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**High levels of urinary complement proteins are associated with chronic renal damage
and proximal tubule dysfunction in IgA nephropathy**

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Running title: Urinary complement proteins in IgAN

Abstract

Aim: Complement activation is involved in the pathogenesis and progression of IgA nephropathy (IgAN); however, the clinical implication of abnormal complement protein levels in serum and urine is not clear. To address this we analysed the correlation between disease

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activity and complement proteins in serum and urine from IgAN patients, and compared to patients with other types of chronic kidney disease (CKD) as well as healthy controls.

Methods: We included 85 Chinese patients with IgAN, 23 patients with non-proliferative CKD, and 20 healthy individuals. Patients were divided according to the Oxford classification of M0E0S0T0 (group 1, n= 20), M1E1S0-1T0-1 (group 2, n=25), M1E1S0-1T2 or M0E0S1T1-2 (group 3, n=40). Complement factor H (CFH), mannose-binding lectin (MBL) and membrane attack complex (MAC; C5b-9) in serum and urine were measured by enzyme-linked immunosorbent assay.

Results: Urinary CFH, C5b-9 and serum CFH were increased in both IgAN and CKD patients compared with healthy controls. The urinary excretion of CFH was the highest in IgAN patients with most tubulointerstitial damage (IgAN group 3). Urinary CFH and MBL levels were significantly higher in IgAN patients with crescents formation (C1-2) than in patients without (C0). Urinary complement protein excretion correlated negatively with estimated glomerular filtration rate, and positively with urinary retinol-binding protein and α 1-microglobulin excretion indicating proximal tubule dysfunction.

Conclusion: Increased urinary excretion of complement proteins in IgAN is related to chronic injury and tubular dysfunction. This warrants caution using urinary complement proteins as markers of disease activity.

Key words: Chronic kidney disease, complement proteins, IgA nephropathy, proximal tubule dysfunction, urinary.

Introduction

IgA nephropathy (IgAN) is the most common, primary glomerulonephritis worldwide, leading to end-stage renal disease (ESRD) in up to 40% of patients within 10-20 years.¹ Although it has been over 40 years since its recognition, the pathogenic mechanisms of IgAN are still unclear. Accumulating evidence indicates that complement activation is involved in the pathogenesis and development of IgAN.^{2,3} Specifically, C3 mesangial co-deposition with IgA1 is the major histopathological feature of IgAN,^{2,4} and properdin,⁵ C4 or C4d,⁶ as well as the membrane attack complex (MAC; C5b-9)⁷ are also frequently detected in renal biopsies. These findings suggest activation of complement system, especially alternative and lectin pathway, participating in the pathological changes of this disease.

Complement factor H (CFH) is one of the most important inhibitory proteins of the alternative pathway by competing with C3 convertase and by acting as a cofactor for factor I in the C3b cleavage.⁸ Mannose-binding lectin (MBL) is a liver derived C-type lectin, which activates the complement cascade by recognizing carbohydrates on the surfaces of many pathogens followed by reaction with MBL-associated serine proteases MASP-1 and MASP-2.^{9,10} Several studies have demonstrated deposition of both CFH^{11,12} and MBL^{10,13} in glomeruli as well as their correlations to the disease severity of IgAN. Recently, a significant elevation of both CFH and MBL in urine from patients with IgAN was observed to be associated with prognosis.^{12,14,15} Yet, the clinical implication of these complement proteins in urine has not been fully elucidated.

The present study sought out to investigate the association between serum and urinary CFH, MBL, complement activation marker C5b-9 and disease severity of IgAN. We measured the levels of CFH, MBL and C5b-9 in serum and urine from IgAN patients with different histopathological phenotypes based on Oxford classification as well as the patients with chronic

kidney disease (CKD) owing to non-proliferative disease. We analysed the association between these complement proteins in serum and urine and clinical as well as histopathological parameters in IgAN patients. In addition, we evaluated the association between proximal tubule damage markers, retinol-binding protein (RBP) and α 1-microglobulin (α 1-MG), and urinary complement protein excretion in patients with IgAN.

Methods

Patients and samples

A total of 85 Chinese patients with IgAN confirmed by kidney biopsy and fulfilling the histopathologic criteria of Oxford classification M0E0S0T0, M1E1S0-1T0-1, M1E1S0-1T2, or M0E0S1T1-2 were enrolled in this cross-sectional study between December 2014 and November 2017 at the First Affiliated Hospital of Zhengzhou University, China. Patients treated with immunosuppressive agents, including prednisone, at the time of kidney biopsy, or with other coexisting renal pathology or recurrent IgAN after kidney transplantation were excluded. Twenty-three patients with biopsy-proven focal segmental glomerulosclerosis (FSGS, n = 9) or hypertensive nephrosclerosis (n = 14) were included as non-proliferative CKD controls and twenty age- and sex-matched healthy individuals were as healthy controls. The study was approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University, China and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in this study.

Clinical data including age, gender, mean arterial pressure (MAP), serum creatinine, 24-hour urinary protein, and medication were recorded at the time of kidney biopsy. The estimated glomerular filtration rate (eGFR) was estimated using the CKD-EPI formula.¹⁶ Early-morning fasting blood and urine samples for assessment of the complement proteins were collected from all included patients on the morning of kidney biopsy. The blood and urine samples were

centrifuged at 2000g and 600g, respectively, for 10 minutes and the sediment was discarded; the supernatant was immediately frozen at -80°C until further analysis.

