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Spotlight on vision

Complement factor H and related proteins in age-related macular degeneration

Facteur complément H et protéines liées dans la dégénérescence maculaire liée à l'âge

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ABSTRACT

Age-related macular degeneration (AMD) is the major cause of legal blindness in the industrialized world. Polymorphisms and recently discovered rare mutations of the Complement Factor H gene have been shown to be strongly associated with AMD. The deletion of CFH-related proteins 1 and 3, proteins that share homologous regions with CFH, is found in protective haplotypes. The following is a critical review of the current state of knowledge of the implication of CFH and CFH-related proteins 1 and 3 in AMD.

RÉSUMÉ

La dégénérescence maculaire liée à l'âge (DMLA) est la principale cause de cécité dans le monde industrialisé. Des polymorphismes ainsi que des mutations rares récemment découvertes du gène du facteur complément H (CFH) se sont révélés être fortement associés avec la DMLA. La suppression des protéines 1 et 3 liées au CHF, qui partagent des régions homologues avec le CFH, a été observée dans des haplotypes protecteurs. Cette contribution constitue une revue critique de l'état actuel des connaissances sur l'implication du CFH et des protéines 1 et 3 liées au CFH dans la DMLA.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of legal blindness in the industrialized world [1]. The pathology is characterized by lesions of photoreceptors, the retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaris [2]. The early onset form of AMD, called age-related maculopathy (ARM), presents large soft Drusen associated with changes in RPE pigmentation but no overt degeneration. ARM can regress (~20%); stay stable for years (~60%), or progress and develop into AMD (~20%) [1]. There are two clinical forms of late AMD: the fast progressive "wet" form defined by choroidal neovascularisation and the more slowly progressive "atrophic" form characterized by RPE atrophy, photoreceptor degeneration and choroidal involution and obliteration [1].

Risk factors, such as age, smoking, high body mass index and a family history of AMD have been shown to predispose subjects to AMD. Extensive genetic studies have identified a number of polymorphisms that are associated with AMD [3]. The rs1061170 polymorphism in the gene of Complement Factor H (CFH) is associated with

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a major risk for AMD development [4–7]. Complement factor H is a negative regulator of the complement alternative pathway, but the nature in which Y402H substitution influences the CFH function that participates in AMD pathogenesis is not certain.

Apart from CFH, there are five Complement factor H related proteins (CFHR1-5) [8]. These proteins are encoded by five distinct genes located in close proximity to the CFH gene on human chromosome 1 [8]. They present a high degree of sequence identity with CFH in some domains, but not in others, conferring some similar and some distinct functional properties of CFH [8]. Several recent studies have shown a protective effect of deletions of Complement Factor H related proteins 1/3 in AMD [9,10]. The following is a concise review of the CFH, CFHR1 and CFHR3 proteins functions and their association with AMD.

2. The alternative complement cascade

The complement cascade is an innate component of the immune system designed to kill pathogens, such as bacteria. Complement components are activated in a cascade on the pathogen's surface and result in the assembly of a pore-forming complex in the pathogen's membrane, the membrane attack complex (MAC), which can kill the pathogen. It is also important in chemotaxis and the opsinization and clearance of apoptotic bodies [11,12]. The key event of the cascade is the assembly of complement factor 3 (C3) and complement factor 5 (C5) convertases, leading to the formation of the MAC complex on the cell surface. The complement cascade can be activated by specific antibodies that are directed to the pathogen surface epitopes, but also via an alternative path in which serum C3 is spontaneously hydrolyzed to form C3a and C3b. C3b can attach itself to the target cell surface and trigger alternative complement activation. On the cell surface, it assembles with properdine and the complement Factor Bb to form a C3 converting enzyme C3bBb that catalyses more C3a and C3b formation from C3. The recruitment of an additional C3b molecule leads to the formation of the C3bBbC3b complex, a C5 convertase that cleaves C5 into C5a and C5b. C5b recruits C6, C7, C8, and C9 to form the MAC. C3a and C5a are potent chemotactic agents and C3b is an important opsonin [11,12]. There are several mechanisms that inhibit alternative, spontaneous complement activation to protect host cells from complement activation and MAC formation. Complement factor H can inhibit the formation of C3bBb by binding C3b and acting as a cofactor of complement factor I (CFI) that degrades already formed C3bBb and inhibits the cascade.

