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EDUCATIONAL REVIEW

Genetics and complement in atypical HUS

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Abstract Central to the pathogenesis of atypical hemolytic uremic syndrome (aHUS) is over-activation of the alternative pathway of complement. Following the initial discovery of mutations in the complement regulatory protein, factor H, mutations have been described in factor I, membrane cofactor protein and thrombomodulin, which also result in decreased complement regulation. Autoantibodies to factor H have also been reported to impair complement regulation in aHUS. More recently, gain of function mutations in the complement components C3 and Factor B have been seen. This review focuses on the genetic causes of aHUS, their functional consequences, and clinical effect.

Keywords Hemolytic uremic syndrome · Transplantation · Complement · Factor H · Factor I · Membrane cofactor protein · Thrombomodulin · Thrombotic thrombocytopenic purpura

Abbreviations

HUS	Hemolytic uremic syndrome
D+ve HUS	Diarrhoeal-associated hemolytic uremic syndrome
aHUS	Atypical hemolytic uremic syndrome
AP	Alternative pathway
CP	Classical pathway
CCPs	Complement control protein modules
RCA	Regulators of complement activation
ESRF	End-stage renal failure
SNPs	Single nucleotide polymorphisms

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Introduction

Hemolytic uremic syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. It has been classified as either diarrhoeal-associated (D+ve) or non-diarrhoeal/atypical (aHUS) [1].

Pathologically, glomerular capillary wall thickening is seen due to endothelial cell swelling and accumulation of material between the endothelial cell and the basement membrane. This narrowing of the vessel lumen, in addition to the platelet and fibrin thrombi, results in occlusion of the glomerular capillaries. Fibrinoid necrosis of the afferent arteriole associated with thrombosis may also be seen. Mesangiolysis occurs early in the disease process and is subsequently replaced by sclerotic changes. Early arterial changes are variable, ranging from only mild endothelial swelling to fibrinoid necrosis with occlusive thrombus formation. Later in the disease process there is mucoid intimal hyperplasia with narrowing of the vessel lumen. Immunofluorescence demonstrates deposition of fibrin or fibrinogen in the glomeruli and in the mesangium as well as within the vessel walls. Granular deposits of complement and immunoglobulins along the capillary loops of glomeruli are occasionally seen [2].

D+ve HUS accounts for the majority of cases and is usually caused by a preceding illness with vero cytotoxin-producing bacteria, most commonly *E. coli* O157:H7 [3]. aHUS is rare, accounting for ~10% of HUS and has a poor prognosis with up to 50% of patients requiring ongoing

dialysis [4]. aHUS occurs at any age and may be sporadic or familial. Guidelines on the diagnosis of aHUS have recently been published [5].

The past decade has revealed aHUS to be a disease characterized by over-activation of the alternative complement pathway (AP) (Fig. 1). Since the initial description of mutations in the gene (*CFH*) encoding the complement regulator factor H in aHUS, mutations in other complement proteins and the presence of autoantibodies against complement regulators have been demonstrated in many cohorts of aHUS patients. In this review, we will discuss the genetic and autoimmune factors which predispose to aHUS and their functional consequences.

Role of complement factor H

Factor H is an abundant serum glycoprotein (~500 μg/ml) which is composed of 20 complement control protein modules (CCPs) [6]. It is the most important fluid-phase regulator of the AP. It acts as a cofactor for factor I-mediated proteolytic inactivation of C3b, competes with factor B for C3b binding, and accelerates the decay of the C3 convertase into its components [7]. These functions are mediated through the N-terminal four CCPs (CCP 1–4) [8]. Factor H also regulates complement on host surfaces by binding to the glycosaminoglycans of endothelial cells and exposed basement membranes via its carboxyl-terminal domain (CCPs 19–20) [9].

Mutations in *CFH* associated with aHUS were first demonstrated by Warwicker et al. [10] and have been demonstrated to be the commonest genetic abnormality found in many cohorts of aHUS patients [11–16]. The majority of mutations are heterozygous and are located in the exons encoding the C-terminal domain of the protein which is responsible for mediating complement protection on the cell surface (Fig. 2). These mutations do not usually result in a quantitative deficiency of factor H.

CFH lies in the RCA gene cluster at chromosome 1q32 and is in close proximity to the genes (*CFHR1–5*) encoding the five factor H-related proteins. The *CFHR*s show a very high degree of sequence identity to *CFH* (Fig. 2) and are thought to have arisen from several large genomic duplications. This

