A Genetic Insight: Factor H-Related 1 in Atypical Hemolytic Uremic Syndrome and C3 Glomerulopathy

A Slagle[1,2], M Jones[1], N Meyer[1], A taylor[1], N Ghiringhelli Borsa[1], C Nester[1], Y Zhang[1], RJH Smith[1,2]
[1]*Molecular Otolaryngology and Renal Research Laboratories, Department of Otolaryngology, University of Iowa*
[2]*Graduate Program in Immunology, University of Iowa*

Introduction

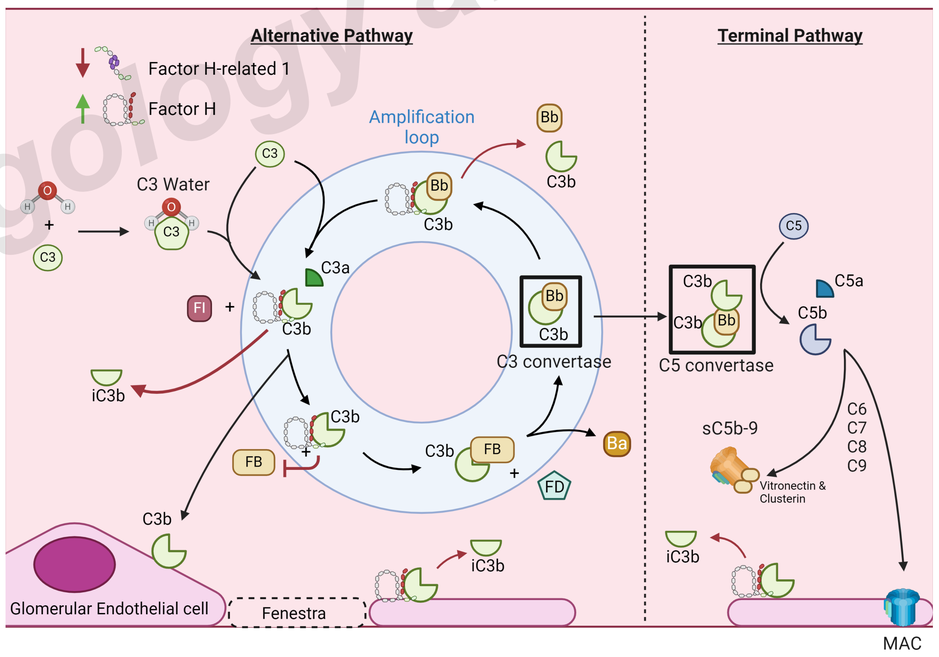

Figure 1. The alternative pathway of Complement. C3 in the fluid phase is spontaneously hydrolyzed to C3(H2O), a process known as tick over. Cleavage of C3 leads to the generation of anaphylatoxin C3a and opsonin C3b, which binds factor B (FB). FB is then cleaved by factor D (FD) to form C3bBb, known as the C3 convertase. C3 convertase is negatively regulated by Factor H (FH), thus preventing complement activity, while FH related proteins (FHRs) are thought to have the opposite effect. FH and FHRs, therefore, functionally compete for complement control. The C3 convertase then proceeds in one of two directions 1) cleave additional C3 molecules into C3a and C3b, creating an amplification loop or 2) interacts with an additional C3b to form the C5 convertase. C5 convertase is the start of the terminal pathway and cleaves C5 into C5a and C5b. C5b binds C6, C7, C8, and several C9s to form the membrane attack complex (MAC) or binds with vitronectin and clusterin to form soluble C5b-9.

Atypical Hemolytic Uremic Syndrome (aHUS) and C3 Glomerulopathy (C3G) are complex diseases that often progress to end stage kidney failure. While both present with distinct clinical features and required different treatment strategies, they share similarities in their pathophysiology - dysregulation of the alternative pathway (AP) of complement. The AP is a continuously active process that is the first line of host defense however, overactivation of the AP can lead to prolonged inflammation. Mediators of the AP, such as Factor H (FH), regulate the amplification loop through short consensus repeats (SCRs) 1-4, known as the regulatory domains, preventing excessive activation (Figure 1). FHR1 however, lacks the regulatory SCRs 1-4 found in FH but is very similar to FH in key binding domains located on the C-terminus. Specifically, the acidic isoform of FHR1 (FHR1*A) is five amino acids different than FH at the C-terminus, while the basic isoform of FHR1 (FHR1*B) is only 2 amino acids different than FH at the C-terminus (Figure 2). These similarities and differences lead us to believe FHR1 functionally competes with FH to enhance complement responses and that FHR1*B posses' stronger competition against FH than FHR1*A. Thus, we hypothesize in complement driven disease such as aHUS and C3G, patients will favor the FRH1*B genotype over the FHR1*A and that FHR1*B will have more robust C3b deposition on cell surfaces compared to FHR1*A due to heightened AP activation. Therefore, we aimed to determine the genotype frequencies of FHR1*A and FHR1*B in aHUS and C3G compared to healthy controls as well as the impact of FHR1*A and FHR1*B on complement activity on cell surfaces.