Histological assessment

For histopathology, sections were routinely stained with haematoxylin and eosin (HE), periodic-acid Schiff (PAS), Masson, periodic-acid-silver methenamine (PASM) and immunofluorescence at the Department of Renal Pathology at the First Affiliated Hospital of Zhengzhou University. Two pathologists who were blinded to patients' data examined the sections. The histopathological changes in the renal biopsy were graded based on the Oxford classification.¹⁷ Accordingly, the included patients were stratified into three groups: 1) M0E0S0T0 representing minimal damage (n=20, group 1); 2) M1E1S0-1T0-1 representing proliferative changes (n=25, group 2) and 3) M1E1S0-1T2 or M0E0S1T1-2 representing sclerotic damage (n=40, group 3). Well-defined cellular or fibrocellular crescents, but not fibrous crescents, were included in the crescent score: C0 equal to no crescents, C1 equal to crescents in less than one fourth of glomeruli, and C2 equal to crescents in over one fourth of glomeruli as described previously.^{18,19} The intensity of staining for C3 was scored as follows: 0, invisible; 1, ambiguous under a low-power lens and apparent under a high-power lens; 2, apparent under a low-power lens and clear under a high-power lens; 3, clear under a low-power lens and bright under a high-power lens.²⁰

ELISA analysis and biochemical tests

Serum and urinary levels of CFH (Abcam, Cambridge, UK), MBL (CUSABIO, Wuhan, China) and C5b-9 (CUSABIO) were measured using commercially available ELISA kits according to the manufacturer' procedure. The absorbance at 450nm was recorded using a microplate reader (EMax Plus, Molecular Devices, Sunnyvale, CA, USA). Each sample was performed in 2-3 replicates with low intra-assay and inter-assay coefficient of variations (%CV < 10-15). The

ratios of urinary CFH, MBL or C5b-9 concentration to creatinine concentration (ng/mg) were calculated and used for analyses, respectively.

Serum creatinine and urinary total protein, creatinine, RBP and α 1-MG were measured using an automatic biochemical analyzer in the Clinical Laboratory Center at the First Affiliated Hospital of Zhengzhou University. Specifically, RBP was measured by Immune Turbidimetry (YIJIE Biology, Ningbo, China) and α 1-MG was measured by Latex Turbidimetry (YIJIE Biology). The ratio of urinary RBP (α 1-MG) concentration to creatinine concentration (μ g/mg) was calculated and used for the analyses.

Statistical analysis

Continuous data are presented as mean \pm SD or median (lower and upper quartiles) while categorical data are presented as absolute frequencies and percentages. Chi-square test was used to compare categorical variables. For normally distributed data, the T-test was used to compare between two groups. Non-normally distributed data set were compared using the Mann-Whitney U-test or Kruskal-Wallis test. The Bonferroni method was applied to correct for multiple comparisons. The correlations among various parameters were analysed using Pearson or Spearman's coefficient. Data with a skewed distribution (urinary CFH, MBL and C5b-9) were logarithmic transformed to make these conform or close to a normal distribution. A P-value of less than 0.05 was considered statistically significant. Data were analysed using the SPSS v.19.0 software (IBM Corp., Armonk, NY, USA).

Results

Demographic and clinical data of all subjects

Demographic and clinical data of the healthy controls, CKD patients and IgAN patients are summarized in Table 1. No significant differences in age, gender, and use of renin-angiotensin

system-blockade were observed between groups. All IgAN patients, including three subgroups, and the CKD patients had significantly higher MAP and 24-hour urinary protein levels when compared with healthy controls, and MAP was higher in IgAN patients of group 3 than in group 1. The serum creatinine levels were higher and eGFR lower in all IgAN patients, including the patients in subgroup 2 and 3, as well as in CKD patients when compared to healthy controls. The IgAN patients of group 3 had significantly higher levels of serum creatinine and lower eGFR as compared with group 1 and 2. Serum creatinine levels and eGFR were normal and comparable to healthy controls in IgAN patients of group 1.

Serum CFH, MBL and C5b-9 levels in IgAN and controls

Serum CFH levels were significantly increased and MBL levels decreased in IgAN patients, including all three subgroups, as well as in CKD patients when compared with healthy controls (Figure 1A and 1B). Serum C5b-9 levels in IgAN patients were significantly higher than in healthy controls; when these patients were divided into subgroups, this difference was only significant in group 3 (Figure 1C). No significant differences in serum CFH, MBL, or C5b-9 were observed between IgAN subgroups (Figure 1).

Urinary CFH, MBL and C5b-9 levels in IgAN and controls

Urinary CFH levels were significantly higher in IgAN patients, including all subgroups, as well as in CKD patients when compared to healthy controls (Figure 2A). Moreover, IgAN patients of group 3 showed higher levels of urinary CFH than group 1 and 2 (Figure 2A). Patients with IgAN, including all subgroups, revealed higher levels of urinary MBL compared to healthy controls (Figure 2B). When combined, all IgAN patients, as well as the patients in group 3 revealed significantly higher levels of urinary MBL than the CKD patients (Figure 2B). Urinary C5b-9 levels were also higher in IgAN patients and in CKD patients compared to healthy controls;

however, when IgAN patients were divided into subgroups, this difference was only significant for group 3 (Figure 2C).