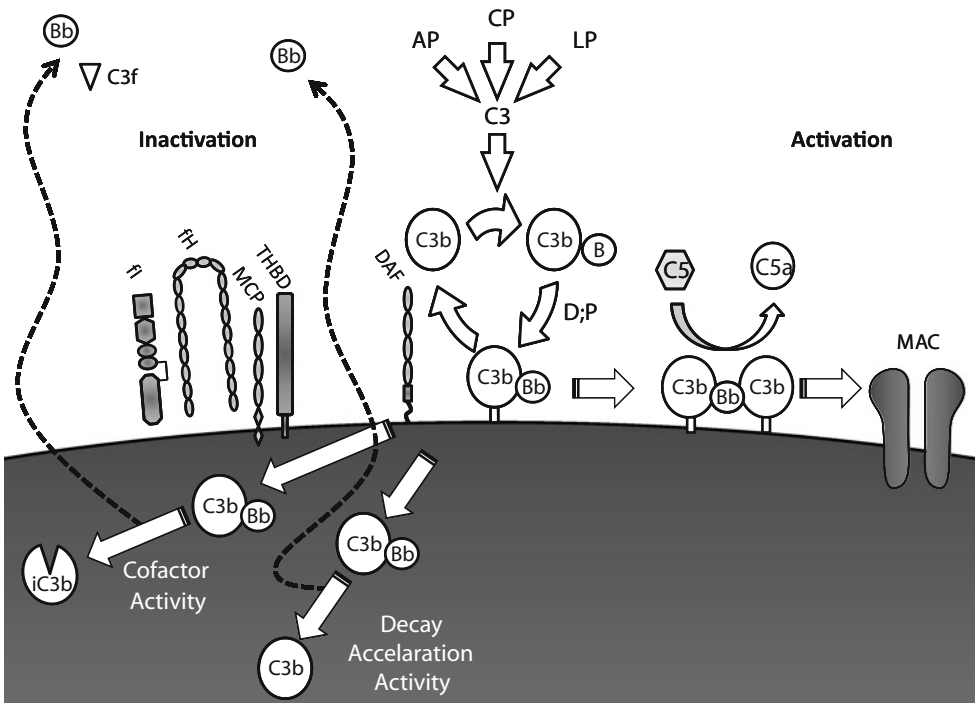

Fig. 1 Complement regulation. The alternative complement pathway (AP) is a positive-feedback amplification loop. The AP constantly undergoes tick over but can also be primed by the classical (CP) and lectin (LP) pathways. The C3b that is formed interacts with factor B (B), which is then cleaved by factor D (D) to form the AP C3 convertase (C3bBb). This enzyme complex is attached to the target covalently via C3b while Bb is the catalytic serine protease subunit. It is stabilized by binding properdin (P). Because C3 is the substrate for this convertase, a powerful feedback loop is created. Unchecked, this will lead to activation of the terminal complement pathway with generation of the effector molecules; the anaphylatoxin C5a and the membrane attack complex (MAC). To limit damage to host cells, the AP is down-regulated by cofactor activity (CA) and decay accelerating activity (DAA). CA results in the permanent inactivation of C3b to iC3b, such that it is no longer capable of binding B and thus cannot form the AP convertase. CA requires both a cofactor protein (factor H [fH] or membrane cofactor protein [MCP]) and a protease, factor I (fI). DAA is the dissociation of the C3/C5 convertases. The decay accelerator protein, in this example decay accelerating factor (DAF), displaces the catalytic Bb from target-bound C3b. However, this C3b can bind another B and then reform the convertase

