Factor H family proteins and human diseases

Mihály Józsi[1] and Peter F. Zipfel[2,3]

[1] Junior Research Group Cellular Immunobiology and [2] Department of Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany; [3] Friedrich Schiller University, Jena, Germany

Corresponding author: Zipfel, P.F. (peter.zipfel@hki-jena.de)

Abbreviations: AMD, age-related macular degeneration; SCR, short consensus repeat domain; CFH, factor H; CFHR, factor H-related; aHUS, atypical hemolytic uremic syndrome; MPGN II, membranoproliferative glomerulonephritis type II; CRP, C-reactive protein

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Abstract

Complement is a major defense system of innate immunity and aimed to destroy microbes. One of the central complement regulators is factor H, which belongs to a protein family that includes CFHL1 and five factor H-related (CFHR) proteins. Recent evidence shows that factor H family proteins (factor H and CFHRs) are associated with diverse and severe human diseases, and are also utilized by human-pathogenic microbes for complement evasion. Therefore, dissecting the exact functions of the individual CFHR proteins will provide insights into the pathophysiology of such inflammatory and infectious diseases, and will define the therapeutic potential of these proteins.

Complement, an innate defense system

Complement is an essential part of innate immunity, and plays a central role in the elimination of microbes, clearing of immune complexes and damaged self cells, and also in modulating the adaptive immune response [1]. Inappropriate complement function, however, leads to various human diseases [2]. Due to its vital role in homeostasis, this network of proteins receives increasing attention from immunologists and clinical oriented researchers. It is therefore of general interest to understand the physiologic function of this defense system at the molecular level.

The complement system consists of ~35 proteins that are present in body fluids or on cell and tissue surfaces, and is activated in a cascade-like manner by three major pathways [1]. The alternative pathway is activated continuously at a low rate by the spontaneous hydrolysis of the central component C3, the lectin pathway is initiated by mannose binding lectin or ficolins that recognize microbial carbohydrates, and the classical pathway is activated by binding of C1q to antigen bound immunoglobulins. Enzymatic steps generate active fragments of complement components, and trigger further amplification. The three pathways merge at the level of C3, which upon activation is cleaved into C3a and C3b. C3a acts as an anaphylatoxin and antimicrobial substance. C3b binds covalently to surfaces, and as opsonin aids phagocytosis of target cells. C3b is also part of the C3 and C5 convertase enzymes, which by cleaving C3 and C5 further amplify complement activation. This step may initiate the terminal pathway, which leads to the assembly of membrane attack complexes that form pores on target cells and cause lysis.

Complement activation, which by the elimination of invading microbes is important for host defense, may also damage host tissues. To prevent this, the human body utilizes fluid-phase and membrane anchored complement regulators. Several regulators act at the level of C3, and either facilitate the disassembly of the C3 convertases (decay accelerating activity) or act as cofactors for the serine protease factor I, which in the presence of a cofactor cleaves and inactivates C3b and C4b. Thus further activation is inhibited. With a concentration of 300-800 mg/L, factor H (CFH) is one of the most abundant human plasma proteins and is an important complement regulator that acts both in fluid phase and on tissue surfaces [3].

The human factor H protein family

CFH is the best characterized member of the CFH protein family. This family of closely related proteins includes the complement regulators CFH and CFH-like protein 1 (CFHL1), as well as five CFH-related proteins CFHR1, CFHR2, CFHR3, CFHR4 and CFHR5 [4]. The individual CFH family proteins share common features, as these plasma glycoproteins are produced primarily in the liver, they are exclusively composed of individually folding domains termed short consensus repeats (SCRs; also known as complement control protein modules), and they show immunological cross-reactivity. The relatedness of the individual proteins is further reflected on the genomic level, by the tandem arrangement of the *CFH* gene and the five *CFHR* genes in the CFH gene cluster on human chromosome 1q32 **(Figure 1A)**.

CFH is composed of 20 SCR domains. Two major functional regions are located at the opposite ends of the protein. The N-terminal four SCR domains (SCRs 1-4) display complement regulatory activity by facilitating the decay of the C3 convertase and acting as a

cofactor for factor I [3]. The C-terminus of the protein (SCRs 19-20) mediates surface binding and target recognition [5]. This C-terminal region includes binding sites for several ligands, such as C3b, C3d, heparin, cell surface glycosaminoglycans and microbial virulence factors [6]. Attached to the surface of host cells and membranes, such as the glomerular basement membrane, CFH inhibits complement activation [7,8]. Although CFH possesses multiple binding sites for C3b and heparin **(Figure 1B)**, apparently the C-terminus represents the major ligand recognition site of the intact protein. This feature is explained by a folded-back conformation of the protein in solution [5].

