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Mutation of complement factor B causing massive fluid-phase dysregulation of the alternative complement pathway can result in atypical hemolytic uremic syndrome

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Abstract

Atypical hemolytic uremic syndrome is an ultra-rare disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury. Its pathogenesis is driven most frequently by dysregulated cell-surface control of the alternative pathway of complement secondary to inherited and/or acquired factors. Here we evaluated two unrelated patients with atypical hemolytic uremic syndrome. The first, a five-year-old Caucasian female, presented at 10

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Disclosure

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months with schistocytes, thrombocytopenia and kidney injury. The second, a 55-year-old Caucasian female, presented at age 31 following caesarean section for preeclampsia. Complement biomarker testing was remarkable for undetectable levels of C3 in both. Circulating levels of C5 and properdin were also low consistent with over-activity of the alternative and terminal pathways of complement. Genetic testing identified a heterozygous novel variant in *CFB* (c.1101 C>A, p.Ser367Arg) in both patients. Functional studies found strong fluid-phase C3 cleavage when normal and proband sera were mixed. Cell-surface C3b deposition was strongly positive when patient serum was supplemented with C3. *In vitro* control of C3 convertase activity could be restored with increased concentrations of factor H. Thus, *CFB* p.Ser367Arg is a gain-of-function pathogenic variant that leads to dysregulation of the alternative pathway in the fluid-phase and increased C3b deposition on cell surfaces. Our study highlights the complexities of complement-mediated diseases like atypical hemolytic uremic syndrome and illustrates the importance of functional studies at the variant level to gain insight into the disease phenotype.

Graphical Abstract

Keywords

Atypical hemolytic uremic syndrome; Complement; Pathogenic variant; Factor B

Introduction

Atypical hemolytic uremic syndrome (aHUS) defines a spectrum of thrombotic microangiopathies (TMA) characterized by hemolytic anemia, thrombocytopenia and acute renal injury not caused by Shiga toxin-producing *E. coli* or ADAMTS13 deficiency.^{1, 2} Pathogenesis is driven primarily by uncontrolled activation of the alternative pathway (AP) of complement on the cell surface.³

At baseline, the AP is constitutively active through spontaneous hydrolysis of C3 to C3(H₂O), a process referred to as 'tick over'.^{4, 5} C3(H₂O) provides a dock for factor B (FB) to form the proconvertase C3(H₂O)B in a Mg²⁺-dependent manner. Factor D (FD) cleavage of FB in the C3(H₂O)B complex generates the active but unstable fluid-phase C3 convertase

C3(H₂O)Bb. The latter cleaves more C3 to ultimately produce the C3b convertase C3bBb, which initiates rapid and indiscriminate deposition of C3b on all nearby surfaces. The amplification process and downstream complement activity are tightly controlled by a host of fluid-phase and membrane-bound complement regulators such as factor H (FH), factor I (FI), complement receptor 1 (CR1, CD35), membrane cofactor protein (MCP, CD46), and decay accelerating factor (DAF, CD55).

Pathogenic genetic variants are identified in ~60% of patients with a clinical diagnosis of aHUS.^{6–10} The list of implicated complement genes includes *CFH* (~25% of patients), *MCP* (~10%), *CFI* (~6%), *C3* (~6%) and *CFB* (~2%), with loss-of-function pathogenic variants in the complement regulators *CFH*, *MCP* and *CFI* being far more common than gain-of-function pathogenic variants in the complement activators *C3* and *CFB*. Pathogenic variants in non-complement genes include thrombomodulin (*THBD*) (~2%), a cofactor for thrombin-induced activation of protein C in the coagulation pathway, and diacylglycerol kinase epsilon (*DGKE*) (~3%), an intracellular lipid kinase that phosphorylates diacylglycerol to phosphatidic acid.¹¹ Acquired drivers have also been identified, the most common being factor H autoantibodies (FHAA), which account for 5–13% of cases. FHAA are associated with the absence of both copies of the *CFHR1* gene, a relatively common genetic finding in the general population.^{12, 13}

The complement biomarker profile of aHUS is subtle with evidence of activity often, but not always, reflected by changes in serum levels of complement proteins and complement cleavage products during the acute phase.^{14–17} Complement C3 levels are normal or marginally low as compared to levels in age-matched controls, and C3a and C5a levels, the cleavage products of C3 and C5, respectively, and soluble C5b-9 levels, which reflect terminal pathway activation, are elevated in the majority of aHUS patients with active disease. During remission, complement biomarkers are usually normal.

