Function Analysis of Recombinant CFB Variants by ImmunoFixation Electrophoresis Assay

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Introduction

Complement factor B (FB) is essential for the amplification of the alternative pathway (AP), which is critical for host defense and elimination of damaged cells. However, overactivation of the AP due to FB variants can lead to complement-mediated diseases, such as atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G).

Here, we developed a modified immunofixation electrophoresis (IFE) assay to assess fluid-phase C3 convertase activity introduced by recombinant FB protein (rFB).

Methods

Recombinant FB proteins

C-terminal HIS-tagged cDNA was synthesized and cloned. rFBs were expressed in Expi 293 cells and affinity purified (Thermofisher).

IFE assay

rFB (10 ng) was introduced to FB-depleted serum (Complement Tech, TX) in a buffer containing 10 mM EGTA and 0.5 mM $MgCl_2$. The mixture was incubated at 37°C for a duration of 1 hour. As a background control, the same mixture was prepared in 10 mM EDTA buffer. After incubation, C3 and its activation products were resolved by agarose electrophoresis and immunoprecipitation and quantified using Image J software.

Results

CFB variants

86 variants was selected from published data, gnomAD, or from Spanish or American databases for C3G and aHUS.

Eight constructs failed to express recombinant proteins (Figure 1). 17 variants showed a gain of function (GOF, Figure 1, red) and 29 variants showed a loss of function (LOF, Figure 1, green) by IFE. The remaining variants demonstrated no alterations in function (Table 1).

Table 1. Summary of Functional Results

Location	Ba	vWFA	SP
Total	**36**	**37**	**13**
GOF	4	10	3
LOF	17	11	1
No change	11	12	9
Failed (expression)	4	4	--

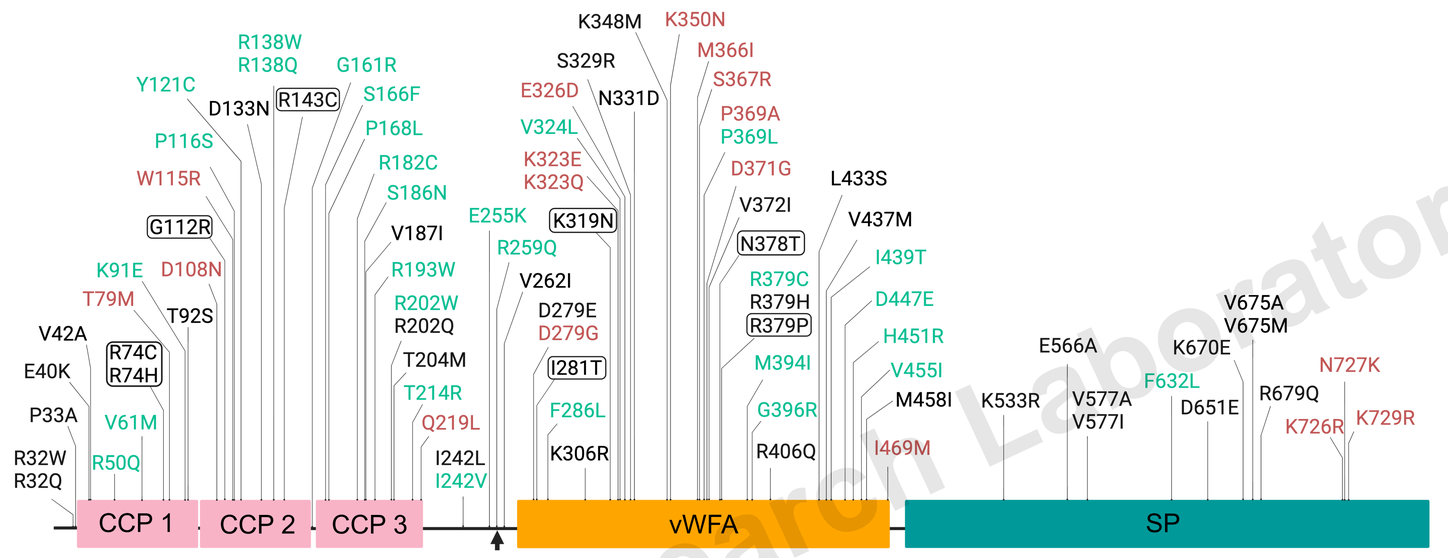


Figure 1. Complement Factor B Domain Overview and Analyzed Variants. (n=86) Eight variant constructs failed to express proteins (boxed). In red are variants with gain-of-function, potentially linked to the pathogenesis of complement-mediated diseases. In green are variants with loss-of-function; variants with no discernible functional impact are in black. CCP: complement-control protein domain; vWFA: von Willebrand type A domain; SP: serine protease domain. The black arrow demarcates the position of the scissile bond which separates the Ba and Bb segments.