The CFHL1 protein, which is derived from the *CFH* gene by means of alternative splicing, is composed of the seven N-terminal domains of CFH and has a unique C-terminal four amino acids extension. Consequently, CFHL1 shares ligand binding and complement regulatory activity with the N-terminus of CFH [9].

The individual CFHR proteins have 4 to 9 SCR domains. A homology alignment of SCRs among CFH family proteins reveals two major conserved regions **(Figure 1B)**. The N-terminal SCRs of all five CFHR proteins are related to each other, and show homology to SCRs 6-9 of CFH. In CFH these three domains include binding sites for heparin, C-reactive protein (CRP), and for several microbial surface proteins. The C-termini of CFHRs are also homologous to each other and to the C-terminal surface binding region of CFH. The extent of identity of the three or two most C-terminal SCRs of the CFHR proteins with the C-terminal domains of CFH ranges from 100%, i.e. SCRs with full sequence identity, to 37% at the amino acid level **(Figure 1)**. Interestingly, the non-human CFHR proteins identified so far show very similar N- and C-termini as their human counterparts, thus indicating related functions [10,11]. The homology of CFHR domains with SCRs of CFH implies similar ligand

binding and functional relatedness. For CFHR3, CFHR4 and CFHR5 C3b binding, for CFHR3 and CFHR5 heparin binding, and for CFHR5 CRP binding was reported [12,13]. (See **Table 1** for known CFHR ligands).

Sequence alignments among CFHR proteins also identify subgroups of related domains, indicating functional redundancy, as well as differences. CFHR1 and CFHR2 show a very similar domain composition, and their two N-terminal domains have almost complete sequence identity. However, the three C-terminal domains of CFHR1, but not of CFHR2, are almost identical with SCRs 18-20 of CFH. CFHR3 and CFHR4 are also similar to each other, again indicating overlapping activities. However CFHR3, but not CFHR4 has a domain homologous to SCR7 of CFH, which in CFH mediates heparin and M protein binding. This might explain why CFHR3, but not CFHR4, binds heparin and M protein [12,14]. Several CFHR domains show rather low level of identity to SCRs of CFH, but are very similar to each other, e.g. SCRs 1-2 of CFHR1, CFHR2 and CFHR5. In addition, CFHR5 differs from the other CFHR proteins by its unique middle region. These structural features may translate into both functional redundancy and unique functions.

Functions of the CFHR proteins

The CFHR proteins lack the potent complement regulatory activity of CFH, i.e. cofactor and decay accelerating activities [12,15]. This difference is consistent with the lack of the N-terminal complement regulatory region of CFH for each CFHR protein. However, a complement modulatory activity, in form of a CFH cofactor enhancing activity was reported for CFHR3 and CFHR4 [12]. The exact mechanism of this synergistic activity is unknown, but is probably related to the C3b binding capacity of the proteins. Similarly, for CFHR5 relatively weak cofactor and decay accelerating activities were described [13].

As all CFHR proteins share a common N-terminal region, a similar function is predicted for these proteins. In addition, the conservation of the C-terminal regions, which are related to the surface recognition region of CFH, suggests that CFH family proteins share surface binding activity, and that some CFHR proteins bind to the same or related ligands, as CFH. However, the sequence differences indicate differences in binding affinities or distinct ligand interactions. CFHR3, CFHR4, CFHR5 and CFH bind C3b, suggesting for each protein a role in C3b inactivation and thus a role in complement regulation. CFH has three C3b binding sites which bind different parts and fragments of C3b [16]. As CFHR3 and CFHR4 also bind the C3d fragments [17], the two proteins may compete with CFH for C3d binding. Based on the common and distinct features of CFH family proteins, three major scenarios are proposed for the role of CFHR proteins on biological surfaces **(Figure 2)**: (i) complement regulatory activity or synergistic activity with CFH, such as described for CFHR3, CFHR4 and CFHR5; (ii) particularly for CFHR1 and CFH, which have almost identical C-terminal binding domains, competition for the same surfaces and ligands may result in less surface bound CFH and, consequently, in lower regulatory activity; and (iii) independent surface binding and independent function for CFHRs and CFH. It will be exciting to define the exact functions and to dissect differences of CFHR proteins in fine tuning of complement activation, and to identify so far unkown unique biological roles.

