr, $p < 0.01$, respectively) (Fig. 1c). An increase in U-FABP4 level was also noticed in DKD patients as compared with normal controls (22.0 [2.5, 68.2] ng/g Cr vs. 0.0 [0.0, 0.0] ng/g Cr). However, the level of U-FABP4 in DKD was much lower than those in patients with active ANCA-GN (50.1 ± 84.2 ng/g Cr vs. 184.3 ± 187.0 ng/g Cr, $p < 0.01$). For 8 patients with sequential urine samples, the level of U-FABP4 was significantly higher in the active stage than in remission (active ANCA-GN vs. ANCA-GN in remission: 115.5 ± 122.9 ng/g Cr vs. 9.4 ± 30.0 ng/g Cr, $p < 0.05$) (Fig. 1d).

Association between Levels of FABP4 and Clinicopathological Parameters in ANCA-GN Patients

Measurements for clinical parameters including serum creatinine, eGFR, blood cell count, hemoglobin, PT, APTT, and urinary albumin/Cr were done at the same time as blood and urinary samples were collected for this

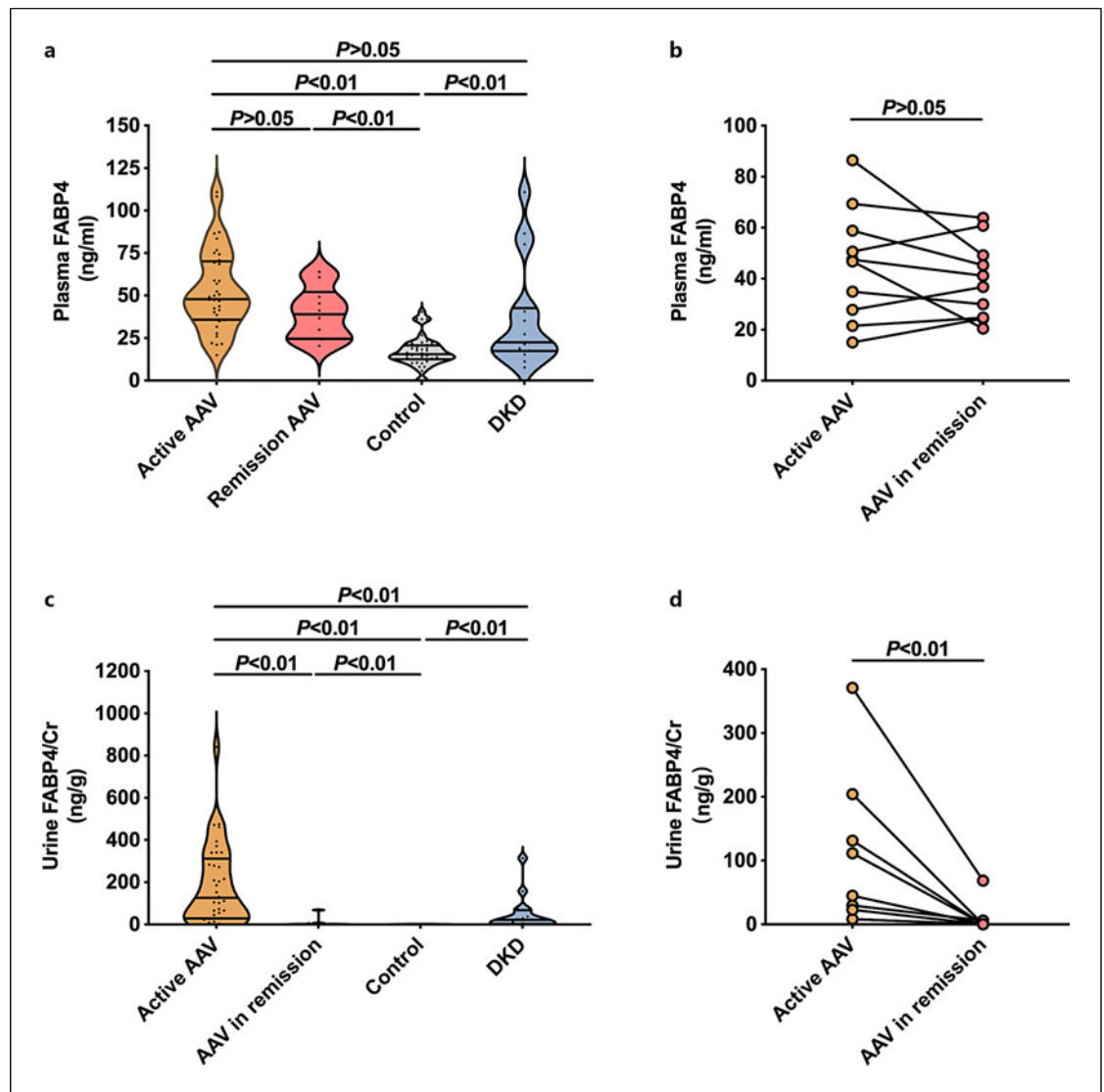
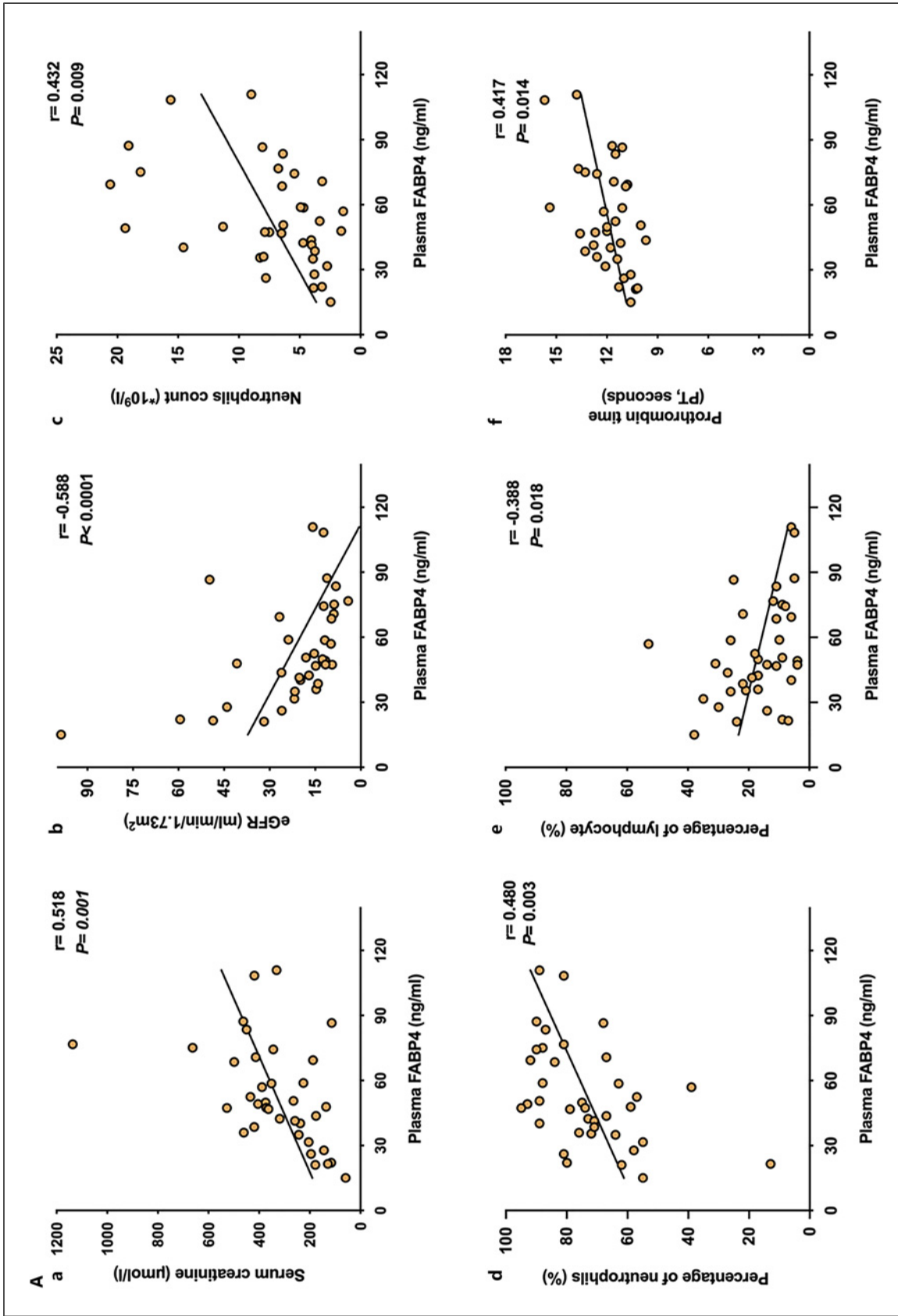


