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Complement Dysregulation and Disease: Insights from Contemporary Genetics

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Abstract

The vertebrate complement system consists of sequentially interacting proteins that provide for a rapid and powerful host defense. Nearly 60 proteins comprise three activation pathways (classical, alternative, and lectin) and a terminal cytolytic pathway common to all. Attesting to its potency, nearly half of the system's components are engaged in its regulation. An emerging theme over the past decade is that variations in these inhibitors predispose to two scourges of modern humans. One, occurring most often in childhood, is a rare but deadly thrombomicroangiopathy called atypical hemolytic uremic syndrome. The other, age-related macular degeneration, is the most common form of blindness in the elderly. Their seemingly unrelated clinical presentations and pathologies share the common theme of overactivity of the complement system's alternative pathway. This review summarizes insights gained from contemporary genetics for understanding how dysregulation of this powerful innate immune system leads to these human diseases.

Keywords

atypical hemolytic uremic syndrome; age-related macular degeneration; alternative complement pathway; C3 glomerulopathies; factor H; CD46; factor I; C3; factor B

INTRODUCTION

First identified in human serum in the late nineteenth century as a heat-labile complement to the heat-stable antibody in mediating bacterial lysis, the early elements of the primeval complement system appeared more than a billion years ago as an innate defense that could coat pathogens and self-amplify (1). The contemporary complement system lies at the interface of innate and adaptive immunity. Although it retains its ancient primary function of opsonization, it has expanded to add features enabling a rapid, highly targeted and powerful defense system against microbes. In less than five minutes, millions of its proteins can attach

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covalently onto bacterial and viral pathogens to opsonize and, in some cases, lyse the microbe. By this process, today's system also serves as a key effector arm of humoral immunity in vertebrates.

Overall, the complement system encompasses six operating strategies. They are (*a*) recognition proteins, such as antibodies and lectins, that specifically identify the target and then efficiently trigger complement activation; (*b*) sequentially acting specific serine proteases, components that enable formation of a key feedback loop for amplification; (*c*) components in plasma that are primarily synthesized by the liver and secreted into the bloodstream (but they are also manufactured locally by monocytes, neutrophils, macrophages, fibroblasts, and many other types of cells); (*d*) regulators in plasma and on cells that maintain homeostasis; (*e*) receptors on host cells that mediate immune adherence and ingestion; and (*f*) a newly identified intracellular complement system.

Because complement activation is designed to be devastating to a pathogen, if misdirected or dysregulated, it can be similarly damaging to self-tissue. Thus, the control of the complement system is tasked to nearly half of its components. These provide stringent regulation of the arsenal. Disruption of this delicate balance, such as by inherited or acquired deficiencies in its activating or control proteins, is increasingly implicated in disease pathology. Indeed, new recognition of the complement system's key role in the immune and inflammatory responses at sites of tissue injury and debris deposition may turn out to be where its greatest impact in clinical medicine will be felt.

Advances in sequencing technology and identification of genetic variants led to the discovery of mutations in components that regulate complement activation in human disease. Striking is the unexpected and remarkable association of mutations in control proteins causing disparate diseases that predominantly affect the young and the old. The first is an acute endothelial injury syndrome called atypical hemolytic uremic syndrome (aHUS), a disease often arising in early childhood. The second, age-related macular degeneration (AMD), features biological debris in the retina and is a disease almost solely of the elderly. New and unequivocal evidence demonstrates that the complement system plays an etiologic role in the pathogenesis of both diseases. An emerging theme over the past decade, particularly from insights gained from genetic studies, is that modulation of complement inhibitory activity predisposes to thrombomicroangiopathies, e.g., aHUS, and to dysfunctional debris handling (AMD). This review focuses on these two seemingly unrelated diseases that share a common theme of overactivity of the complement system, in particular, its alternative pathway (AP).

COMPLEMENT PATHWAYS

Nearly a billion years ago, elements of the complement system most likely consisted of a rather simple innate immune system initiated by proteolysis of a C3-like protein containing a thioester bond. Upon disruption of this bond by proteolytic cleavage, the larger fragment (C3b) binds covalently to the target to mediate opsonization, and the smaller peptide (C3a) elicits a local inflammatory response (1–4). This original pathway persists in the modern day and is rather inappropriately named the AP. It provides a straightforward, enzymatically

based recognition and effector defense against pathogens, especially bacteria. A key feature is C3 activation, which leads to the formation of a covalent ester or amide linkage to membrane structures of pathogens.

Evolution ramped up the potency of this host defense system by initially employing lectins and then antibodies, which more specifically target a pathogen. These recognition proteins select a target and then trigger complement system effector mechanisms of membrane perturbation (opsonization and lysis) and incitement of a proinflammatory response. Because of its increasing efficiency and amplifying capacity, control mechanisms were required to maintain homeostasis by focusing the attack on pathogens but minimizing damage to self-tissue. An additional attribute (and arguably along with covalent binding they represent the system's two most remarkable features) is that it works in seconds! Once a vascular system developed in evolution (from a pathogen's perspective, a fertile growth medium and a means of transit), a rapidly activating host defense system became a necessity.

The modern-day complement system encompasses an arsenal of over 60 proteins, which includes plasma and cellular components. Indeed, complement systems similar to those in mammals have been identified across phyla, including birds, fish, amphibians, reptiles, and others. The complement system consists of three major activation cascades: the alternative, classical, and lectin pathways. Each is uniquely engaged, but they share the two common goals of modifying the target membrane and promoting the inflammatory response (**Figure 1*a***).

The AP has the capacity to deposit on a pathogen without need for prior contact/exposure. This innate immune, self-amplifying defense mechanism results from the lability of an internal thioester bond. A small amount of auto-activated C3 [the so-called C3 tick over that represents a thioester-hydrolyzed form of C3 termed C3(H_2O)] is continuously generated in blood at 1 to 2%/h. If it deposits on healthy self-tissue or ticks over in the fluid phase, host regulatory proteins immediately inactivate it. Evidence of this AP turnover is that a red blood cell contains on average several hundred C3d fragments covalently bound to its membrane (5). In addition, a small percentage of serum albumin migrates at approximately 100 kDa, not 55, because of the attachment to it of a C3d fragment (6). However, if it deposits on a microbe, activated C3 can be swiftly amplified by engaging two proteases, factor B (FB) and factor D, along with a stabilizing protein, properdin (P), to create the powerful AP C3 convertase (**Figure 1*b***). Contained within the AP is an efficient feedback or amplification loop for the generation of large amounts of C3b to opsonize a pathogen.

The classical pathway (CP) was discovered in ~1890, hence its name. It is primarily triggered by antibodies (IgM and IgG subclasses 1 and 3) following their attachment to antigen. The C1q subcomponent links C1 to the Fc portion of Ig. This initiates a cascade featuring multiple proteolytic cleavage steps beginning with autoactivation of C1r that cleaves C1s (both subcomponents of C1). C1s, in turn, cleaves C4 and C2. The newly generated C4b and C2a fragments combine to form the CP C3 convertase. Similar to the AP C3 convertase, this bimolecular enzymatic complex also converts C3 into C3a and C3b.

In an analogous process, the lectin pathway (LP) generates a C3 convertase, but in this case, the lectins attach to sugar moieties, e.g., mannose residues, and mannose-binding lectin-associated serine proteases become substitutes for the C1 enzymatic subcomponents. The targets here are sugars relatively unique to the pathogen.

The central role played by C3 convertases to generate C3b illustrates the importance of the latter to deposit on pathogens and then serve as a ligand for receptors. Furthermore, addition of C3b to a C3 convertase in all three pathways generates a C5 convertase (switches the substrate specificity from C3 to C5) that cleaves the homologous C5 protein into a potent anaphylatoxin (C5a) and the larger C5b, which engages the membrane attack complex (MAC) (reviewed in 7).

In summary, the complement system accomplishes its goal of self-defense against pathogens as it (*a*) focuses attack on microbial membranes via the AP-, CP-, and LP-generated convertases; (*b*) amplifies with great speed and magnitude (more than a million molecules of C3b can fix onto a bacterium in two to three minutes); and (*c*) produces an inflammatory microenvironment at the site of attachment.

REGULATION OF COMPLEMENT ACTIVATION

Because the complement system provides a rapidly activated and potent surveillance mechanism for the host, strict control is required to avoid damage to self. Thus, inhibition of complement activation is mediated by host regulators in the fluid phase (plasma) as well as on cells. Control is aimed at each of the major steps in the pathway: initiation, amplification (leading to C3 and C5 cleavage), and formation of the MAC or C5b–C9.

Control at the critical central step of C3b generation is provided by the orchestrated interplay of a genetically, structurally, and functionally related multigene family called the regulators of complement activation (RCAs), which are tightly clustered on chromosome 1 at q3.2 (8–10). The cell membrane-associated proteins are membrane cofactor protein (MCP; CD46), decay-accelerating factor (DAF; CD55), complement receptor 1 (CR1; CD35), and complement receptor 2 (CR2; CD21). The plasma patrolling inhibitors are factor H (FH) and C4b-binding protein (C4BP).

