Disease-associated N-terminal Complement Factor H Mutations Perturb Cofactor and Decay-accelerating Activities*

Isabell C. Pechtl1, David Kavanagh3, Nicola McIntosh1, Claire L. Harris1, and Paul N. Barlow1,2

From the 1Schools of Chemistry and Biological Sciences, University of Edinburgh, Edinburgh EH9 3JJ, Scotland, United Kingdom, the 2Institute of Human Genetics, University of Newcastle, Newcastle NE1 3BZ, United Kingdom, and the 3Department of Infection, Immunity, and Biochemistry, University of Cardiff School of Medicine, Cardiff CF14 4XN, Wales, United Kingdom

Many mutations associated with atypical hemolytic uremic syndrome (aHUS) lie within complement control protein modules 19–20 at the C terminus of the complement regulator factor H (FH). This region mediates preferential action of FH on self, as opposed to foreign, membranes and surfaces. Hence, speculation on disease mechanisms has focused on deficiencies in regulation of complement activation on glomerular capillary beds. Here, we investigate the consequences of aHUS-linked mutations (R53H and R78G) within the FH N-terminal complement control protein module that also carries the I62V variation linked to dense-deposit disease and age-related macular degeneration. This module contributes to a four-module C3b-binding site (FH1–4) needed for complement regulation and sufficient for fluid-phase regulatory activity. Recombinant FH1–4V62 and FH1–4I62 bind immobilized C3b with similar affinities (K_D = 10–14 μM), whereas FH1–4I62 is slightly more effective than FH1–4V62 as cofactor for factor I-mediated cleavage of C3b. The mutant (R53H)FH1–4V62 binds to C3b with comparable affinity (K_D ~ 12 μM) yet has decreased cofactor activities both in fluid phase and on surface-bound C3b, and exhibits only weak decay-accelerating activity for C3 convertase (C3bBb). The other mutant, (R78G)FH1–4V62, binds poorly to immobilized C3b (K_D > 35 μM) and is severely functionally compromised, having decreased cofactor and decay-accelerating activities. Our data support causal links between these mutations and disease; they demonstrate that mutations affecting the N-terminal activities of FH, not just those in the C terminus, can predispose to aHUS. These observations reinforce the notion that deficiency in any one of several FH functional properties can contribute to the pathogenesis of this disease.

Surface deposition and self-propagation of the activation-specific opsonic complement-protein fragment, C3b, is tightly regulated. Effective control minimizes damage to host tissue by the potentially destructive complement system (1–3). At least three diseases, age-related macular degeneration (AMD)2 (4–7), dense-deposit disease (DDD) (4, 8), and atypical hemolytic uremic syndrome (aHUS) (9–15), are linked to deficiencies in complement regulatory processes. Mutations and polymorphisms associated with one or more of these three pathologies occur in genes for at least six complement proteins. Two of these, C3 (16) (the precursor of C3b) and factor B (FB) (17), participate directly in the C3b-generating amplification cascade of the “tickover” or alternative pathway (AP). The remainder are regulatory proteins that help to down-regulate and inactivate C3b: C4b-binding protein (18), membrane cofactor protein (15, 19, 20), factor I (FI) (21) and complement factor H (FH) (9, 10). In addition to dysregulation of the complement cascade caused by mutations in its components or regulators, autoantibodies to FH have been described in patients with AMD (22) and aHUS (15, 23–25) that prevent association of FH with cell surfaces.

Both AMD and DDD feature tissue-specific accumulations of extracellular deposits in affected tissues. Macular drusen are an early hallmark of AMD, a common cause of blindness among the elderly (26). Complement regulators occur within drusen, as do activation-derived protein fragments from the amplification stages and terminal pathway of complement (26, 27). The dense deposits of DDD, a severe, predominantly pediatric, kidney disorder (28), accumulate in the glomerular basement membrane, and their protein content likewise strongly suggests complement activation. Like DDD, aHUS is a rare inheritable chronic kidney disease caused by complement hyperactivity; but aHUS is characterized by microvascular endothelial cell activation, thrombocytopenia, and hemolytic anemia leading to end-stage renal failure (29).