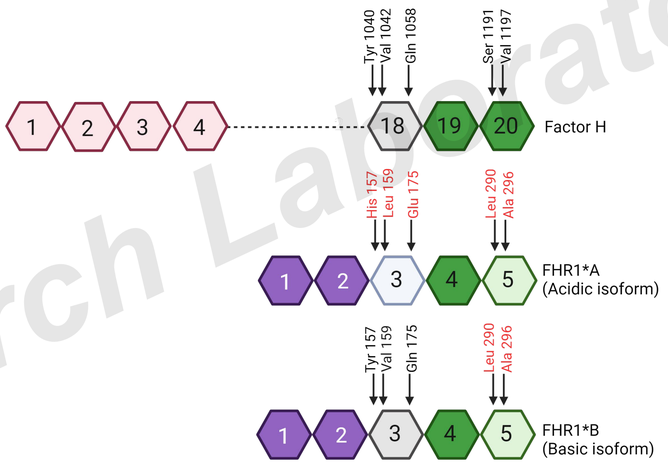

Figure 2. Schematic of FH, FHR1*A, and FHR1*B. FH is comprised of 20 SCRs, of which SCRs 1-4 mediate regulation of the AP and SCRs 19-20 mediate cell surface recognition and binding. The FHR1*A and FHR1*B SCRs are aligned with corresponding related SCRs of FH. SCR 3 of FHR1*B is identical to SCR 18 of FH while SCR 3 of FHR1*A is three amino acids different than SCR 18 of FH, indicated in red. Additionally, SCR 5 of both FHR1*A and FHR1*B are only 2 amino acids different than SCR 20 of FH, indicated in red.

Methods

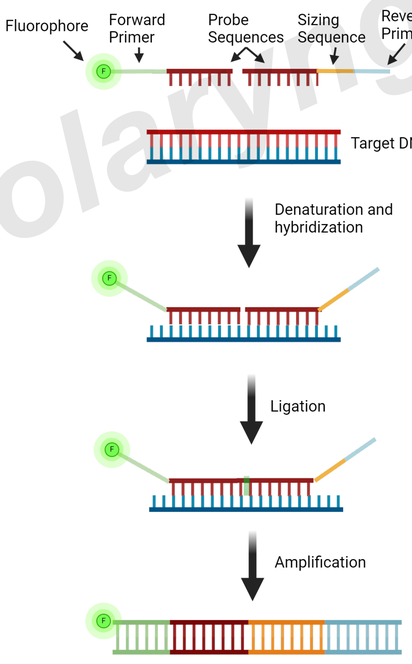

Figure 2. Multiplex ligation-dependent probe amplification. Multiplex ligation-dependent probe amplification (MLPA) is a molecular biology technique used to amplify targeted DNA sequences to detect copy number variants (CNVs) such as gene deletions and duplications. In this study, oligonucleotide probes were designed against the *CFH* gene cluster and were labeled with a fluorophore. Genomic DNA was isolated from C3G patients and denatured through heating of the DNA. The probes were then added to the denatured target DNA to hybridize to complementary sequence on the denatured target DNA. Next, the enzyme ligase joined adjacent probes hybridized to the target DNA to form one long probe. Polymerase chain reaction (PCR) was performed to amplify the target DNA. The final steps include gel electrophoresis to separate DNA fragments by size followed by fluorescence detection to quantify the amount of target DNA.

