Assessing *CFI* Variants Using a Complement Factor I Functional Assay

Renee X Goodfellow*, Nicole C Meyer*, Sarah M Roberts*, Cobey JH Donelson, Nicolo Ghiringhelli Borsa, Amanda O Taylor, Carla M Nester, Richard JH Smith, Yuzhou Zhang

Molecular Otolaryngology and Renal Research Laboratories, Carver College of Medicine, University of Iowa

Background

Complement factor I (FI), encoded by *CFI*, is a serine protease essential for regulating complement activation. In the presence of cofactors like factor H (FH), Membrane Cofactor Protein (MCP/CD46), C4 Binding Protein (C4BP), or soluble Complement Receptor 1 (sCR1), FI cleaves C3b and C4b to limit C3 convertase formation. Rare *CFI* variants cause FI deficiency through either reduced protein expression (classified as Type I deficiency) or impaired activity despite preserved protein levels (classified as Type II deficiency). Functional assessment is therefore important for defining the pathogenicity of *CFI* variants in complement-mediated disease.

Genetic Analysis and Expression Data Identify Candidates for Functional Assessment

69 patients carrying rare variants (RVs, MAF<1%) in *CFI* were identified: 63 heterozygous, 4 compound-heterozygous, and 2 homozygous cases, representing 57 distinct RVs. Based on low FI expression as measured by ELISA (Quidel) or Radial Immunodiffusion (The Binding Site), 19 variants were classified as Type I.

Cofactor-Dependent Assay Measures C3b Inactivation by Patient-Derived FI

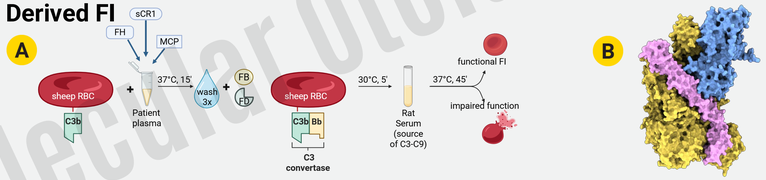

Figure 1: C3b-coated sheep erythrocytes were incubated with patient serum or plasma diluted 1:32 and supplemented with purified cofactors at 37°C for 15 minutes (A). Residual surface C3b was quantified by adding factor B, factor D, and rat serum as a source of terminal complement components to induce hemolysis. Higher hemolysis indicated impaired FI-mediated C3b inactivation. Molecular model (B) shows the interaction of C3b (yellow), FH (pink) and FI (blue).

FI Function as a Linear Correlation to Expression Level in Patients with no RVs Regardless of Cofactor Used

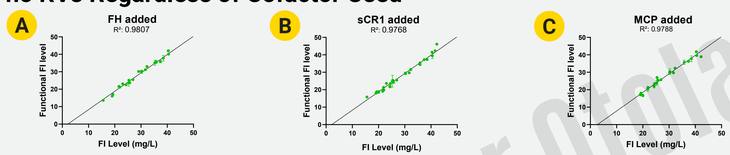

Figure 2: Cofactor-dependent assays using plasma from patients without RVs and normal FI levels showed inactivation of C3b by cofactors: FH (A), sCR1 (B), or MCP (C). A strong linear correlation between normal FI expression and function (R^2>0.97) was observed for each cofactor.

Patients with Complement-Mediated Disease Carry Type I and Type II Rare *CFI* Variants