Sequence variations in the gene for complement regulator FH are linked to all three diseases (30). FH (31) is an abundant plasma glycoprotein (350–600 mg/liter) that regulates the AP of complement. It thereby ensures that soluble C3 levels will not become exhausted by excessive complement activation in the fluid phase and that C3b levels on surfaces are prevented from self-propagating. A single-nucleotide polymorphism

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†1 From whom correspondence should be addressed: Joseph Black Chemistry Bldg., University of Edinburgh, King’s Buildings, West Mains Rd., Edinburgh EH9 3JJ, United Kingdom. Fax: 44 (0)131 650 7056; E-mail: Paul.Barlow@ed.ac.uk.

2 The abbreviations used are: AMD, age-related macular degeneration; aHUS, atypical hemolytic uremic syndrome; AP, alternative pathway; CCP, complement control protein module; DDD, dense-deposit disease; FB, factor B; FD, factor D; FH, factor H; FH1–4, factor H modules 1–4; FI, factor I; IH50, 50% inhibition of hemolysis; NHS, normal human serum; SNP, single-nucleotide polymorphism; SPR, surface plasmon resonance.
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(SNP) leading to substitution of His for Tyr at FH position 402 (Y402H) confers increased risk of AMD and DDD (4–7); another SNP encoding Ile (rather than Val) at position 62 confers decreased risk of these diseases (4). But the very great majority of variations in FH predisposing to aHUS are single-point missense mutations in the C-terminal segment of the protein (11).

No definitive explanation of why particular mutations or SNPs predispose to one disease rather than another exists despite significant knowledge of structure-function relationships of FH (32). FH (see Fig. 1a) is composed entirely of 20 CCP modules (33, 34) that although similar in sequences and structures (35) are diverse in function (36–38). The N-terminal four CCPs (that encompass the V62I SNP) are necessary and sufficient to perform two principal FH activities: cofactor for FI-mediated destruction of fluid-phase C3b, and accelerated decay of the C3b-containing complexes that enzymatically activate C3 to C3b (39, 40) in the AP. The seventh CCP (wherein lies the Y402H SNP) may, through binding to glycosaminoglycans or other polyanions (38, 41, 42), assist FH to dock onto self-surfaces (as opposed to foreign ones) requiring protection from complement. CCP modules 19 and 20, the site of nearly all aHUS-linked missense mutations, encompass additional strong C3b-binding and surface-binding sites. The C terminus likely helps FH distinguish self from foreign surfaces on the basis of polyanionic carbohydrates (43–45). It is vital for controlling the AP of complement on cell surfaces but is not a requirement of fluid-phase AP regulation.

Mice in which wild-type FH was genetically replaced with a truncated version (missing CCP modules 16–20) developed aHUS-like symptoms (46). Notably, mice in which FH had been knocked out altogether and in which plasma C3 levels are consequently severely depleted due to lack of fluid-phase regulation, develop DDD-like pathology (47). It is also notable that those disease-linked FB and C3 mutations that differentially affect susceptibility to regulation by fluid-phase regulators or cell surface regulators, predispose to DDD, or to aHUS, respectively (17, 48). Together, these findings suggest that aHUS could arise when defective host surface protection coincides with a level of fluid-phase complement regulation that is sufficient to avoid significant depletion of plasma C3 and factor B levels. In such a scenario, sufficient C3 and FB are available to activate the complement cascade on inadequately protected host surfaces, with pathogenic consequences.

We set out to characterize aHUS-linked mutations R53H (49) and R78G (50) that occur within the N-terminal CCP of FH and are not involved in self-surface recognition. We show that these mutations disrupt function but that they do so by different mechanisms because R53H, but not R78G, bind normally to C3b.