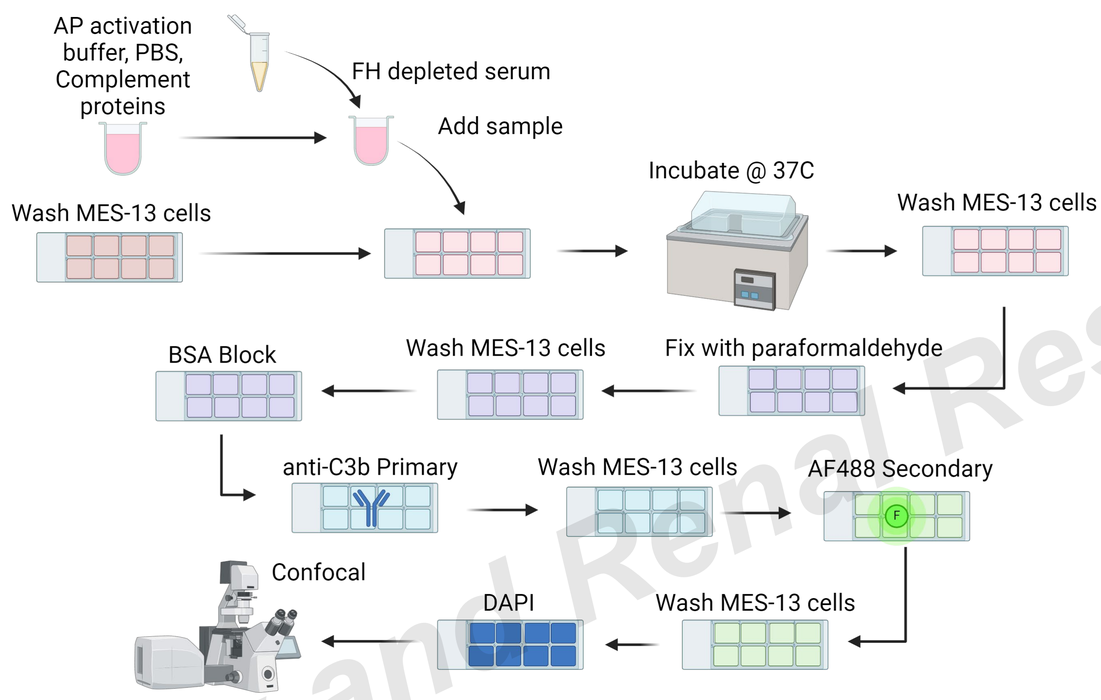

Figure 3. C3b deposition assay. The C3b deposition assay measures complement-mediated C3b deposition on mouse mesangial cell (MES-13) surfaces through generation of the AP C3 convertase. To begin MES-13 cells are washed followed by samples preparation in low binding 96 well plates. Samples are comprised of AP activation buffer, 1XPBS, FH depleted serum, FH, and FHR proteins at various concentrations. Immediately following sample preparation, samples are pipetted onto MES-13 cells and incubated at 37C. Cells are then washed with AP activation buffer and fixed using paraformaldehyde. Following fixation cells are blocked with BSA, incubated with a primary anti-C3b antibody and then an AF488 secondary antibody. Slides are mounted using aqua mount DAPI. Images are taken using a Lecia Confocal microscope with a 10X objective and analyzed using ImageJ software.

	Iowa control (n = 505)	Iowa C3G (n = 273)	Iowa aHUS (n = 227)
Genotype Frequencies			
A/A	0.20 (100)	0.26 (71)	0.14 (31)
B/B	0.11 (55)	0.12 (32)	0.13 (29)
Del/Del	0.06 (32)	0.05 (13)	0.14 (31)
A/Del	0.20 (100)	0.18 (48)	0.16 (36)
B/Del	0.17 (88)	0.10 (26)	0.14(32)
A/B	0.26 (130)	0.30 (81)	0.29 (66)
A/A/B	0.0 (0)	0.0 (0)	0.009 (2)
A/A/A	0.0 (0)	0.004 (1)	0.0 (0)
A/A/A/A	0.0 (0)	0.004 (1)	0.0 (0)

Table 1. FHR1 isoform frequencies in C3 glomerulopathy and atypical hemolytic uremic syndrome patients compared to controls. A control population (n = 505) and patients with C3G (n = 273) or aHUS (n = 227) were ascertained from the Iowa database. FHR1*A, FHR1*B, and homozygous (hmz) and heterozygous (het) *CFHR1* deletions (Δ*CFHR1*) were resolved by MLPA. P-values <0.05 were considered significant (Fisher's exact test). Numbers in parentheses indicate number of participants in the study. FHR1*A and FHR1*B isoforms were observed in: control FHR1*A 430 alleles, FHR1*B 328 alleles; C3G FHR1*A 272 alleles, FHR1*B 171 alleles; aHUS FHR1*A 164 alleles, FHR1*B 157 alleles. Hmz and het Δ*CFHR1* were identified in: control hmz-Δ*CFHR1* 64 alleles, het-Δ*CFHR1* 188 alleles; C3G hmz-Δ*CFHR1 2*6 alleles, het-Δ*CFHR1* 74 alleles; aHUS hmz-Δ*CFHR1 62* alleles, het-Δ*CFHR1* 68 alleles. The C3G cohort has a significantly lower het-Δ*CFHR1* frequency compared to controls. The aHUS cohort has a significantly higher Δ*CFHR1* frequency compared to controls and C3G. Copy number differences were also observed with >2 copies of *CFHR1* found in both the C3G and aHUS cohorts. *Fishers' exact tests: 1) Iowa Control vs Iowa C3G p-value = 0.0115, 2) Iowa Control vs Iowa aHUS p-value = 0.00001, and 3) Iowa C3G vs Iowa aHUS p-value = 0.00019*