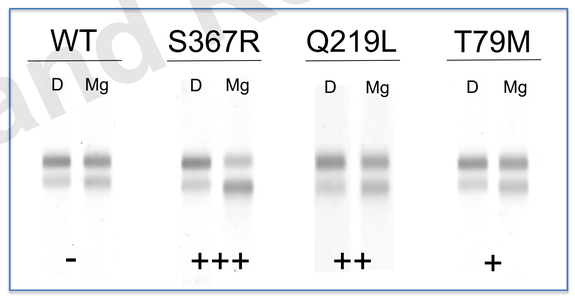
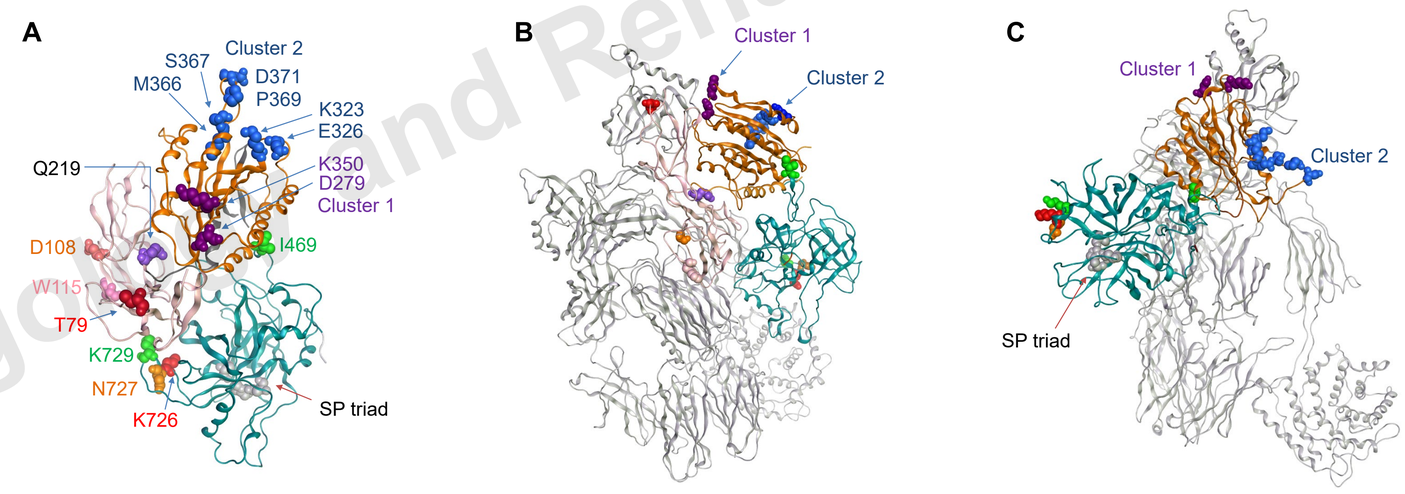


Figure 2. Demonstration of Gain-of-Function (GOF) Variants and Their Influence on C3 Activation. C3 was activated in FB-depleted serum by the introduction of recombinant proteins in the presence of both EGTA and magnesium (Mg lane). The same mixture was prepared in EDTA buffer (D lane) and served as the background control. C3AP refers to C3 activation product.

Table 2. Gain-of-Function Variants

cDNA	Protein	MAF*	CADD score	Domain	FP Activity**	Grade
c.236C>T	T79M	5.4E-05	20.3	CCP1	0.123	+
c.322G>A	D108N	8.1E-06	23.8	CCP2	0.149	++
c.343T>C	W115R	1.2E-05	18.48	CCP2	0.138	+
c.656A>T	Q219L	novel	40	CCP3	0.187	++
c.836A>G	D279G	novel	26	vWFA	0.321	+++
c.967A>C	K323Q	novel	15.87	vWFA	0.323	+++
c.967A>G	K323E	novel	16.35	vWFA	0.275	+++
c.978A>C	E326D	8.1E-04	0.001	vWFA	0.150	++
c.1050G>C	K350N	novel	23	vWFA	0.364	+++
c.1098G>A	M366I	2.4E-05	25.4	vWFA	0.157	++
c.1101C>A	S367R	novel	10.99	vWFA	0.424	+++
c.1105C>G	P369A	4.7E-05	0.212	vWFA	0.136	+
c.1112A>G	D371G	novel	14.22	vWFA	0.394	+++
c.1407C>G	I469M	1.1E-04	23.3	vWFA	0.158	++
c.2177A>G	K726R	4.1E-06	19.08	SP	0.135	+
c.2181C>G	N727K	novel	12.66	SP	0.132	+
c.2186A>G	K729R	9.0E-05	0.026	SP	0.124	+

* Data from gnomAD; ** Fluid-phase activity (nl 0.082 – 0.115)



FB (PDB: 2OK5) C3 pro-Convertase (PDB: 2WXJ) C3 Convertase (PDB: 2WIN)

Figure 4. FB GOF variants. A) FB; B) C3 pro-convertase; C) C3 convertase. C3b (grey) and FB (colored ribbons to highlight distinct domains as shown in Figure 1).

Discussion

- IFE can be used to detect GOF variants in FB and quantify effects on activation of C3
- Likely pathogenic GOF rare variants in FB are distributed across its three domains: Ba, vWFA and SP (Figure 1). This distribution suggests a correlation between these variants and different disease-causing mechanisms. Specifically:

1. GOF variants within the Ba domain tend to enhance the stability of the C3 pro-convertase. For instance, variants like T79M, D108N, and W115R are positioned at the surface that interacts with C3b on C3 proconvertase.
2. In the vWFA domain, GOF variants stabilize C3 convertase (cluster 1) or confer resistance to regulation of C3 convertase (cluster 2).
3. In the SP domain, GOF variants enhance serine protease activity. This enhancement may be due to their proximity to the serine protease center - H526, D576 and S699.

Conclusion

The presence of GOF variants in different FB domains underscores their contribution to a diverse array of pathogenic mechanisms.

Table 3. Mechanism of Actions for GOFs

Domain	Functional impacts	Variants
Ba	Increased interaction with C3b	T79M, D108N, W115R
vWFA	Affect Mg^{2+} adhesion site	D279G, K350N (cluster 1)
vWFA	Resistance to regulation	K323E, K323Q, E326D, M366I, S367R, P369A, D371G (cluster 2)
SP	Increased enzymatic activity	K726R, N727K, K729R

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