EXPERIMENTAL PROCEDURES

Preparation of Plasmids (Including DNA)—Native DNA coding for human FH (1) was amplified from the human universal QUICK-Clone cDNA library (Clontech) and cloned into pCR4Blunt-TOPO vector (Invitrogen). The DNA coding for FH CCP modules 1–4 (i.e., residues 19–263) was amplified from this FH cDNA and inserted into the yeast expression vector pPICZαB (Invitrogen). In protein production trials, the resultant recombinant protein underwent proteolytic degradation before or during purification; this difficulty was circumvented by fusion with tags as follows. DNA coding for a C-terminal His₉₆ tag and an N-terminal myc tag (EQKLISEEDL) were fused to the DNA encoding FH residues 19–263 cDNA in pPICZαB using the QuikChange site-directed mutagenesis kit (Stratagene) with the following forward primers: GGATGGC- GTCCGTTGCTCATGTGAACATCATCATCATTAGTCTAGAACA (for C-terminal His₉₆) tag and GAGGCTGAAGCTGAGGGAGGAACAAAAACTCTCTCA- GAAGGATCTGGAAGATTGCAATGAACTTCCTCCA (for N-terminal myc tag). This yielded the more stable protein product, FH₁–₄V₆₂. Single-site mutations were subsequently introduced (QuikChange) to create (R53H)FH₁–₄V₆₂, FH₁–₄I₆₂, and (R78G)FH₁–₄V₆₂; thus, identical N-terminal and C-terminal modifications were present in all versions of CFH₁–₄ in the present study.

Following plasmid preparation, KM71H Pichia pastoris cells (Invitrogen) were transformed using electroporation (Bio-Rad GenePulser II), and 1 ml of ice-cold 1 m sorbitol was added. Cells were incubated at 30 °C for 2–3 h without shaking. Selection of P. pastoris clones containing the expression plasmid was achieved by streaking transformed yeast onto YPDS (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) dextrose, 1M sorbitol, 1% (w/v) agar) plates containing 100–300 µg·ml⁻¹ zeocin. Cell colonies that grew on 300 µg·ml⁻¹ zeocin plates, consistent with the presence of multiple copies of the gene in the transformed cells, were screened for protein expression.

Protein Production and Purification—Expression was carried out at 30 °C in either 2-liter baffled flasks or a fermentor. For flask expression, 5-ml starter cultures of buffered minimal glycerol enriched with casein amino acids (1% w/v) were transferred to 500 ml of buffered minimal glycerol (with casein amino acids) and incubated for 48 h. For the fermentor, starter cultures (500 ml of buffered minimal glycerol) were transferred into 3.8 liters of buffered minimal glycerol enriched with 1% (w/v) casein amino acids and containing antifoam and 0.5% (w/v) PTM1 salts in a 5-liter fermentation vessel. Following incubation for 16–18 h, recombinant expression was induced with buffered minimal methanol, enriched with 1% (w/v) casein amino acids (for baffled flasks and fermentor growths) and 0.5% (w/v) PTM1 salts (fermentor growths only) as before. After 3–4 days of further incubation with methanol feeds (to 0.5% v/v) every 24 h, cells were pelleted by centrifugation, and the supernatant containing the secreted recombinant protein was filtered (0.22 µm) and its pH adjusted to 7.5. The supernatant was applied manually to an XK 16/20 column packed with 25 ml of IMAC-Sepharose 6 Fast Flow resin (GE Healthcare), charged with NiSO₄₃. Its-tagged target proteins were eluted (3 ml/min) with an imidazole gradient, emerging at ~210 m M imidazole. Size-exclusion chromatography on a HiLoad 16/60 Superdex 75 chromatography column (GE Healthcare) was used as a second purification step. Further purification was accomplished using a 1.7-ml Tricorn MonoQ (GE Healthcare) anion-exchange chromatography column in 20 mM sodium carbonate buffer, pH 9.0, containing 1 mM EDTA. The column was eluted with a gradient to 1 M NaCl; the target proteins emerged at 400
mm NaCl. Protein identity was confirmed by Fourier transform ion-cyclotron resonance mass spectrometry (Bruker) or on a quadrupole time-of-flight mass spectrometer (Q-TOF; Micromass). Mass spectrometry-derived masses agreed with theoretical masses taking into account disulfide formation, the myc tag at the N terminus and the His6 tag at the C terminus, and the erratic presence of an N-terminal Asp-Gly dipeptide that is an artifact of incomplete cleavage of the signal sequence used to promote secretion of recombinant protein. Protein concentrations were estimated using absorbance at 280 nm and calculated extinction coefficients (4,7870 M·cm⁻¹).