The expression levels of CFHRs seem to vary in different tissues. Due to the high similarity of the proteins to each other and to CFH, at present only a few specific antibodies are available, making it difficult to determine the concentration of each CFHR protein in body fluids. Changes in concentrations, however, can be determined using a comparative approach. In the middle-ear effusion fluid of patients with otitis media with effusion, where

inflammation and complement activation is observed, the concentration of CFHR proteins is enhanced in comparison to plasma [18]. In autistic persons CFHR1 levels are increased in the brain [19].

The plasma concentrations of CFHR proteins are comparable to that of other complement components. CFHR1 has an apparent plasma concentration of about 50-100 µg/ml, which is comparable to that of terminal pathway components, like C6, C7 and C8. This concentration is about 10-20% of that of CFH and translates into an approximately threefold molar difference. Even though CFHR5 plasma levels are relatively low (ca. 5 µg/ml) [13], CFHR5 is identified in complement containing deposits in the kidney, which suggests a local role in complement regulation [20]. Various expression levels of CFHR mRNA were detected also in different mouse organs [21]. Altogether these data suggest that CFHR proteins might be locally important.

Factor H family proteins in human diseases

Mutations, polymorphisms as well as large deletions within the CFH gene cluster are associated with several human diseases (summarized in **Table 2)**, such as the kidney diseases atypical hemolytic uremic syndrome (aHUS) and membranoproliferative glomerulonephritis type II (MPGN II), and the retinal disease age-related macular degeneration (AMD). Based on the overlapping functions of the individual CFHR proteins, common pathophysiological principles are proposed for these human disorders in terms of defective local complement regulation.

Atypical hemolytic uremic syndrome (aHUS). aHUS is a severe kidney disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Mutations in complement genes account for approximately 50% of aHUS cases [22]. The known mutations affect the genes coding for CFH, membrane cofactor protein, factor I, factor B and C3 [23]. The mutations result in defective alternative pathway regulation, which leads to complement mediated tissue damage in the kidney.

The majority of *CFH* mutations are heterozygous and affect the C-terminal surface binding region. Mutant CFH proteins show reduced binding to C3b, heparin and endothelial cells [6]. In addition, ~10% of aHUS patients have autoantibodies that bind to the most C-terminal domain of CFH and, by blocking CFH binding, they inhibit complement regulation on the cell surface [24,25]. Recently, a mouse model for CFH-associated aHUS was reported. Mice that express a truncated CFH molecule, which lacks the surface recognition region (i.e. ΔSCRs 16-20) develop aHUS spontaneously. Thus demonstrating that defective surface recognition of CFH eventuates in aHUS [26].

In some aHUS patients, large genomic deletions were found. The missing chromosomal fragments are flanked by duplicated regions with more than 98% sequence identity, suggesting that the deletion is caused by non-allelic homologous recombination (Figure 1A). Two chromosomal breakpoints have been defined so far. One breakpoint results in deletion of *CFHR3* and *CFHR1* [27]. Such a homozygous CFHR1 and CFHR3 deficiency occurs in 10-16% of aHUS patients as compared to 2-4% of control groups, and heterozygous deletions are also more frequent among aHUS patients (35% versus 9%) [27]. The absence of CFHR1 and CFHR3 proteins in plasma may affect fine tuning of the complement cascade. However, the deletion of the *CFHR1* and *CFHR3* genes in healthy individuals indicates that

the lack of these proteins can at least be partially compensated for. Interestingly, this deletion predisposes for the development of anti-CFH autoantibodies, which block CFH recognition function [28]. The second breakpoint within the same duplicated regions results in deletion of exons 22 and 23 of *CFH*, the complete *CFHR3* gene and exons 1-4 of *CFHR1*. This leads to a hybrid *CFH-CFHR1* gene, whose protein product has SCRs 1-18 derived from the *CFH* gene, and the two C-terminal domains from *CFHR1* [29]. This hybrid CFH-CFHR1 protein has defective recognition functions. Given the presence of several duplicated sequences within this gene cluster **(Figure 1A)**, further deletions and genomic rearrangements are predicted.

Additional changes that affect this gene cluster have been reported. *De novo* gene conversion between *CFHR1* and *CFH* results in the exchange of two residues within SCR20. Such a mutant CFH protein shows reduced binding to heparin and to cell surfaces [30]. Five heterozygous *CFHR5* gene variants have also been identified in aHUS patients (9 out of 45) and/or in healthy controls (4 out of 80) [31].