Fig. 1. Levels of plasma and U-FABP4 in patients with ANCA-GN and healthy controls. **a** The levels of P-FABP4 in patients with active ANCA-GN (active AAV, $n = 37$), remission stage of ANCA-GN (remission AAV, $n = 10$), normal controls (control, $n = 41$), and diabetic kidney disease (DKD, $n = 15$). **b** The change of P-FABP4 level in the active stage and remission stage of ANCA-GN. **c** The level of U-FABP4 in

patients with active ANCA-GN (active AAV, $n = 37$), remission stage of ANCA-GN (remission AAV, $n = 10$), normal controls (control, $n = 41$), and diabetic kidney disease (DKD, $n = 15$). **d** The change of U-FABP4 level in the active stage and remission stage of ANCA-GN. Bars represent the means $\pm$ SD. AAV, antineutrophil cytoplasmic antibody-associated vasculitis.

study. Correlation analysis showed moderate correlation between P-FABP4 and serum creatinine ($r = 0.518$, $p = 0.001$), eGFR ($r = -0.588$, $p < 0.001$), blood neutrophil count ($r = 0.432$, $p = 0.009$), blood neutrophil ratio ($r = 0.480$, $p = 0.003$), PT ($r = 0.417$, $p = 0.014$) in patients with ANCA-GN. Meanwhile, a significant but mild correlation between P-FABP4 and blood lymphocyte ratio was also noted ($r = -0.388$, $p = 0.0018$) (Fig. 2A).