Thus, IgAN is associated with increased serum CFH and C5b-9, decreased serum MBL, as well as increased urinary excretion of all three complement proteins when compared to healthy controls. The same pattern was observed for CKD patients with non-proliferative disease except that these patients did not have significantly elevated serum C5b-9 and urinary MBL. There were no correlations between the levels of these complement proteins in urine and respective levels in serum from patients with IgAN (Figure S1).

Serum and urinary complement proteins, and crescent score in IgAN

The percentage of IgAN patients with crescents (C1-2) was significantly higher in both group 2 and 3 when compared to group 1 (Table 2), and patients with crescents (C1-2) had a lower eGFR than patients without (C0; 63.59 ± 35.52 versus 84.97 ± 39.08 ml/min/1.73m², $P = 0.01$). There was no significant difference in the glomerular deposition of C3 between IgAN subgroups (Table 2), or between the patients with C0 and C1-2 (data not shown). The serum CFH levels were significantly decreased in IgAN patients with C1-2 compared with the patients with C0 (1.12 [0.9, 1.5] versus 1.42 [1.13, 1.76] mg/ml, $P = 0.02$), while IgAN patients with C1-2 had significantly higher levels of urinary CFH and MBL than the patients with C0 (CFH/Cr: 220.49 [113.26, 375.42] versus 128.56 [67.62, 216.1] ng/mg, $P = 0.007$; MBL/Cr: 6.28 [2.44, 17.72] versus 3.06 [1.62, 5.82] ng/mg, $P = 0.02$). There were no significant differences in the levels of serum MBL, serum C5b-9 or urinary C5b-9 between the two groups (data not shown).

Correlations between urinary complement proteins, eGFR and proteinuria in IgAN

Urinary CFH, MBL and C5b-9 levels correlated inversely with eGFR in patients with IgAN (Figure 3A). The 24-hour urinary protein excretion significantly correlated with urinary CFH, but

not with urinary MBL or C5b-9 (Figure 3B). The findings show that in patients with IgAN the urinary excretion of complement proteins CFH, MBL and C5b-9 is associated with renal function (eGFR) while the correlation with total proteinuria is weak (CFH only). In patients with CKD no significant correlations were observed between urinary excretion of all three complement proteins and eGFR (Figure 3C). However, urinary CFH and MBL, but not C5b-9 correlated positively with 24-hour urinary protein excretion (Figure 3D).

Correlations between urinary complement proteins and markers of proximal tubule dysfunction

The urinary excretion of both RBP (Figure 4A-4C) and α 1-MG (Figure 4D-4F) correlated significantly and positively with the urinary excretion of CFH, MBL and C5b-9, but negatively with eGFR in patients with IgAN (Figure S2). A similar, positive correlation of urinary CFH, MBL and C5b-9 with the two tubular damage markers was observed in CKD patients (Table 3). Both RBP and α 1-MG are freely filtered in the glomerulus and reabsorbed by megalin, which is highly expressed in proximal tubule and serves as a scavenger for proximal tubule uptake of filtered proteins.²¹⁻²³ Thus, urinary excretion of RBP and α 1-MG may be considered markers of proximal tubule dysfunction. Together, the findings support the notion that the increased urinary excretion of these complement proteins correlates with proximal tubule dysfunction, and possible megalin dysfunction, in both IgAN and CKD.

Discussion

The present study shows 1) that IgAN is associated with increased levels of serum CFH and C5b-9 as well as decreased levels of serum MBL compared to healthy controls; however, similar changes in serum CFH and MBL were observed in CKD patients; 2) the levels of complement proteins (except MBL) in urine from both IgAN patients and CKD patients were higher than in healthy controls; 3) urinary CFH and MBL levels were significantly higher in IgAN

patients with crescent formation than in the patients without; and 4) the urinary complement protein excretion correlated inversely with eGFR and was strongly associated with tubular damage markers (RBP and α 1-MG) in patients with IgAN.

Complement activation has been associated with the pathogenesis of IgAN as part of the four-hit model: Increased circulating levels of galactose-deficient IgA1 (Hit 1) followed by synthesis and binding of antibodies directed against galactose-deficient IgA1 which form immune complexes accumulating in the glomerular mesangium (Hits 2 and 3), and finally activation of mesangial cells inducing proliferation and secretion of inflammatory mediators (Hit 4).^{2,24} Complement is activated, mainly via alternative and lectin pathway,² by the deposition of immune complexes and by the activation of mesangial cells, which eventually contributes to kidney tissue damage. CFH and MBL are important complement proteins associated with the alternative or lectin pathway. Previous studies have demonstrated elevated levels of urinary CFH, MBL and C5b-9 correlating with the degree of renal damage, suggesting that complement activation may occur within the urinary space; in line with this, it has been proposed that urinary complement components could be useful biomarkers of kidney injury in IgAN.^{12,14,15,25} However, no direct comparison with other types of kidney injury has been performed and the concomitant analysis of complement proteins in serum was not included.