Herein, we report a novel variant in *CFB* (c.1101 C>A, p.Ser367Arg) identified in two unrelated patients. Biomarker testing showed undetectable levels of C3 suggesting AP dysregulation. Functional studies confirmed that the FB variant confers on C3 convertase resistance to FH-mediated decay leading to complement dysregulation in the fluid phase and on the cell surface.

Cases

Case 1

A previously healthy infant presented for acute care at age 10 months due to the onset of swelling of the face, feet and hands in the setting of a diarrheal illness. Laboratory studies during the first week of hospitalization were remarkable for hemolytic anemia, thrombocytopenia and renal insufficiency (creatinine 1.15 mg/dL); stool was negative for Shiga toxin-producing *E. coli* (Table 1). After receiving fresh-frozen plasma for presumed aHUS, she developed airway edema requiring emergent tracheostomy for short-term airway support. Eculizumab treatment was initiated with improvement in the thrombocytopenia although the renal insufficiency persisted and necessitated continuous renal replacement therapy. A renal biopsy at two weeks after clinical disease onset showed evidence of acute

and chronic thrombotic microangiopathy (TMA) with glomerular thrombi, endothelial swelling, mesangiolysis, and duplication of the glomerular basement membranes (GBMs) (Figure 1a – c). Approximately one week later, renal function began to recover. The patient now has normal renal function (creatinine 0.3 mg/dL) on standard dosing eculizumab. Chronic medications also include penicillin and enalapril. Serum C3 levels remain undetectable despite excellent control of her disease.

Of note, the family history is remarkable for two distant cousins with aHUS who are also on chronic eculizumab therapy with daily oral penicillin prophylaxis (Figure 2a). The younger of the second cousins presented to her primary care provider in 2014 with severe hypertension and visual changes. Evaluation revealed renal failure (creatinine 4.0 mg/dL), hemolytic anemia, thrombocytopenia and low C3. She had a positive anti-C3 direct antiglobulin test and was initially treated with plasma exchange for presumed thrombotic thrombocytopenic purpura but was diagnosed with atypical hemolytic uremic syndrome based on normal ADAMTS 13 activity. With eculizumab therapy, her renal failure resolved and on chronic eculizumab infusions, she has maintained normal renal function (creatinine 1.1 mg/dL) without evidence of hemolysis. About 18 months after the younger sister was diagnosed with aHUS, the older sister presented with shortness of breath, severe hypertension, renal failure (creatinine 4.2 mg/dL), hemolytic anemia, thrombocytopenia and low C3. She also had a positive anti-C3 direct antiglobulin test. Renal biopsy revealed thrombotic microangiopathy. She was diagnosed with aHUS and receives chronic eculizumab infusions. Her renal function has returned to normal (creatinine 0.94 mg/dL).

Case 2

A 31-year-old female underwent caesarean section at 37 weeks of gestation for signs and symptoms consistent with preeclampsia (Table 1). Postoperatively, she developed thrombocytopenia, hemolytic anemia and renal failure necessitating the start of dialysis. In addition, she developed heart failure (ejection fraction 36%). The patient received 17 sessions of plasma exchange. Kidney biopsy revealed acute TMA with fibrin-platelet thrombi and thickened GBMs with focal double contours (Figure 1d – h). Hemodialysis was discontinued after 3 months.