As for U-FABP4, it showed positive correlation with serum creatinine ($r = 0.596$, $p < 0.0001$), urinary albumin/Cr ($r = 0.523$, $p = 0.001$), blood neutrophil ratio ($r = 0.386$, $p = 0.018$), PT ($r = 0.583$, $p = 0.001$) and APTT ($r = 0.364$, $p = 0.034$) in patients with ANCA-GN (Fig. 2B-a, c, e-g). A mild negative correlation was also noticed between U-FABP4 and patients' hemoglobin level ($r = -0.398$, $p = 0.015$) (Fig. 2B-d). Notably, an obvious negative



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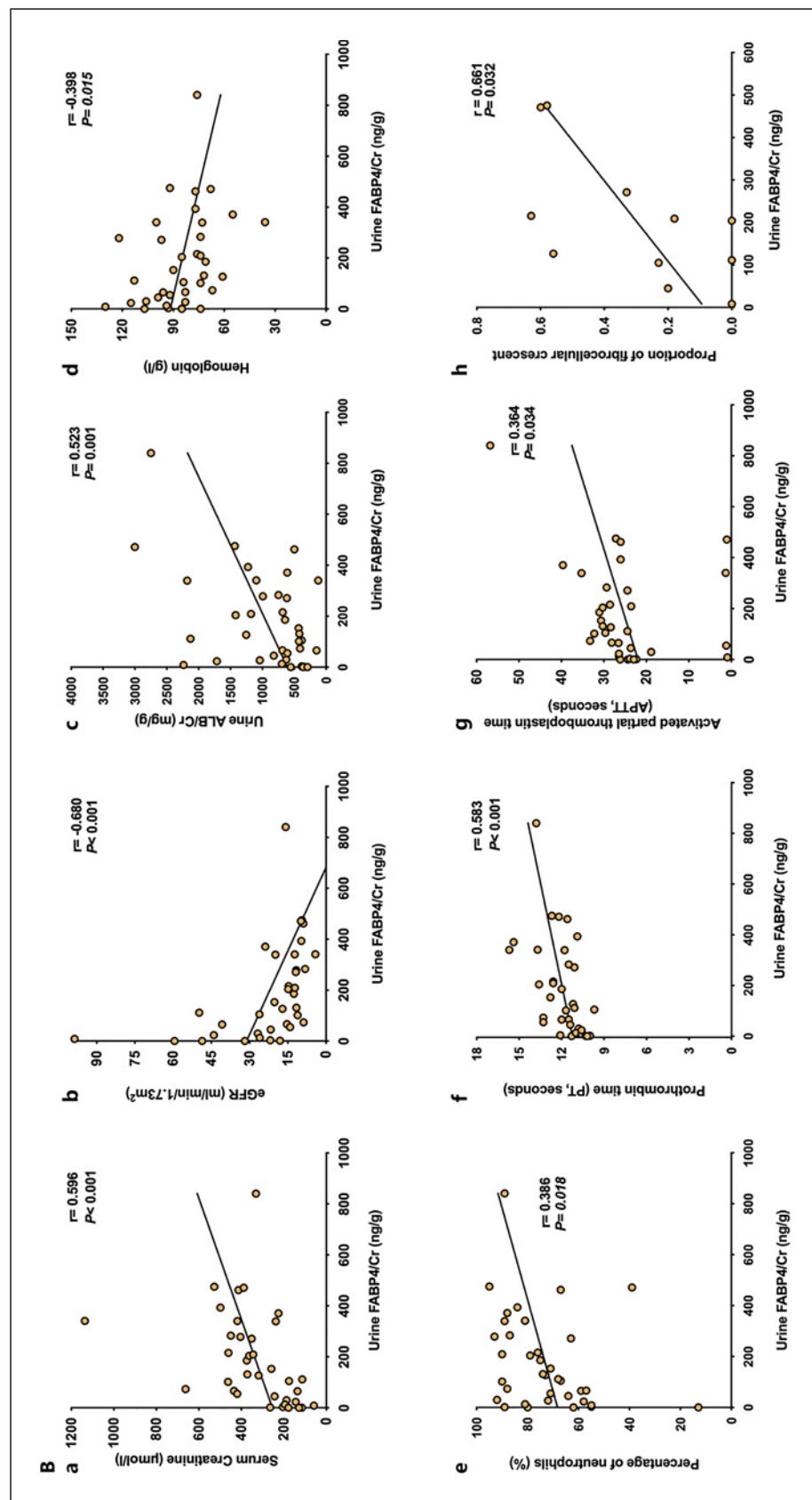


Fig. 2. Association between FABP4 levels and clinicopathological parameters of patients with ANCA-GN. **A-a** Association between P-FAPB4 and serum creatinine. **A-b** Association between P-FAPB4 and eGFR. **A-c** Association between P-FAPB4 and blood neutrophil count. **A-d** Association between P-FAPB4 level and blood neutrophil percentage. **A-e** Association between P-FAPB4 level and blood lymphocyte percentage. **A-f** Association between P-FAPB4 level and PT. **B-a** Association between U-FAPB4 level and serum creatinine. **B-b** Association between U-FAPB4 level and eGFR. **B-c** Association between U-FAPB4 and urine ALB/Cr. **B-d** Association between U-FAPB4 and hemoglobin. **B-e** Association between U-FAPB4 and blood neutrophil percentage. **B-f** Association between U-FAPB4 and PT. **B-g** Association between U-FAPB4 and APTT. **B-h** Association between U-FAPB4 and fibrocellular crescent proportion. eGFR, estimated glomerular filtration rate; PT, prothrombin time; ALB, albumin; Cr, creatinine; APTT, active partial thromboplastin time.