**Measuring Decay Acceleration Activity on Sheep Erythrocytes**—C3b-coated sheep erythrocytes were prepared as described previously (51). Cells were resuspended to 1% (v/v) in AP buffer (5 mM sodium barbitone, pH 7.4, 150 mM NaCl, 7 mM MgCl₂, 10 mM EGTA), and the AP convertase (C3bBb) was formed on the cell surface by incubating with FB (7 μg/ml) and FD (0.2 μg/ml; Complement Technologies) at 37 °C for 15 min. Cells (100 μl) were incubated with 50 μl of the FH1–4 samples in PBS/10 mM EDTA for 20 min. Lysis was developed by adding 50 μl of normal human serum (NHS) depleted of FB and FH (4%, v/v NHSΔBΔH) (51) in PBS/10 mM EDTA and incubating at 37 °C for 60 min. To determine the amount of lysis, cells were pelleted by centrifugation, and hemoglobin release was measured at 410 nm. Controls included 0% lysis (buffer only) and 100% lysis (0.1% (v/v) Nonidet P-40), and the observed cell lysis was calculated as a percentage as described previously (51).

**Measuring Cofactor Activity on Sheep Erythrocytes**—To test FH1–4 cofactor activity, washed EA-C3b cells were resuspended to 2% (v/v) in AP buffer and incubated with an equal volume of a range of concentrations of each of the CFH1–4 samples and 2.5 μg/ml FI (Complement Technology) for 15 min at 25 °C. After three washes in AP buffer, a 50-μl aliquot of cells (2%) was mixed with 50 μl of FB (14 μg/ml) and FD (0.2 μg/ml) and then incubated for 15 min at 25 °C to form AP convertase on the remaining C3b. Lysis was developed by adding 50 μl of NHSΔBΔH (4%, v/v) in PBS/10 mM EDTA and incubating at 37 °C for 30 min. Percentage lysis was calculated as above.

**Binding Affinity for C3b by Surface Plasmon Resonance**—The binding affinity of the disease-associated CFH1–4 mutants was monitored by surface plasmon resonance (SPR) using a Biacore T100 instrument (GE Healthcare). A Biacore series S-carboxymethylated dextran (CM5) sensor chip (GE Healthcare) was prepared by immobilizing on it human C3b (Complement Technology) using standard amine coupling. The reference surface of the chip was prepared by performing a mock coupling in the absence of any protein. Experiments were performed at 25 °C and 30 μl/min flow rate. Duplicate injections were performed for selected samples (concentrations 0.5–20 μM) in 10 mM HEPES-buffered saline with 3 mM EDTA and 0.05% (v/v) surfactant P20 (i.e. HSBEp +) (GE Healthcare). A contact time of 90 s was used (this achieved steady-state conditions for most samples) followed by dissociation using running buffer for 600 s. Between sample injections, chips were regenerated by two 45-s injections of 1 M NaCl. Data were processed using the BIAevaluation software (GE Healthcare). Data from the reference cell and a blank (buffer) injection were subtracted and dissociation constants calculated using steady-state fitted model and background-subtracted traces of the individual injections fitting.

**Measurement of Decay Acceleration Activity by SPR**—Decay-accelerating activity was measured in real-time using a Biacore T100 instrument as described previously (17, 52). All proteins were gel-filtered into the running buffer (HEPES-buffered saline containing 0.5% (v/v) surfactant P20 and 1 mM MgCl₂) prior to analysis. Briefly, 900 resonance units of C3b were thio-coupled to the CM5 sensor chip (52). Subsequently, a mixture of FB (110 μg/ml) and FD (1 μg/ml) was flowed (20 μl/min) over the surface for 120 s to form C3 convertase. After allowing the convertase to decay naturally for 120 s, the FH1–4 sample (0.5 μM or 0.17 μM) was flowed across the surface for 90 s, and convertase decay was visualized in real time. Between injections, surfaces were regenerated using 10 mM sodium acetate buffer, pH 4, containing 1 M NaCl. Data were evaluated using BIAevaluation 4.1 (GE Healthcare). As a control, construct was flowed over the bare surface and binding data subtracted from the decay histogram to account for CFH1–4 binding to the C3b surface.

