Elevated Factor H-related 1 and Factor H-related 5 Associate with End Stage Kidney Disease in C3 Glomerulopathy

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Background

C3 Glomerulopathy is an ultra-rare kidney disease

- Caused by dysregulation of the alternative pathway
- Characterized by C3b deposition in the glomerulus
- Considered a multifactorial disease
- 50% of patients progress to end-stage kidney disease (ESKD) within 10 years

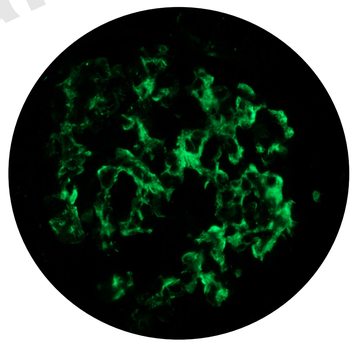

Figure 1. C3b deposition in the glomerulus. A kidney biopsy from a patient with C3G shows prominent immunostaining for C3 in the virtual absence of staining for other immunoreactants.

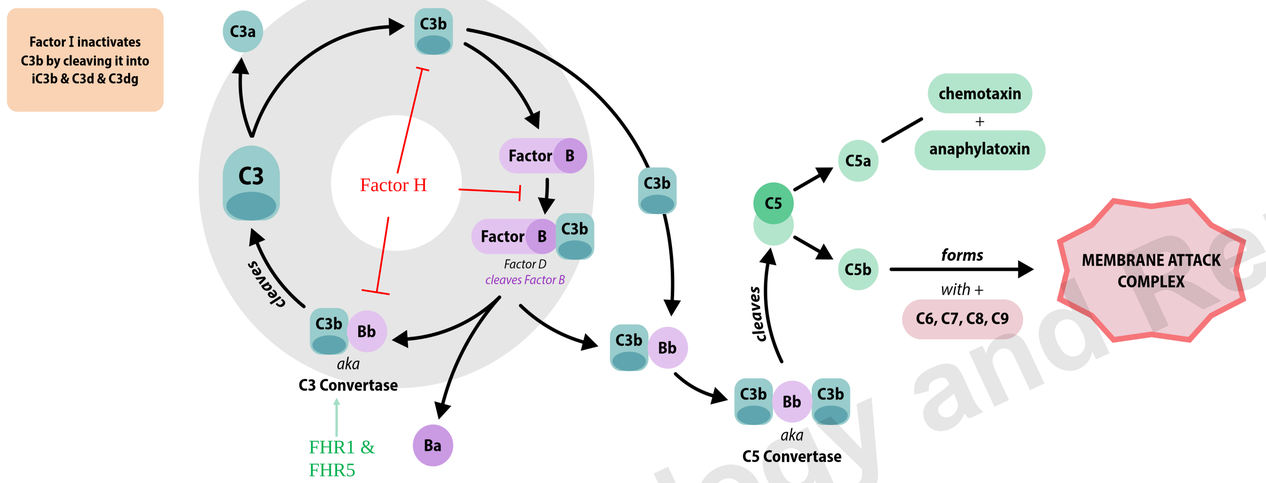

Figure 2. The alternative pathway of complement. C3 in the fluid phase is spontaneously hydrolyzed to C3(H2O), a process known as tick over. C3 leads to the generation of C3b, which binds factor B (FB). FB is then cleaved by factor D to form C3bBb, known as the C3 convertase. C3 convertase is negatively regulated by FH, thus preventing complement activity, while FHRs have the opposite effect. FH and FHRs, therefore, functionally compete for complement control. When C3 convertase interacts with an additional C3b, C5 convertase is formed. C5 convertase cleaves C5 into C5a and C5b. C5b binds C6, 7, 8 and several C9s to form membrane attack complex or soluble C5b-9 (sC5b-9).