Table 3. Associations of plasma and U-FABP4 levels with end-stage kidney disease and death

Outcomes	Plasma FABP4	U-FABP4
<i>ESKD</i>		
Events, <i>n</i>	12	12
Hazard ratio (95% CI)		
Model 1: unadjusted	1.12 (0.56–2.24)	1.67 (0.89–3.12)
Model 2: model 1+eGFR	0.49 (0.14–1.76)	1.20 (0.54–2.65)
<i>Death</i>		
Events, <i>n</i>	4	4
Hazard ratio (95% CI)		
Model 1: unadjusted	3.73 (1.15–12.13)	2.76 (1.07–7.12)
Model 2: model 1+eGFR	3.72 (1.14–12.17)	2.93 (1.05–8.19)

ESKD, end-stage kidney disease; eGFR, estimated glomerular filtration rate; CI, confidence interval.

correlation between U-FABP4 and eGFR ($r = -0.680$, $p < 0.0001$), the important indicator of renal function in ANCA, was observed (Fig. 2B-b). Besides, in 11 patients who underwent kidney biopsy, U-FABP4 also showed an obvious positive correlation with the proportion of crescentic glomeruli in the renal specimens ($r = 0.661$, $p = 0.032$) (Fig. 2B-h). Another interesting finding was that we found a significant positive correlation between P-FABP4 and U-FABP4 ($r = 0.635$, $p < 0.001$) (online suppl. Fig. S1).

Association of Plasma and U-FABP4 Concentration with Patient Outcomes in ANCA-GN

During follow-up period, a total of 12 incident ESKD events (median follow-up duration, 34.0 [12.8–38.0] months) and 4 all-cause death events (median follow-up duration, 35.5 [33.0–37.0] months) occurred. Table 3 shows the hazard ratios (HRs) with 95% CI for the correlations of P-FABP4 and U-FABP4 with outcomes. Both P-FABP4 and U-FABP4 were significantly correlated with all-cause death of ANCA-GN patients. Every 1-SD increase in FABP4 measurement was significantly associated with higher risk of death (HR 3.73, 95% CI [1.15–12.13] and HR 2.76, 95% CI [1.07–7.12] for P-FABP4 and U-FABP4, respectively). The correlations were neither reversed nor strengthened after adjusting for eGFR (HR 3.72, 95% CI [1.14–12.17] and HR 2.93, 95% CI [1.05–8.19] for P-FABP4 and U-FABP4, respectively).

Renal FABP4 Expression in Patients with ANCA-GN

To investigate the kidney expression of FABP4 in patients with ANCA-GN, kidney tissue sections from 56 patients were stained and scored for FABP4. The clinical

and histopathological data of all 56 patients were listed in Table 3. The results of immunohistochemical examination showed that FABP4 was abundantly expressed in the glomeruli of patients with ANCA-GN while minimal FABP4 staining was found within some capillaries of glomeruli in normal controls (0.015 ± 0.012 vs. 0.004 ± 0.003 , $p < 0.001$) (Fig. 3a, b). Tubulointerstitial staining of FABP4 was presented in almost all kinds of tubules and infiltrating cells in the kidney specimens of ANCA-GN patients. However, little FABP4 expression was detected in the limited tubules of normal sections (0.053 ± 0.026 vs. 0.011 ± 0.010 , $p < 0.001$) (Fig. 3a, c).

Further immunofluorescence double staining for FABP4 and markers of the glomerular intrinsic and infiltrating cells was performed to detect FABP4 positive cells in the kidneys of ANCA-GN patients. In the glomeruli, co-location of FABP4 with CD31 and α -SMA was observed, suggesting the expression of FABP4 in glomerular endothelial cells and mesangial cells (online suppl. Fig. S2A). Meanwhile, in the tubulointerstitial compartment, it was noticed that FABP4 co-located with CD16b, CD3, and CD68, indicating that infiltrating neutrophils, lymphocytes, and monocytes/macrophages might also be the source of tubulointerstitial FABP4 expression (online suppl. Fig. S2B).

Association of Renal FABP4 Expression with Clinicopathological Parameters in Patients with ANCA-GN

Correlation analysis between immunohistochemical expression of renal FABP4 and clinicopathological parameters was carried out in 56 ANCA-GN patients. We found significant but mild correlations between