Members of the RCA family utilize two processes to maintain homeostasis. One is called cofactor activity. This refers to proteolytic cleavage of C3b and C4b. In this process, a cofactor protein (such as FH or CD46) binds to those fragments, then the plasma serine protease factor I (FI) can proteolytically inactivate them (**Figure 2**). The fragments generated may serve as ligands for other CRs that facilitate clearance of targets. The second regulatory mechanism is called decay-accelerating activity whereby the catalytic domain (serine protease) of a C3 or C5 convertase is disassociated. Both mechanisms prevent activation of the powerful feedback loop on self; however, cofactor activity is a permanent fix as the cleaved C4b or C3b cannot regenerate a convertase. By contrast, following decay-accelerating activity, C4b or C3b remains intact and can rebind a newly generated partner protease.

To overcome the host complement system and establish an infection, opportunistic pathogens (especially those that invade the bloodstream) commonly produce virulence factors that enable coating of their membranes with host complement inhibitors or synthesize their own regulatory mimics (reviewed in 11).

COMPLEMENT SYSTEM DYSREGULATION AND DISEASE

The role of the AP in mediating human disease is increasingly being recognized (12, 13). For example, aHUS commonly occurs in the setting of a heterozygous loss-of-function mutation in one of three complement regulators: FH, MCP, or FI (reviewed in 14) (see **Table 1**). Furthermore, over the last decade, sequencing studies have demonstrated a link between both common and rare variants in AP proteins and the risk of AMD development (14).

Atypical Hemolytic Uremic Syndrome

Endothelial cell injury triggers aHUS, a rare thrombomicroangiopathy (TMA) (14–16). The disease usually has a rapid onset and is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure.

In the 1970s and 1980s, several kindreds were reported in which low levels of FH were associated with a familial form of HUS (17, 18). The probands did not have a total absence of FH, in which the AP turns over in plasma to exhaustion, but the FH and C3 levels were low (17). Subsequently, it was the pioneering work of Goodship and his team in Newcastle, England, that broke the field open (reviewed in 18). They initially used microsatellite polymorphism genotyping to map variants to the RCA gene cluster on the long arm of chromosome 1 (1q32). They then sequenced candidate regulatory genes in this cluster, and in several families, it was noted that a variant in the *CFH* gene was associated with aHUS. This key report was published in 1998 (17). Moreover, in other families, they identified variants in *MCP*, including one in a Dutch family in which three brothers carried the variant and all had aHUS (17–20). They contacted us about this variant as we had previously performed extensive mutagenesis studies to define the C3b-binding site on this membrane inhibitor (21). The variant they described was a serine to proline change at position 206 of the mature protein (21). We had already mutated this same serine to an alanine, and it had approximately a 90% loss in C3b cofactor activity (21). This family along with two others carrying *MCP* variants were reported in 2003 (20). There have now been several hundred variants identified in the *CFH* gene and over 60 in *MCP* (15, 22, 23). Many loss-of-function variants in FI and a few gain-of-function variants in C3 and FB have also been described (reviewed in 15, 22, 24).

The common theme in the case of the regulators is a loss-of-function leading to haploinsufficiency. In these cases, the protein is generally (*a*) not synthesized, (*b*) synthesized but not secreted, or (*c*) secreted into the blood in normal amounts but is dysfunctional. A normal complement system is finely tuned and prepared to properly deal with injury. In aHUS patients, the simple explanation is that an excessive degree of AP activation takes place in the setting of dysfunctional control, leading to microthrombi (especially the vulnerable kidney with its glomeruli).

Further evidence to support this line of reasoning is that a subset of patients with aHUS (7–10%) have function-blocking autoantibodies to FH (25), and approximately 5% have a gain-of-function mutation in C3 or FB (26). In the latter, the mutations are commonly in the binding site for the cofactor proteins (FH and MCP). A major advance over the past 15 years in complement research was obtaining the crystal structure of C3b (27–30). In addition, the group led by the Gros laboratory (30) recently published the crystal structures of FH and MCP bound to C3b.

Disease penetrance is approximately 50%, suggesting that an environmental trigger is necessary in many cases (18). In 50 to 70% of children, this appears to be an infection, whereas in adults pregnancy is a known predisposing factor. However, in many cases in children and adults, the precipitating event is unknown. Disease severity tends to be greater with *CFH* or *CFI* than *MCP* mutations (18).

C3 glomerulopathy (C3G) is a newly defined clinical entity that includes both dense deposit disease (previously defined as membranoproliferative glomerulonephritis type II) and C3 glomerulonephritis (31). This group of glomerular diseases shares a common pathogenesis of dysregulation of the AP. Similar to aHUS, in ~50% of cases, C3G features mutations in (or antibodies to) AP components (reviewed in 16). Thus, both entities overlap in this way as well as phenotypically. This became evident when the patients who developed C3G were previously diagnosed with aHUS or vice versa. Further evidence came from the finding that one member of a family with complement mutation developed aHUS and that others developed C3G (16).

Age-Related Macular Degeneration

AMD affects more than 50 million individuals worldwide and is the leading cause of blindness in the elderly in developed countries (32, 33). This slowly progressive, degenerative ophthalmological disease of the retina usually manifests after 60 years of age. Studies performed in the last decade in humans (particularly genetic analyses) and in experimental animals have been the major sources of new information (reviewed in 34 and 35).

A number of genetic alterations are associated with increased risk of developing AMD, and many reside in genes encoding the complement cascade (32–37). These variants span the allelic spectrum of disease from common variants (exhibiting relatively low risk) to rare variants with complete penetrance. The prevailing hypothesis is that an overly exuberant inflammatory response, driven by an inadequately regulated complement cascade, significantly contributes to the pathophysiology of AMD.

In AMD, loss of central vision commonly occurs secondary to disruption of photoreceptor cells in the macula (**Figure 3**). There are two types of AMD: dry (atrophic) and wet (neovascular or exudative). Most cases start as the dry type, and 10–20% progress to the more severe vision-compromising type, wet type. The latter results from growth of abnormal blood vessels from the choroid into the macula that tend to break and leak, resulting in severe macular damage. A characteristic feature of the more common dry type of AMD is the presence of debris, known as drusen, in the retina (**Figure 3**). Complement activation on

this debris has been demonstrated for more than 15 years (38, 39) but did not receive much notoriety until genetic associations with regulators of the AP exploded on the scene with four seminal publications in 2005 (reviewed in 34–36).

The spectrum of diseases, which includes aHUS (and related C3G), as well as AMD, can be better understood by examining how regulators and C3b interact. A recent publication presented the crystal structure of C3b in complex with FH, MCP, DAF, and CR1 (30). It sheds light on how structure affects function and, in this case, dysfunction. The report also contains a summary of all the mutations involving, for example, *MCP* or *CFH*, either found in aHUS or produced experimentally to define the binding site. Thus, one can now map variants/mutations on these structures. Depending upon the mutation, about 50% are not synthesized or secreted, and about 50% are secreted but dysfunctional. In either case, the patients are haploinsufficient, a common anomaly in both aHUS and AMD.

FACTOR H MUTATIONS

FH is an abundant 155-kDa plasma complement regulatory protein (**Figure 4**). Composed entirely of 20 complement control protein (CCP) repeats of approximately 60 amino acids each, it structurally resembles beads on a string in which the number of connector/linker amino acids varies from 3 to 8. The CCPs of FH are grouped as three functional domains. CCPs 1–4 contain the C3b-binding site that carries out CA. CCPs 6–8 bind to glycosaminoglycans (GAGs). Repeats 19–20 facilitate the binding of FH to damaged cell membranes and cell debris via an interaction with GAGs and already deposited C3b/C3d. Each of these discrete regions is known to play a role in human disease pathology. The mutations associated with pathologic syndromes have helped inform us about the distinct roles that these domains play in regulation of the AP. Mutations in FH have been described in AMD, aHUS, and C3G. Of interest and unexplained, although the same mutation may be found in more than one disease, more commonly, they appear to be preferentially restricted to one disease (40).

The initial genetic studies in AMD identified a common single-nucleotide polymorphism (SNP) in the *CFH* gene. The SNP is associated with an increased risk of developing AMD (41–44). This SNP results in the amino acid change Y402H (using the protein sequence numbering system containing the signal peptide); Y402H is in position 384 in the mature plasma protein. Heterozygous individuals for the Y402H polymorphism have an approximately two- to threefold increased risk of developing AMD and homozygotes an approximately five- to sixfold increased risk (34, 36, 41, 43–46). About 30% of people of European descent carry at least one copy of the 402H risk allele. Interestingly, Y402H is also associated with dense deposit disease, but there is no association with aHUS (47).

Y402H is in the FH6–8 region, which binds to GAGs (**Figure 4**). Structurally, the Y402H polymorphism occurs in the seventh CCP domain and does not alter the overall conformation of the protein (48). Y402H, however, does alter the binding of FH to a number of ligands (49), notably C-reactive protein (50), *Streptococcus* M protein (51), and sulfated polyanions (52) such as GAGs. FH interacts with GAGs of debris and cell surfaces (53). The Y402H polymorphism disrupts the CCP 6–8 region of FH from efficiently binding GAGs in

Bruch's membrane (53). Decreased localization to Bruch's membrane, the site of drusen deposition, would presumably lead to poorly controlled complement turnover and an excessive local, chronic inflammatory response. A proposed explanation for the high prevalence of the Y402H polymorphism is that the 402H allele provides in early life a survival advantage against streptococcal and probably other infections including *Yersinia pestis* (51). The underlying mechanism is that the FH-binding protein of *Streptococcus* has a much lower affinity for H402 than Y402, and therefore the organism has a decreased ability to block the AP. In other words, the allele frequency of 402H likely has been increasing in the population because it protects against death from streptococcal infections early in life.