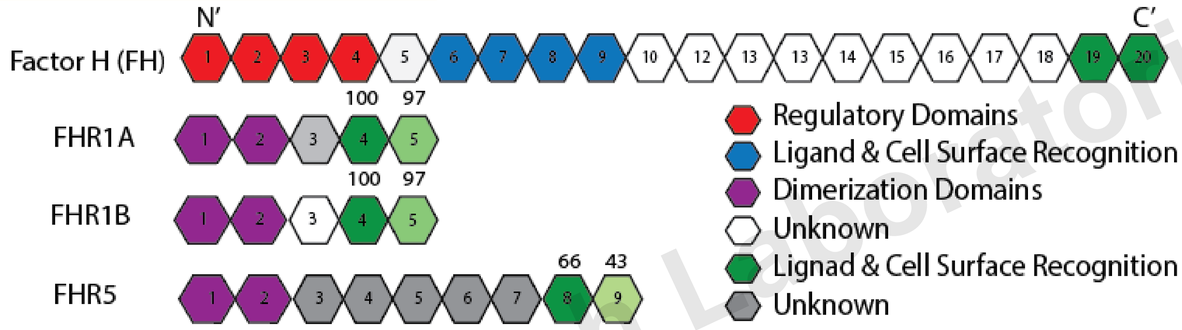

Figure 3. The human factor H family. Human factor H (FH) is comprised of 20 short consensus repeats (SCRs). SCRs 1-4 are regulatory domains that regulate the formation of the C3 convertase. SCRs 6-9 are weak ligand and cell surface recognition domains, while SCRs 19-20 strongly recognize ligands and cell surfaces such as the glomerular basement membrane (GBM). Human factor H-related 1 (FHR1) and factor H-related 5 (FHR5) contain unique dimerization SCRs (1-2) and C'-terminal ligand and cell surface recognition SCRs that are similar to SCRs 19-20 of FH. Hexagons represent SCRs, numbers above the hexagons represent amino acid similarity percentages to FH SCRs 19-20, and A and B represent the acid and basic forms of FHR1.

Stage of Kidney Disease	Glomerular Filtration Rate	Kidney Function
Stage 1	≥90ml/min	Normal
Stage 2	60-89ml/min	Mild
Stage 3	30-59ml/min	Moderate
Stage 4	15-29ml/min	Severe
Stage 5	<15ml/min	End Stage Renal Disease

Table 1. Stages of chronic kidney disease. Chronic kidney disease (CKD) as observed in C3G patients is divided into five stages: 1) normal or high CKD glomerular filtration rate (GFR) ≥90ml/min, 2) mild CKD GFR 60-89ml/min, 3) moderate CDK 30-59ml/min, 4) severe CKD GFR 15-29ml/min, and 5) ESKD <15ml/min. GFRs for adults were calculated using age, gender, creatinine, and race while pediatric GFRs were calculated using creatinine, height, and gender.

Hypothesis & Methods

Hypothesis: The concentration of FHR1 and FHR5 will be higher in more severe stages of chronic kidney disease.

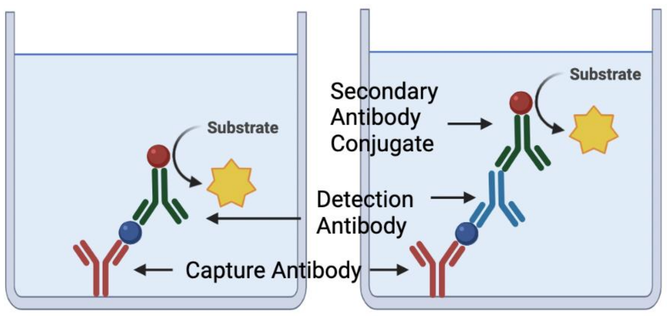

Figure 4. Sandwich enzyme-linked immunosorbent assay (ELISA). All ELISAs performed are colorimetric sandwich ELISAs in which a capture antibody is followed by the addition of patient serum/plasma; a detection antibody specific to the complement protein being detected is then added. If necessary, a secondary antibody conjugate is included for colorimetric detection. Measurements were read at 450nm, with peak absorbance spectra of 3,3',5,5'-tetramethyl-benzidine (TMB) and stop solution hydrochloric acid.