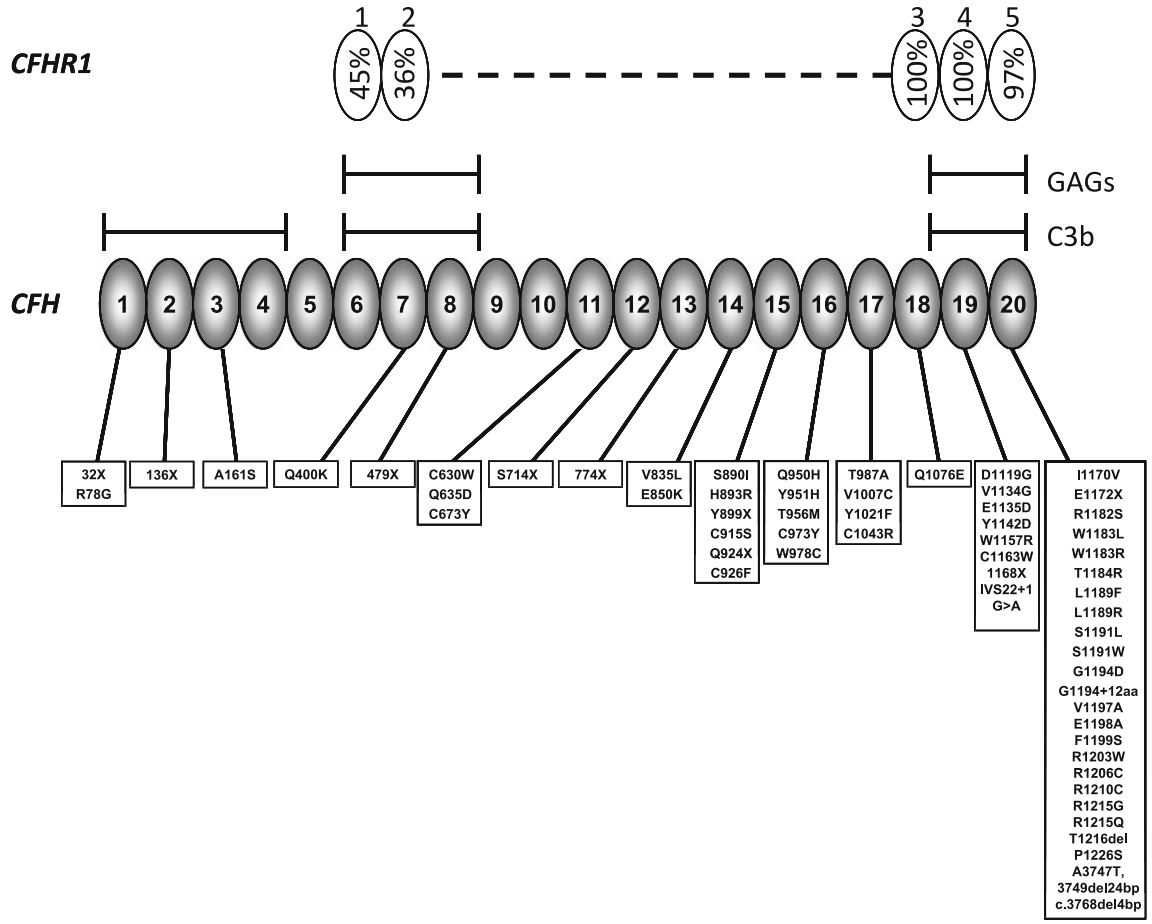

Fig. 2 Complement factor H and complement factor H-related 1. The figure demonstrates the 20 CCP modules of factor H (*bottom*) and the five CCP modules of factor H-related protein 1 (*top*). The homology of each factor H-related protein 1 CCP to the corresponding factor H CCP is given as a percentage in the factor H-related protein 1 figure. Note the homology between CCP18 of factor H and CCP 3 of factor H-related protein 1 is given for the basic isoform. An acidic isoform differs by three amino acids [62]. The glycosaminoglycan and C3b binding sites of factor H are indicated on the diagram. Mutations in *CFH* reported in aHUS are listed below the figure

homology predisposes to both gene conversion and genomic rearrangements through non-allelic homologous recombination (NAHR). Heinen et al. [17] showed that the aHUS-associated factor H mutations S1191L, V1197A, and combined S1191L/V1197A had arisen through gene conversion between *CFHR1* and *CFH*. Venables et al. showed that a hybrid (fusion) gene comprising the 21 N-terminal exons of *CFH* and the 2 C terminal exons of *CFHR1* has arisen through NAHR and is associated with aHUS [18].

The functional consequences of aHUS-associated factor H mutations have been studied, particularly those which cluster in the C-terminal domain of the protein. Structural analysis has shown that all such mutants are folded and have only very localized structural perturbations [19]. Functional analysis has shown varied consequences on the binding to heparin, C3b, and endothelial cells (Table 1). However, all aHUS-associated factor H mutants show impaired complement regulation at the cell surface using erythrocyte lysis assays [19–21]. Thus it is hypothesized that these C-terminal mutants fail to control complement activation at the glomerular endothelium particularly where basement membrane is exposed by the fenestrated endothelium. Renal biopsy data from an aHUS patient with a C-terminal mutant showed reduced factor H binding to renal endothelium compared to wild-type, in keeping with this hypothesis [22]. Additionally, it has been demonstrated that aHUS-associated C-terminal factor H mutants have reduced ability to bind to platelets, resulting in complement activation on the surface of platelets. This in turn causes platelet activation with aggregation and release of tissue-factor-expressing micro-particles [23]. Thus complement activation on the glomerular vasculature and on platelets is thought to result in the pro-coagulant phenotype which leads to aHUS.

As most *CFH* mutations associated with aHUS are heterozygous, it has been postulated that these mutations may exert a dominant negative effect [24]. Recent studies have suggested that factor H may exist in monomer-dimer

Table 1 Structural and functional consequences of mutations in aHUS

Mutant	CCP	Structural changes	C3b/d binding	Heparin binding	Endothelial cell binding	Hemolysis assay	Reference
D1119G	19	Local	↓[e]	↔	↔[a]	↓[c]	[19, 86]
Y1142C	19	ND	ND	ND	ND	↓[d]	[21]
W1157R	19	ND	↓	↓	ND	ND	[87]
E1172X	20	ND	↓	↓	ND	ND	[88, 89]
R1182S	20	Local	↓	↓	ND	↓[c,d]	[19, 20]
W1183R	20	Local	↑	↑	ND	↓[c]	[19]
W1183L	20	Local	↓	↓[e]	↓[a,b,e]	↓[c]	[19, 86, 87, 90]
T1184R	20	Local	↑[e]	↑	↑[a]	↓[c]	[19, 86]
L1189R	20	Local	↑	↑	↑[a]	↓[c,d]	[19, 20, 86]
L1189F	20	Local	↑	↑	ND	↓[c,d]	[19, 20]
S1191W	20	ND	ND	ND	ND	↓[d]	[20]
S1191L	20	Local	↑[e]	↔	ND	↓[c,d]	[17, 19]
S1191L/V1197A	20	Local	↑[e]	↔	ND	↓[c,d]	[17, 19]
V1197A	20	ND	↓[e]	↓[e]	ND	↓[d]	[17, 20, 87, 90]
E1198K	20	ND	ND	ND	↓[b]	↓[d]	[22]
R1210C	20	Local	↓	↓[e]	↓[b]	↓[c]	[19, 20, 87, 89, 90]
R1215G	20	Local	↓	↓	↓[b]	↓[c]	[19, 87, 89]
R1215Q	20	ND	↔	↓	↔[a]	ND	[86]
P1226S	20	ND	↓	↓	ND	ND	[87]