As might be expected, rare mutations in *CFH* also are associated with disease. An amino acid change, R1210C, is a missense mutation in the CCP 19–20 region of the protein. In the initial study of AMD patients, it was enriched in AMD cases compared to controls (1.4% versus <0.1%, respectively) (54). The R1210C mutant is highly penetrant and also leads to an earlier age of onset compared to the overall AMD population (mean age of onset 65 versus 71 years). This association of the R1210C mutant with AMD has now been shown by several groups (see below) and has been reported in aHUS patients (55, 56) as well as in C3G (40, 57).

R1210C is a missense mutation in CCP 20, the domain responsible for FH localizing to sites of complement activation through C3b/C3d and polyanionic binding (**Figure 4**). Functional studies of the recombinantly produced R1210C protein identified a defect in its binding to GAGs on cell membranes (55, 56). These studies were initially performed with a truncated protein (CCPs 8–20). Western blot analysis under nonreducing conditions identified a higher molecular weight band in the serum of R1210C carriers (210 versus the expected FH molecular weight of 150 kDa), likely related to the presence of having an extra cysteine in CCP 20 (58). Further analysis demonstrated that the R1210C protein forms a complex with albumin through a disulfide bridge (58). The interaction of the R1210C FH-albumin heterodimer with immobilized C3b is also impaired. Thus, this mutant in FH affects its complement regulatory function, particularly on debris or a membrane, and thereby could lead to increased AP activation at sites of debris deposition (40).

More recently, two other rare variants in *CFH* were identified through whole-exome sequencing of families with AMD (59). They were selected on the basis of a high burden of disease but a low load of known genetic risk. In these families, the R53C and D90G missense variants were highly penetrant. R53C has also been reported in the setting of aHUS and glomerulonephritis (60, 61). Upon functional assessment, each had decreased regulatory activity. Although R53C exhibited normal binding to C3b by surface plasmon resonance, it had reduced decay-accelerating activity for the AP C3 convertase. In addition, R53C and D90G displayed a defect in FH-mediated cofactor activity. Together, these rare variants (minor allele frequency <0.1%) support the concept that FH is key to maintaining a balance between complement activation and regulation in the retina (62). Indeed, in a recent report, the burden of rare FH variants (≤1% minor allele frequency) was assessed (**Table 2**) (63). In this study, 65 missense, nonsense, or splice-site mutations were identified in a population with advanced AMD (A-AMD). Rare variants with a minor allele frequency ≤1% and all singletons were enriched in the A-AMD cases. Remarkably, variants in *CFH* increased the

risk as the allele frequency decreased. These rare variants tended to be located in functional domains of FH (**Table 2**) or led to low serum levels of FH (63) (**Table 3**). These data demonstrate that there is a diversity of variants in FH and that there is an enrichment of variants in the functional domains of FH associated with A-AMD.

FH mutations are the most common genetic association in aHUS, identified in 20–30% of patients (64). Many mutations in aHUS patients are localized to CCPs 19–20 and do not usually result in a quantitative deficiency of FH. Instead, they disrupt GAG interactions and/or C3b and C3d binding, resulting in impairment of AP homeostasis at the target surface. Interestingly, these mutated proteins are usually able to control complement activation in the fluid phase. However, mutations have also been identified in FH 1–4 (23) in aHUS, and this is a hot spot for mutations in A-AMD (63). This region is responsible for the cofactor activity of the protein, and thus, these mutations lead to impaired regulation both at the cell surface and in the fluid phase.

Acquired deficiencies in FH also occur in the setting of aHUS. Autoantibodies toward FH have been reported in 5–10% of aHUS patients (64, 65). A genetic defect, namely a deletion in *CFHR1* and *CFHR3* (complement FH-related 1 and 3), predisposes to development of FH autoantibodies (66). However, although a homozygous deletion state is common in the general Caucasian population (approximately 5%), only a rare minority develops autoantibodies to FH (67).

An interesting observation from these studies is the overlapping, yet distinct, effect of mutations in FH. Even the same mutation, in different patients, leads to a different disease. For example, the R53C mutation in CCPs 1–4 results in defective decay-accelerating activity and impaired cofactor activity. In some patients, this led to development of AMD, and others developed kidney disease. A pathophysiologic explanation for these differences is not available.

MEMBRANE COFACTOR PROTEIN MUTATIONS

CD46 is a widely expressed type I transmembrane regulatory protein that protects host cells from complement attack. It performs this function by serving as a cofactor for the FI-mediated proteolytic inactivation of C3b and C4b deposited on cells (**Figure 2**). It is an intrinsic regulator in that it only carries out cofactor activity on C3b or C4b bound to the same cell on which it is expressed. Furthermore, it does not bind C3b or C3b-bearing complexes found in the fluid phase (plasma). More than 60 disease-associated CD46 mutations have been identified; most all of these are rare and deleterious, and associated with the development of aHUS (22) (**Figure 5**).

CD46 was originally identified as a complement regulator because of its ability to bind to C3b and C4b (68). Surface radiolabeled lysates of human peripheral blood cell populations were assessed employing C3b affinity chromatography. Three protein species were detected: CR1, CR2, and CD46. CD46 possessed a rather unique electrophoretic profile in that it consisted of variable quantities of two heterogeneous protein species of 51–58 kDa (lower

band) and 59–68 kDa (upper band). The CR1 and CR2 proteins migrated at approximately 220 kDa and approximately 140 kDa, respectively.

The cloning and characterization of the *MCP* gene determined that it is an alternatively spliced approximately 43-kb gene consisting of 14 exons and 13 introns located in the RCA cluster (9, 68). Although a partial duplication of exons 1–4 (*MCP*-like) exists, there is no evidence for its expression. CD46 is expressed as four major alternatively spliced isoforms (9). Most cells express all four isoforms, but in a genetically controlled ratio in which 65% of the population predominantly expresses the upper-band isoforms, approximately 30% express equal quantities of each, and approximately 5% predominantly express the lower-band isoforms (68). A few cell-specific isoform expression patterns have also been identified, but their significance is unclear (8, 68).

Common to all isoforms are four N-terminal repeating domains called CCP modules (**Figure 5**). Within each of these approximately 60 amino acid domains are 10–16 highly conserved amino acids. The CCPs largely house the sites for complement regulation. CCPs 1, 2, and 4 each have one site for N-linked glycosylation. Next is an alternatively spliced region for *O*-glycosylation called the STP domain (enriched in serine, threonine, and proline residues). Although there are three exons coding for this region (A, B, and C), the four most common isoforms express B and C (representing the upper band phenotype with 29 residues) or C alone (representing the lower band phenotype with 14 amino acids). Next is the juxtamembranous segment of 12 amino acids, followed by a hydrophobic transmembrane domain and a charged cytoplasmic anchor. The C terminus of CD46 is also alternatively spliced, expressing one of two nonhomologous tails of 16 or 23 amino acids that differ in signaling motifs. Thus, the four major isoforms are termed CD46-BC1, -BC2, -C1, and -C2.

CD46 is increasingly recognized for other important roles in linking innate and adaptive immune responses (69–71). Aside from its primary designation as a complement regulator, CD46 is also known as a pathogen magnet. Because of its nearly universal expression on most cells (with the notable exception of erythrocytes), CD46 is a target for multiple pathogens that employ it as a receptor to gain cellular entrance or to co-opt its signaling capabilities or internalization mechanisms (reviewed in 72). Nine human-specific pathogens exploit CD46, including four viruses (measles virus, adenovirus groups B and D, and herpesvirus 6) and five bacterial species (*Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Streptococcus pyogenes*, *Escherichia coli*, and *Fusobacterium nucleatum*).

Pathogenic microbes may express human-like proteins that mimic complement proteins to subvert host defenses. For example, poxviruses control host complement by secreting CD46-like proteins that share 30–40% homology (73, 74). They consist of three to four CCPs that structurally and functionally mimic CD46 and CD55. These virulence factors attach to GAGs via their heparin-binding sites and downregulate complement attack on the virus. In other cases, bacteria may coat themselves with fluid-phase regulators, such as is accomplished by streptococcal and neisserial species, which bind FH to their surfaces (75).

CD46 also has emerged as a key sensor of immune activation and an important modulator of adaptive immunity (70). These studies have been extensively reviewed, especially as they relate to human T cell biology (69–71). For example, stimulation of the C3a receptor and CD46 by T cell– generated C3a and C3b during T cell receptor engagement is a requirement for the induction of Th1 cells. CD46-deficient or C3-deficient patients are not able to generate normal Th1 responses, although Th2 cell responses are unchanged (76). The contribution of local complement activation to normal human Th1 cell generation is achieved at least partially via CD46-mediated regulation of the IL-2 receptor assembly, the induction of protein kinase B phosphorylation, and the regulation of *IL2* promoter activity (76). Furthermore, CD46 is linked to a pathway for metabolic reprogramming involving CD46-mediated Th1 cell activation (CYT-1) and contraction (CYT-2) (77). However, it is unclear how these other functions of CD46 may play out in human disease, but interesting and provocative data are available relative to multiple sclerosis and inflammatory arthritis (78, 79).

In addition, variants of *MCP* have been increasingly associated with inflammatory disorders, especially those characterized by development of thrombi in small blood vessels, particularly aHUS (22, 23, 80–83). The majority of aHUS disease-associated mutations occur in the four CCPs. An in-depth analysis dissecting the active sites of CD46 by mutation modeling demonstrated how critical amino acids at binding sites lead to a loss of function (21).