We found that serum and urinary levels of CFH and urinary levels of C5b-9 were increased in both IgAN patients and other CKD patients when compared with healthy controls. Higher urinary levels of the three complement proteins were associated with a chronic histopathological phenotype in patients with IgAN. We did not identify any correlations between serum CFH and MBL with eGFR or proteinuria (data not shown). However, significant inverse correlations between the three urinary complement proteins (CFH, MBL and C5b-9) and eGFR were observed in IgAN. Taken together, our findings identified a potential association between

increased urinary levels of complement proteins and chronic renal damage. Urinary excretion of the three complement proteins is closely associated with the degree of renal function. While this could suggest accelerated complement activation with increasing disease severity, it is more likely a reflection of sclerotic renal damage, such as glomerulosclerosis and tubular atrophy, rather than active glomerular disease.

Recently, crescentic lesions have been added to the updated version of Oxford classification and biopsy reporting will provide a MEST-C score.¹⁹ IgAN patients with cellular or fibrocellular crescents were shown to be at risks of a poor renal outcome.¹⁸ In the present study, an elevation of both urinary CFH and MBL levels was observed in the patients with crescents formation (C1-2) compared to the patients without (C0). Further studies are necessary to assess the possible implications of this in relation to disease activity and complement activation.

Previous studies have shown an association between urinary complement proteins and tubular damage markers, including β 2-microglobulin, in IgAN as well as other types of proteinuric renal diseases.^{15,26} In line with this, we found significant correlations between the three complement proteins (CFH, MBL and C5b-9) in urine and the tubular damage markers, urinary RBP and α 1-MG, both known to be ligands to the endocytic receptor megalin.²¹⁻²³ Similar correlations were observed for CKD patients. Based on these findings, we speculate that urinary excretion of these complement proteins may be associated with proximal tubule dysfunction, and possible reduced megalin activity.

The source of urinary complement proteins remains unclear. Recent studies suggest that urinary CFH and MBL may derive from deposited CFH and MBL in renal tissue from patients with IgAN.^{12,14} Unfortunately, we did not have the opportunity to measure the renal deposition of these complement proteins in the patients. The urinary excretion of complement proteins did not

correlate with their respective levels in serum, but correlated negatively with eGFR and only weakly with proteinuria, which supports the notion that urinary complement proteins may not derive from the filtration of plasma proteins alone. It has been suggested that the kidney has the capacity to synthesize most of the complement components.^{27,28} Greater amounts of CFH transcript were observed in tubular fraction than in glomerular or medullar fractions of normal human kidney, and interferon-gamma has been shown to induce biosynthesis of CFH by human proximal tubular epithelial cells.²⁹ Thus, it is possible that the urinary complement proteins, at least in part, may be derived from intrarenal synthesis, and excretion to the urinary space.

There are some limitations in our study. Firstly, it was a single-center study with a single ethnic group and a limited number of patients. Secondly, this study is cross-sectional and does not allow us to evaluate disease progression in relation to serum or urinary complement protein levels. Thus, further studies are needed to evaluate if changes are associated with disease susceptibility or represent the consequence of such. It would definitely be interesting to carry out a study with follow-up, allowing assessment of the prognostic implications of our findings. Moreover, we did not measure all complement factors, and other complement proteins potentially involved in IgAN need to be examined.

Conclusions

In conclusion, our study suggests that increased urinary excretion of complement proteins in IgAN is related to chronic injury and proximal tubule dysfunction. This suggests caution using urinary complement components as markers of active glomerular disease. Further studies are important to clarify the pathophysiological mechanism underlying urinary complement excretion in IgAN.

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Declaration of interest

The authors have no conflicts of interest to declare.

References

1. Berthoux FC, Mohey H, Afiani A. Natural history of primary IgA nephropathy. *Semin Nephrol.* 2008; **28**:4-9.
2. Maillard N, Wyatt RJ, Julian BA, *et al.* Current understanding of the role of complement in IgA nephropathy. *J Am Soc Nephrol.* 2015; **26**:1503-12.
3. Daha MR, van Kooten C. Role of complement in IgA nephropathy. *J Nephrol.* 2016; **29**:1-4.
4. McCoy RC, Abramowsky CR, Tisher CC. IgA nephropathy. *Am J Pathol.* 1974; **76**:123-44.
5. Wyatt RJ, Julian BA. IgA nephropathy. *N Engl J Med.* 2013; **368**:2402-14.
6. Espinosa M, Ortega R, Gomez-Carrasco JM, *et al.* Mesangial C4d deposition: a new prognostic factor in IgA nephropathy. *Nephrol Dial Transplant.* 2009; **24**:886-91.
7. Miyamoto H, Yoshioka K, Takemura T, Akano N, Maki S. Immunohistochemical study of the membrane attack complex of complement in IgA nephropathy. *Virchows Arch A Pathol Anat Histopathol.* 1988; **413**:77-86.
8. Noris M, Remuzzi G. Overview of complement activation and regulation. *Semin Nephrol.* 2013; **33**:479-92.
9. Turner MW. Mannose-binding lectin: The pluripotent molecule of the innate immune system. *Immunol Today.* 1996; **17**:532-40.