ND not done; Endothelial cell binding relates to either [a] mGEnC-1 [86] or [b] HUVEC [22, 88, 89] binding. Hemolysis assay using [c] recombinant proteins to compete with full-length *CFH* on human erythrocytes [19] or [d] using patient serum on sheep erythrocytes [20]. To amalgamate these different hemolysis assays, the *arrows* indicate the effect on cell surface complement regulation produced by the mutation. [e] Indicates contradictory results

equilibrium, but that up to 95% will be monomeric in isolation in serum [25]. However, oligomerization of factor H via glycosaminoglycans [26] or C3d [27] on cell surfaces has been described. The extent to which oligomerization of mutant factor H with wild-type may interfere with the complement regulatory function has yet to be established.

The *CFH* knockout mouse ($Cfh^{-/-}$) and a transgenic mouse lacking the C-terminal region of factor H ($Cfh^{-/-}\Delta16–20$) have proved illuminating [28]. The $Cfh^{-/-}$ mouse has very low C3 levels due to uncontrolled turnover of the alternative pathway and has a renal phenotype similar to membranoproliferative glomerulonephritis (MPGN) [29]. This is similar to the phenotype of the factor H-deficient Norwegian–Yorkshire pig [30]. In contrast, the $Cfh^{-/-}\Delta16–20$ mouse has higher plasma C3 levels than the $Cfh^{-/-}$ mouse, and spontaneously develops aHUS, not MPGN [31]. Thus, this mouse model provides the first in vivo evidence that the *CFH* mutations seen in aHUS impair endothelial cell surface recognition, resulting in local complement dysregulation, while controlling the alternative pathway in plasma. Goicoechea et al. [32] have also crossed the $Cfh^{-/-}\Delta16–20$ with a C5-deficient mouse to investigate the role of C5 activation in the pathogenesis of aHUS. These $C5^{-/-}$ $CFH^{-/-}\Delta16–20$ mice do not develop aHUS, suggesting a critical role downstream of C3b generation in aHUS.

Mutations in membrane cofactor protein (*MCP*: CD46)

Membrane cofactor protein (MCP: CD46) is a membrane glycoprotein present on the surface of all cells with the exception of erythrocytes [33]. It consists of an extracellular segment comprising 4 CCPs, an alternatively spliced STP region and a group of 12 amino acids of unknown function. This is followed by a transmembrane domain and an alternatively spliced cytoplasmic tail [33] (Fig. 3). As with *CFH* and the *CFHR*s, the gene (*MCP*, *CD46*) encoding MCP is located in the RCA cluster. MCP acts as a cofactor for factor I in the proteolytic inactivation of C3b and C4b bound to host cells.

Mutations in *MCP* have been demonstrated in up to 15% of cohorts of aHUS patients [13, 34–37]. The majority of *MCP* mutations in aHUS are heterozygous (~75%), although homozygous or compound heterozygous mutations are reported [33]. Most *MCP* mutations cluster in the four extracellular complement control protein domains of *MCP* (Fig. 3), the region critical of complement regulation [33].

Around 75% of *MCP* mutations result in decreased cell surface expression and a quantitative deficiency in complement regulation [33]. Functional analysis of the remaining mutations has demonstrated a qualitative defect in complement regulation. Ligand binding and cofactor assays of two

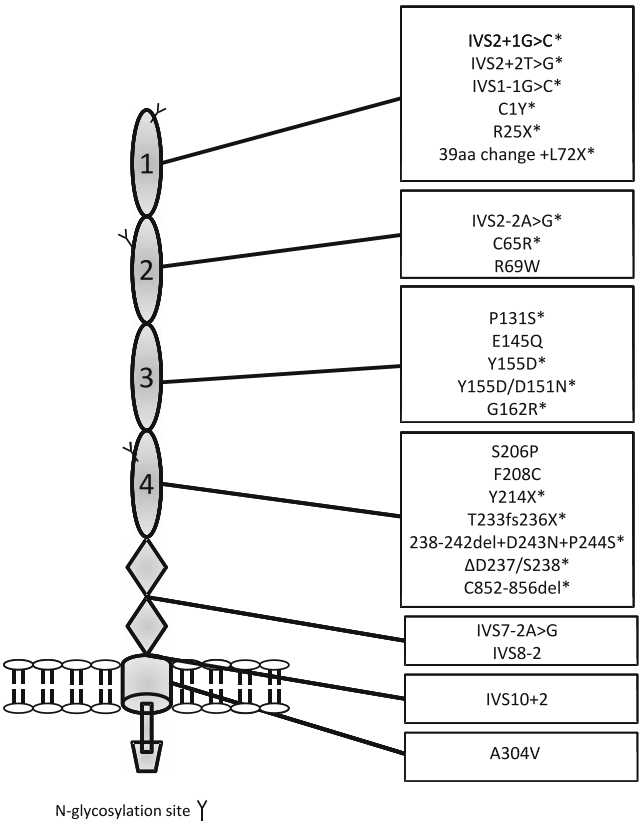

Fig. 3 Membrane cofactor protein. The figure demonstrates clustering of mutations in the four extracellular CCP domains of membrane cofactor protein in aHUS. * denotes mutations resulting in decreased cell surface expression of membrane cofactor protein