Because some publications differed in the numbering of the residues of CD46, a recent publication updated the numbering designation of mutations in the literature to include the signal peptide and all exons of the protein, as recommended by the Human Genome Variation Society (22). This review also has adopted that format.

CD46 mutations predominantly occur in the CCP regions, although other areas have been identified (**Figure 5**). Two mutations have been described in the promoter region, 4 in the signal peptide, 13 in CCP 1, 9 in CCP 2, 14 in CCP 3, 13 in CCP 4, 1 in the STP region, 4 in the transmembrane domain, and 1 in the cytoplasmic tail (CYT-1). Although 52 mutations were associated with the development only of aHUS, 13 were linked to aHUS and/or other diseases (such as C3G, miscarriage, systemic lupus erythematosus, or preeclampsia) (22).

Mutations in MCP that predispose to aHUS, initially described in 2003 (20, 84), are present in 10–20% of aHUS patients. Most of these mutations are missense, but nonsense and splice-site variants have also been identified (reviewed in 15, 22, 23, 81, 85–87). Most mutations are rare or novel and deleterious. In addition, the majority of mutations are heterozygous, leading to a haploinsufficient state. For approximately 75% of cases, the mutant protein is not expressed. For the remainder, the aberrant protein, albeit expressed, has a major reduction in or lacks complement regulatory function. In these patients, the common alteration is reduced C3b binding causing a defect in cofactor activity, which in turn impairs the regulation of the AP and its feedback loop (22).

Additionally, a specific MCP SNP haplotype in its promoter, termed the MCPggaac haplotype, may have reduced transcriptional activity (23, 88). Usually, this has been

associated with an increased risk of aHUS, primarily in the setting of a causative variant of another inhibitor such as FH or FI.

In contrast to mutations in *CFH* or *CFI*, kidney transplantation of CD46-deficient patients has a high rate of success because the transplanted organ carries a normal level of CD46 protein (23, 89–91).

Hypomorphs (i.e., genetically based changes resulting in functionally deficient complement inhibitors) account for approximately 50% of the attributable genetic risk for AMD. Although no CD46 mutations have been directly associated with AMD, several studies suggest CD46 may play a role (reviewed in 35). CD46 has been identified in drusen (35). Lower expression of CD46 is observed in AMD patient monocytes (35), and CD46 expression is reduced by cigarette smoke extract (smoking has been directly linked to AMD development) (35).

Animal models have played a crucial role in our understanding of AMD pathology. They provided tools necessary to understand the etiopathogeneses of this disease and proved to be critical for preclinical studies testing new therapeutic agents (reviewed in 35). A mouse model of AMD has been developed based on the finding that mice express CD46 (mCD46) in only two tissue-specific sites: the eye and spermatozoa (92). Conversely, a unique rodent transmembrane protein known as Crry (complement-receptor 1–related gene/protein y) has both CD46 and CD55 activities and is considered a functional homolog on most cells in rodents (93).

A mouse model in which CD46 was knocked out ($cd46^{-/-}$) demonstrated increased levels of MAC and vascular endothelial growth factors in the retina and choroid (92). Additionally, these knockout mice also developed more severe retinal damage in a laser-induced model of AMD. However, human genetic studies have not identified an association between CD46 mutations and AMD. Furthermore, a spontaneously occurring mouse model of AMD has been described (94–96). This genetic model demonstrates a multifocal, bilateral spontaneous choroidal neovascularization, which leads to early, persistent neovascular lesions that result in death. The precise trigger for the neovascularization is unknown, but growth of the lesions and their leakage is driven by vascular endothelial cell growth factor-A and by a local inflammatory response.

FACTOR I MUTATIONS

FI is an ~90-kDa serine protease of plasma that cleaves C3b to iC3b in the presence of a cofactor protein (FH, CD46, or CR1) bound to the C3b (**Figure 2**). FI is a heterodimer consisting of two polypeptide chains linked by a single disulfide bond (**Figure 6**). iC3b does not have the capacity to engage the players in the AP. The fully processed form of FI protein consists of a heavy-chain (50 kDa) and a light-chain (38 kDa) peptide linked together covalently by a disulfide bond. Although the heavy chain is multimodular, the light chain consists only of the serine protease domain. FI concentration in blood is low (approximately 45 μg/ml), and it is mainly produced by hepatocytes, and also by monocytes and fibroblasts (97, 98).

At least 14 *CFI* gene mutations, associated with aHUS, have been functionally evaluated relative to their effects on protein expression, secretion, and function (97). All mutations were heterozygous. Six were generated by premature stop codons and eight by amino acid substitutions. As observed in other complement regulators and components, the presence of premature stop codons primarily affected protein secretion, whereas amino acid substitutions altered either secretion or function.

A common polymorphism near the 3′ region of the *CFI* gene located on chromosome 4 is a risk variant for AMD (98, 99). A specific functional effect has not been found. By contrast, a large number of *CFI* genetic variants have been described in patients with A-AMD (**Figure 5**). In a large cohort of Caucasian patients with A-AMD, 7 to 8% carried a rare variant in *CFI* (versus 2.3% in controls) (98). Fifty-nine variants in 140 cases were identified. An excess of rare variants was observed in the catalytic domain. These were predicted to be deleterious with 13 already having been described in aHUS (98). Of note, about 50% of the rare variants had FI antigenic levels below 30 μg/ml (normal 45 ± 15) and thus were haploinsufficient (reviewed in 23).

The combination of rare variants in *CFH* and *CFI* accounted for approximately 10% of all cases of A-AMD in the Seddon study (99) of approximately 1,500 patients. These and other data on the AP described in this article explain why alteration in the activity profile of the AP (i.e., its downregulation) is such an attractive therapeutic approach. These results also raise the intriguing question of whether other common inflammatory diseases will have rare variants, such as Alzheimer's disease (AD), atherosclerosis, or osteoarthritis (degenerative joint disease). In all of these cases, for example, complement activation fragments are deposited at the lesion sites. In AMD, thanks to modern genetics, we now know that the complement system is a key contributor to disease pathogenesis. In summary, it is a loss of function in regulators of the AP that is causative in approximately 50% of AMD patients and an even higher percent in aHUS patients—two examples of how haploinsufficiency causes human disease.

C3 MUTATIONS

C3 is the most abundant complement protein in human blood plasma. Its gene is located on chromosome 19. Following synthesis as a single polypeptide chain of 1,641 amino acids, it is processed and subsequently secreted into blood as a glycosylated two-chain protein (**Figure 7**).

A mosaic protein, C3 consists of multiple modules and includes a core of eight homologous macroglobulin domains (similar to the α2-macroglobulin protein family) (100). Interestingly, there is no sequence homology among the MG domains, yet all exhibit a fibronectin-type 3-like core fold. Domains MG1–MG5 are formed by residues of the β-chain, whereas domains MG7 and MG8 are formed by the α-chain. Additionally, MG6 is formed by residues of both chains. C3 is rich in disulfide linkages. One disulfide bridge covalently connects both chains, another is an inter-domain bridge, and eleven are intradomain bonds. A central feature of C3 is its reactive thioester moiety, which provides an

ingenious mechanism to transfer a plasma protein onto a membrane. The other modules help define the structural conformation of C3 and possibly play other roles.

Activation of the classical, lectin, and APs results in cleavage of C3 by an enzymatic complex called a C3 convertase. This reaction generates C3b and the anaphylatoxin C3a. Newly generated C3b can form an AP C3-convertase thereby providing a feedback amplification loop (**Figure 1*b***).

In a healthy host, activation of C3 in plasma is maintained at a very low level, and deposition of C3b is limited to pathogens by multiple RCA proteins, often in concert with FI. Recent three-dimensional structures of C3b complexed with regulators provide insights into the functional domains of FH, CD46, and CR1 (30).

Mutations in C3 have been described in reports of aHUS (101, 102), C3G, and AMD (26, 98, 103). In a recent study, a total of 48 C3 variants were identified in 130 aHUS patients from France, Italy, the United Kingdom, and the United States (26). Of these 48, 27 were novel variants, which underwent mapping and functional analyses. Sixteen of 27 resulted in impaired binding to MCP, and 17 of 27 demonstrated decreased FH binding. The decrease in binding to MCP and FH correlated with decreased cofactor activity.

Another report of 14 aHUS patients carrying the same C3 mutation (R139W) (101) established that it had an increased affinity for FB and a decreased binding to CD46. A novel C3 mutation (V1636A) has been described in one Swedish kindred with three affected individuals having aHUS (102). The mutation, located in a FB-binding site, led to enhanced C3b binding to FB and resulted in the formation of a more potent C3 convertase resistant to decay by FH and DAF. Interestingly, all three patients had late onset disease and clinical features and pathological findings suggesting a TMA as well as C3G.

aHUS patients with C3 mutations often (approximately 50%) rapidly progress to end-stage renal disease, and a few are reported to have a high frequency of cardiac and neurologic events (101). In these patients, inefficient regulation of the mutant C3b by FH and CD46 causes a secondary gain of function of the C3 convertase because of the reduced rate of C3b cleavage. Complement activation is thereby amplified, leading to endothelial damage and thrombosis.