Following the acute kidney injury, her medical history was significant for hypertension, gout, high frequency hearing loss of unknown origin and bilateral cataracts although she had no evidence of drusen. At 37 years of age, a second kidney biopsy was done to evaluate a serum creatinine of 1.7 mg/dL (eGFR 38 ml/min/1.73m²). There was evidence of chronic TMA with extensive GBM duplication and sclerosis, advanced tubular atrophy and arteriolar smooth muscle hyperplasia with concentric intimal thickening (Figure 1i – l). Immunofluorescence revealed focal, segmental, and nonspecific irregular deposits of IgA 1+, IgM 3+, C3 3+, C1q 0.5+, and kappa 1+. On electron microscopy, there was ischemic glomerular collapse with segmental expansion of the subendothelial space with partial mesangial interposition and deposition of new basement membrane material. No electron dense deposits were identified and no tubuloreticular inclusions were seen.

At age 55, with a creatinine of 4.01 mg/dL (eGFR 12 ml/min/1.73m²), she underwent preemptive unrelated living donor kidney transplantation with perioperative administration

of eculizumab. A one-month protocol biopsy revealed mild diffuse acute tubular injury, negative C4d staining and no evidence of rejection or recurrent TMA. Seven months post-transplant, serum creatinine is 1.47 with no evidence of recurrent aHUS on biweekly eculizumab. Serum C3 levels remain undetectable. Her family history is negative for other persons with aHUS (Figure 2b).

Results

Genetic analyses

Targeted massively parallel sequencing identified a novel variant in exon 8 of *CFB* (c.1101C>G; p.Ser367Arg) in both patients (Figure 2a and b).¹⁸ The variant has a scaled combined annotation dependent depletion (CADD) score 11.46, which is modestly low (a scaled CADD score of 10 indicates that the variant is amongst the top 10% of deleterious variants in the human genome, a score of 20 means that a variant is amongst the top 1% of deleterious variants and so forth). Patient 2 also carries a rare variant in *CFH* (c.2850G>T; p.Gln950His), which has a minor allele frequency of 0.39% (gnomAD). Copy number variation (CNV) over the *CFH-CFHR* region determined by multiplex ligation-dependent probe amplification (MLPA) showed that both patients are hemizygous for *CFHR1*.¹⁸ Patient 1 carries the del*CFHR1-CFHR4* CNV while patient 2 carries the more common del*CFHR3-CFHR1* CNV. Patient 1 also carries risk haplotypes for aHUS in *CFH* (*CFH-H3*) and *CD46* (*ggaac*), while Patient 2 does not (Figure 2).

Pathway and biomarker analyses

Pathway analysis showed that alternative and classical pathway activity (APFA and CH50 assays, respectively) is abolished in both patients, consistent with the low C3 levels. The normal hemolytic assay using non-sensitized sheep erythrocytes reflects the lack of C3 and is a false negative (Table 2). In addition to the low C3, the elevation of C3c supports on-going poorly regulated activation of the AP. In Patient 1, there are also multiple biomarker changes consistent with terminal pathway dysregulation (low properdin, low C5 and high sC5b-9). In Patient 2, the only indication of terminal pathway activity is the marginal elevation of sC5b-9. With respect to regulators of complement activity, FH is borderline low in Patient 1 but well within the normal range in Patient 2, implying that the *CFH* variant p.Gln950His in heterozygosis does not affect expression.

Acquired drivers of the disease

Both patients were negative for acquired drivers of complement-mediated disease (*i.e.* FHAA, FBAA, nephritic factors to C3, C4 and C5 convertases).¹⁹ In addition, both patients were negative for circulating monoclonal antibodies by serum immunofixation electrophoresis (IFE).

Abnormal fluid phase AP activity

Fluid-phase AP activity was measured using a modified IFE (C3-IFE) assay by combining normal human serum (NHS) with patient serum under AP activation-permissive conditions (AP-APC). A very strong band of C3 cleavage products was seen with the NHS-patient serum mix but not when NHS alone was used (Figure 3a). The assay remained strongly

positive when IgG-depleted patient serum was used but was negative with patient-derived IgG and NHS (Figure 3b and c). These results are consistent with unregulated fluid-phase activity of C3 convertase conferred by the *CFB* c.1101 C>A, p.Ser367Arg variant.