10. Liu LL, Liu N, Chen Y, *et al.* Glomerular mannose-binding lectin deposition is a useful prognostic predictor in immunoglobulin A nephropathy. *Clin Exp Immunol.* 2013; **174**:152-60.
11. Miyazaki R, Kuroda M, Akiyama T, Otani I, Tofuku Y, Takeda R. Glomerular deposition and serum levels of complement control proteins in patients with IgA nephropathy. *Clin Nephrol.* 1984; **21**:335-40.
12. Zhang JJ, Jiang L, Liu G, *et al.* Levels of urinary complement factor H in patients with IgA nephropathy are closely associated with disease activity. *Scand J Immunol.* 2009; **69**:457-64.
13. Endo M, Ohi H, Ohsawa I, Fujita T, Matsushita M, Fujita T. Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy. *Nephrol Dial Transplant.* 1998; **13**:1984-90.
14. Liu LL, Jiang Y, Wang LN, Liu N. Urinary mannose-binding lectin is a biomarker for predicting the progression of immunoglobulin (Ig)A nephropathy. *Clin Exp Immunol.* 2012; **169**:148-55.
15. Onda K, Ohsawa I, Ohi H, *et al.* Excretion of complement proteins and its activation marker C5b-9 in IgA nephropathy in relation to renal function. *BMC Nephrol.* 2011; **12**:64.
16. Levey AS, Stevens LA, Schmid CH, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009; **150**:604-12.
17. Cattran DC, Coppo R, Cook HT, *et al.* The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int.* 2009; **76**:534-45.
18. Haas M, Verhave JC, Liu ZH, *et al.* A multicenter study of the predictive value of crescents in IgA nephropathy. *J Am Soc Nephrol.* 2017; **28**: 691-701.
19. Trimarchi H, Barratt J, Cattran DC, *et al.* Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. *Kidney Int.* 2017; **91**:1014-21.
20. Zhang Y, Yan X, Zhao T, *et al.* Targeting c3a/c5a receptors inhibits human mesangial cell proliferation and alleviates IgA nephropathy in mice. *Clin Exp Immunol.* 2017; **189**:60-70.

21. Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: From experimental models to human disease. *Kidney Int.* 2016; **89**:58-67.
22. Leheste JR, Rolinski B, Vorum H, *et al.* Megalin knockout mice as an animal model of low molecular weight proteinuria. *Am J Pathol.* 1999; **155**:1361-70.
23. Christensen EI, Moskaug JO, Vorum H, *et al.* Evidence for an essential role of megalin in transepithelial transport of retinol. *J Am Soc Nephrol.* 1999; **10**:685-95.
24. Suzuki H, Kiryluk K, Novak J, *et al.* The pathophysiology of IgA nephropathy. *J Am Soc Nephrol.* 2011; **22**:1795-803.
25. Liu M, Chen Y, Zhou J *et al.* Implication of urinary complement factor H in the progression of immunoglobulin A nephropathy. *PLOS ONE.* 2015; **10**:e0126812.
26. Nagamachi S, Ohsawa I, Suzuki H, *et al.* Properdin has an ascendancy over factor H regulation in complement-mediated renal tubular damage. *BMC Nephrol.* 2014; **15**:82.
27. Song D, Zhou W, Sheerin SH, Sacks SH. Compartmental localization of complement component transcripts in the normal human kidney. *Nephron.* 1998; **78**:15-22.
28. Zhou W, Marsh JE, Sacks SH. Intrarenal synthesis of complement. *Kidney Int.* 2001; **59**:1227-35.
29. Gerritsma JS, Gerritsen AF, De Ley M, van Es LA, Daha MR. Interferon-gamma induces biosynthesis of complement components C2, C4 and factor H by human proximal tubular epithelial cells. *Cytokine.* 1997; **9**:276-83.

Figure legends

Figure 1. Serum CFH, MBL and C5b-9 levels in patients with IgAN or CKD, and healthy controls. (A, B) Serum CFH levels were significantly increased and MBL levels decreased in IgAN patients, including all subgroups, and in CKD patients compared with healthy controls. (C) Serum C5b-9 levels were significantly increased in patients with IgAN compared with healthy controls; the levels were significantly higher in IgAN patients of group 3 than in healthy controls. CFH, complement factor H; MBL, mannose-binding lectin; C5b-9, membrane attack complex; IgAN, IgA nephropathy; CKD, chronic kidney disease. Healthy controls, n = 20; CKD patients, n = 23; IgAN patients, n = 85; IgAN-Group 1, IgAN patients with M0E0S0T0, n = 20; IgAN-Group 2, IgAN patients with M1E1S0-1T0-1, n = 25; IgAN-Group 3, IgAN patients with M1E1S0-1T2 or M0E0S1T1-2, n = 40. Each box plot represents the median and the 25th and 75th percentiles. Whiskers represent 1.5 times interquartile range. Differences among various groups were compared using Kruskal-Wallis test. The Bonferroni method was applied to correct for multiple comparisons. **P* (vs. Healthy controls) < 0.01.