The role of C3 mutation (D923G) in the pathogenesis of a C3G has also been described (103). This novel variant was identified in a 53-year-old woman and in her 26-year-old identical twin sons. All three individuals had a biopsy-proven diagnosis of dense deposit disease. The functional characterization demonstrated that the mutant C3 generated an active AP-C3 convertase that was resistant to decay by FH. In addition, the mutant C3b and $C3(H_2O)$ were resistant to proteolysis by FI in the presence of FH.

More recently, a single rare variant in C3 (K155Q) has been associated with a high risk for AMD (98, 104, 105). This variant was resistant to inactivation by FH. In a subsequent analysis, another C3 variant (K65Q), which had been previously identified in aHUS, was found to be significantly associated with AMD (34).

R102G is a common SNP in C3 that is associated with risk of AMD (106). This polymorphism results in impaired FH binding thereby leading to decreased cofactor activity. The R102G has also been associated with dense deposit disease (107).

FACTOR B MUTATIONS

FB is encoded by the *CFB* gene on chromosome 6 and expressed as a 90-kDa single-chain plasma glycoprotein (**Figure 8**). Assembly of the AP convertase begins with the association of FB with C3b in the presence of Mg^{++} . This association permits FB to be cleaved at a single site by factor D, producing two fragments, Ba and Bb. Although Ba dissociates from the complex, Bb and Mg^{++} remain bound to C3b. The resulting active AP C3 convertase, C3bBb, can catalyze C3 cleavage. Furthermore, association of C3bBb with an additional C3b yields the AP C5 convertase, $C3b_2Bb$, which cleaves C5.

Although rare, FB mutations occur in aHUS with a frequency of 0–4% in patients from different cohorts (reviewed in 24). A number of mutations have been identified, but many have not yet reported functional data. Some FB mutations cause a gain of function in forming an overactive convertase resistant to decay. Other mutations resulted in reduced ligand binding or functional activity. However, most FB mutations have not yet been definitely linked to disease pathogenesis (reviewed in 24).

C4b-BINDING PROTEIN MUTATIONS

C4BP is a 570-kDa plasma glycoprotein that serves primarily as a fluid phase regulatory protein of both the classical and lectin pathways (108). It inhibits formation and accelerates decay of the CP/LP C3 convertase (C4b2a). It also serves as a strong cofactor for the FI-mediated degradation of C4b and a weak cofactor for the cleavage of C3b. C4BP consists of seven identical α-chains and a single β-chain. Each α-chain has eight CCPs, whereas the β-chain has three. Another function of C4BP is its capacity to bind with high affinity to the vitamin K–dependent anticoagulant protein S, the serum amyloid P component, and heparin (108).

C4BP is encoded by two genes, *C4BPA* and *C4BPB* (coding for the α- and β-chains, respectively). A SNP in the α-chain of C4BP (R240H) was associated with aHUS in cohorts from the United Kingdom and France (109). Functional analyses of the modeled protein demonstrated normal expression and function relative to CP and LP regulation. However, the R240H SNP was not able to efficiently regulate the AP (109). Because many of the aHUS patients bearing this polymorphism also carried mutations to other complement genes associated with aHUS, it is possible that this change represents an additive risk factor for aHUS. However, this association was not confirmed in a separate Spanish cohort of patients (110).

DECAY ACCELERATING FACTOR

DAF (CD55), another member of the RCA family, is a 70-kDa membrane protein that is widely distributed on most cells. Similar to its sister protein, CD46, DAF is also composed of four CCPs, followed by a region for *O*-linked glycosylation. However, it is attached to

cells via a glycophosphatidyl inositol (GPI) anchor. DAF is a potent inhibitor of complement activation at the step of C3 convertase formation. It decays (disassociates) the protease from the CP/LP C3 convertase (C2a) and the AP C3 convertase (Bb) thereby inactivating the enzyme complex. Of note, the decayed protease is inactive. It cannot rebind to the C4b or C3b covalently attached to a target. However, a newly minted C2 or FB can now transiently bind to the substrate of C4b or C3b, respectively, to begin to regenerate another C3 convertase. Thus, in this sense, DAF is a temporary fix in that the enzyme can reform. This is in contrast to CD46, which provides a permanent fix via cofactor activity in which the C4b or C3b is converted to C4d or iC3b, respectively, and these proteolytic fragments cannot reform a convertase. Perhaps this is the reason why no common or rare variant in DAF has been identified in aHUS (111), AMD, or C3G. However, DAF is more efficacious than MCP against the CP in several types of complement challenge assays, and both are strong inhibitors of AP C3 convertase (reviewed in 112, 113). It is a puzzle though as to why DAF is not incriminated in the aforementioned diseases analogous to MCP: Both are abundantly expressed, intrinsically acting (only function if present on same cell being attacked by complement) membrane regulatory proteins, and they work cooperatively on convertases (112). Nevertheless, these data clearly point out that cofactor activity is the primary regulatory profile that is deficient in both aHUS and AMD.

COMPLEMENT THERAPEUTICS

Atypical Hemolytic Uremic Syndrome

Until recently, plasma procedures have been the mainstay for the management of aHUS (18). The rationale behind this treatment is to reduce the quantity of a mutant protein by plasmapheresis and then deliver a functionally normal protein by plasma infusion. Lack of improvement in hematological parameters (platelet count, hemoglobin, and lactate dehydrogenase) is considered a nonresponse to therapy, and plasma exchange is usually discontinued. There are no randomized controlled trials of plasma therapy in aHUS, but overall the efficacy has been poor (114, 115).

Eculizumab is a humanized monoclonal antibody directed against the terminal pathway protein C5 (part of the MAC) (**Figure 9**). It binds to and blocks the N-terminal cleavage of C5 into two effector molecules, C5b and C5a, thereby preventing formation of the MAC, C5b-9, and the liberation of C5a (116, 117). Animal studies (118–120) demonstrated that a FH-deficient mouse crossed with a C5-deficient mouse did not develop aHUS, suggesting a critical role for components downstream of C3b in aHUS development and thus a strong experimental rationale for the use of eculizumab in aHUS. The drug was originally approved by the US Food and Drug Administration (FDA) in 2007 for the treatment of the complement-dependent hemolytic disease paroxysmal nocturnal hemoglobinuria. In September 2011, it was approved by the FDA to treat aHUS. The use of this C5 complement inhibitor in aHUS was first reported in two separate cases in 2009 (121, 122). Since these initial reports, clinical experience relative to the use of eculizumab has been communicated in approximately 50 case reports and two prospective phase 2 trials (123), as well as in a subsequent two-year follow-up study (124). These initial trials demonstrated that eculizumab inhibited a complement-mediated TMA and was associated with improvement

in renal function in approximately 85% of aHUS patients. The two-year follow-up analysis established that the early clinical benefits achieved by eculizumab treatment of aHUS were maintained at two years. Recent evidence suggests that earlier initiation of treatment leads to improved results relative to kidney function (125).

Patients with aHUS who progress to end-stage renal disease are candidates for renal transplantation. However, there is a recurrence risk of >50% with graft loss occurring in 80 to 90% of the cases. The outcome though can vary depending on the complement abnormality. The risk of recurrence is 70–90% with a *CFH* mutation, 50–80% with a *CFI* mutation, and 40–70% with a *C3* mutation (64). The recurrence rate is low in patients with *MCP* mutations because the allograft expresses normal membrane-bound MCP. Recently published data also show that eculizumab is effective for the treatment of aHUS recurrence after a kidney transplant (126) and as prophylaxis before transplantation in patients with a known pathogenic mutation.

Similar to aHUS, studies performed in animal models of C3G and on the identification of genetic mutations in complement proteins in patients with C3G suggest that blocking complement should be beneficial in this disease as well (127–131). The pathophysiology of C3G suggests that a C3 convertase inhibitor, which would limit C3 breakdown product deposition on basement membranes, would be a strong therapeutic option. To date, no drug is commercially available that specifically inhibits the early components of the AP required for C3 convertase. Consequently, the anti-C5 antibody has been tried with some success. It appears to be a beneficial therapy for a subset of these patients; however, the available data concerning the use of eculizumab in C3G remain relatively scarce. The information is mostly in the form of case reports and one small prospective trial (131). These limited data suggest that eculizumab is effective in approximately 60% of patients with C3G and that it is most efficient in treating rapidly progressive and crescentic forms of C3G. Formal prospective studies are needed to assess the effect of eculizumab as a therapeutic agent in C3G. Renal transplantation in patients with C3G has a high rate of recurrence in the allograft (reported to be greater than 50%). Eculizumab has been shown to be beneficial in a few patients with recurrence of C3G after a kidney transplant (117).

Treatment with a Monoclonal Antibody to C5

As indicated above, the monoclonal antibody eculizumab targets the terminal pathway protein C5, a component of the MAC. This prevents the proinflammatory effects of C5a and the cytolytic effects of the MAC. Because it acts downstream of C3, eculizumab preserves the autoimmune protective and immune-enhancing functions of the opsonin C3b and the anaphylatoxin C3a.

Treatment with eculizumab is usually initiated as soon as thrombotic thrombocytopenic purpura (TTP) is ruled out by measurement of ADAMTS13 (the von Willebrand factor-cleaving protease) activity.

Dosing of therapy

Monoclonal antibody therapy is initiated early to offer the best chance to recover renal function (125). Genetic testing is performed in patients with aHUS and C3G and, depending

upon the clinical scenario, treatment with eculizumab is usually initiated early. The drug is administered intravenously and has a half-life of approximately 11 days. The recommended dosage regimen is intravenous administration of 900 mg weekly for four doses, then 1,200 mg at week five, followed by 1,200 mg every two weeks. In patients receiving plasmapheresis, a supplemental dose is administered after each plasma exchange.

