A Functional Assay to Assess the Impact of Missense Variants in Complement Factor I

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Background

Complement Factor I (FI) is a serine protease that plays a vital role in regulating three complement pathways. In conjunction with its cofactors – complement factor H (FH), membrane cofactor protein (MCP or CD46), soluble complement receptor 1 (sCR1) or C4 binding protein (C4BP) – FI inactivates either C3b or C4b through proteolytic cleavage on cell surfaces and in the fluid phase.

FI deficiency, arising from genetic variation that can reduce expression or affect functions, leads to unchecked complement activation. However, using the American College of Medical Genetics and Genomics (ACMG) criteria, most *CFI* rare variants (RVs) identified in patients remain classified as variants of unknown significance (VUS). This classification reflects the fact that functional evidence is scarce and inconsistent, segregation or *de novo* data are limited, and allele frequencies do not meet pathogenicity thresholds, partly due to incomplete penetrance.

Previous studies have sought to determine the functionality of mutant FI protein using recombinant constructs, cell culture, and/or splicing assays. Herein, we present a cell-based hemolytic assay to directly assess the functional impact of *CFI* RVs.

Patients and FI level

We identified 68 patients carrying 51 *CFI* variants that were VUSs or with a minor allele frequency <1%. Twenty variants resulted in low FI expression (Type I) while 31 exhibited normal levels. FI levels were measured by ELISA (Quidel) or Radial Immunodiffusion (The Binding Site).

1 Novel Functional Assays

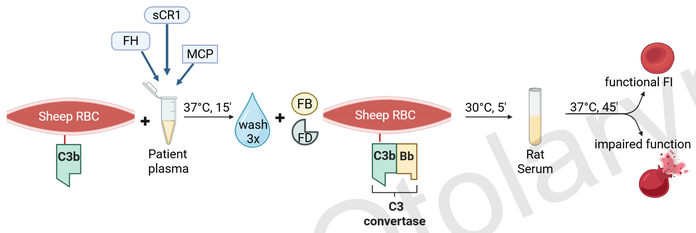

Figure 1. Cofactor-Dependent Factor I Functional Assay. Patient serum or plasma was diluted (1:32) to eliminate endogenous FH activity; and incubated with C3b-decorated sheep erythrocytes supplemented with one of three FI cofactors: FH, sCR1, or MCP. Cells were washed ×3 in GVB-Mg-EGTA to remove unincorporated serum/cofactor. FB and FD were added to enable formation of C3 convertase. The addition of rat serum allowed the membrane attack complex to form. Lysis was measured @ A415.

2 FI Level–function Correlation

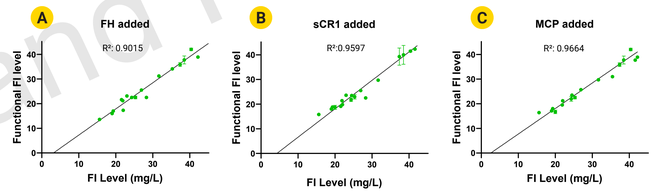

Figure 2. FI levels by ELISA and cofactor-dependent assays. FI levels in patients without *CFI* variants were measured using ELISA and cofactor-dependent assays with FH (A), sCR1 (B), or MCP (C). A strong correlation was observed between FI expression and function.

3 Experimental Controls

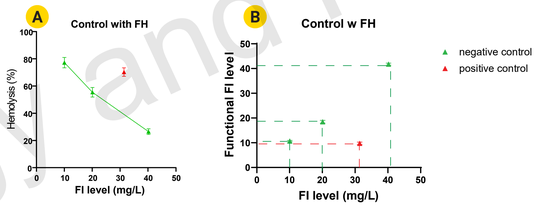

Figure 3. Standard Curve and Positive/Negative Controls.

A. In each experiment, plasma sample from a patient carrying no known *CFI* pathogenic variants was used to create a standard curve using 40, 20 and 10 mg/L FI. Additionally, a patient carrying a heterozygous p.(Arg336Gly) variant was used as a positive control. Calculated FI function tightly correlated with patient plasma levels of FI.

B. Positive and negative controls showed consistency over time, suggesting that calculated functional FI levels (dashed lines) were consistent across experiments, independent of sheep erythrocyte characteristics.