C3b deposition assay

C3b deposition on cell surfaces was measured by adding 20% patient sera in AP-APC buffer to cultured MES-13 cells under two conditions—with supplemental C3 and without supplemental C3. Under the first condition, in the absence of C3, no cell-surface deposition of C3b was observed (Figure 4, left). However, when patient serum was supplemented with purified C3 (to correct for the lack of C3 in patient serum), cell-surface deposition of C3b was robust (Figure 4, middle). Addition of sufficient amounts of purified FH restored complement control in a dose-dependent manner (Figure 4, right two columns). These results are consistent with increased activity of C3 convertase with mutant Bb, the regulation of which is dependent on the concentration of FH.

Mutant FB constructs activate complement in the fluid phase and on the cell surface

Purified recombinant mutant FB p.R367 or wildtype (WT) FB p.S367 was added to NHS (Supplementary Figure 1S). The C3-IFE assay was repeated: mutant FB p.R367 conferred activity to C3 convertase in the fluid phase (Figure 5a). To test surface regulation, mutant FB p.R367 or WT FB p.S367 was added to NHS before incubating with cultured cells: C3b deposition was observed only in the mutant FB p.R367-supplemented condition. These results confirm the gain-of-function effect of mutant FB p.R367 (Figure 5b).

Mutant FB confers high resistance to FH-mediated decay accelerating activity

To study impact of regulatory proteins, C3 convertases were built on C3b-coated sheep erythrocytes using FD and mutant or WT FB in the absence or presence of complement regulators (Supplementary Figure 2S). In the absence of regulators, C3 convertase with mutant Bb had a longer half-life (slower spontaneous decay) as compared to WT C3 convertase (Figure 6a). When FH was added, C3 convertase with mutant Bb had a slower decay that could be restored by increasing the concentration of FH (Figure 6b–d). Regulation was moderately impaired with soluble CR1 and DAF (Figure 6e and f). These results demonstrate that mutant FB p.R367 increases C3 convertase activity by rendering the convertase resistant to FH-mediated DAA.

FH variant p.Gln950His does not affect FH function

The functional consequence of FH p.His950 in patient 2 was studied using cell-based DAA and cofactor activity (CA) assays. Neither DAA nor CA was affected by FH p.His950 (Supplementary Figure 3S). These results indicate that this genetic variant is a benign polymorphism.

Discussion

Herein we report two unrelated patients with aHUS both of whom carry a novel variant in *CFB* c.1101C>G; p.Ser367Arg that increases the affinity of FB for C3b. The result is a C3 convertase with mutant Bb that has slower natural decay activity and significantly impaired

FH-mediated DAA. The *in vivo* consequence is massive consumption of serum C3 due to sustained fluid-phase activity.

Both the extremely low C3 levels and the novel variant in *CFB* drew our attention and prompted these studies. In our experience, extremely low C3 levels (<0.2 g/L) are typically associated with C3 glomerulopathy (C3G), another ultra-rare complement-mediated renal disease characterized by fluid-phase dysregulation of the AP.²⁰ In fact, of 523 patients in our database with either C3G or aHUS, C3 levels less than 0.2 g/L were reported in 69 (13.2%) patients, all of whom carry the diagnosis of C3G with the exception of these two cases. In addition to low serum C3 levels, elevation of C3c and sC5b-9 in these two patients supported on-going activity of the alternative and terminal complement pathways. C3 levels did not normalize with disease quiescence, which is typical for aHUS and implies a state of continued fluid-phase dysregulation of the AP.^{14–17}

FB is a 90-kDa single-chain zymogen that interacts with C3(H₂O) or C3b to form the proconvertases C3(H₂O)B or C3bB. In both complexes, cleavage of FB by FD releases Ba with the subsequent formation of C3(H₂O)Bb or C3bBb, which are active but unstable C3 convertases that undergo spontaneous or regulator-mediated decay through the dissociation of Bb. Once dissociated, Bb loses serine protease activity and cannot re-associate with C3b. Bb, itself, is comprised of two globular domains: a vWA domain and a serine protease domain. The vWA domain on Bb serves as the point-of-contact for C3b through a Mg²⁺ adhesion site (MIDAS) that forms after the release of Ba. Several FB pathogenic variants surrounding MIDAS dramatically increase Bb affinity for C3b (D279G, F286L, K350N).