Duration of treatment

Eculizumab is well tolerated and relatively safe. The optimal duration of therapy is unclear. The almost prohibitive cost of this drug and other issues mean that the duration of treatment should be tailored based in part at least on individual complement genetics. Treatment may be needed lifelong in patients with pathogenic mutations known to be associated with poor outcomes (for example, FH loss-of-function or C3 gain-of-function mutations), whereas withdrawal of eculizumab is more likely to be successful in patients with isolated MCP mutations (23). The duration of treatment in patients with a mutation of unknown functional consequence and in those with no identified genetic abnormalities remains a largely unexplored issue. These remaining questions underscore the necessity to continue therapeutic studies, in particular to employ larger-scale, prospective randomized trials. Ultimately, the aim is to balance the risk of side effects (see below) and high cost of the drug against this agent and other effective treatment programs. There is much yet to learn about the duration, frequency, and magnitude of treatment with eculizumab.

Adverse effects

The most frequently reported adverse reactions with eculizumab are hypertension, headache, upper respiratory tract infection, urinary tract infection, nausea, vomiting, diarrhea, anemia, and leukopenia. The relationship, if any, of these relative minor side effects to drug activity is unclear.

The predominant concern is a life-threatening infection with *N. meningitidis* (due to blockage of the terminal complement pathway). A meningococcal vaccination must be administered to everyone undergoing treatment with eculizumab. Physicians need to be aware that the standard vaccine does not protect against the B serotype of the bacteria nor many of the infrequent (rare) serotypes. The Centers for Disease Control and Prevention (www.cdc.gov) now recommends serotype B *Neisseria* vaccine in patients receiving eculizumab as well as the standard *Neisseria* vaccine. In addition, appropriate antibiotics should be used for at least 14 days if there is not enough time to wait for the immune response. Moreover, suggestive symptoms of a bacteremia or septicemia should necessitate urgent investigation and antibiotic therapy.

CONCLUSIONS AND PERSPECTIVES

Over the last decade, a remarkable advance has been the elucidation of the role of mutations in complement regulators and components in aHUS, AMD, and C3G. Next-generation sequencing has led the way to these discoveries, but functional assessments are the critical factors in definitively associating pathogenesis with genetic variants.

Most exciting has been the development and approval by the FDA of the monoclonal antibody, eculizumab, as the new standard of care for treatment of aHUS. Challenges remain, however, because eculizumab is costly and the duration of treatment remains uncertain and warrants further prospective studies. The use of eculizumab in C3G should also be prospectively addressed. Furthermore, given the increasing number of mutations in the complement regulatory proteins identified in aHUS and C3G and the heterogeneity in the mechanisms leading to dysregulation of the AP, there is a need for further assessment of the genetic variants of unknown significance. As yet, no complement inhibitor has been approved to treat AMD.

These analyses coupled with the anticipated new developments of complement therapeutics will help establish patient-tailored therapies based on each patient's specific alteration. The future holds much promise for the further delineation of complement-disease associations and for novel complement-targeted therapeutic agents.

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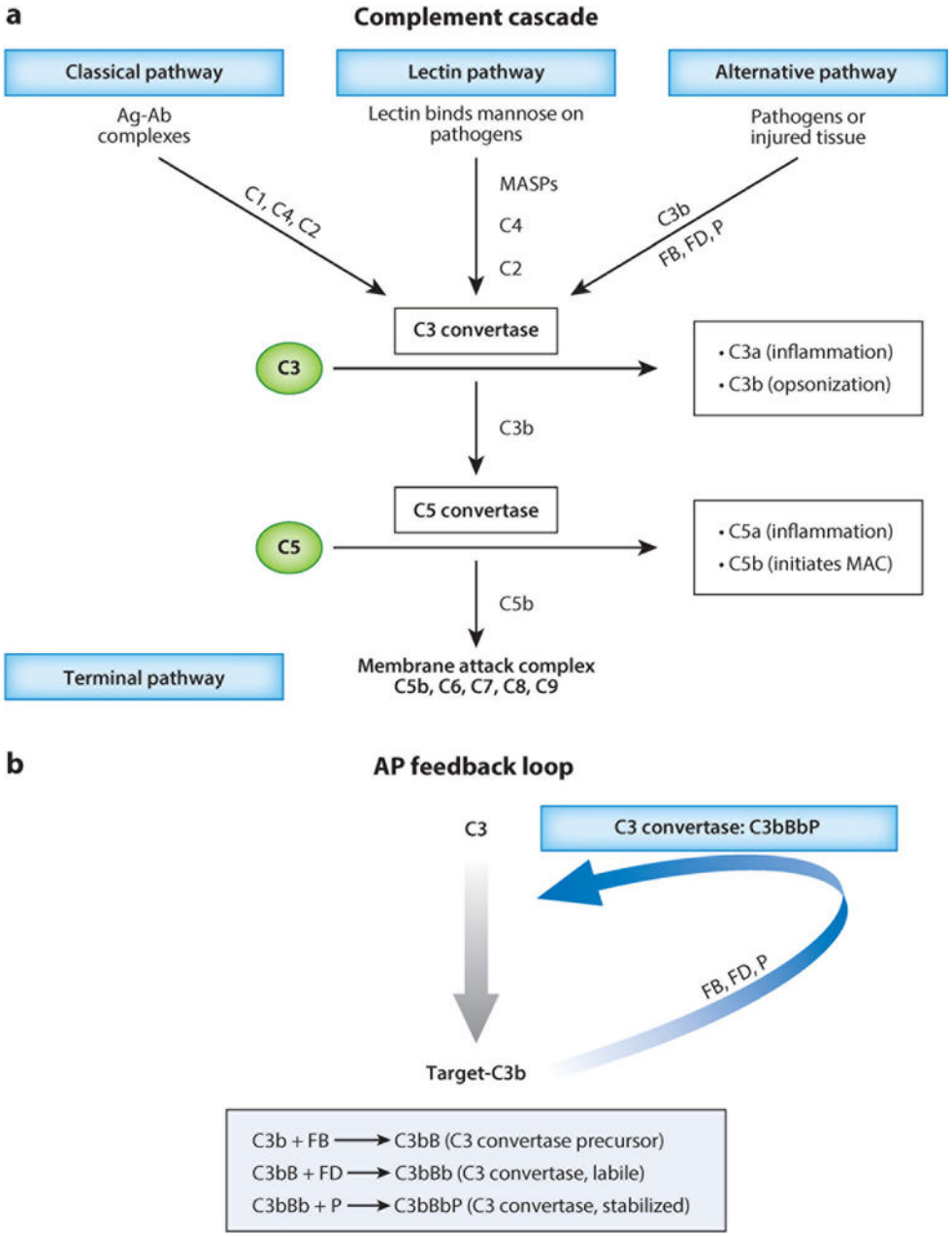

Figure 1.

The complement cascades. (*a*) The three pathways of complement activation are shown. Although each is independently triggered, they all merge at the step of C3 activation. The classical pathway (CP) is initiated by the binding of antibody (Ab) to antigen (Ag) and the lectin pathway (LP) by the binding of lectin to an oligosaccharide. The alternative pathway (AP) turns over continuously, generating small amounts of $3(H_2O)$ (the thioester-hydrolyzed form of C3) and C3b, which it amplifies in the presence of pathogens or injured tissue. $C3(H_2O)$ behaves like C3b in that it can bind factor B (FB) to initiate the AP (see below). Activation of the complement system leads to inflammation (release of anaphylatoxins C3a and C5a), opsonization (the coating of targets with C3b and/or C4b), and membrane

perturbation (formation of the membrane attack complex, MAC). (*b*) The AP's feedback loop of complement activation. First, C3b attaches to the surface of a target, such as a pathogen or self-debris. Next, C3b binds to FB, and then factor D (FD) cleaves FB to form the AP C3 convertase (i.e., an enzyme that cleaves C3 to C3b). This convertase is stabilized by the binding of properdin (P). The C3 convertase triggers the amplification loop via the generation of more C3b. The initial C3b can be generated by the CP and LP or tick over [formation of C3(H_2O), which can also bind FB to trigger the AP]. Abbreviations: MASPs, mannose-binding lectin-associated serine proteases; C3(H_2O), C3 with a cleaved thioester bond. Modified with permission from Liszewski MK, Atkinson JP. Complement Pathways. In UpToDate, Post TW (Ed), UpToDate, Waltham, MA, accessed on 4–10-15. Copyright © 2016 UpToDate, Inc.

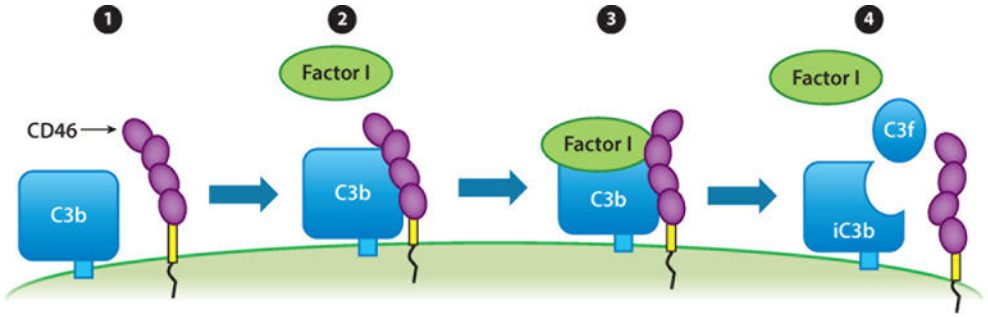

Figure 2.