Results

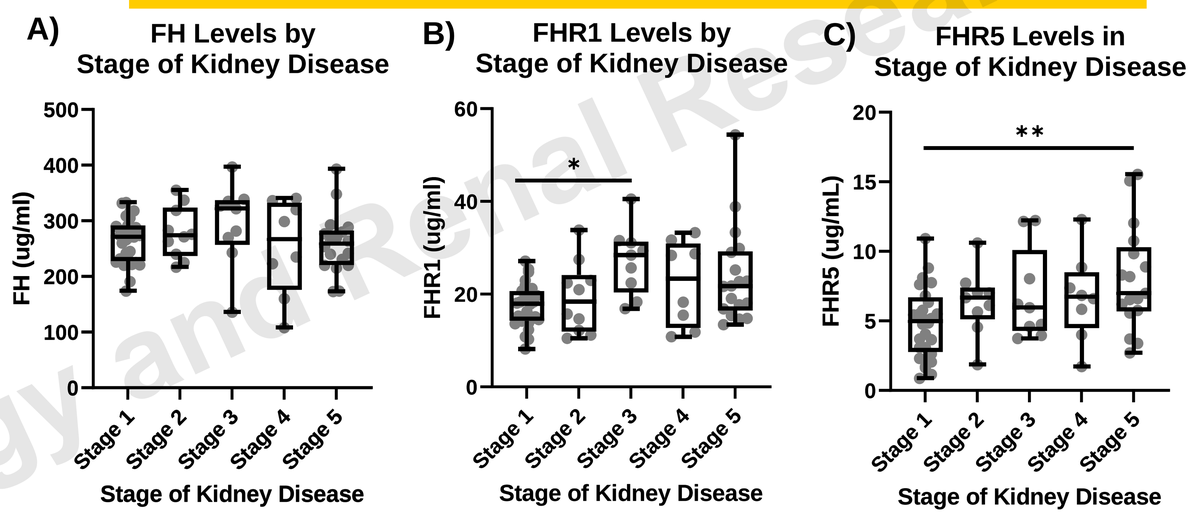

Figure 5. Factor H, Factor H-related 1, and Factor H-related 5 concentrations in C3G patients across stages of chronic kidney disease. Serum and/or plasma were collected from C3G patients with two copy numbers across the *CFH-CFHR5* genomic region. The stage of CKD for each patient was determined as stated in Table 1. Proteins concentrations were measured by ELISAs (ug/ml). **(A)** FH concentrations are not significantly different across stages of CKD. **(B)** FHR1 concentrations trend upward with more severe CKD (Kruskal-Wallis with multiple comparisons, p-value = 0.05). **(C)** FHR5 concentrations significantly increase with more severe CKD (Kruskal-Wallis with multiple comparisons, p-value = 0.01).

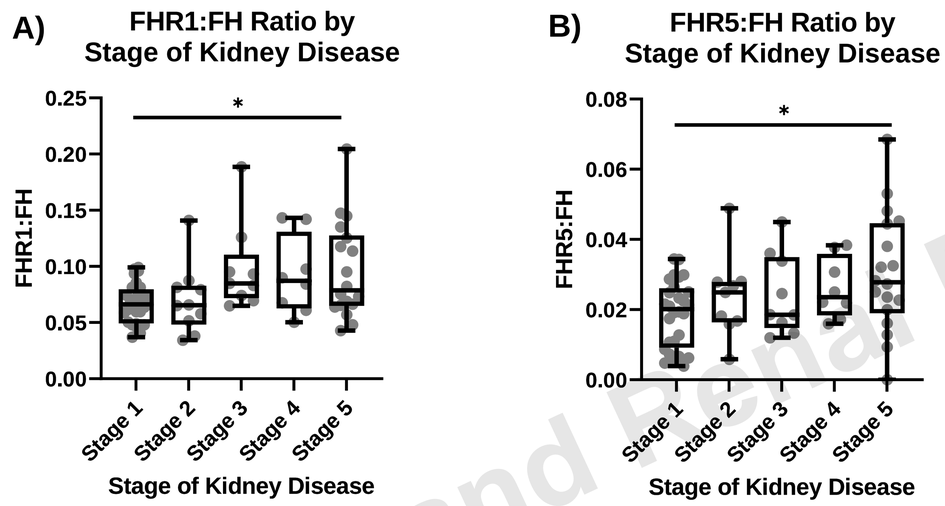

Figure 6. Complement protein ratios FHR1:FH and FHR5:FH in C3G patients across stages of chronic kidney disease. Box plots show the ratios of FHR1:FH and FHR5:FH from C3G patients with two copy numbers across the *CHF-CFHR5* genomic region. The stage of CKD for each patient was determined as stated in Table 1. **(A)** The ratio of FHR1: FH significantly increases with more severe CKD (Kruskal-Wallis with multiple comparisons, p-value = 0.05). **(B)** The ratio of FHR5:FH significantly increases with more severe CKD (Kruskal-Wallis with multiple comparisons, p-value = 0.05).

Conclusions

- FHR1 and FHR5 concentrations increase with more severe chronic kidney disease
- The ratios FHR1:FH and FHR5:FH increase with more severe chronic kidney disease
- FHR1:FH and FHR5:FH ratios maybe potential biomarkers for severity of disease

Acknowledgements/ References

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