3. Structure and functions of complement factor H

CFH is very abundant in plasma ($\sim 500\ \mu g/mL$) and is mainly synthesized by hepatocytes. It is a soluble serum factor that binds to cell surfaces under certain conditions. In the normal healthy eye, CFH is expressed in RPE cells but little in retinal neurons [13]. Mononuclear phagocytes, such as microglial cells and macrophages can also express CFH (www.immgen.org [14]) and contribute significantly to the local concentration of CFH in inflamed tissues [15–18].

The CFH gene is located on human chromosome 1q32. It produces two transcripts, a full-length mRNA encoding a 1231 amino acid protein composed of 20 globular short consensus repeat domains (SCR, also called complement control protein modules or Sushi domains) and an alternatively spliced mRNA coding for a 431 amino acid protein, the CFH-like protein 1, that contains only SCR 1 to 7 [8]. SCR domains are formed by 60 amino acids and have many activities and properties related to:

- the inhibition of complement activation [19,20];
- cell surface receptor binding [21,22];
- binding to compounds, such as C3b [23], glycosaminoglycans [24], monomeric C-reactive protein (CRP) [24,25], and malondialdehyde [26].

SCR7 (which contains the Y402H polymorphism) and SCR19–20 mediate cell adhesion through binding to Complement 3 Receptor (CR3, a heterodimer formed by CD11b/CD18 [22]), glycoaminoglycans [27–29] and the opsonin C-reactive protein (CRP) [25]. Glycoaminoglycans are present on virtually all cell surfaces of the body. CRP binds to necrotic and apoptotic cells, and CR3 is strongly expressed by certain leukocytes. CFH binding to the cell surfaces can inhibit complement activity and protect the cells from complement-induced cell death. CFH also mediates the phagocytosis of opsonized cell debris [11].

SCRs 1–4, SCRs 12–14, and SCRs 19–20 bind C3b [19,27,30]. C3b-binding can mediate CFH adhesion on cell surfaces if C3b is fixed on the membrane and inhibits C3bBb formation [23]. The C3b-binding of SCRs 1–4 is important for the complement factor I (CFI) cofactor activity, accelerating the decay of C3bBb convertase [19,20].

CFH does not homodimerize or heterodimerize with CFHR proteins [31]. The absence of CFH in the blood leads to uncontrolled complement activation and a rapid consumption of C3 and CFB as demonstrated in $CFH^{-/-}$ mice [32].

4. Structure and functions of CFHR1 and CFHR3

Similar to CFH, CFHR1 is produced by hepatocytes and present in the plasma at equimolar concentrations compared to CFH ($2.1\ \mu M$) [10]. Contrary to CFH, its expression in retina, in RPE, and in mononuclear phagocytes is very low [15] (www.immgen.org [14])

CFHR1 is encoded by a gene in close proximity to the CFH gene. The protein contains five SCRs. SCRs 1–2 present low sequence identities with the glycoaminoglycan-binding SCR6–7 of CFH and SCRs 3–5 are highly similar to the CFH SCRs 18–20 that mediate C3b-binding and cell adhesion. The first two SCRs share a dimerization motif leading to the formation of homodimers and heterodimers with CFHR5 and CFHR2 [31]. CFHR1 does not contain the CFH SCRs 1–4, which are necessary for complement factor I cofactor activity, and therefore, lacks C3 convertase decay activity [33]. However, CFHR1 binds C3b and could inhibit the complement cascade by limiting the availability of C3b

and the formation of the C3 convertase [10]. Thus, contrary to CFH, CFHR1 cannot accelerate the decay of existing C3 convertase, but can nevertheless inhibit its formation. CFHR1 can also act downstream from CFH by inhibiting the C5 convertase as well as the assembly and membrane insertion of the MAC [34]. *In vitro*, CFHR1 inhibits complement-induced hemolysis and chemotaxis but not as efficiently as CFH [10]. As a consequence, CFHR1 competes with CFH cell surface binding and, lacking the CFI cofactor activity of CFH, is more permissive of MAC formation and the hemolysis of erythrocytes *in vitro* [31].