Complement regulation: cofactor activity. In Step 1 of this example, C3b becomes covalently bound to a target (e.g., an endothelial cell). In Step 2, the membrane-anchored host protein, CD46, locates and binds to the C3b. In Step 3, the plasma serine protease factor I (FI) binds to the C3b/cofactor protein complex. In Step 4, FI proteolytically cleaves C3b to iC3b (a product that cannot participate in the feedback loop) and C3f (the small degradation fragment) and thereby halts further complement activation. Modified with permission from Liszewski & Atkinson (22). Published by BioMed Central ©2015.

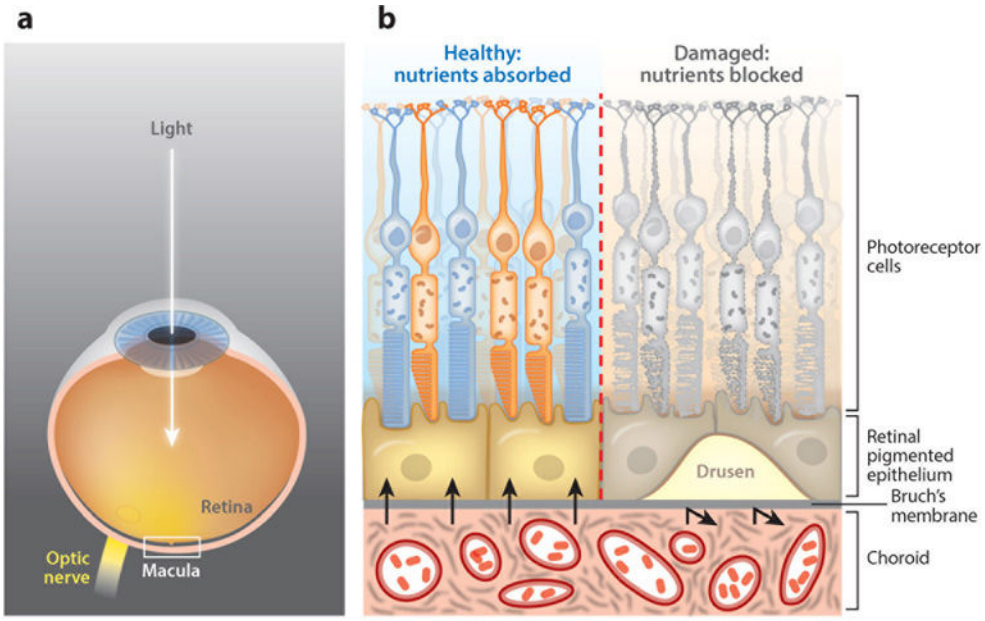

Figure 3.

Cross-sectional diagrams of the human eye. (*a*) Macroscopic schematic picture of the human eye. (*b*) Microscopic schematic picture of the retina in health and in age-related macular degeneration (AMD). Subretinal drusen accumulate; this accumulation may block nutrients and thereby damage the photoreceptor cell layer, leading to atrophy. In theory, the primary process in AMD could be excessive photoreceptor damage, retinal pigment epithelial dysfunction, an alteration in Bruch's membrane, or vascular damage to the choroid. Modified from Schramm et al. (34). Published by Elsevier © 2014.

Figure 4.

Factor H (FH) in age-related macular degeneration (AMD) and atypical hemolytic uremic syndrome (aHUS). FH is a 155-kDa plasma protein composed of 20 repeating units, called complement control proteins (CCPs), consisting of approximately 60 amino acids, linked together by 3–8 amino acids. The sites involved in ligand binding and attachment to membranes bearing glycosaminoglycans (GAGs) are identified. A common variant (Y402H) associated with AMD risk is located in the seventh CCP. The C terminus of FH (repeats 19 and 20) is particularly associated with aHUS, as 60% of all FH mutants occur in these two CCPs, which mediate both ligand and GAG binding. In contrast, mutations causing a regulatory dysfunction in CCPs 1–4 are commonly observed in both aHUS and AMD. Abbreviations: CA, cofactor activity; DAA, decay-accelerating activity; SNP, single-nucleotide polymorphism. Modified from Schramm et al. (34). Published by Elsevier © 2014.

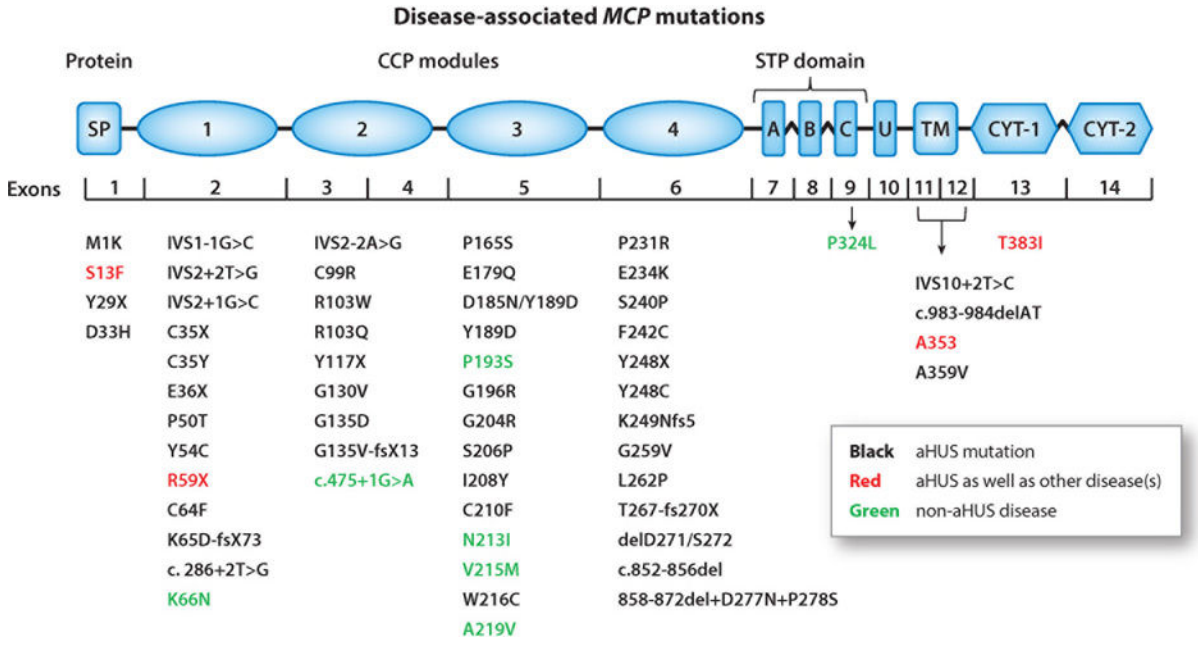

Figure 5.

Disease-associated membrane cofactor protein (MCP) (CD46) mutations. The schematic depicts the CD46 protein, genomic organization, and disease-associated mutations. CD46 has a 34-amino acid signal peptide (SP). The mature protein consists of four complement control protein (CCP) repeats that house the sites for its regulatory cofactor activity. This is followed by an alternatively spliced region for *O*-glycosylation (segments A, B, C), a short 12-amino acid segment of undefined function (U), a transmembrane domain (TM), and one of two alternatively spliced cytoplasmic tails (CYT-1 or CYT-2). The gene consists of 14 exons and 13 introns for a minimum length of 43 kb. More than 90% of the mutations are rare (gene frequency less than 1%) and deleterious. Note that in a majority, the protein is not synthesized (splice site, stop, and cysteine mutations) or secreted, whereas in the others, it is expressed but dysfunctional (commonly missense mutations). A majority of mutations for aHUS and for other disorders (such as systemic sclerosis, systemic lupus erythematosus, and pregnancy-related disorders) occur in the four CCPs. Mutations and their locations are listed below the exons. Modified with permission from Liszewski & Atkinson (22). Published by BioMed Central © 2015.

Figure 6.