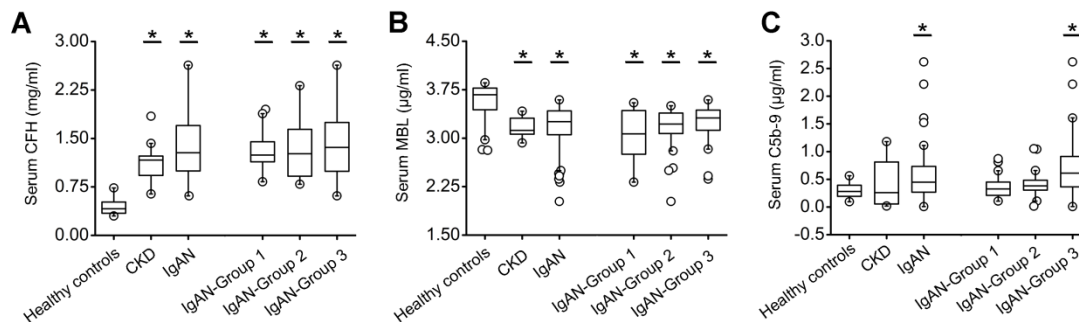


Figure 2. Urinary CFH, MBL and C5b-9 levels in patients with IgAN or CKD, and healthy controls. (A) Urinary CFH levels were significantly increased in IgAN patients, including all subgroups, and in CKD patients compared with healthy controls; the levels were significantly higher in IgAN patients of group 3 than in group 1 and 2. (B) Urinary MBL levels were significantly increased in patients with IgAN, including all subgroups, compared with healthy controls; the levels were significantly higher in all IgAN patients and the subgroup 3 than in CKD patients. (C) Urinary C5b-9 levels were significantly increased in all IgAN patients, including subgroup 3, and in CKD patients compared with healthy controls. CFH, complement factor H; MBL, mannose-binding lectin; C5b-9, membrane attack complex; IgAN, IgA nephropathy; CKD, chronic kidney disease; Cr, creatinine. Healthy controls, n = 20; CKD patients, n = 23; IgAN patients, n = 85; IgAN-Group 1, IgAN patients with M0E0S0T0, n = 20; IgAN-Group 2, IgAN patients with M1E1S0-1T0-1, n = 25; IgAN-Group 3, IgAN patients with M1E1S0-1T2 or M0E0S1T1-2, n = 40. Each box plot represents the median and the 25th and 75th percentiles. Whiskers represent 1.5 times interquartile range. Differences among various groups were compared using Kruskal-Wallis test. The Bonferroni method was applied to correct for multiple comparisons. **P* (vs. Healthy controls) < 0.01; #*P* (vs. CKD) < 0.01; §*P* (vs. IgAN-Group 1 and 2) < 0.001.

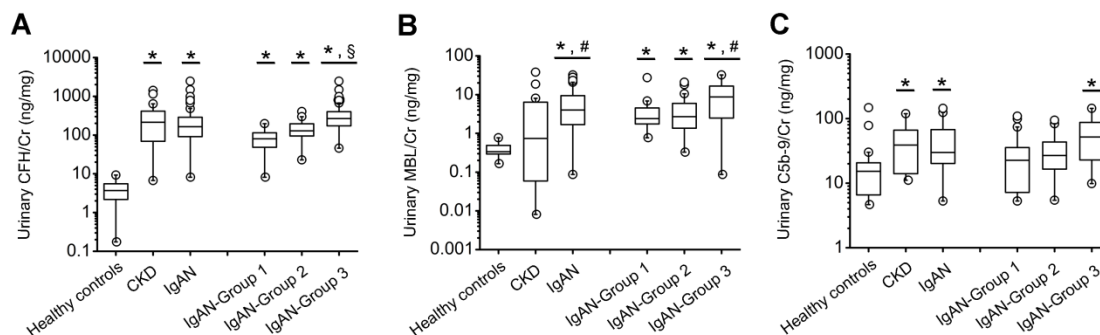


Figure 3. Correlations between urinary complement proteins and clinical parameters of patients with IgAN or CKD. (A) Urinary CFH, MBL, and C5b-9 levels correlated inversely with eGFR in patients with IgAN. (B) Urinary CFH levels significantly correlated with 24-hour urinary protein excretion in patients with IgAN whereas the levels of MBL and C5b-9 did not correlate with it. (C) No significant correlations were observed in CKD patients between eGFR and the levels of urinary CFH, MBL or C5b-9. (D) Urinary levels of CFH and MBL, but not C5b-9 significantly correlated with 24-hour urinary protein excretion in CKD patients. IgAN, IgA nephropathy; CKD, chronic kidney disease; CFH, complement factor H; MBL, mannose-binding lectin; C5b-9, membrane attack complex; eGFR, estimated glomerular filtration rate. The ratios of urinary CFH, MBL or C5b-9 to creatinine (Cr) were presented using logarithmic scale to make these conform or close to a normal distribution. The correlation among various parameters was analysed using Pearson or Spearman's coefficient.