The S367R variant is also located in the vWA domain although it is remote from the MIDAS (Supplementary Figure 4S). It is, however, in close proximity to another gain-of-function pathogenic variant K323E/Q. K323 and S367 lie within 8 Å of each other but neither is in direct contact with C3b (PDB 2WIN and 2WXJ). Consistent with this location, K323E/Q and S367R only slightly increase the affinity of FB for C3b. Both K323E/Q and S367R increase resistance of C3bBb to FH-mediated decay.

How is the *CFB* c.1101C>G; p.Ser367Arg pathogenic variant related to the final clinical phenotype in these patients? Our data show that FB p.R367 requires higher concentrations of FH for effective DAA (Figure 6c and d), an observation that may explain the phenotypic variations in disease severity: Patient 1, who developed disease as a 10-month-old, has borderline low FH levels, while in Patient 2, who developed aHUS post-partum, FH levels are twice as high. The difference can be explained at least in part by the *CFH-H3* risk haplotype found in Patient 1. *CFH-H3* is associated with lower FH levels and predisposes to complement dysregulation. FH levels directly impact C3 convertase tick-over activity and C3b deposition on cell surfaces. At high FH concentrations such as in Patient 2, surface regulation of C3 convertase with mutant Bb was maintained until the disease was triggered by pregnancy. In the C3b deposition assay, we used 20% serum spiked with FH to a final concentration of 50 mg/L (left panel in Figure 3), which is equivalent to a plasma concentration of 250 mg/L. Both patients are also hemizygous for *CFHR1*, which is known to be contributory to the aHUS phenotype.²¹ But why do these patients have aHUS and not C3G?

In general, aHUS is considered a disease in which there is defective protection of host cells in the context of normal fluid-phase complement activity.²² These two cases challenge this paradigm as both patients have defects of complement regulation in the fluid phase and on cellular surfaces. Interestingly, the same amino acid variation resulting from a different nucleotide change (*CFB* c.1099A>C; p.Ser367Arg) has been reported in a Japanese patient who had C3G and not aHUS.²³ That patient, a 9-year-old girl, developed asymptomatic proteinuria and hematuria associated with non-detectable serum C3 levels and a markedly elevated sC5b-9. Renal biopsy confirmed the diagnosis of C3G. A brother and the patient's mother also had hypocomplementemia, and while the brother was asymptomatic, the mother was diagnosed with membranoproliferative glomerulonephritis type I as a teenager. As compared to our cases, one important difference is that the C3G patient carries two other ultra-rare genetic variants—one in *CFI* (c.603A>C, p.R201S; minor allele frequency 0.0011) and the other in *C3* (c.2746G>A, p.V916I; minor allele frequency 0.000054). In the presence of the *CFB* p.Arg367 mutant protein, these variants may drive the phenotype to C3G, as it has been shown that multiple rare variants, especially in the *C3* and *CFB* genes, are more likely to be identified in C3G patients as compared to aHUS patients.¹⁸

In conclusion, *CFB* p.Ser367Arg is a novel gain-of-function pathogenic variant associated with aHUS. This amino acid change leads to persistent fluid-phase dysregulation of the AP by conferring resistance of C3 convertase with mutant Bb to FH-mediated DAA. These two cases highlight the complex consequence of pathogenic variants in the *CFB* gene and the contextual importance of other genetic and complement biomarkers (i.e. FH levels) in defining the ultimate disease phenotype in a given patient. The underlying fluid-phase dysregulation of the AP in the two cases we have described also calls into question the pathogenesis of aHUS and C3G, and suggests that the final disease phenotype is based on a host of modifying genetic and perhaps environmental conditions.