Schematic diagram of the protein structure of factor I (FI). FI is synthesized as a single polypeptide chain of 90 kDa. Following removal of the signal peptide (SP), FI is proteolytically processed into a heavy and a light chain linked by a disulfide bond. The noncatalytic heavy chain consists of several modules that share homology to the FI membrane attack complex domain (FIMAC), the scavenger receptor cysteine-rich domain (SRCR), and two low-density lipoprotein receptor domains (LDLR1 and LDLR2). The light chain comprises the serine protease domain that contains the expected catalytic triad of His-362, Asp-411, and Ser-507, responsible for its cleavage activities. Modified from Schramm et al. (34). Published by Elsevier © 2014

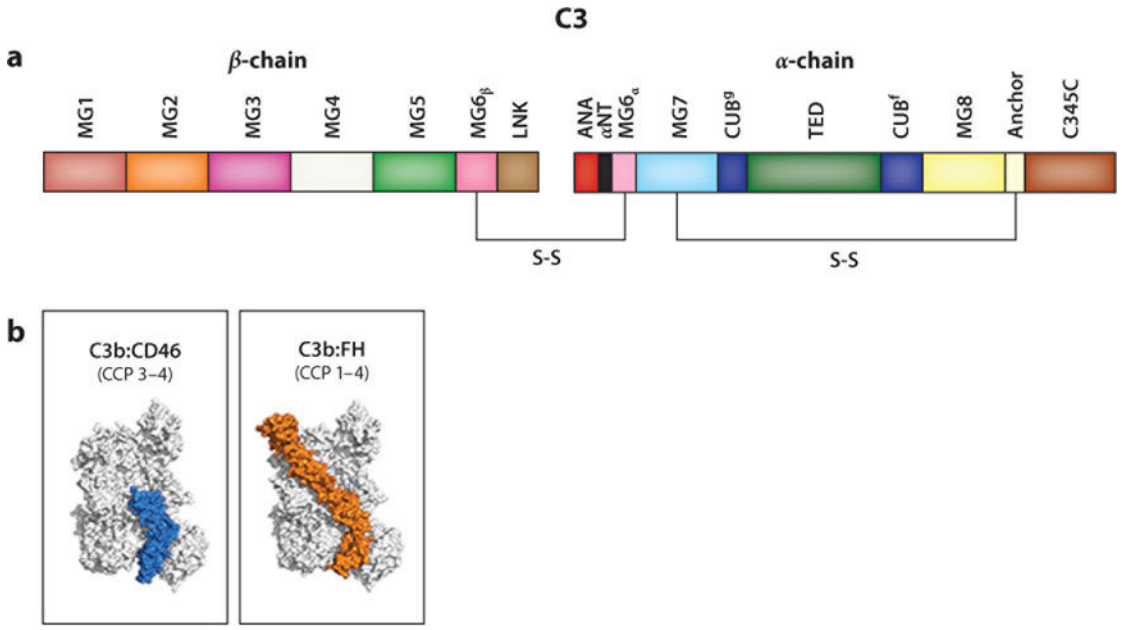

Figure 7.

Protein structure of C3. (*a*) C3 is synthesized as a single precursor chain. Following proteolytic processing, the mature structure of C3 consists of two disulfide-linked polypeptide chains (α and β). Features include eight macroglobulin domains (MG) with approximately 100 amino acids in each and a linker domain (LNK). The N terminus of the α-chain consists of an anaphylatoxin domain (ANA), an αNT module, a CUB domain (C1r/C1s, Uefg, Bmp1), a thioester-containing domain (TED) allowing for C3 attachment to a target, an anchor domain, and a C-terminal C345C segment. Modified with permission from Schramm et al. (34) and published by Elsevier © 2014. (*b*) Surface representation of the three-dimensional structures of C3b bound by membrane cofactor protein (MCP) (*blue*) and factor H (FH) (*orange*). Abbreviation: CCP, complement control protein. Reprinted from Forneris et al. (30) and published by Wiley © 2016.

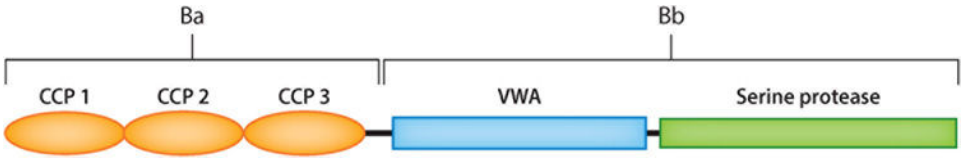

Figure 8.

Schematic diagram of the protein structure of factor B. Factor B is a mosaic glycoprotein composed of three types of protein modules. The N-terminal region (the Ba fragment) features three complement control protein (CCP) modules. This is followed by a von Willebrand factor type A (VWA) domain (a ligand and metal-binding site). The C terminus is a serine protease domain similar to that of trypsin. The capacity to cleave C3 is acquired through C3 convertase assembly (C3bBb) (See **Figure 1*b***).

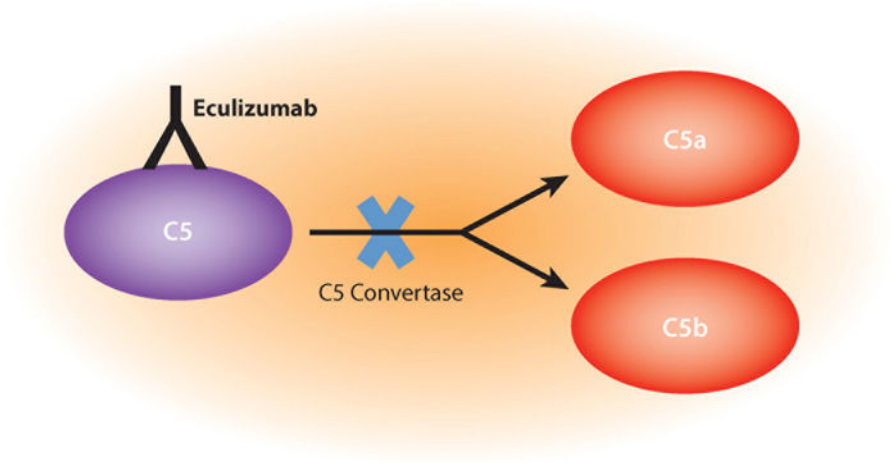

Figure 9.

Therapeutic inhibition of C5 by eculizumab. Eculizumab is a humanized recombinant murine monoclonal antibody approved for treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. The antibody binds C5 near the cleavage site and thereby prevents its enzymatic activation by the C5 convertase into C5b and the proinflammatory peptide C5a. The upstream immune-enhancing and autoimmune protective functions of C3 remain intact with this agent.

Table 1

Mutations in complement system proteins associated with atypical hemolytic uremic syndrome[a]

Protein (kDa)	Synthesis site (location)[b]	Function	Mutation %
FH (155)	Liver (plasma)	Cofactor protein for C3b: DAA for AP and CP/LP convertases containing C3b	20–30
MCP (51–68)	Cells (wide distribution)	Cofactor protein for C3b and C4b	10–20
FI (90)	Liver (plasma)	Protease to cleave C3b and C4b (requires a cofactor protein)	5–15
C3 (190)	Liver (plasma)	Cleavage produces C3b, an opsonin; C3b is also part of the AP C3 and C5 convertases and CP and LP C5 convertases	5–15
FB (90)	Liver (plasma)	AP protease for cleavage of C3 and C5; Bb fragment cleaves C3 to C3b and C3a and C5 to C5b and C5a	<5
C4BP (570)	Liver (plasma)	Cofactor protein for C4b; DAA for CP/LP C3 and C5 convertases	≤1

[a] Modified with permission from Wiley & Sons © 2014, Liszewski & Atkinson (14).

[b] The five plasma proteins are also synthesized and secreted by a variety of cell types, but those in plasma are largely derived from the liver.

Abbreviations: AP, alternative pathway; CA, cofactor activity; CP, classical pathway; DAA, decay-accelerating activity; FB, factor B; FH, factor H; FI, factor I; LP, lectin pathway; MCP, membrane cofactor protein (CD46)

Table 2

Advanced age-related macular degeneration cases are enriched for rare variants in the functional domains of the *CFH* gene[a,b]

Sites	*n*[c]	Cases (%)[d]	Controls (%)[d]	Odds ratio (95% CI)
CCPs 1–4	27	34 (2.0)	4 (0.5)	4.30 (1.70–13.58)
CCPs 6–8	10	11 (0.7)	2 (0.3)	1.60 (0.40–8.68)
CCPs 19–20[e]	5	24 (1.4)	2 (0.3)	5.10 (1.60–25.91)
Functional domains[f]	42	69 (4.1)	8 (1.1)	4.20 (2.10–9.50)

[a] Modified and reprinted with permission from Triebwasser et al. (63) and the Association for Research in Vision and Ophthalmology © 2015.

[b] This table defines a minor allele frequency as ≤0.5%. A total of 1,665 cases and 752 controls were tested.

[c] This is the number of distinct variants observed in the cases and controls, and the total number identified is the sum of the number of cases and controls.

[d] Number in parentheses is the percent of individuals with that phenotype.

[e] For the majority of cases in CCP19 and CCP20, one rare variant known to be a highly penetrant risk variant (R1210C) was present in 16 cases and one control.

[f] Functional domains include missense mutations in CCPs 1–4, 6–8, and 19–20 (see **Figure 4**).

Abbreviations: CCP, complement control protein (repeat or module); CI, confidence interval.

Table 3

Serum levels of factors H and C3 in advanced age-related macular degeneration cases carrying rare *CFH* variants[a]

Variant	Effect	FH (μg/ml)[b]	C3 (mg/ml)[b]
C192F	Loss of cysteine	205	1.30
Y277X	Nonsense	180	1.03
C431S	Loss of cysteine	200	1.11
chr1:196642295; T>C	Splice site	247	1.70
chr1:196648924; G>A	Splice site	161	0.82

[a]Modified and reprinted with permission from Triebwasser et al. (63) and the Association for Research in Vision and Ophthalmology © 2015.

[b]Sixty phenotyped controls had a median factor H (FH) concentration of 312 μg/ml with a 10th percentile of 253 μg/ml and a 90th percentile of 420 μg/ml. Each variant was carried by a single case. The mean C3 concentration in serum for 40 controls from the cohort was 1.10 mg/ml with a 10th percentile of 0.86 mg/ml and a 90th percentile of 1.55 mg/ml. Note that the FH levels were all low, consistent with these patients being haploinsufficient for FH. Also, the individuals with the two lowest FH levels had the lowest C3 levels.

Abbreviation: *CFH*, factor H gene.