Membranoproliferative glomerulonephritis type II (MPGN II). MPGN II is a severe kidney disease characterized with electron-dense deposits within the glomerular basement membrane, and mesangial cell proliferation. Apparently, MPGN II is associated with inappropriate complement regulation, and MPGN patients show low C3 plasma levels and enhanced amount of C3 activation products in plasma [32]. Several conditions have been described to cause MPGN II [32]: (i) the absence of CFH in plasma, due to mutations of essential cysteine residues that result in a block of protein secretion; (ii) secretion of a mutated CFH protein which shows reduced regulatory activity in plasma [33]; (iii) autoantibodies against CFH that block regulatory function in fluid phase; and (iv) autoantibodies against the C3 convertase (also termed C3 nephritic factor), which stabilizes

the C3 convertase and enhances complement activation [32]. The majority of CFH genetic mutations identified so far appear in homozygous or compound heterozygous form, resulting in lack of CFH function in plasma. CFH deficient pigs [34] and CFH knockout mice [35] develop glomerulonephritis due to uncontrolled complement activation in plasma. Interestingly, for some MPGN II patients ocular lesions have been reported, indicating formation of related deposits at the glomerular basement membrane and the Bruch's membrane [32].

For *CFHR5* three allelic variants are reported which are more frequent in MPGN II patients, as compared to healthy controls [36]. The distribution of the CFHR5 protein correlates with that of other complement activation products in glomerular immune deposits, which indicates a local regulatory function of CFHR5 in the kidney [20]. ~~In addition, the rat homolog of human CFHR5 is also expressed in the kidney and displays complement regulatory activity [36].~~

Age-related macular degeneration (AMD). AMD is a leading cause for irreversible vision loss in developed countries and affects millions of elderly individuals worldwide. AMD is associated with immune deposits (drusen) formed between retinal pigment epithelial cells and Bruch's membrane [37]. Proteomics and histological analyses demonstrate the presence of complement proteins and complement activation products in drusen, suggesting a local role for complement in the pathophysiology of this disease [38]. A recent study has demonstrated that during inflammation complement components accumulate in the eye [39].

In AMD patients both protective and disease-associated variants of *CFH* were reported. In particular a Tyr402His exchange within SCR7 of CFH and CFHL1 strongly increases the

risk for AMD [40]. Both the CFH_{H402} and $CFHL1_{H402}$ variants show reduced binding to heparin and CRP [41,42]. The protective role of CFH in the eye is also shown by the analysis of aged CFH knockout mice, which develop ocular lesions similar to those seen in human patients [43].

Curiously, and in contrast to aHUS, the deletion of the *CFHR1* and *CFHR3* genes has a protective effect in AMD [44,45]. The frequency of homozygous *CFHR1/CFHR3* deletion shows large variation between different ethnic groups, and occurs in 17.3% of African populations, 15.9% of African Americans, 6.8% of Hispanic, 4.7% of Caucasian, and 2.2% of Chinese ethnic group [45]. In line with this, AMD is more rare among African Americans, compared to Caucasian and Chinese populations [46]. Based on the high sequence similarity of the C-terminal surface binding regions of CFHR1 and CFH, CFHR1 may act as a competitor for CFH. By inhibiting CFH surface attachment, CFHR1 may reduce the local complement inhibitory and anti-inflammatory activity (Figure 2C). Thus, the lack of CFHR1 in the eye may result in enhanced protection against complement activation.

For AMD, mutations in the complement components C3, factor B and C2 have also been reported [47,48]. In addition, the complement activation products C3a and C5a were shown to contribute to neovascularization in the diseased eye [49]. These data altogether indicate that complement dysregulation contributes to the development of AMD.

Infectious diseases. Numerous human-pathogenic microbes, including bacteria, fungi, viruses and parasites, recruit host complement regulators from plasma and utilize these regulators for complement and immune evasion [50]. This exploitation of host regulators by microbes is a rapidly developing research field, and because the number of known

CFH/CFHL1 binding microbes and proteins is rapidly expanding [50], here we focus on CFHR proteins.

Several *Borrelia* species, the causative agents of Lyme borreliosis, bind CFH and for *Borrelia burgdorferi* CFH binding seems to correlate directly with serum resistance [51,52]. The five identified borrelial CFH binding proteins, which all can be expressed by one strain, show different binding affinities and bind also CFHL1 (CRASP1 and CRASP2) or CFHR1 (CRASP3, CRASP4 and CRASP5) [53]. CRASPs 3-5 are members of the polymorphic Erp (OspE/F-related proteins) family, whereas CRASP1 and CRASP2 show no sequence similarity. The structure of CRASP1 of *B. burgdorferi* was determined and shown to form homodimers, with a cleft between the monomers, that possibly allows binding of the C-termini of CFH and CFHL1 [54]. Similarly, CFHR1 binds to CRASPs also via its C-terminus, and for CRASP5 a competition between CFHR1 and CFH for binding was demonstrated, which was accompanied with reduced CFH mediated complement regulatory activity [53]. Thus, CFHR1 can be advantageous for the host as it prevents CFH binding to the pathogen **(Figure 2B)**. As these microbial proteins bind the host ligands with different affinities, and as their expression is developmentally regulated and varies during the infection process, it is hypothesized that regulation of CRASP gene expression and protein translocation helps the pathogen to optimally adapt to its host.