4 Functional Effects of *CFI* RVs

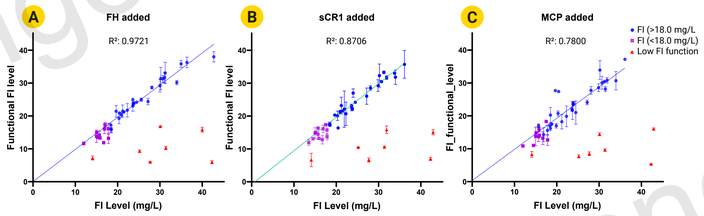

Figure 4. Cofactor-Dependent Assays Reveal Type II RVs.

The functional impact on FI cofactor activity with either FH (A), sCR1 (B), or MCP (C) in patients carrying at least one *CFI* RV. Normal FI level and function (blue circles) or low FI level with normal function (purple squares) are noted. Eight patients with seven distinct RVs had reduced cofactor function, despite normal expression (red triangles), indicating Type II variants (red stars in Figure 5).

5 Impact of Rare *CFI* Variants

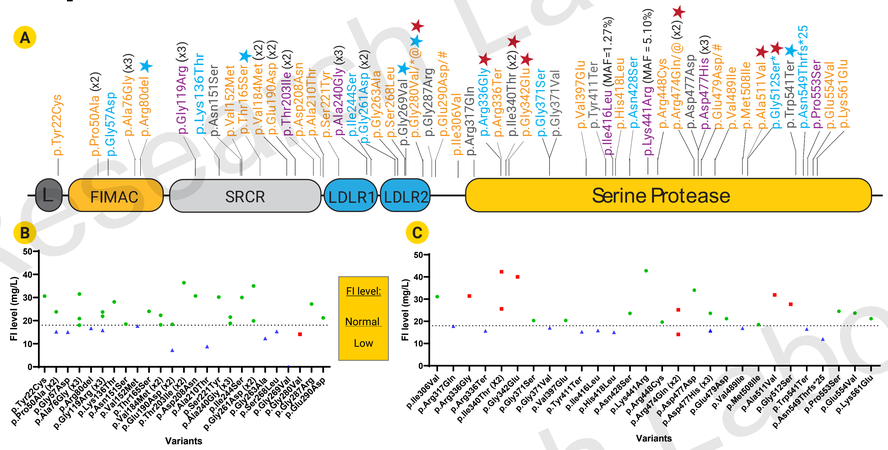

Figure 5. *CFI* Rare Variants Show Variable Expression and Function. Factor I is composed of 5 domains (FIMAC, Factor I Membrane Attack Complex; SRCR, Scavenger Receptor Cysteine-Rich; two LDLRs, Low-Density Lipoprotein Receptor; and the Serine Protease domain), with RVs occurring in all domains. The L, leading sequence is cleaved.

A. Patients who carry VUSs in *CFI* in this cohort had a variety of phenotypes (orange, TMA; blue, C3G; purple, both C3G and TMA; gray, other). Novel variants are noted with a blue star. Variants with impaired cofactor function are noted with a red star. Compound heterozygotes are also indicated (@, *, or #).

B. And C. RVs in *CFI* can result in either reduced, type I (blue triangles), or normal FI expression (green circles); the normal threshold is noted by dashed line. In this study, we demonstrate that 7 variants: p.(Gly280Val), p.(Arg336Gly), p.(Ile340Thr), p.(Gly342Glu), p.(Arg474Gln), p.(Ala511Val), and p.(Gly512Ser) have impaired cofactor function despite normal levels (Type II variants, red squares).

Discussion and Conclusions

1. **Our novel assays effectively demonstrate the functional impact of missense variants on FI cofactor activity which can lead to dysregulation of the alternative pathway.**
2. **In our population, 47% of patients with VUSs in the serine protease domain have normal FI levels, but 30% of these have impaired cofactor function.**
3. **The p.(Gly119Arg) variant is relatively common (present in 9 patients; tested in 3). This variant can be seen with normal and low FI levels but consistently retained normal cofactor function. Our previous study demonstrated impaired splicing for this variant. Given its location in the SRCR domain, the absence of an effect on protease function is plausible.**
4. **Importantly, low FI expression alone should not be considered causative of disease. For example, in our population there are patients who carry the p.(Pro50Ala) variant and have normal or low expression, but cofactor function does not appear to be impaired.**
5. **These findings highlight the need for multiple complementary approaches to assess the pathogenicity of *CFI* variants, as no single test can establish causality for all variant types.**

Acknowledgments

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