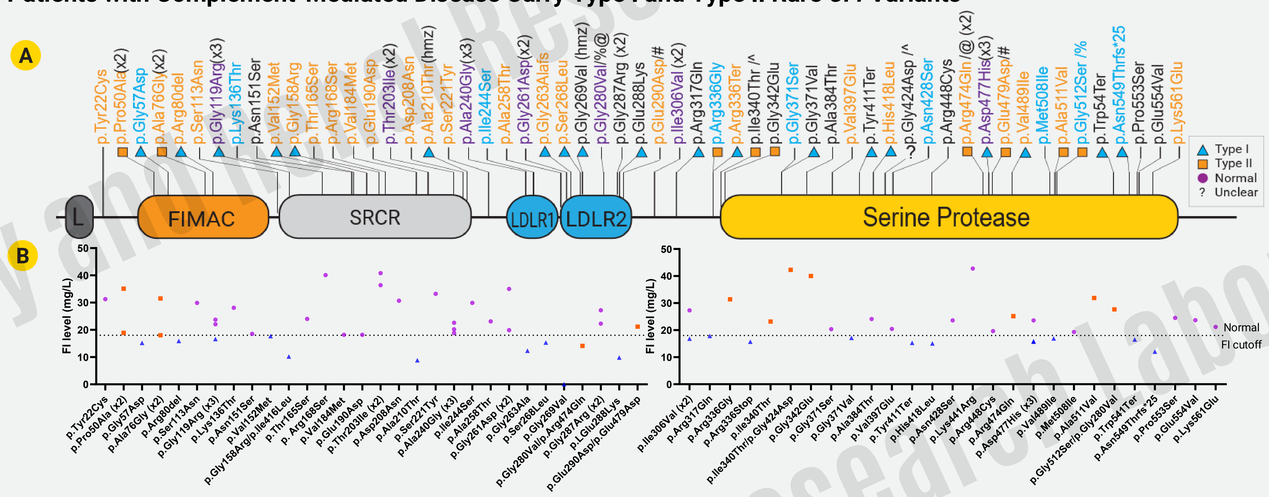

Figure 3: *CFI* domains and rare variants in patients with complement-mediated disease (A) showing Type I (n=19, blue triangles) and Type II (n=13, orange squares) variants. Compound heterozygotes are noted with symbols (%, @, #, ^). Low FI level (normal cutoff indicated by dashed line) identifies Type I variants (B). The FI functional assay increased our diagnostic rate by demonstrating impaired function using the patient's own sample (C). While these variants contribute to a variety of disease phenotypes (D), they do not cluster in a particular domain as shown in the schematic. A molecular model (E) shows type II variants in orange.

Cofactor-Dependent Assays Demonstrate Functional Impact of Type II RVs

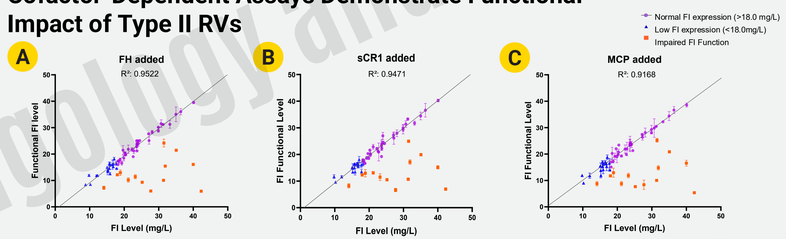

Figure 4: FI functional assays in conjunction with individual cofactors: FH (A), sCR1 (B), or MCP (C). Patients with normal expression and function closely followed the standard curve (purple circles) as expected. One-third of patients with RVs and normal FI level showed impaired cofactor function (Type II variants, orange squares). Patients with Type I variants (low expression, blue triangles) held tightly to the standard curve as only the unaffected allele is measured.

Conclusion

- Our study highlights the importance of including functional assays along with FI level measurement to assess the pathogenicity of *CFI* variants, as Type II variants can be missed using level alone. We were able to give positive diagnoses to an additional one-third of patients with normal FI levels.
- In cases where a patient is compound heterozygous for multiple rare variants, additional studies are needed to determine pathogenicity. One such example is the patient with p.Ile340Thr/p.Gly424Asp. FI function was impaired, but it is unclear if one or both variants are causative (Figure 3A/B).
- While previous studies have shown pathogenic variants clustering in the SP domain, our data indicate Type I and II variants are found across domains.

Acknowledgments

We thank the patients and families who made this work possible.

Supported in part by NIH R01 DK110023.