mutations with normal expression (S206P; F208C) demonstrated reduced C3b binding and cofactor activity [13, 33, 34]. These assays of complement regulatory activity failed to reveal any defect in two further normally expressed variants (R69W; A304V), however further analysis of function using cell surface assays demonstrated deficient control of the alternative pathway [38]. Only one mutation (E145Q) so far described has affected only C4b cofactor activity and the surface expression of this mutant was increased [33, 35].

Complement factor I

Factor I is a highly specific serine protease that acts through its proteolytic activity to control complement activation. It consists of a catalytic light-chain disulfide bonded to a heavy chain of unknown function. Factor I cleaves the α chains of C3b and C4b in the presence of its cofactor proteins: factor H for C3b [39, 40]; C4-binding protein (C4BP) for C4b [41, 42]; and MCP [43] and complement receptor 1 (CR1; CD35) [44, 45] for both. By inactivating C3b and C4b through limited proteolytic cleavage, factor I prevents the formation of the C3 and C5 convertases and thus down-regulates the AP and classical pathway (CP). Factor I is a serum glycoprotein predominantly synthesized by the liver. Unlike many other complement regulatory genes *CFI* does not reside in the RCA cluster at 1q32 but is located at chromosome 4q25.

CFI mutations have been reported in 2–12% of aHUS patients [13, 46–50]. The mutations are seen throughout the molecule but are most commonly seen the serine protease domain (Fig. 4).

In vitro analysis of aHUS-associated mutations has demonstrated that the majority of mutations in *CFI* result in a quantitative defect. Analysis has shown that even in those mutations which have been shown to decrease secretion, the serum factor I level can be within the normal range [47]. Factor I is an acute-phase protein and this is probably responsible for the large variation seen in factor I levels in normal individuals (39–100 μg/ml) [51]. A minority of mutations in *CFI* result in a qualitative defect in complement regulation. Functional analysis of these mutations demonstrated a loss of alternative and classical cofactor activity, both in the fluid phase and on cell surfaces [50, 52]. However, analysis of some *CFI* variants, using recombinant proteins, has failed to demonstrate a functional consequence (e.g. G243D [53]). It is possible that they are not involved in the pathogenesis of disease or alternatively, the defect may be too subtle to be detected by currently available assays. This emphasizes the difficulty in attributing disease causality to mis-sense mutations.

Thrombomodulin

Thrombomodulin (THBD) is a key component of the protein C anticoagulation pathway and facilitates the activation of protein C by thrombin [54]. Additionally, it enhances thrombin-mediated activation of plasma procarboxypeptidase B (TAFI) an inhibitor of fibrinolysis that also inactivates the complement-derived anaphylatoxins C3a and C5a. THBD has recently been shown to down-regulate the AP of complement by accelerating factor I-mediated inactivation of C3b in the presence of co-factors [55]. Mutations in the gene (*THBD*) encoding thrombomodulin have recently been shown to predispose to atypical HUS. These mutations resulted in a loss of cofactor activity [55].

Factor B (*CFB*) mutations

Mutations have also recently been described in factor B (CFB) although they are rare in the cohorts so far described (0–3%) [56–58]. Factor B carries the catalytic site of the complement AP convertase (C3bBb) and as such, unlike previously described mutants associated with aHUS, acts as