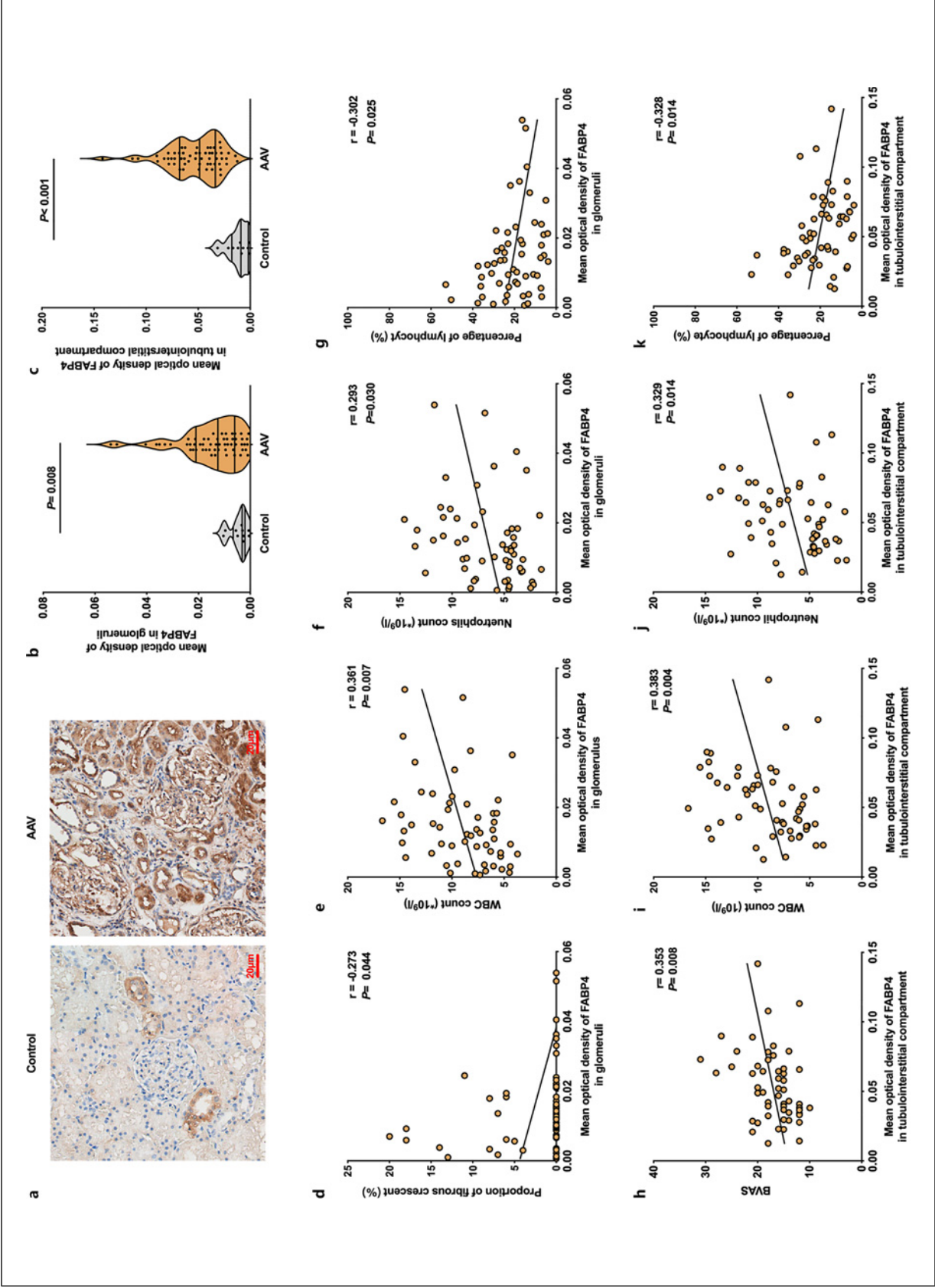
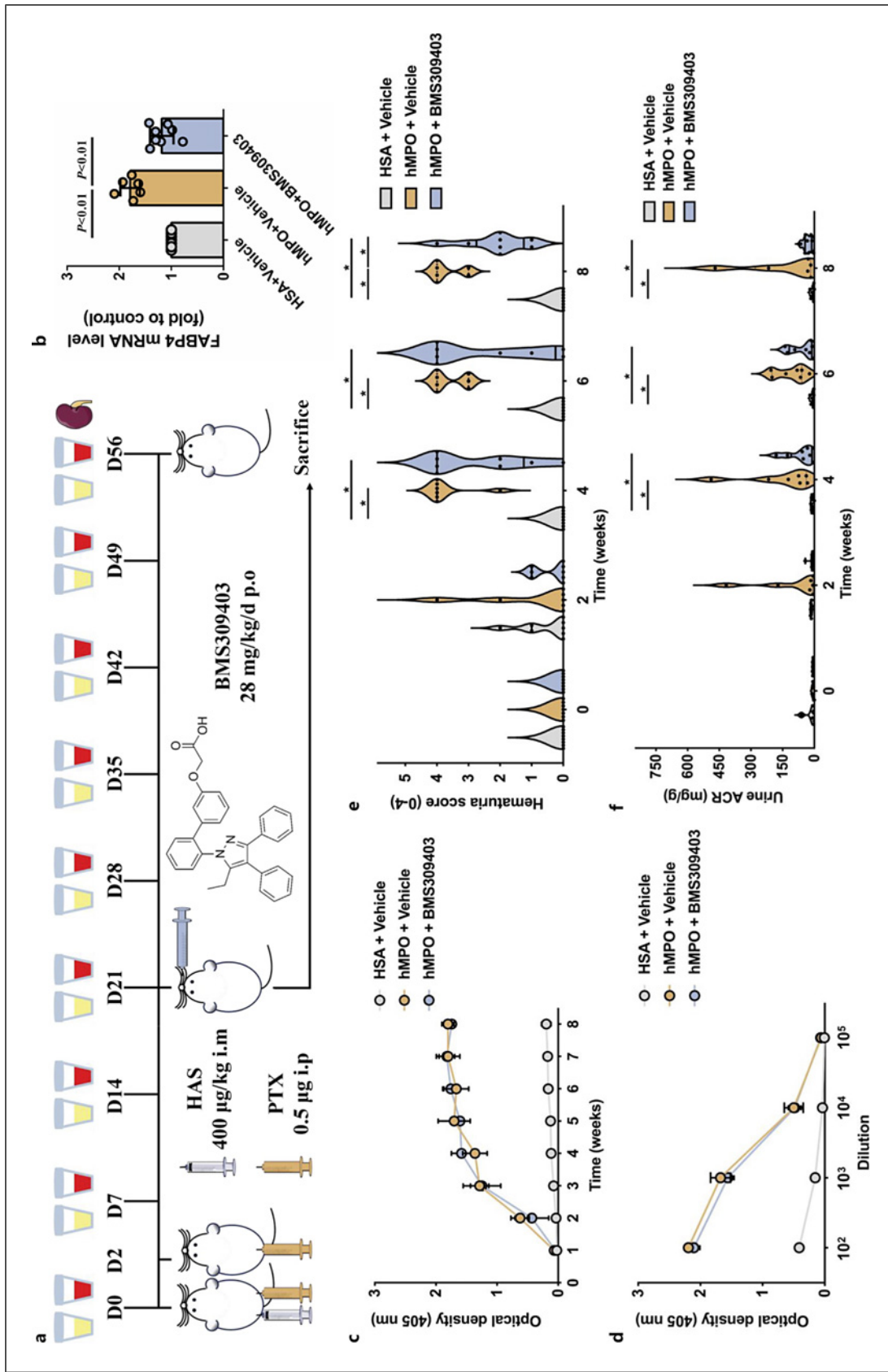