Methods

Participants.

Participants gave informed consent for this study, which was approved by the Institutional Review Board of Carver College of Medicine at the University of Iowa.

Genetic Analysis.

After extracting DNA from peripheral blood lymphocytes, targeted massively parallel sequencing was performed using a genetic panel that included 10 thrombotic microangiopathy associated genes (*C3*, *CFB*, *CFD*, *CFH*, *CFHR5*, *MCP*, *CFI*, *DGKE*, *THBD*, *MMACHC* and *ADAMTS13*) as described.^{18, 24} Variants were confirmed by Sanger sequencing. MLPA was performed over the *CFH-CFHR5* region using in-house probes.²⁵

Complement Analyses.

Complement biomarkers were assayed using a customized in-house panel that measures pathway activity, protein and breakdown products, and autoantibodies.²⁶ Paraproteins were excluded by serum immunofixation electrophoresis.

Fluid-phase convertase activity.

A modified IFE assay was used to assess fluid-phase convertase activity.¹⁹ C3 degradation products were quantified after 1:1 mixing patient serum (or IgG fractions) with normal human serum in a buffer containing 10mM EGTA and 0.5mM MgCl₂. The same mixture in 10mM EDTA buffer was used as background control. IgG depletion and isolation were carried out using Protein G agarose (Seracare, Milford, MA) with 1:1 diluted serum sample (PBS).

C3b deposition assay.

C3b deposition was quantified on MES-13 cells (derived from mouse kidney mesangial cells, ATCC CRL-1927) in the presence of EGTA and Mg⁺⁺ (0.5mM) by visualization using Alexa-488 labeled anti-C3 antibody 7C12.²⁷

Recombinant FB proteins.

Plasmids containing HIS-tagged *CFB* were generously gifted by Alexion Pharmaceuticals, Inc. Mutagenesis (*CFB* c.1101C>G) was performed using the QuickChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Inc). All constructs were sequenced confirmed. Recombinant proteins (mutant FB p.Arg367 and WT FB p.Ser367) were produced in HEK293 cells (Sigma-Aldrich, Inc) and purified using the HisPurTM Ni-NTA purification kit (Thermo Fisher Scientific, Inc).

Decay Accelerating Activity Assay.

C3b was deposited on sheep erythrocytes using FH-depleted and FB-partially inactivated serum.¹⁹ C3 convertase was assembled with recombinant FB (mutant or WT) and FD (purchased from Complement Technology, Tyler, TX) under EGTA and Mg²⁺ conditions, and was allowed to decay for 0, 2.5, 5, 8, and 12 minutes. At each time point, residual C3 convertase activity was titrated out by adding rat-serum in EDTA as a source of C3-9. To assess regulator activity, FH (Complement Technology), soluble CR1 (Celldex Therapeutics, Hampton, NJ) or DAF (Origene, Rockville, MD) was added at a concentration of 10 nM after the formation of C3 convertase. FH- mediated DAA of C3 convertase with mutant Bb was measured in the presence of increasing amounts of FH (10 to 200 nM) (Supplementary Figure 2S).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8.2 (GraphPad, San Diego, CA). All data are presented as the mean $\pm$ SD. For 2-group comparison, the unpaired 2-tailed Student's *t* test was used. A *P* value <0.05 was considered to be statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative renal biopsy images.