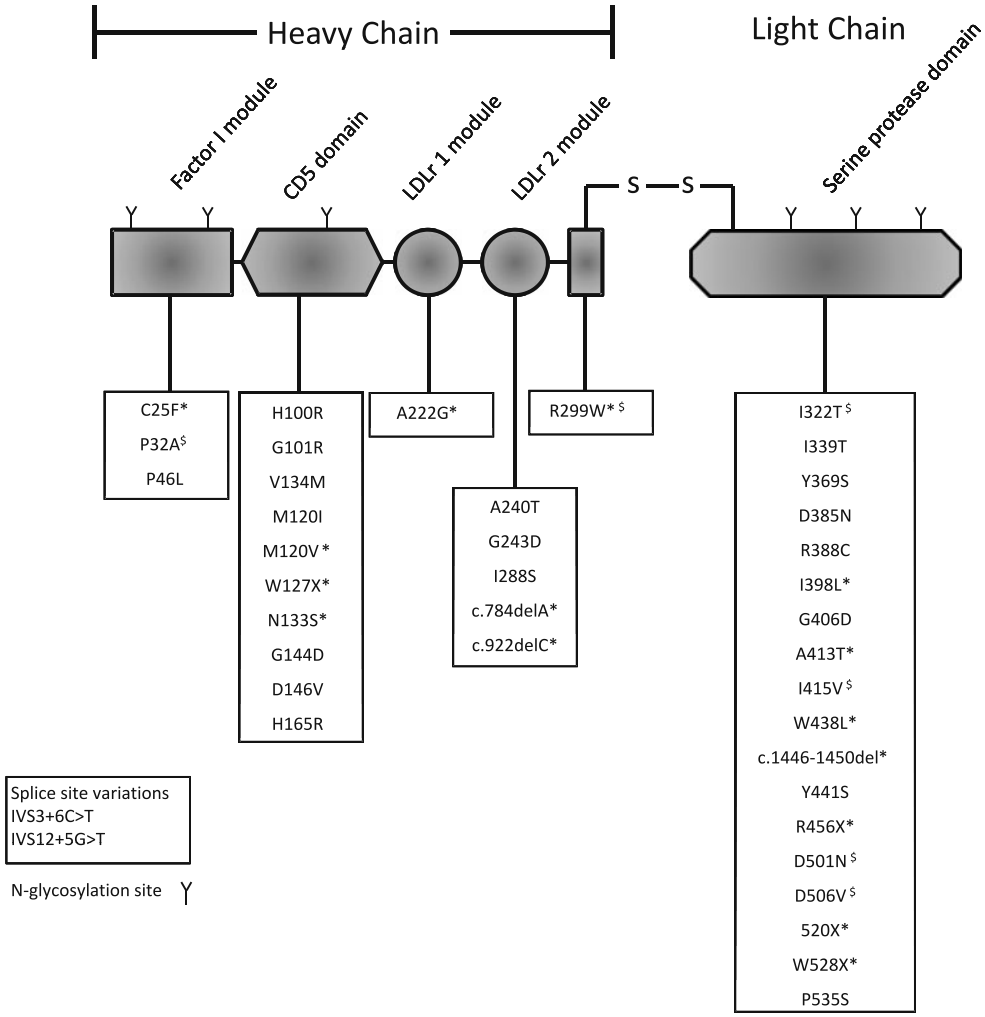

Fig. 4 Complement factor I. The figure demonstrates the modular structure of factor I with aHUS-associated mutations below. $ denotes factor I mutants that are secreted but have been demonstrated to have decreased activity. * denotes factor I mutants that have impaired secretion

an activator of complement, not a regulator. Mutations in *CFB* associated with aHUS, are therefore activating mutations. Goicoechea de Jorge et al. [56] elegantly described two mechanisms through which these separate mutations led to increased complement activation. One mutant (F286L) showed enhanced formation of the C3bB proenzyme, which will result in a more active enzyme in vivo. The other mutant (K323E) formed a C3bBb enzyme more resistant to decay by the complement regulators decay accelerating factor (DAF; CD55) and factor H. This also caused increased enzyme activity [56]. The two mutations described in the French cohort were also located in the von-Willebrand type A domain, suggesting that this is a hotspot for mutations in aHUS. These mutations were both demonstrated to be more active than the wild-type protein, resulting in increased complement deposition on human glomerular endothelial cells [58].

C3 mutations

C3 is the central component of the complement cascade, critical to activation by the classical, lectin, and alternative pathways. C3 is cleaved to form the anaphylatoxin C3a and C3b, which is highly reactive and can bind to cell surfaces via its reactive thioester. C3b can then interact with factor B in the presence of factor D to form the alternative pathway convertase, thus introducing a positive-feedback amplification loop.

Recently mutations in C3 have been described in aHUS patients [59, 60]. Functional analysis of five of the nine mutations so far described has revealed decreased binding to MCP with a consequent decrease in the ability of MCP to act as a cofactor to inactivate C3b [60]. Thus, in vivo these will act as gain of function mutations. In two of the mutations, no functional impairment could be demonstrated and in another two, the mutations resulted in a decreased secretion of C3. How impaired secretion of C3 fits into the current model of complement over-activation in aHUS is yet to be resolved.

Autoantibodies to factor H/factor H-related 1

In addition to the genetic abnormalities described in aHUS, autoantibodies to factor H have also been linked to disease

in 5–10% of aHUS patients [61–66]. Available data would suggest that the onset of disease or disease recurrence correlates with the presence of factor H autoantibodies. The titre of these antibodies may also spontaneously decline with time.

Jozsi et al. demonstrated using recombinant fragments of factor H that the autoantibodies in five patients bound to the C-terminus of the molecule [65]. This is an area of factor H that is responsible for cell surface protection and is a hotspot for mutations. Factor H autoantibodies have been demonstrated to impair the binding of factor H to C3b and are associated with increased hemolysis of sheep erythrocytes in patient plasma [65]. In the Newcastle cohort, the majority of the autoantibodies also bound to CCPs19–20 [61].

An 80-kb-long genomic deletion of *CFHR*1 and *CFHR*3 has been associated with an increased risk of aHUS [66]. It was subsequently demonstrated that this complete deficiency of *CFHR*1 and *CFHR*3 was strongly associated with factor H autoantibodies [64]. More detailed analysis has subsequently revealed aHUS patients with *CFHR*1 deficiency resulting from point mutations in *CFHR*1 [62] or from a deletion incorporating *CFHR*1 and *CFHR*4 [61, 62]. Thus the association between the *CFHR*1/*CFHR*3 deletion and the presence of autoantibodies in aHUS is probably related to the absence of CFHR1. It should be noted, however, that deficiency of CFHR1 is not a prerequisite for formation of autoantibodies as Moore et al. describe three patients with no evidence of deficiency of CFHR1 or CFHR3 and high titres of autoantibodies [61].