M proteins of streptococci have a protruded fibrillar structure with both conserved and variable regions that bind a number of host proteins, including complement regulators, plasminogen, IgG and albumin [55]. CFH, CFHL1 and CFHR3, but not CFHR4, are bound by the M5 protein [14]. There is a large number of serotypes with distinct M protein sequences, which differ in their ability to bind CFH. It has been proposed that M protein bound CFH

family proteins promote complement evasion of steptococci. However, phagocytosis resistance of M protein expressing streptococci is also mediated by other factors [55] and is not necessarily dependent on CFH [56]. In addition, *Streptococcus pyogenes* binds both CFHR1 and CFH with the Scl1 surface protein [57]. Multiplicity and coordinated simultaneous expression of such surface proteins that bind several host molecules are likely important for immune evasion of pathogens. The relevance of the individual CFH family proteins for complement evasion of streptococci needs to be evaluated in functional assays.

Expression of the Por1A protein enables *Neisseria gonorrhoeae* to bind CFH and CFHR1, and weakly also CFHL1. Por1A binding is specific to human CFH and contributes to human-restricted pathogenicity [58]. The Tuf protein, a translation elongation factor of *Pseudumonas aeruginosa*, binds CFH, CFHL1 and CFHR1. It has been shown that CFH retains its complement regulatory activity when bound to Tuf [59]. *Leptospira interrogans* binds CFH and CFHR1, but not CFHL1, with the LenA (or LfhA) outer surface protein [60]. Although *L. interrogans* has five additional paralogs of LenA, only one of them binds CFH [61]. The mold *Aspergillus fumigatus* binds CFH, CFHL1 and CFHR1 but the microbial ligands have not yet been identified [62]. Furthermore, CFHR3 and CFHR4 were shown to bind to C3b-opsonized pneumococci [12], and may cooperate with CFH for complement inhibition **(Figure 2A)**.

Thus, a wide range of structurally and functionally different microbial surface molecules bind CFH family proteins. The binding sites generally show a preference, however, for SCR7 of CFH and CFHL1, and the C-termini of CFH and CFHR1. Because of the highly related structure of the CFHR proteins, it is expected that additional CFHRs, like CFHR2 and CFHR5, bind to human-pathogenic microbes. Such a surface decoration allows the pathogen

to utilize specific and distinct host proteins for immune- and, in particular, for complement evasion. CFHR proteins might be advantageous for the pathogen, in the case of cooperation with CFH, or advantageous for the host, when they interfere with CFH binding to pathogens. An additional, so far poorly explored aspect is the possible role of acquired CFH family proteins in cellular adhesion [63,64]. This could be advantageous for the pathogens to facilitate contact with host cells.

Conclusion

Although the exact functions of CFHR proteins are still not well defined, their involvement in various diseases clearly indicates a specific and important role for this group of proteins. The organs that are mainly affected in these diseases, the glomerular basement membrane in the kidney and the Bruch's membrane in the eye, have in common that they lack endogenous complement regulators, and rely on bound CFH for complement inhibition. Based on their known synergistic (CFHR3, CFHR4 and CFHR5) and antagonistic (CFHR1) effects on CFH function, the CFHR proteins are probably involved in fine tuning of local complement activation. The lessons learned from human diseases, where deficiency of CFHR1 and CFHR3 is a risk factor in one disease (aHUS), and a protective factor in another disease (AMD), suggests that a similar dichotomy in CFHR function on pathogens might exist. The structural differences also imply distinct roles, possibly unrelated to complement, for the individual proteins. To decipher these unique functions is the major challenge for future work. Expanding our knowledge on the genetics, tissue expression and physiological functions of the CFHR proteins will certainly help to better understand the role of these proteins in the pathomechanisms of the discussed diseases and in the immune evasion of pathogenic microbes.

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Open questions box

A general role for the CFHR proteins in the regulation of complement activation is emerging, but there are still many important questions regarding their direct functions.