CFHR3 is present in plasma at 1–1.6 μM [10]. Similar to CFHR1, it is not significantly expressed in mononuclear phagocytes (www.immgen.org [14]). Its expression in retina and RPE is unknown. The protein contains five SCRs. Contrary to CFHR1, its SCR1–3 present high similarities with the glycoaminoglycan-binding SCR6–8 of the CFH and efficiently bind to glycoaminoglycans and C3b. CFHR3 SCRs 4–5 exhibit low similarity with the cell surface and C3b-binding SCRs 19–20 of CFH. CFHR3 does not contain the dimerization motif found in CFHR1 [31]. Similarly to CFHR1, CFHR3 inhibits complement-induced *in vitro* erythrocyte hemolysis, but does so less efficiently than CFH; it thus might act as a competitive antagonist of CFH [10].

5. Genetic studies of CFH and CFHR1 and CFHR3 in AMD

Family history is an important risk factor of AMD. First-degree relatives of patients with AMD are four times as likely to develop late AMD [35]. Linkage and polymorphism studies have identified several genetic CFH and CFHR alterations that are associated with AMD.

In 2005, the common single nucleotide polymorphism (SNP) rs1061170 of the CFH gene (minor allele frequency (MAF) of 0.3) was shown to be strongly associated with late AMD in Caucasian populations [4–7]. In Asian populations, rs1061170 is a rare polymorphism (MAF < 0.1) [36] and association studies therefore necessitate larger study groups. The association of rs1061170 with AMD could at first not be established in an underpowered study of Japanese patients [37] but was later demonstrated in a larger case-control study [38].

Interestingly, the Y402H polymorphism of CFH is not only associated with late AMD, but also strongly associated with early age-related maculopathy (ARM), which is characterized by large-sized soft Drusen and RPE pigmentation changes in the absence of marked degeneration or neovascularization [39–41]. Furthermore, the Y402H polymorphism of CFH is associated with the progression of Drusen size [41] and progression from ARM to AMD [40–42]. Large studies have shown that it is more strongly associated with geographic atrophy than with neovascular AMD [43,44].

Rs1061170 leads to the substitution of tyrosine 402 for histidine in SCR7 (Y402H) [4,5]. This substitution has been shown to induce differences in CFH's affinity to glycoaminoglycans [29], malondialdehyde [26], and to oxidize low-density lipoproteins (oxLDL) [45]. These changes in biological activity have all been proposed to be at the origin of the association of rs1061170 with AMD, but they are much debated. Genetically, it is not clear to what extent rs1061170 and the Y402H protein modification are directly responsible for the increase in AMD risk or whether they are markers of a haplotype that plays an important role in the increase in AMD risk. A detailed analysis of the CFH gene region in large case-control studies has suggested that several SNPs are associated more strongly with AMD and that no single polymorphism could account for the contribution of the CFH locus to disease susceptibility [5,46]. The strongest association with AMD susceptibility in one of these studies was actually found in a haplotype that does not affect the CFH protein sequence [46]. This observation would suggest that the associated haplotypes modulate AMD risk, not because they disrupt CFH protein function, but because they are important in regulating the expression of CFH, other nearby genes, or both [46]. On the other hand, other studies did not find any statistically significant association between haplotypes not containing the rs1061170 risk variant [41]. These results would suggest that the substitution of tyrosine 402 for histidine in SCR7 and its possible implication in CFH cell surface binding is indeed directly implicated in AMD pathogenesis.

Recently, high throughput sequencing in a larger case-control study has led to the discovery of a rare high-risk CFH haplotype containing an R1210C mutation in SCR20 [47]. Similar to Y402H, the R1210C mutation in SCR20 affects CFH surface binding to anionic surfaces and C3b [48], suggesting that altered CFH cell surface binding is implicated in AMD pathogenesis.