Fig. 3. Renal FABP4 expression and association with clinicopathological parameters in patients with ANCA-GN. **a** Representative immunohistochemical images of FABP4 in the kidneys of ANCA-GN patients and normal controls. **b, c** The quantification of immunohistochemical staining of FABP4 in the glomerular and tubulointerstitial compartment of ANCA-GN patients and normal controls, respectively. **d–g** The association of glomerular FABP4 expression with the proportion of fibrous crescent, WBC count, neutrophil count, and lymphocyte percentage, respectively. **h–k** The association of tubulointerstitial FABP4 expression with BVAS score, WBC count, neutrophil count, and lymphocyte percentage, respectively. BVAS, Birmingham Vasculitis Activity Score; WBC, white blood cell. Bars represent the means $\pm$ SD.



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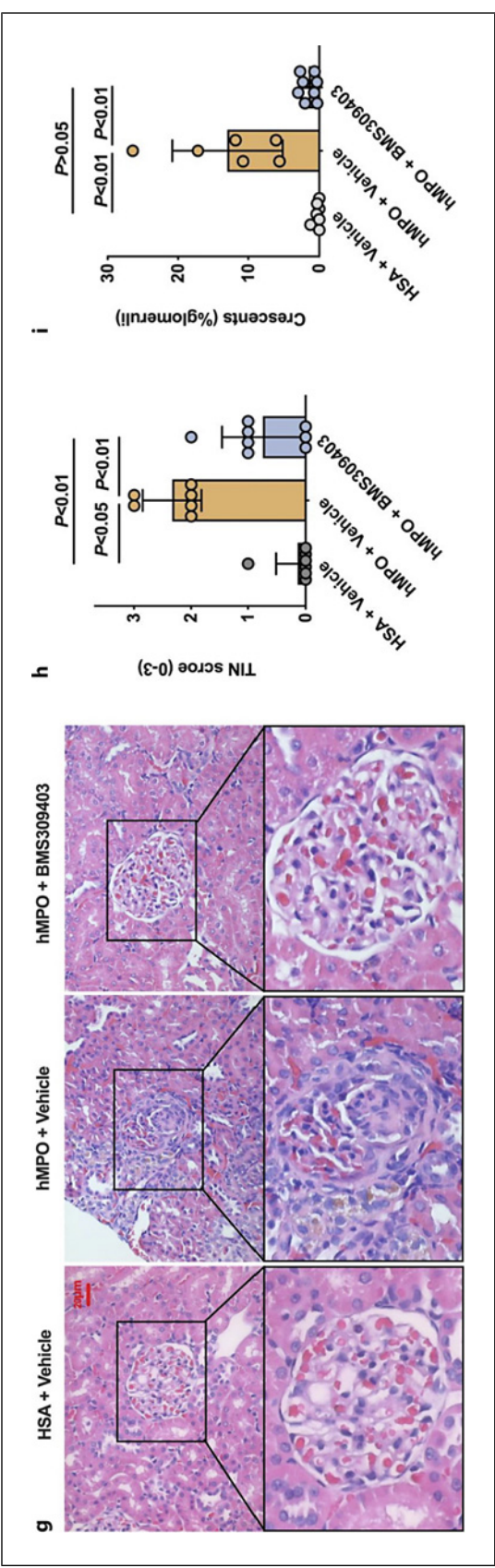


