Circulating complement factor H–related protein 5 levels contribute to development and progression of IgA nephropathy

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IgA nephropathy (IgAN) is a disease associated with activation of the complement system. But the factors influencing complement activation in IgAN are not fully understood. Complement factor H (FH) is an essential negative regulator of complement C3 activation. Complement factor H–related protein (FHR)-5 shares high sequence similarity with factor H. However, unlike factor H, on binding to activated C3 it enables further activation to proceed. Previously, we reported the contribution of rare variants of the *CFHR5* gene to IgAN susceptibility. Here we compared circulating levels of FHR-5 in 1126 patients with IgAN and regular follow-up with those of 153 unrelated healthy individuals to explore the relationship of FHR-5 levels with IgAN development and progression. Circulating FHR-5 levels were significantly elevated in patients with IgAN compared to healthy individuals (median 4.55 [interquartile range 3.58 to 5.85] μg/ml vs 3.19 [interquartile range 2.55 to 3.92] μg/ml). Higher circulating FHR-5 levels were associated with a lower estimated glomerular filtration rate, hypertension, and severe Oxford-T and Oxford-C scores. High FHR-5 levels were independently and significantly associated with a risk of developing either a 30% decline in the estimated glomerular filtration rate or end-stage renal disease (hazard ratio, per standard deviation increment of natural square root transformed FHR-5 of 1.226; 95% confidence interval: 1.106-1.359). Thus, the circulating FHR-5 level is an independent risk factor for IgAN progression.

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IgA nephropathy (IgAN) is the commonest pattern of primary glomerulonephritis around the world.[1] Although the precise pathogenesis of IgAN is still unclear, the multi-hit model is currently the most widely accepted mechanism for IgAN. The model implicates the binding of galactose-deficient IgA1 molecules and anti-glycan antibody to form circulating immune complexes that accumulate in the glomerular mesangium and induce renal injury.[2] Complement component C3 is observed in both circulating immune complexes and glomerular deposits accompanied with IgA in IgAN,[3–5] suggesting a role for complement activation in IgAN. Moreover, the degree of mesangial C3 deposits was reported to predict renal outcome in patients with IgAN, which suggested a role for complement activation in IgAN progression.[6] However, the regulatory factors for complement activation in IgAN remain incompletely understood.

Complement factor H (FH) is an essential negative regulator of complement C3 activation through the alternative pathway and the C3b amplification loop.[7] In addition, 5 complement factor H–related proteins (FHR-1, FHR-2, FHR-3, FHR-4, FHR-5), which share high sequence similarity to FH exist.[8,9] Unlike FH, the FHR proteins do not contain complement regulatory domains. However, both FH and the FHR proteins contain C3b binding domains. *In vitro* studies have demonstrated that FHR-1, FHR-2, and FHR-5 could function as competitive antagonists of FH for the binding to C3b. This process, termed FH deregulation, could impair the ability of FH to negatively regulate C3 on surfaces such as the mesangium, and potentially enhance C3 deposition.[10,11]

The contribution of FH and FHRs to IgAN aroused considerable interest after chromosome location 1q32, which contains the *CFH*, *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5* genes, was identified as an IgAN-susceptible locus in a genome-wide association study.[12,13] In addition, the high clinical similarity between IgAN and CFHR5 nephropathy, which is caused by internal duplication of the *CFHR5* gene, implicated a specific role for FHR-5 in IgAN pathogenesis.[14,15] We previously screened the coding sequence of the *CFHR5* gene in IgAN patients and demonstrated the contribution of rare variants of the *CFHR5* gene in IgAN

susceptibility.[16] Additionally, Medjeral-Thomas *et al.* recently observed elevated circulating FHR-5 levels in IgAN, and an association between elevated FHR-5 levels and progressive IgAN.[17] These data suggested that FHR-5 could influence both susceptibility to IgAN and its severity.

In order to elucidate the contribution of FHR-5 levels to IgAN, we measured circulating FHR-5 levels in a large cohort of IgAN patients with regular follow-up, and we explored the relationship of FHR-5 levels with IgAN phenotypes and progression.

RESULTS

Demographic, clinical, and histological characteristics of patients with IgAN

We defined the time of renal biopsy as baseline for our recruited patients with IgAN. Among the 1126 recruited patients with IgAN, 569 (50.5%) were male and 557 (49.5%) female, with a median age of 33 (interquartile range [IQR] 26–42) years (Table 1). At the time of renal biopsy, the median proteinuria was 1.30 (IQR 0.69–2.52) g/24 hours, and average estimated glomerular filtration rate (eGFR) was 82.73 ± 30.56 ml/min per 1.73 m^2. In total, 551 (49.0%) IgAN patients presented with hypertension, defined as blood pressure over 140/90 or taking antihypertensive medications to prevent hypertension. Except for 21 patients with less than 8 glomeruli in biopsy specimens, the pathological lesions of 1105 patients were graded using the Oxford classification (MESTC scores). Mesangial hypercellularity (M1), endocapillary hypercellularity (E1), and segmental glomerulosclerosis (S1) were found in 739 (66.9%), 498 (45.1%), and 759 (68.7%) patients, respectively. For tubular atrophy and interstitial fibrosis (Oxford-T) and crescent (Oxford-C) lesions, T0, T1, and T2 were found in 715 (64.7%), 278 (25.2%), and 112 (10.1%) patients, while C0, C1, and C2 were found in 494 (44.7%), 491 (44.4%), and 120 (10.9%) patients. All patients were regularly followed up, with a median follow-up time of 43.5 (IQR, 24.0–79.0) months. During follow-up, 511 (45.4%) of 1126 patients received corticosteroids and/or immunosuppressive agents, and 373 (33.1%) reached the composite end point, defined as 30% eGFR decline or end-stage renal disease (ESRD).