1. There are fragmental data on tissue expression, distribution and concentration in body fluids for the individual CFHR proteins. Due to the high sequence similarity on both the nucleotide and amino acid levels, currently CFHR specific DNA probes and antibodies are not available. Determining the amounts of CFHR proteins is hampered by the immunological cross-reactivity between the CFHR proteins and CFH/CFHL1. It will be a challenge to analyze both the relative CFHR mRNA levels and CFHR protein concentrations in various tissues and to correlate these parameters in order to evaluate the biological relevance of the individual CFHRs.
2. An association with plasma lipoproteins has so far been demonstrated for CFH, CFHR1, CFHR2, CFHR4 and CFHR5. Thus, CFH family proteins may participate in lipid transport or may regulate lipid homeostasis. The relevance of lipoprotein association needs to be addressed further.
3. It needs to be demonstrated in functional assays if CFHR proteins indeed modulate CFH activity to a biologically relevant extent. Analysis of the activity of these proteins on human tissues and in complement resistance of microbes will be helpful to understand the involvement of CFHR proteins in inflammatory processes and infectious diseases.

4. More data are needed on the functions of the CFHR proteins in order to understand their physiological roles. Especially CFHR1 and CFHR2 are poorly characterized to date, but also more detailed analysis of the other CFHR proteins in functional assays and the identification of new ligands will be informative. These studies are expected to define novel and unique functions for the members of this group of proteins.

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Figure legends

Figure 1. The human complement Factor H gene cluster and structure of the various proteins.

(A) The human Factor H (CFH) gene cluster includes six genes which show a consecutive arrangement on human chromosome 1q32. This cluster spans a region of 415 kbp and includes the *CFH* and five CFH-related *CFHR* genes. For the *CFH* and the *CFHR4* genes two transcripts have been identified, which are derived by alternative splicing and which encode related but distinct proteins (CFH and CFHL1, and CFHR4A and CFHR4B, respectively). This cluster includes homologous repeat regions (marked as A1-C2) which due to non-allelic homologous recombination events can result in deletion of large chromosomal fragments. Depending on the site of recombination as indicated by either the solid or the dotted lines, a large genomic deletion is observed in this cluster which either results in a *CFH:CFHR1* hybrid gene (solid line) or the deletion of *CFHR3* and *CFHR1* (dotted line). These deletions predispose to atypical hemolytic uremic syndrome.

(B) The CFH family proteins are plasma glycoproteins, which are exclusively composed of short consensus repeat (SCR) domains, which are common among complement regulatory proteins. The individual SCR domains of the CFHR proteins share high sequence identity with each other and with SCRs of the complement regulator CFH. Homologous domains identified by sequence similarity are indicated by vertical alignment, and the numbers above each SCR indicate the identity to the corresponding domain in CFH at the protein level. All CFHRs contain domains related to the C-terminal surface and ligand recognition region (SCRs 19-20) and to the middle region (SCRs 6-9) of CFH. CFHR proteins lack SCRs homologous to the complement regulatory domains (SCRs 1-4) of CFH. The conservation of the N- and C-termini among the CFHR proteins is indicative of related or even overlapping

functions. For CFH, the localization of binding domains for C3b and its fragments,as well as for heparin, and C-reactive protein (CRP) are indicated.

Figure 2: The related C-terminal regions of CFHRs with the C-terminal CFH surface binding region indicate binding to common or related surface structures.

The C-termini representing the 2-3 C-terminal SCRs of the five human CFHR proteins are highly related to the C-terminal surface binding region of CFH. This homology suggests surface binding activity for each CFHR protein. Three major scenarios can be postulated, which may occur both in host tissues and on microbial surfaces, as microbes express distinct surface proteins that can simultaneously bind CFH, CFHL1, CFHR1 or CFHR3, such as streptococcal M proteins or the CRASP proteins of *Borrelia spp*.

(A) Independent surface binding with functional synergism. The CFHR3 and CFHR4 proteins have been shown to enhance complement inhibitory activity of CFH, but alone lack regulatory activity.

(B) Competition for surface binding via the same ligand was shown for CFHR1 and surface bound CFH. In this scenario CFHR1 reduced the regulatory activity of CFH at the surface. Thus, CFHR1 (and possibly other CFHR proteins) can modulate the local activity of CFH. This may explain why the absence of CFHR1 and CFHR3 is protective against the development of the ocular disease age related macular degeneration (AMD).

(C) Independent surface binding and independent function. Upon surface binding, the distinct CFH family proteins may display independent but similar activities (such as the weak complement regulatory activity of CFHR5, which might be important in the kidney), or exert different functions. As CFHR proteins also have significant differences in sequence and domain structure, they may possess so far undiscovered biological activities, which are unrelated to the complement system.