**Cofactor Assay in Fluid Phase**—A dilution series fluid-phase assay (53) was used to measure cofactor activity for FI-mediated proteolytic cleavage of C3b. FI, FH, and C3b were all purchased from Complement Technology. For the positive control, FI (0.045 μg) and of C3b (3 μg) were mixed with 0.3 μg of FH (i.e. 0.1 μM) in a final reaction volume of 20 μl of PBS; the negative control was prepared in the same way but replacing FH1–4 with PBS. Experimental reaction mixtures contained a range of FH1–4 concentrations in place of FH. Reaction mixtures were vortexed and incubated in a water bath (37 °C) for 15 min. An aliquot of 2 × reducing SDS buffer (7.5 μl NuPage sample buffer (4×) (Invitrogen), 3 μl of NuPage reducing agent (1×) (Invitrogen) in a final volume of 30 μl of ddH₂O was added to stop the reaction, and samples were heated prior to SDS-PAGE. The FI/FH-catalyzed α’-chain cleavage was followed by visualizing the products and unused substrates using Coomassie Blue staining.

**RESULTS**

**Truncated Human FH Constructs Were Produced in P. pastoris**—The preparation from patients’ plasma of purified FH mutants, in a form free of other sequence variants, presents logistical and technical difficulties. In the current work, we avoided these issues by examining functional consequences of disease-linked N-terminal FH sequence variations in the context of recombinant, truncated FH constructs consisting only of CCPs 1–4 (with an N-terminal myc tag and a C-terminal His₆ tag; see Fig. 1, b and c) (FH1–4). The allotypic variants FH1–4V62 and FH1–4I62 were successfully produced in P. pastoris (Fig. 1c). The availability of both of these “wild-type” variants allowed comparison of their functional activities (see below) to establish a benchmark for subsequent studies of (R53H)FH1–4V62 and (R78F)FH1–4V62 that were also produced in P. pastoris (Fig. 1c). Not only was recombinant FH1–4 produced and purified in very much higher yields than recombinant FH (54, 55), but also its use in the current study avoided the complicat-
The presence of the second, stronger, C3b-binding site of FH in CCPs 19 and 20.

FH1–4I62 Is Slightly More Active than FH1–4V62 although Both Bind C3b Equally Well

In fluid-phase cofactor assays (Fig. 2) both wild-type variants of FH1–4 promoted cleavage of the C3 α-chain by FI, although FH1–4V62 was less efficient in this respect than FH1–4I62. An assay for cofactor activity for FI-mediated cleavage of C3b on cell surfaces similarly demonstrated that both variants are active although FH1–4V62 is less efficient than FH1–4I62 (Fig. 3). SPR studies demonstrated that FH1–4I62 (KD = 14 μM) binds no better to C3b (amine-coupled to a Biacore CM5 sensor chip) than does FH1–4V62.

FIGURE 1. Sequence variants of FH analyzed in the current study. a, modular composition of full-length human FH (each oval corresponds to a CCP module) highlighting functional sites and disease-associated mutations and polymorphic variations. b, constructs, bearing myc and His tags, produced in P. pastoris and analyzed for functional activity in the current study. c, location of Arg53, Ile62, and Arg78 within the co-crystal structure (57) of a CFH1–4C3b complex (Protein Data Bank ID code 2WII). Note that Arg53 is surface-exposed in this complex, Ile62 is buried within CCP 1 of CFH1–4, and Arg78 participates in an intermolecular H-bonded contact. d, reducing SDS-PAGE of variants used in this study.