The two C-terminal CCPs (4 and 5) of CFHR1 are almost identical to CCPs19–20 of factor H (Fig. 2) and it is not surprising therefore that autoantibodies to CCPs19–20 of factor H also bind to CCPs 4–5 of *CFHR*1 [61]. It has been suggested that the antibodies generated in the absence of *CFHR*1 are different to those in its presence [62] and it is interesting that one of the patients with two copies of *CFHR*1 had antibodies against factor H CCPs1–4 [61], the region of the molecule responsible for cofactor and decay accelerating activity. Although most individuals with autoantibodies and *CFHR*1 deficiency do generate autoantibodies to CCP19–20, antibodies to this epitope are seen in the presence of *CFHR*1 [61].

Other complement genes

A mutation (Q433P) in the gene (*Clu*) encoding clusterin has been described in one family with aHUS [67]. Clusterin is a serum regulator of the terminal pathway of complement, it is predominantly produced by the liver, but is also released by activated platelets [68, 69]. In addition to the *Clu* mutation, the patient also had a functionally significant *MCP* mutation. This was of paternal origin while the *Clu* mutation came through the maternal line. Functional analysis of the mutant clusterin revealed decreased binding to C5b-7 and reduced complement regulation in a hemolytic assay. Serum from this patient also induced complement deposition on platelets and their activation. Although this mutation has been demonstrated to be functionally significant, aHUS only occurred in the presence of an *MCP* mutation known to predispose to aHUS. This is in keeping with the hypothesis that multiple concurrent factors may be necessary in individual patients for disease manifestation [70]. Mutation screening of other complement genes has been undertaken in aHUS. *DAF* is another complement regulatory gene located in the RCA cluster. It encodes a widely expressed membrane bound regulatory protein which accelerates the decay of either C4bC2a or C3bBb and the corresponding C5 convertases. Mutation screening of DAF in two cohorts has shown only one mutation which did not impair regulatory activity [56, 71]. *Complement receptor 1* (CR1) is a cell surface complement regulator with cofactor and decay accelerating activity for both the AP and CP. In the one reported cohort of patients, no mutations were detected [36]. Screening of the genes (CFHR1–5) encoding the factor H-related proteins in one cohort of aHUS individuals failed to reveal mutations [37] while no causative mutations in *CFHR*5 were demonstrated in a separate cohort [72]. However, Abarrategui-Garrido have shown that deficiency of factor H-related protein 1 is in some aHUS patients secondary to point mutations in *CFHR1*. They also described a novel variant of *CFHR1*, which is probably a result of gene conversion between *CFH* and *CFHR1*. This variant is strongly associated with aHUS [62].

Incomplete penetrance

Incomplete penetrance has been reported for all the genes associated with aHUS. For mutations in *CFH*, *CFI*, *MCP*, and *CFB* penetrance is ~50% while in the limited number of C3 mutations described to date, the penetrance is lower [59].

It has now been demonstrated that for disease to manifest in an individual a combination of mutations, risk haplotypes, and single nucleotide polymorphisms (SNPs) must be present [13, 31, 64, 66, 73–77]. The *CFH* risk haplotype for aHUS (*CFH*–H3) contains a SNP in the region of *CFH* responsible for cofactor activity. Functional analysis has demonstrated that the risk variant, *CFH*-Val_{62}, has a subtle decrease in cofactor activity compared to the protective variant, in keeping with the minor structural differences between these SNPs [8, 78].

Similar risk haplotypes have also been described in *MCP* [73, 74] with a haplotype termed *MCPggaac* conferring a two-fold increased risk of aHUS compared with controls [74]. This contains two SNPs in the *MCP* promoter and reporter gene assays suggests that these haplotype differences may reduce transcriptional activity in the risk haplotypes by

25% [74, 75]. In vivo differences have not been demonstrated between the haplotypes, however, and it is possible that this is only revealed at times of cellular activation [74, 75].

C4b binding protein is the predominant regulator of the classical pathway of complement in the fluid phase [79, 80]. In addition to classical pathway activity, *C4BP* is also a weak regulator of the alternative pathway, acting as a cofactor for the factor I mediated cleavage of C3b. It is comprised of 7 identical α chains and a single β chain. *C4BP* is encoded by two genes, *C4BP*A and *C4BP*A in the RCA cluster. A SNP in *C4BP* (R240H) was associated with aHUS in cohorts from the UK and France [77]. Functional analysis of the change demonstrated normal secretion of the protein and normal ability to regulate classical and lectin pathways. The R240H SNP was, however, unable to down regulate the alternative pathway as efficiently as wild-type [77]. Many of the aHUS patients with this polymorphism also carried mutations in other complement genes associated with aHUS, and it is possible that this change is an additive risk factor for aHUS. However, this association was not confirmed in a separate Spanish cohort of patients [81].

The *CFHR*1/*CFHR*3 deletion is another risk factor for the development of aHUS as described previously.