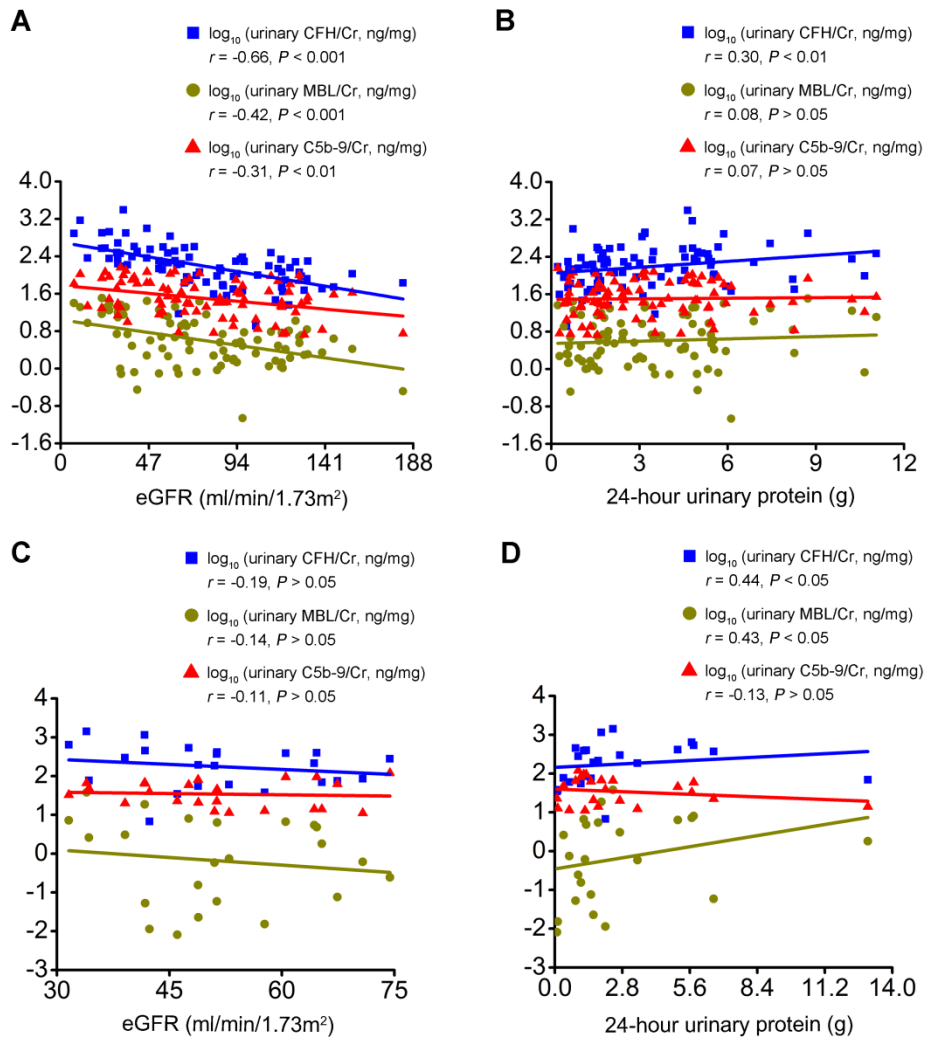


Figure 4. Correlations between urinary excretion of complement proteins and tubular damage markers, i.e., established megalin ligands: RBP and α 1-MG, in patients with IgAN.

The urinary excretion of RBP (A-C) and α 1-MG (D-F) significantly and positively correlated with the urinary excretion of CFH, MBL and C5b-9 in patients with IgAN. IgAN, IgA nephropathy; RBP, retinol-binding protein; α 1-MG, α 1-microglobulin; CFH, complement factor H; MBL, mannose-binding lectin; C5b-9, membrane attack complex. The ratios of urinary CFH, MBL or C5b-9 to creatinine (Cr) were presented using logarithmic scale to make these conform or close to a normal distribution. The correlation among various parameters was analysed using Spearman's coefficient.

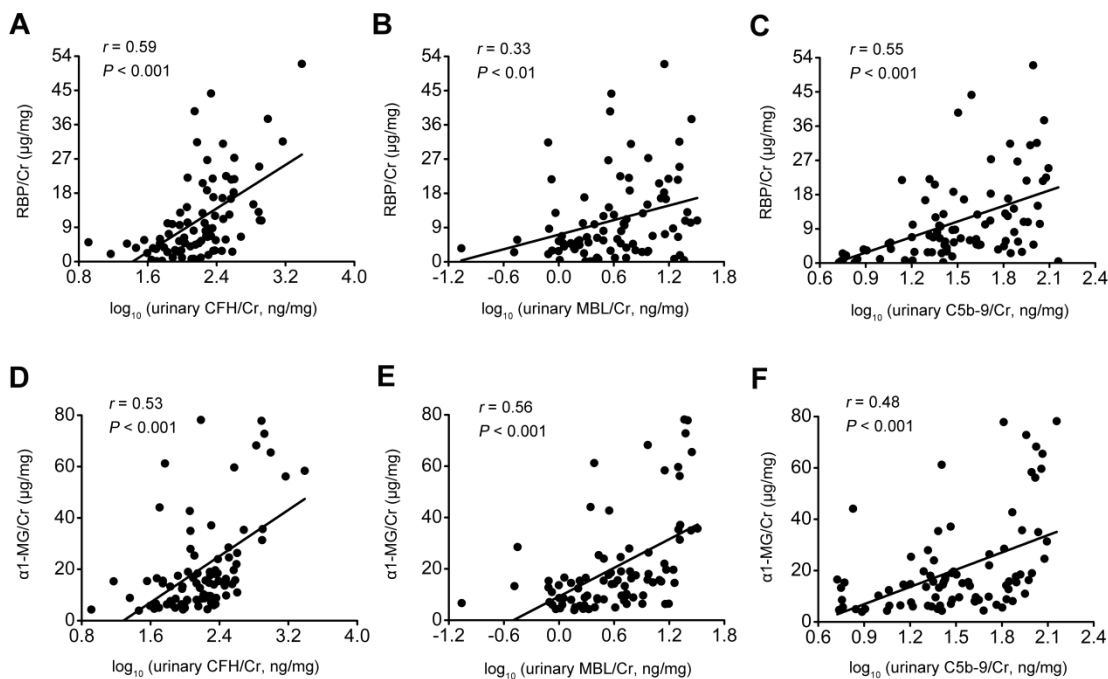


Table 1. Demographic and clinical data of all subjects.