FIGURE 2. Fluid-phase cofactor activity. FI and C3b were incubated for 10 min with a series of concentrations of the two wild-type FH1–4 variants and the two aHUS-linked mutants. Subsequently, the loss of the intact C3b α-chain and appearance of its FI cleavage products (43-kDa and 68-kDa fragments of iC3b α'-chain) were visualized by SDS-PAGE. Gels are labeled according to the concentrations of FH1–4 variant/mutant in the incubation mixture. It is apparent that in the range 75–300 nM, (R78G)FH1–4V62 promotes production of less 68-kDa iC3b α'-chain fragment than (R78G)FH1–4I62 or the wild-type variants. The presence of 25 nM FH1–4I62, but not of 25 nM FH1–4V62 (or the mutants), produces detectable 68-kDa iC3b α'-chain fragment.
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Despite this unimpaired affinity for C3b, (R53H)FH1–4\textsuperscript{V62}, like (R78G)FH1–4\textsuperscript{V62}, proved nearly 3-fold less active than wild-type FH1–4\textsuperscript{V62} as a cofactor for FI-catalyzed C3b cleavage on sheep erythrocyte surfaces (IH\textsubscript{50} 1.67 \textmu M versus 0.64 \textmu M; Figs. 2 and 3). The (R53H)CFH1–4\textsuperscript{V62} mutant was also less active in fluid-phase cofactor assays. The ability to decay C3bBb of (R53H)CFH1–4\textsuperscript{V62} was also severely impaired compared with wild-type protein as measured both by hemolysis assay (IH\textsubscript{50} 346 nM; Fig. 5a) and in real time by SPR (Fig. 5b).

**DISCUSSION**

The life-threatening kidney disease aHUS has been discussed extensively with respect to mutations that prevent the C terminal of complement FH from mediating selective regulatory action on self (rather than foreign) surfaces. We set out to discover whether a causal link could also be established between aHUS and mutations in the N-terminal module of FH, which is not involved in binding directly to surfaces.

It was important to first consider the widespread V62I polymorphism of FH. Ile\textsuperscript{62} has been reported to be protective (versus Val\textsuperscript{62}) for aHUS (46) as well as AMD (4) and DDD (4, 46), an effect ascribed to its superior cofactor activity (51). Indeed our results, obtained using FH1–4\textsuperscript{V62} and FH1–4\textsuperscript{I62}, agree with previous findings showing that twice the concentration of full-length FH\textsuperscript{V62} relative to full-length FH\textsuperscript{I62}, was needed to achieve 50% inactivation of C3b by FI in fluid-phase and on cell surfaces (51). These authors also found that FH\textsuperscript{I62} binds slightly more tightly to C3b (K\textsubscript{D} = 1.0 \mu M) than does FH\textsuperscript{V62} (K\textsubscript{D} = 1.3 \mu M), consistent with higher cofactor activity. We, however, observed no difference in C3b binding between FH1–4\textsuperscript{V62} and FH1–4\textsuperscript{I62}. Thus, the effect of I62V on C3b binding by FH is not exaggerated in the FH1–4 setting despite FH1–4 having only one C3b-binding site whereas full-length FH contains an additional, stronger, C3b-binding site.

We additionally showed that both FH1–4\textsuperscript{V62} and FH1–4\textsuperscript{I62} variants accelerate decay of the C3bBb complex with equivalent activity. This also agrees with data from Tortajada et al. (51), demonstrating similar relative decay accelerating activities for both wild-type variants of full-length FH. In summary, the new data establish that the CFH1–4\textsuperscript{V62} and CFH1–4\textsuperscript{I62} variants function efficiently in complement regulation with the CFH1–4\textsuperscript{V62} variant showing slightly better cofactor activity. Subtle differences between the two versions are consistent with their minor structural differences (56) and their relatively weak associations with disease propensity at least until old age. These studies provided a useful baseline for functional analyses of the mutants.