(a-c) Native kidney biopsy for patient 1. Hematoxylin and eosin (H&E) shows several glomeruli with intracapillary thrombi (arrows) and fragmented red blood cells incorporated into the capillary walls and mesangial areas, consistent with acute thrombotic microangiopathy (a). The glomeruli demonstrate architectural alterations including mesangiolysis and duplication of the glomerular basement membranes (arrows), consistent with chronic thrombotic microangiopathy (PAS) (b). Electron microscopy demonstrates endothelial cell swelling (arrows) and duplication of the glomerular basement membranes with cellular interposition (c). (d-h) Native kidney biopsy for patient 2. Low power view shows well-preserved renal cortex with back-to-back tubules and minimal interstitial fibrosis (trichrome) (d). H&E shows afferent arteriole with fibrin-platelet thrombus (*) (e). (f-h) High power view shows well-preserved renal cortex with back-to-back tubules and minimal interstitial fibrosis (trichrome) (f). H&E shows afferent arteriole with fibrin-platelet thrombus (*) (g). Electron microscopy demonstrates endothelial cell swelling (arrows) and duplication of the glomerular basement membranes with cellular interposition (h). (i-l) High power view shows well-preserved renal cortex with back-to-back tubules and minimal interstitial fibrosis (trichrome) (i). H&E shows afferent arteriole with fibrin-platelet thrombus (*) (j). PAS shows architectural alterations including mesangiolysis and duplication of the glomerular basement membranes (arrows) (k). Electron microscopy demonstrates endothelial cell swelling (arrows) and duplication of the glomerular basement membranes with cellular interposition (l).

Glomerular capillaries occluded and distended by thrombi with fragmented erythrocytes (H&E) (**f**). Jones methenamine silver demonstrates frequent duplication of the glomerular basement membranes (**g**). Electron microscopy also shows double contour formation (*) (**h**). (i-l) Transplant biopsy for patient 2. Low power view shows progressive interstitial fibrosis and tubular atrophy (H&E) (**i**). Jones silver stain highlights numerous globally sclerosed glomeruli (**j**). Some of the intact glomeruli demonstrate segmental sclerosis with solidification and adhesion to Bowman capsule (H&E) (**k**). Segmental sclerosis with matrix accumulation and broad adhesion formation (trichrome) (**l**). All bars equal 100 μ M except c, g, h (5 μ M for c and h; 50 μ M for g).

Figure 2. Pedigrees.

Patient 1 is a 5-year old Caucasian female who presented with schistocytes, thrombocytopenia and low serum C3 levels at 10 months of age. Genetic testing identified heterozygosity for a novel *CFB* variant (c.1101 C>A, p.Ser367Arg). This variant was also identified in her healthy father, healthy grandmother, and two affected second cousins (**a**). Patient 2 is a 55-year old Caucasian female who presented with aHUS at the age of 31 immediately after pregnancy (**b**). Genetic testing identified the same *CFB* variant and a variant of unknown significance in *CFH* (c.2850G>T, p.Gln950His heterozygote). The genotypes of FH risk alleles are shown under each individual. H* denotes a rare haplotype.

Figure 3. AP activation by patient serum.

Large amounts of C3 activation products (C3AP) are seen under AP activation permissive conditions (APAPC, lane Mg) but not with EDTA (lane D). C3AP result from cleavage of the C3 alpha chain (release of C3a) followed by a major conformational change. C3AP is not seen in the control (normal) (a). The same experiment is repeated with patient IgG-depleted serum and with the isolated IgG, respectively. C3AP is present under APAPC (lane Mg) when mixing IgG- depleted PS with NHS (b). A negative result is obtained when mixing patient-derived IgG and NHS (c). AP activation is independent of immunoglobulins (neg: negative).

Figure 4. C3b Deposition Assay.

No C3b deposition is observed when patient serum (PS) is used alone (left column).

Addition of C3 (250 $\mu\text{g/mL}$) to PS leads to deposition of C3b but not in the control (normal, bottom). When both C3 (250 $\mu\text{g/mL}$) and FH (10 $\mu\text{g/mL}$) are provided, C3b deposition is minimal (center, right column). With addition of more FH (50 $\mu\text{g/mL}$), C3b deposition is not detectable (right column). These results show that the cleavage of C3 by C3 convertase with mutant Bb can be prevented with sufficiently high levels of FH, suggesting that the mutant FB protein impairs normal decay accelerating activity. Bar = 100 μm for all figures.