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Figure 1
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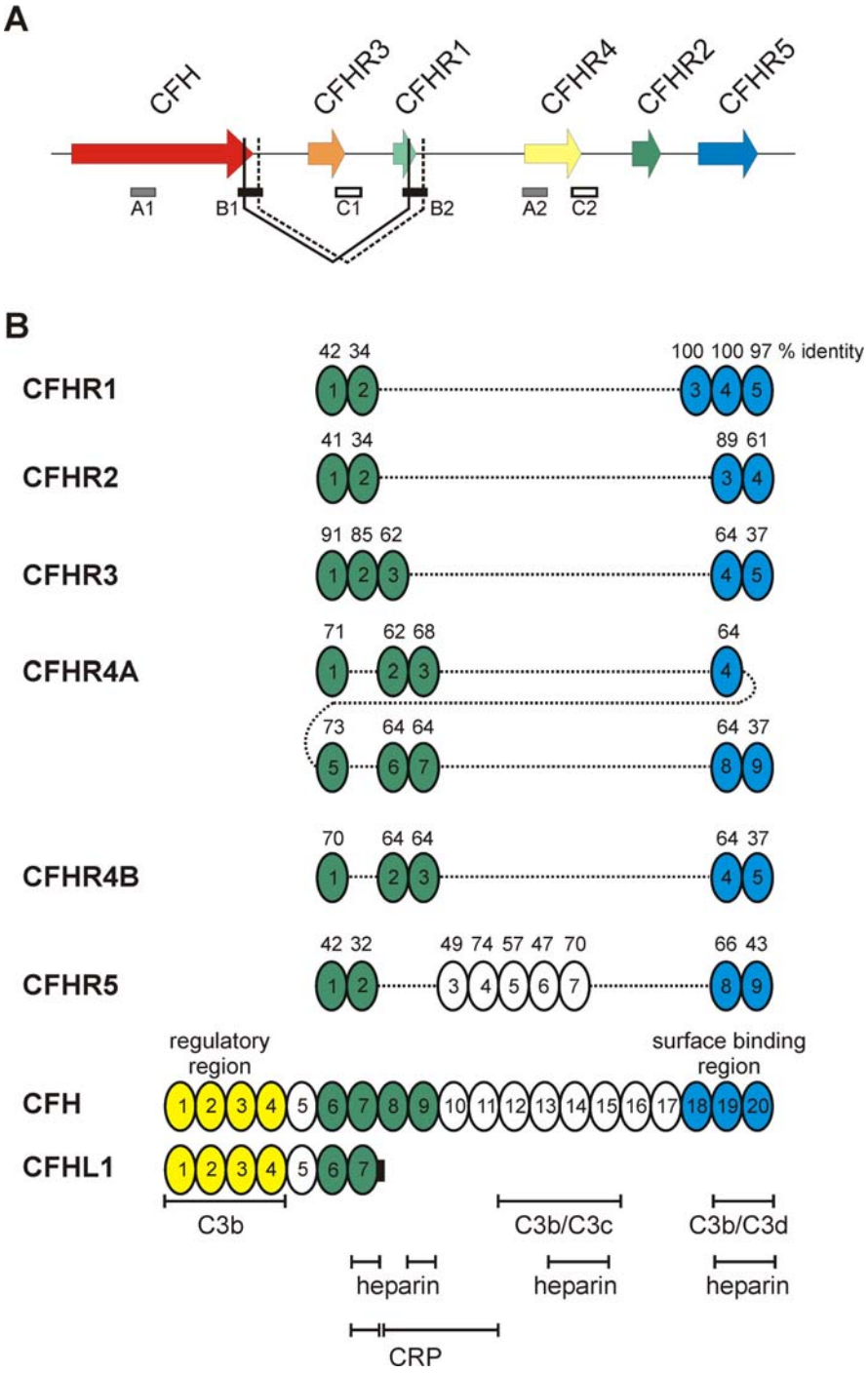

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Figure 2
Józsi and Zipfel 2007