Circulating FHR-5 levels are elevated in IgA nephropathy

Circulating FHR-5 levels in patients with IgAN (median 4.55 μg/ml, IQR 3.58–5.85) were significantly higher compared with healthy controls (median 3.19 μg/ml, IQR 2.55–3.92, $P < 0.001$, Figure 1a). Next, we investigated the correlation of FHR-5 levels with clinical and pathological manifestations in patients with IgAN. In the whole IgAN cohort, the FHR-5 levels showed weak but significant negative correlation with eGFR (correlation coefficient –0.159, $P < 0.001$, Figure 1b). However, firstly, when we compared IgAN patients with normal renal function (chronic kidney disease stage 1 and eGFR > 90 ml/min per 1.73 m^2) with healthy controls, circulating FHR-5 levels remained significantly elevated in the IgAN cohort (Figure 1c). Secondly, no correlation was found between circulating FHR-5 levels and eGFR in our diabetic nephropathy cohort, who presented with highly variable levels of eGFR (Figure 1d). This indicated that the raised FHR-5 levels in IgAN were not due to changes in eGFR. FHR-5 levels in patients with IgAN also showed significant but weak correlation with proteinuria (correlation coefficient 0.074, $P = 0.014$, Figure 1e) and were higher in IgAN patients with hypertension (Figure 1f).

We also noted differences in FHR-5 levels when we compared IgAN patients based on the Oxford classification (MESTC scores) (Figure 2). FHR-5 levels differed between patients when stratified according to Oxford-E, Oxford-T, and Oxford-C lesions (Figure 2b, d, and e), but not when stratified according to either Oxford-M (Figure 2a) or Oxford-S lesions (Figure 2c). FHR-5 levels were higher in patients with Oxford E0 (4.75 μg/ml, IQR 3.64–5.96, $P = 0.020$) compared with Oxford E1 (median 4.34 μg/ml, IQR 3.55–5.57). Patients with severe Oxford T2 (4.86 μg/ml, IQR 3.65–6.20) and T1 (4.96 μg/ml, IQR 3.96–6.29) lesions showed higher FHR-5 levels than those with Oxford T0 (4.38 μg/ml, IQR 3.46–5.53). FHR-5 levels showed a step-wise correlation with glomerular crescents. FHR-5 levels were higher in patients with Oxford-C2 (median 5.42 μg/ml, IQR 4.09–6.90) than Oxford-C1 (median 4.65 μg/ml, IQR 3.65–5.75), and lowest in patients with Oxford-C0 (median 4.29 μg/ml, IQR 3.35–5.65).

Circulating FHR-5 levels were significantly higher in male IgAN patients (median 4.98 μg/ml, IQR 3.93–6.18) compared with female IgAN patients (median 4.15 μg/ml, IQR 3.25–5.33, $P < 0.001$, Figure 3a). Because FHR-5 levels did not differ between male and female healthy controls (Figure 3b), the higher level in the male IgAN patients may reflect more severe disease (Table 1).

Clinical and histological features of IgAN patients when stratified according to circulating FHR-5 levels

Considering the highly variable clinical and pathological manifestations in patients with IgAN, and in order to explore the association of circulating FHR-5 levels with IgAN phenotypes, we divided patients into 4 equal groups according to the quartiles of the FHR-5 distribution (Table 1). Group 1 to group 4 were defined as IgAN patients with circulating FHR-5 levels of <3.576 μg/ml, 3.576 to 4.546 μg/ml, 4.546 to 5.850 μg/ml, and >5.850 μg/ml, respectively. We found that IgAN patients in groups with high FHR-5 levels showed more severe clinical and pathological manifestations than those with lower FHR-5 levels. From group 1 to group 4, patients had gradually decreased eGFR (87.63 ± 28.73 ml/min per 1.73 m^2 vs. 84.78 ± 29.41 ml/min per 1.73 m^2 vs. 84.30 ± 31.23 ml/min per 1.73 m^2 vs. 74.19 ± 31.26 ml/min per 1.73m^2; $P < 0.001$), increased incidence of hypertension (40.6% vs. 48.6% vs. 48.8% vs. 50.6%; $P < 0.001$), and increased proportion of higher Oxford T-scores (T1/T2: 49 [17.8%]/26 [9.4%] vs. 60 [21.6%]/27 [9.7%] vs. 76 [27.5%]/24 [8.7%] vs. 93 [33.8%]/35 [12.7%]; $P < 0.001$) and Oxford C-scores (C1/C2: 114 [41.3%]/16 [5.8%] vs. 122 [43.9%]/21 [7.6%] vs. 141 [51.1%]/32 [11.6%] vs. 114 [41.5%]/51 [18.5%]; $P < 0.001$). With regard to proteinuria and Oxford-E scores, differences