Figure 5. Mutant FB p.Ser367Arg activates the AP.

Complement activation in the fluid phase. Purified mutant or WT FB was spiked into NHS and IFE was repeated. Results show that the mutant FB directly activates complement the AP in the fluid phase (**a**). Complement activation on the cell surface. Mutant FB or WT FB was added into NHS before incubating with cultured cells. C3b deposition was seen only with the mutant FB and not with WT FB (**b**). Bars = 100 μ m.

Figure 6. Resistance to FH-mediated decay.

Spontaneous decay. C3 convertase containing mutant Bb has a slightly longer half-life than C3 convertase with WT Bb (a). C3 convertase containing mutant Bb significantly resists FH regulation thus decreasing FH-mediated decay accelerating activity (DAA) (b). Increasing amounts of FH (from 10 nM to 200 nM) control activity of C3 convertase containing mutant Bb (c). C3 convertase activities with various amounts of mutant FB and FH are shown (d). The impairment of DAA is moderate with sCR1 and DAF (e and f, respectively). Mean $\pm$ SD; n=3 for each group for all figures. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 by unpaired 2-tailed t test.

Table 1.

Clinical data at presentation

	Patient 1	Patient 2
Gender	Female	Female
Age (current)	5 y	55 y
Onset (age)	10 m	31 y
Extrarenal manifestations		HFrEF, HTN
Chief complaint		
Diarrhea	Yes	No
Hemoglobin (g/dL, 12–15.5 [*])	5.6	7.3
Platelets (cells/L, 150–450)	109	55
LDH (IU/L, 140–280)		2360
BUN (mg/dL, 7–20)	16	92
Creatinine (mg/dL, 0.5–1.1 [*])	0.35 to 1.15 [#]	7.5
Direct antiglobulin test		n/a
Hematuria	Yes	n/a
Proteinuria	Yes	3+
Stool culture	Negative	n/a
Treatment	FFP, CRRT, eculizumab	Hemodialysis x 3 months
Outcome	Complete renal recovery; ongoing low C3	CKD leading to unrelated living donor kidney transplantation at age 55; ongoing low C3

LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CKD, chronic kidney disease; CRRT, continuous renal replacement therapy; FFP, fresh frozen plasma; HFrEF, heart failure with reduced ejection fraction; HTN, hypertension; n/a, not available;

^{*} normal reference values for females;

[#] patient 1 presented with significant edema and the initial creatinine of 0.35 mg/dL climbed to a peak of 1.15 institution of CRRT and resolution of edema

Table 2.

Pathway activities, biomarkers and autoantibodies

Assay (normal range)	Patient 1	Patient 2
Pathways		
AP Function Assay (50–130%)	0	0
CH50 (>70 U/ml)	13	14
Hemolytic (< 3%)	0	0
Biomarkers		
C3 Level (0.9–1.8 g/L)	< 0.16	< 0.16
C3c Level (<1.5 mg/L)	2.4	3
C4 Level (0.15–0.57 g/L)	0.22	0.3
FB Level (22–50 mg/dL)	41.5	36.2
Ba Level (<1.2mg/L)	1.5	3.8
Bb Level (<2.2 mg/L)	4.2	2
Properdin (10–33 mg/L)	5.8	12.4
C5 Level (10–21 mg/dL)	11.9	17.1
Soluble C5b-9 (<0.3 mg/L)	0.92	0.34
FI Level (16–30 mg/L)	23.5	40.2
FH Level (180–420 mg/L)	183	323
Acquired Drivers of Disease		
FBAA* (< 200 AU) use serum	< 50	< 50
FHAA# (< 200 AU) use serum	< 50	< 50
C3Nef (<20%) use IgG	10%	11%
C5Nef (<20%) use IgG	17%	12%
C4Nef (<20%) use IgG	11%	10%
Monoclonal proteins	Neg	Neg

* FBAA = Factor B autoantibodies;

FHAA = FH autoantibodies