The poor affinity we observed between (R78G)FH1–4\textsuperscript{V62} and C3b agrees with inferences based on a co-crystal structure of C3b and CFH1–4 (57) in which Arg\textsuperscript{78} in the N-terminal CCP forms an H-bonded interaction with Glu\textsuperscript{732} in the α'N-terminal domain of C3b (Fig. 1c). *In vivo*, loss of affinity of the (R78G)FH N terminus for C3b could impair its ability to control AP convertases both in fluid phase and on cell surfaces. Therefore, the complement-regulating function of (R78G)FH1–4\textsuperscript{V62} was assessed and found to be very poor both in terms of cofactor and decay-accelerating activities. Taken together, these data support a disease mechanism in which (R78G)FH is function-
ally compromised by poor affinity for C3b, potentially resulting in severely impaired complement regulatory function and explaining low C3 levels in this patient (50). The physiological significance and consequences of these observations depend on the levels of the various proteins and whether or not they are locally present in sub-saturating concentrations. Very often an

![Figure 4](image-url)

**FIGURE 4. Measurements of affinity for C3b.** SPR response curves for concentration series (0.05, 0.1, 0.3, 0.6, 1.3, 2.5, 5.0, 10, and 20 μM; duplicate traces that did not overlay marked with *) of FH1–4V62 (a), FH1–4I62 (b), (R53H)FH1–4V62 (c), and (R78G)FH1–4V62 (d), respectively, flowed over C3b (2,000 response units) immobilized by amine coupling on a CM5 sensor chip. Plots of response (units) versus concentration, used to estimate $K_d$ values, are shown for FH1–4V62 (e), FH1–4I62 (f), (R53H)FH1–4V62 (g), and (R78G)FH1–4V62 (h).
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ahUS patient with a disease-linked CFH gene also has a normal version of the CFH gene; haploinsufficiency, caused by mis-
sense or nonsense mutations in just one allele, has previously
been associated with aHUS in many individuals (14, 58), illus-
trating the delicate balance between complement activation
and regulation required for maintenance of homeostasis and
health.

The unperturbed C3b-binding ability of a second aHUS-
linked mutant, (R53H)FH1–4V62, is consistent with the struc-
tural integrity of this mutant as reported previously on the basis
of an NMR study (56), and with the location of Arg53, within the
crystallized C3b-CFH1–4 complex, on an exposed face of FH
rather than adjacent to C3b (Fig. 1c) (57). Hence, the dimin-
ished cofactor activity of (R53H)FH1–4V62 cannot be explained
by the lower affinity for C3b as was the case with the R78G
mutant. An alternative explanation is that Arg53 lies within a
putative interaction site for FI, consistent with a previous sug-
gestion that FI binds to CCPs 1–3 within FH as well as to the
C345C domain of C3b (57). The decay accelerating activity of
(R53H)FH1–4V62 is also very poor. Given the wild-type-like
affinity of (R53H)FH1–4V62 for C3b, these data suggest that
impaired ability to accelerate decay of the convertase compo-
ents may be due to weakened association with Bb. Thus, the
side chain of Arg53 may participate in interactions with both Bb
and FI, implying overlap between binding sites for these ligands
within CCP module 1 of FH. The functionally deficient
(R53H)FH mutant would likely compete with wild-type FH for
binding C3b in heterozygous individuals and could thereby fur-
ther diminish complement regulatory capacity.

In summary, both N-terminal FH mutants examined in the
current study are defective in their ability to control the AP C3
convertase in the fluid phase and on cell surfaces, but the
molecular mechanisms underlying the dysregulation caused by
these mutant proteins are distinct. Thus, despite differences in
their C3b-binding properties, both mutations have similar out-
comes with respect to complement regulation and are associ-
ated with similar disease symptoms. It follows that a causal link
between mutations and disease is the simplest explanation of
these data. Previous functional analyses of C-terminal FH
mutants have suggested that multiple mechanisms are respon-
sible for defective cell surface regulation in aHUS (59–64);
these include decreased binding to C3b/C3d and/or to glyco-
saminoglycans and altered oligomerization of FH on the sur-
face. Thus, the current observations greatly strengthen the
hypothesis that a wide range of defects in FH activity can con-
tribute to a similar disease phenotype.

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