Variables	Healthy controls n = 20	CKD n = 23	IgAN n = 85	IgAN-Group 1 n = 20	IgAN-Group 2 n = 25	IgAN-Group 3 n = 40
Age (year)	35 (27.5, 44)	44 (33, 51)	38 (27, 47)	36.5 (22, 46.3)	32 (24, 51)	39 (29, 47)
Gender, male, n (%)	11 (55.0)	14 (60.9)	50 (58.8)	12 (60.0)	11 (44.0)	27 (67.5)
MAP (mmHg)	89 (86, 92)	117 (96, 137)*	101 (93, 113)*	96 (90, 101)*	98 (92, 109)*	107 (99, 116)* [†]
Serum creatinine ($\mu\text{mol/L}$)	62 (54, 71.8)	142 (105, 156)*	94 (69.5, 157)* [§]	64.5 (55.3, 80.8) [#]	78 (63.5, 93)* [#]	166 (108.5, 226.5)* ^{†,‡}
eGFR (ml/min/1.73m ²)	117.4 (107.6, 123.7)	51 (41.8, 64.3)*	71.3 (43.3, 111.6)* [§]	117.9 (92.1, 129.5) [#]	94 (71.9, 115.3)* [#]	40 (30.5, 60.3)* ^{†,‡}
24-hour urinary protein (g)	0.04 (0.01, 0.05)	1.6 (0.97, 3.43)*	2.89 (1.47, 4.93)*	1.86 (0.61, 3.69)*	2.47 (1.34, 5.29)*	3.46 (1.72, 5.24)*
Treated with RAS blocker, n (%)	--	7 (30.4)	18 (21.2)	3 (15.0)	8 (32.0)	7 (17.5)

CKD, chronic kidney disease; IgAN, IgA nephropathy; MAP, mean arterial pressure; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system; group 1, patients with M0E0S0T0; group 2, patients with M1E1S0-1T0-1; group 3, patients with M1E1S0-1T2 or M0E0S1T1-2; patients with focal segmental glomerulosclerosis or hypertensive nephrosclerosis were included in CKD controls group. Values were described by absolute frequencies and percentages for categorical variables and median (lower and upper quartiles) for quantitative variables. Statistical analysis was done using Chi-square test (categorical variables) or Kruskal-Wallis test (quantitative variables) for comparisons between groups. The Bonferroni test was used for multiple comparison correction and the level of significance was of $P < 0.017$ or $P < 0.005$. * $P < 0.005$ vs. Healthy controls; [§] $P < 0.017$ vs. CKD; [#] $P < 0.005$ vs. CKD; [†] $P < 0.005$ vs. IgAN-Group 1; [‡] $P < 0.005$ vs. IgAN-Group 2.

Table 2. Histopathological characteristics of IgAN patients in different groups.

	Group 1 n = 20	Group 2 n = 25	Group 3 n = 40	P Value
Crescent scores				0.01
C 0, n (%)	19 (95)	10 (40)	22 (55)	
C 1-2, n (%)	1 (5)	15 (60)*	18 (45)*	
Glomerular deposition of C3				0.05
Score 0, n (%)	7 (35)	8 (32)	11 (27.5)	
Score 1, n (%)	9 (45)	7 (28)	6 (15)	
Score 2-3, n (%)	4 (20)	10 (40)	23 (57.5)	

Values were described by absolute frequencies and percentages for categorical variables.

IgAN, IgA nephropathy; group 1, patients with M0E0S0T0; group 2, patients with M1E1S0-1T0-1; group 3, patients with M1E1S0-1T2 or M0E0S1T1-2. Statistical analysis was done using Chi-square test for comparisons between groups. The Bonferroni test was used for multiple comparison correction and the level of significance was of $P < 0.017$. * $P < 0.017$ vs. Group 1.

Table 3. Correlations of urinary complement proteins with RBP and α 1-MG excretion in patients with CKD.

	Log₁₀ (CFH/Cr) n = 23		Log₁₀ (MBL/Cr) n = 23		Log₁₀ (C5b-9/Cr) n = 23	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Urinary RBP/Cr (μ g/mg)	0.76	<0.001	0.57	0.004	0.55	0.006
Urinary α 1-MG/Cr (μ g/mg)	0.64	0.001	0.61	0.002	0.55	0.007

CFH, complement factor H; MBL, mannose-binding lectin; C5b-9, membrane attack complex; CKD, chronic kidney disease; RBP, retinol-binding protein; α 1-MG, α 1-microglobulin; The ratios of urinary CFH, MBL or C5b-9 to creatinine (Cr) were presented using logarithmic scale to make these conform or close to a normal distribution. The correlation among various parameters was analysed using Pearson or Spearman's coefficient.