Fig. 4. FABP4 inhibitor BMS309403 alleviated kidney histopathological lesion and hematuria in EAV. **a** Diagram of the animal study. **b** The mRNA expression of FABP4 in the kidneys of mice. **c** The relative optical density value of hMPO-specific antibodies. **d** The gradient diluted serum of rats at week 8 and their relative optical density value were adopted to detect the production of hMPO-specific antibodies. **e** Effect of BMS309403 on hematuria at week 8. **f** Effect of BMS309403 on proteinuria in EAV rats. **g** H&E staining of kidney sections. **h** Effect of BMS309403 treatment on tubulointerstitial nephritis (TIN) score. **i** Effect of BMS309403 treatment on the fraction of glomerular crescent in mice kidneys. Data represent means $\pm$ SD from 6 to eight rats per group; $*p < 0.05$ compared with HSA- or hMPO-treated rats.

glomerular FABP4 expression and fibrous crescent proportion ($r = -0.273$, $p = 0.04$) (Fig. 3d), as well as tubulointerstitial FABP4 expression with the BVAS score ($r = 0.353$, $p = 0.008$) (Fig. 3h). Moreover, both glomerular and tubulointerstitial FABP4 expression level showed similar correlation with the level of white blood cell count ($r = 0.361$, $p = 0.007$; $r = 0.383$, $p = 0.004$, for glomerular and tubulointerstitial FABP4, respectively) (Fig. 3e, i), neutrophil count ($r = 0.293$, $p = 0.030$; $r = 0.329$, $p = 0.014$, for glomerular and tubulointerstitial FABP4, respectively) (Fig. 3f, j), and lymphocyte percentage ($r = -0.302$, $p = 0.025$; $r = -0.328$, $p = 0.014$, for glomerular and tubulointerstitial FABP4, respectively) (Fig. 3g, k) in patients with ANCA-GN. In addition, an obvious correlation between the expression level of glomerular FABP4 and tubulointerstitial FABP4 in ANCA-GN patients was noticed ($r = 0.68$, $p < 0.0001$) (online suppl. Fig. S3).

Renal Effects of FABP4 Inhibition in an Animal Model of EAV

To further elucidate the role of FABP4 in ANCA-GN, we introduced a well-established EAV model by immunizing susceptible rat strains with hMPO [29]. The FABP4 inhibitor, BMS309403, was given by gavage to evaluate the effect of FABP4 inhibition in ANCA-GN [30]. The diagram of the animal study was depicted in Figure 4a, and the results showed that FABP4 expression was significantly increased in the kidneys of immunized rats, which was inhibited by the treatment of BMS309403 (Fig. 4b). Moreover, the production of hMPO-ANCA obviously increased from week 2 and stabilized from week 5 in all hMPO-immunized rats (Fig. 4c). At week 8, the titers of hMPO-ANCA in all hMPO-immunized rats reached a value of 1:10,000 and no difference was noticed regarding hMPO-ANCA level upon BMS309403 treatment (Fig. 4d). By week 4, all the hMPO-immunized rats had developed hematuria and proteinuria (Fig. 4e, f). In the rats treated with BMS309403, there was less hematuria at week 8 as compared with those treated with vehicle (2.0 [1.0, 3.0] vs. 4.0 [3.0, 4.0], $p < 0.05$) (Fig. 4e). Histopathological assessment showed that crescents were found in all hMPO-immunized rats (Fig. 4g). The rats treated with BMS309403 exerted a significant lower proportion of crescents than vehicle ($1.53\% \pm 1.12\%$ vs. $13.02\% \pm 7.82\%$, $p < 0.05$) (Fig. 4h). The assessment of the tubulointerstitial lesions showed that the EAV rats treated with BMS309403 had a lower TIN score compared with vehicle (1.0 [0.0–1.0] vs. 2.0 [2.0–3.0], $p < 0.01$) (Fig. 4i), suggesting ameliorated renal histopathological injury after BMS309403 treatment.

Discussion

Accurate biomarkers for early detection and monitor of ANCA-GN are crucial for optimizing treatment and improving patient outcome [9, 31, 32]. The present study demonstrated the potential of a lipid chaperone protein, FABP4, as a novel biomarker in ANCA-GN. An increasing number of studies have highlighted the critical role of FABP4 in autoimmune diseases and several types of kidney diseases via regulating inflammation, lipid metabolism and angiogenesis [30, 33–35]. However, the expression and function of FABP4 in ANCA-GN remains unclear.

In the current study, we noticed abnormally elevated FABP4 expression level in the plasma, urine, and kidney samples of active ANCA-GN patients, and FABP4 inhibition significantly improved kidney injury in hMPO-induced vasculitis rats. The levels of FABP4, especially U-FABP4, were significantly higher in patients with active ANCA-GN than patients in remission and normal controls. Further analysis during follow-up showed that the level of U-FABP4 in active patients decreased dramatically once the patient reached remission, suggesting that U-FABP4 might be a sensitive indicator for the reflection of disease activity in ANCA-GN. Additionally, U-FABP4 showed a relatively strong correlation with eGFR and the proportion of crescentic glomeruli, suggesting that U-FABP4 might also be indicative of the severity of kidney injury in ANCA-GN. For the prognosis analysis, U-FABP4 was significantly associated with all-cause death of patients, which potentiates it a promising predictor of death in ANCA-GN. To sum up, there exists correlation between U-FABP4 concentration and disease activity, severity, and prognosis in ANCA-GN. Meanwhile, the detection of U-FABP4 was simple and non-invasive, making it an ideal biomarker for the monitor of ANCA-GN [15].