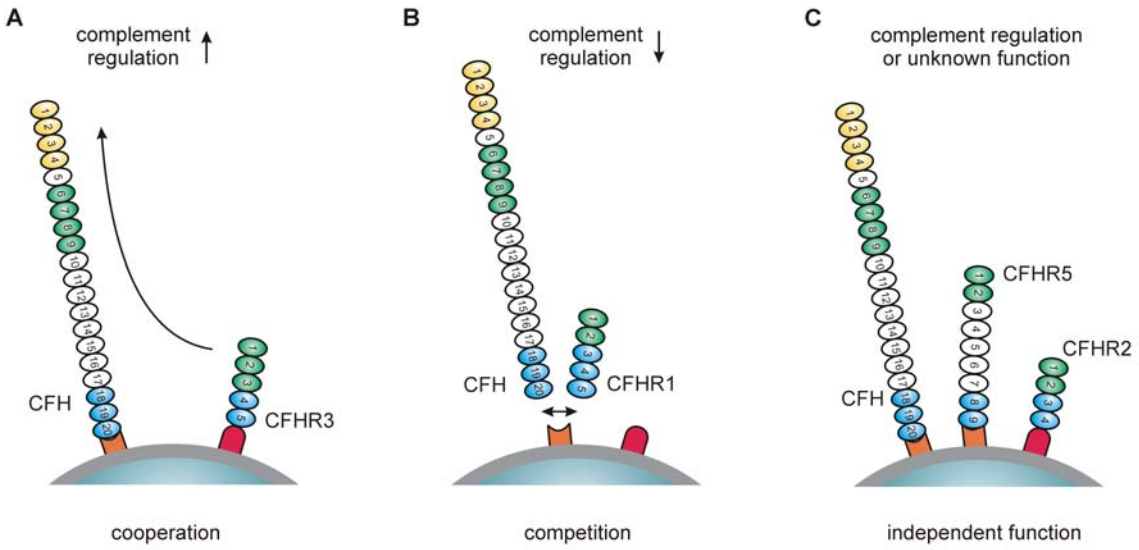

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Table 1. Characteristics of human CFH family proteins

Protein	Size	Appearance in plasma	Known host ligands	Binding to Pathogens	Function	References
CFH	150 kDa	glycoprotein, associated with HDL and LDL	C3b, C3c, C3d, heparin, CRP, and others	M protein, BbCRASPs 1-5 and multiple microbial ligands	cofactor and decay accelerating activity, cellular adhesion	3-6, 14, 16-18, 50-53, 57-64, 66
CFHL1	42 kDa	glycoprotein	C3b, heparin, CRP	M protein, BbCRASPs 1-2 and multiple microbial ligands	cofactor and decay accelerating activity, cellular adhesion	4, 9, 14, 18, 50, 51, 53, 58, 59, 62
CFHR1	37 kDa 43 kDa	glycosylated isoforms, associated with HDL		BbCRASPs 3-5, multiple microbial ligands		4, 15, 18, 19, 53, 57-60, 62, 65, 66
CFHR2	24 kDa 29 kDa	non-glycosylated and glycosylated isoforms, associated with HDL				4, 18, 65, 66
CFHR3	36-50 kDa	multiple bands (likely differently glycosylated forms)	C3b, C3d, heparin	M protein	enhances CFH cofactor activity	4, 12, 14, 17, 18
CFHR4	42 kDa (CFHR4B) 86 kDa (CFHR4A)	likely splice variants, associated with chylomicron, LDL and VLDL	C3b, C3d		enhances CFH cofactor activity	4, 12, 17, 18, 66-68
CFHR5	65 kDa	associated with HDL	C3b, heparin, CRP		weak cofactor and decay accelerating activities	4, 13, 18, 20

HDL: high density lipoproteins, LDL: low density lipoproteins, VLDL: very low density lipoproteins, BbCRASP: complement regulator acquiring surface protein of *Borrelia burgdorferi*, CRP: C-reactive protein

Table 2. Disease-associated mutations and polymorphisms in CFH family genes

	aHUS Atypical Hemolytic Uremic Syndrome	**MPGN II Membranoproliferative Glomerulonephritis type II**	**AMD Age-related Macular Degeneration**	**references**
CFH	heterozygous mutations (>75% in SCRs 18-20), *CFH:CFHR1* hybrid gene, gene conversion	homozygous or compound heterozygous mutations - mutations in Cys residues result in a block of protein secretion, - deletion of K224 in SCR4, - Y402H polymorphism	V62I, Y402H, T493R polymorphisms, intronic polymorphisms	23, 32, 33, 40, 44, 45
CFHR1	deletion of *CFHR1*, *CFH:CFHR1* hybrid gene, gene conversion		deletion of *CFHR1* is protective	27, 29, 30, 44, 45, 46
CFHR3	deletion of *CFHR3*		deletion of *CFHR3* is protective	27, 44, 45, 46
CFHR5	L66F* (SCR1), K126N* and InsA573/197Stop (SCR3), R338H* (SCR6), M496R* (SCR9)	-249T/C and -20T/C promoter polymorphisms, P46S exchange in SCR1		31, 36

* The numbering of amino acids in ref. 31 starts after the 18 amino acid-length signal peptide.