Results

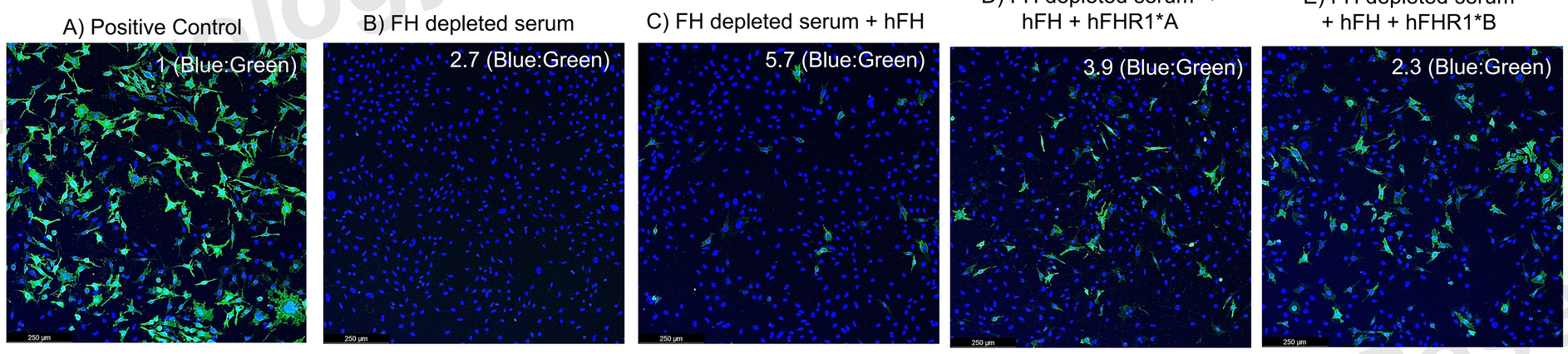

Figure 4. C3b Deposition on mouse mesangial cells. Mouse mesangial cells (MES-13) were incubated with positive control serum or FH depleted serum spiked with human FH (hFH) and human FHR1*A (hFHR1*A) or human FHR1*B (hFHR1*B). C3b deposition is seen in green and MES-13 nuclei in blue. Amount of C3b deposition was quantified using a ratio of nuclei to C3b in ImageJ. A) MES-13 cells incubated with positive control serum. B) MES-13 cells incubated with FH depleted serum which uses up C3b within second of activation preventing visualization of C3b deposition on cell surfaces. C) MES-13 cells incubated with FH depleted serum and 165ug hFH shows low C3b deposition compared to FH depleted serum alone. D) MES-13 cells incubated with FH depleted serum, 165ug hFH, & 10ug hFHR1*A increased C3b deposition compared to FH depleted serum with 165ug hFH. E) MES-13 cells incubated with FH depleted serum, 165ug hFH, & 10ug hFHR1*B increased C3b deposition compared to FH depleted serum with 165ug hFH.

Conclusions

- The acidic isoform of FHR1 (FHR1*A) is favored in C3G
- aHUS favors homozygous deletions of FHR1
- Increased copy numbers of FHR1 are associated with development of chronic kidney disease.
- FHR1 enhances C3b deposition on MES-13 cells.
- FHR1*B results in increased C3b deposition compared to FHR1*A on MES-13 cells.

Discussion

These findings suggest a potential underlying genetic predisposition for development of chronic kidney disease. Increased copy numbers of FHR1 predispose the kidney microenvironment to chronic inflammation by tipping the balance in favor of complement activation. Heighten AP activation can result in increased deposition of complement fragments, such as C3b, on cell surface which in turn creates a pro-inflammatory state. This proinflammatory state overtime results in damage to cell surfaces and therefore, decreased kidney function typical of C3G and aHUS.

Acknowledgements& References

This work was sup-ported by NIH Grant R01 DK110023 (CMN and RJS) and T32AI 007485 (KJ). We also appreciate the assist-ance provided by the Graduate Program in Immunology at the University of Iowa, Iowa City, IA. Images were created using Biorender.com.