Thus, there are now described a number haplotypes and SNPs which act in concert with mutations in complement genes and inhibitory autoantibodies. Only when an unfavorable group of risk factors co-segregate will aHUS develop. Even then the disease may not manifest until middle age, suggesting that a trigger is required to reveal the latent complement regulatory deficiency. These precipitating events are hypothesized to be the endothelial cell insults which have historically been associated with aHUS [82]. In an Italian cohort of patients with *MCP* mutations, infection precipitated the onset of disease in all individuals. In those with factor H mutations, 70% of cases were preceded by infection, and pregnancy and drugs each accounted for 4%. For *CFI* mutations 40% were preceded by pregnancy and 60% were precipitated by infection [13].

Thus, the environmental trigger initiates the positive feedback loop of complement and in susceptible individuals unable to control complement turnover, disease will manifest.

Genotype: phenotype effects

The prognosis of patients correlates to a certain extent with the genetic defect. Those with *CFH* mutations carry the worst outcome. Within a year after disease onset, 60–70% [13, 49] of patients with *CFH* mutations die or reach ESRF. This genotype: phenotype is not absolute, however. This was best illustrated by analysis of 13 individuals carrying the same mutation (R1210C) [76]. As a whole the group did poorly, eight patients developed end-stage renal failure and one patient had chronic kidney disease, but there was one individual with this mutation who had a single episode of aHUS with no long-term sequelae. It is noteworthy that of this group only this individual did not have additional genetic risk factors for aHUS. Thus, although mutations in *CFH* are a poor prognostic indicator, it appears that other genetic risk factors do modify disease outcome.

In comparison to those with *CFH* mutations, the outcome of patients with *MCP* mutations is good, with ~80% remaining dialysis-independent [13, 49]. Recurrence of disease in *MCP*-associated aHUS is frequent however. In the minority of cases with *MCP* mutations that do go onto develop ESRF, it is likely that additional genetic modifiers are present.

In those with *CFI*-associated aHUS, the prognosis is intermediate between *CFH* and *MCP*-associated aHUS. A report from an Italian cohort of patients suggested that in those with *CFI*-associated aHUS, >60% of patients developed ESRF [13]. In the French cohort, in those with mutations in *CFI*, around 50% of patients died or progressed to ESRF within 2 years of the initial episode of aHUS, while ~30% of individuals recovered with no disease recurrences [49]. Bienaime et al. demonstrated that the severity of disease was influenced by additional genetic factors. In particular, those with an associated deletion of *CFHR*1 had a significantly worse outcome [50].

To date, all those individuals with factor H autoantibodies are children [61, 63] and less than 50% have gone on to develop ESRF [61, 63].

Definite clinical correlations have yet to emerge in those with *CFB* and C3 mutations due to the limited number of cases so far reported. The outcome of patients with *CFB* mutations would appear poor, however, with the majority of patients developing ESRF. Of the 14 individuals reported with C3-associated aHUS, eight developed ESRF and one developed chronic renal impairment.

In addition to pre-determining the outcome of aHUS in the native kidneys, the genetic predisposition also affects the aHUS recurrence rate after renal transplantation. The complement regulatory defect in those with mutations in the membrane bound, *MCP*, is corrected by an allograft bearing wild-type *MCP* and so the recurrence rate is low. In those with mutations in *CFH* and *CFI*, which are produced in the liver, a renal transplant does not correct the defect so the recurrence rate is high [83–85].

Summary

Although many different alterations in complement genes have been reported to predispose to aHUS, the downstream consequence of all is over-activation of the alternative pathway of complement on the glomerular vasculature. It is

increasingly becoming clear that a combination of mutations, SNPs, and haplotypes are required for disease to manifest upon exposure to an environmental trigger. The genetic predisposition also determines the prognosis after the initial episode and following renal transplantation.

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Questions

(Answers appear following the reference list)

1. Familial atypical HUS
   a) Is completely penetrant.
   b) Has been causally associated with Eculizumab treatment.
   c) Is predisposed to by mutations in complement factor H.
   d) Always presents in childhood.
   e) Never recurs following renal transplantation.
2. In atypical HUS
   a) Complement factor B mutations are the commonest genetic cause.
   b) Individuals with membrane cofactor mutations never have disease recurrence.
   c) Individuals who present during pregnancy never have complement mutations.
   d) Complement factor H mutations are the rarest genetic cause.
   e) Autoantibodies to factor H have been associated with disease.
3. Atypical hemolytic uremic syndrome is associated with mutations in complement regulatory genes. A low recurrence rate of aHUS post renal transplantation is associated with mutations in which of the following genes?
   a) Complement factor I
   b) Complement factor H
   c) Membrane cofactor protein
   d) Complement factor B
   e) C3
4. In aHUS, autoantibodies to complement factor H
   a) Do not result in impaired complement regulation at host cell surfaces.
   b) Are associated with a deletion of the CFHR1 and CFHR3 genes.
   c) Always bind to the C-terminal region of complement factor H.
   d) Never cross react with the complement factor H-related 1 protein.
   e) Cannot be removed by plasma exchange.

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Answers:

1. c
2. e
3. c
4. b