The elevation in the concentration of U-FABP4 has already been reported by several previous investigations. Tanaka, et al. [15] noticed a mild increase of U-FABP4 concentration in patients with IgA nephritis and mild change disease, a moderate increase in patients with membrane nephropathy, and a great increase in patients with DKD [36]. The discrepancy in the increment of U-FABP4 in different types of kidney diseases suggests that it might be a useful tool for differential diagnosis of kidney diseases. The result of the present study was in consistent with the conclusion that the levels of U-FABP4 in patients with DKD were higher than those in healthy controls [37]. Moreover, we found significantly higher level of U-FABP4 in ANCA-GN patients than those in

DKD patients. Early evidence indicated that the level of U-FABP4 was associated with albuminuria and might serve as a novel biomarker of glomerular damage [38]. In line with this, we noticed a moderate correlation between U-FABP4 concentration and urinary albumin level in patients with ANCA-GN. Meanwhile, hematuria of rats as induced by immunization was significantly improved after FABP4 inhibition, altogether demonstrating that FABP4 is an important indicator of glomerular injury whereas the mechanism still needs further explored.

The source of the elevated U-FABP4 concentration in ANCA-GN was also another important question. Generally, FABP4 was thought to be secreted by adipocytes and macrophages in the circulation, and with its small molecular weight and net positive surface electrostatic potential across the portal region, it could easily pass through the glomerular filtration barriers and then re-absorbed by proximal tubule epithelial cells [39, 40]. In the present study, we found a positive correlation between P-FABP4 and U-FABP4 in patients with active ANCA-GN, which suggested that the circulation might be part of the source of U-FABP4. However, it was also noticed that U-FABP4 concentration decreased dramatically with the remission of disease while the elevated P-FABP4 remains barely changed. Moreover, the concentration of U-FABP4 was much higher in active AAV than those of DKD while P-FABP4 levels shows no difference between patients with active AAV than those of DKD. This inconsistency indicated that the circulation was not the only source of U-FABP4, and the kidney itself might be the major source of the elevated U-FABP4 in patients with active ANCA-GN. As a matter of fact, studies had reported the ectopic expression of FABP4 in glomerular endothelial cells and intrinsic macrophages as induced by glomerular injury [41]. In our study, immunofluorescence results identified the expression of FABP4 both on kidney intrinsic cells (including glomerular endothelial cells, mesangial cells, and tubules) and the infiltrating cells (including neutrophils, lymphocytes, and macrophages) of ANCA-GN patients. Therefore, it could be assumed that both the circulation and kidney itself contributed to the elevated level of U-FABP4 in patients with active ANCA-GN, whereas the kidney might play the major part.

Importantly, our findings found significantly elevated levels of FABP4 in the serum, urine, and kidney samples of ANCA-GN patients. However, whether the upregulated FABP4 expression is an adaptive change in the disease state or a driving force in the development of disease remains unknown. In other autoimmune disease, such as rheumatoid arthritis, it has been reported that upregulated FABP4 ex-

pression could promote the proliferation, migration, invasion of M1-polarized macrophages in mice, and the production of inflammatory cytokines in endothelial cells and fibroblast-like synoviocytes, which exacerbated synovitis, angiogenesis, and cartilage degeneration in the progression of rheumatoid arthritis [20]. Meanwhile, in various kidney diseases, FABP4 was found to be involved in the development of disease by orchestrating mechanisms of inflammation, apoptosis, and so on [30, 36]. In our animal experimental study, we found that the inhibition of FABP4 by BMS309403 could effectively alleviate the development of ANCA-GN by reducing crescent formation and improving hematuria in EAV rats, which indicated the pathogenic role of FABP4 in ANCA-GN. Our findings suggested that FABP4 might serve as a potential therapeutic target in ANCA-GN but the underlying mechanism needs further investigation.

Our study also has some limitations. First, this is a single cohort study; we do not know whether FABP4, especially U-FABP4, still shows clinical correlation in ANCA-GN patients of other ethnics. Second, the sample size in our study was relatively small, and because of this, the correlations between U-FABP4 and some clinical parameters might be ignored. Hence, we do hope in the future there will be more relevant investigations with multi-center set and larger sample size to add more evidence.

Statement of Ethics

This study was in compliance with the Declaration of Helsinki and approved by the Ethics Committee of West China Hospital of Sichuan University, Approval No. 2016-149, and written informed consent was obtained from all participants.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

Lu Cheng and Qian Ren: conceptualization, methodology, software, formal analysis, investigation, animal study, writing – original draft, and visualization. Jing Liu: investigation, formal analysis, and

resources. Mei-Lian Yu: resources and animal study. Rong-Shuang Huang: methodology and formal analysis. Fan Guo: animal study. Min Shi and Ping Fu: resources. Liang Ma: conceptualization, methodology, writing – review and editing, and supervision. Shen-Ju Gou: conceptualization, methodology, resources, writing – review and editing, and supervision.

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author upon reasonable request.

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