Analysis of the CFH gene also led to the discovery of a protective CFH polymorphism Rs800292 [5,41] that leads to the substitution of valine 62 for isoleucine in SCR2 (V62I). The protective CFH I62 form has been shown to increase C3b-binding and enhance CFI cofactor activity compared to CFH V62 [49]. Interestingly, and contrary to CFH Y402, the "risk" CFH V62 is associated only with late AMD [5,41], not with ARM [41].

Analysis of the CFH containing the 1q25-q31 locus also revealed a deletion of CFHR1 and CFHR3 in protective haplotypes [9,50,51]. This association has been shown to be independent of the CFH Y402H polymorphism [10]. CFHR1 and CFHR3 are believed to be partial CFH antagonists *in vitro* [10], but their presence in AMD-affected eyes and their interaction with CFH in patients remains speculative.

Polymorphisms of several other genes in the alternative complement pathway, including CFI [52], CFB [53], and C3 [54], have also been shown to be associated with AMD and are likely to affect the alternative complement cascade independently of CFH function.

6. Pathomechanistical considerations

Taken together, there is extensive and detailed genetic evidence for an association of AMD and polymorphisms of the CFH gene and the *CFHR1* and *CFHR3* genes, but also of other genes of the complement system. We are only beginning to understand how alterations in CFH function, or more generally, how the alternative complement system is implicated in cellular and molecular events

involved in ARM and AMD. Before we will be able to develop efficient complement-based strategies for treating AMD, we will have to find answers to important pathomechanistical questions: Is the alternative complement system systemically and/or locally activated in AMD patients? Is this activation the result of polymorphisms in components of the complement system, or does it reflect inflammatory and immune-mediated events in AMD patients? Is the CFH Y402H substitution a cause of complement activation in AMD? Is there a lack of CFH and complement inhibition in the eyes of AMD patients, and does it lead to complement-induced cell death, increased chemotaxis or problems in debris phagocytosis? Which cells are the targets of complement-mediated cell death or signaling? The past decade has produced a number of responses, but many questions remain unanswered.

Several lines of evidence suggest that AMD is associated with the activation of the complement system. Systemically, plasma complement components and activation fragments are elevated in AMD patients [55–57]. It is however not clear whether the systemic increase of activated complement components is the result of polymorphisms in AMD, the result of inflammatory and immune-mediated events in AMD patients, or both. Several studies have shown an increase of activated complement factors in the blood of patients not carrying the Y402H containing risk haplotype [55–57]. In some studies, but not in others [55], a further increase was associated with the Y402H polymorphism [56]. These conflicting results are difficult to interpret. Studying complement components in patients with a more detailed haplotype analysis might allow one in the future to determine the influence of the CFH Y402H polymorphism and other SNPs on complement activation in AMD patients. Recently, Heurich et al. demonstrated that combining the "risk" CFH 62V with the "risk" variants of C3 and FB increases complement activation *in vitro* six-fold, independently of the CFH Y402H polymorphism [58]. The increase of complement components and activation fragments in AMD patients might therefore be independent of the CFH Y402H polymorphism and due to other SNPs or inflammatory events in AMD.

In the eye, complement factor constituents, including CFH [5], are invariably found in the Bruch's membrane and on the RPE and choriocapillaries of AMD patients [59], but do not seem to be found in the photoreceptor cell layer or the inner retina [5,59]. The localization of these factors in soft Drusen is more variable and factor dependent [59,60]. The presence of other serum proteins, such as albumin, in the Bruch's membrane and the choriocapillaris of AMD patients might suggest that the deposits of activated complement proteins originate from the serum rather than a local activation of the cascade [59].

An elegant method for evaluating whether plasma or local polymorphic CFH is important in AMD development involved the study of liver transplant patients. Interestingly, AMD was associated with recipient CFH Y402H genotype but not with the donor's CFH Y402H genotype [61]. Furthermore, in liver transplant patients, AMD was not associated with systemic complement activity. These results suggest that polymorphic plasma CFH is not important for disease pathogenesis. These findings might suggest that local intraocular complement activity is of greater importance in AMD pathogenesis.

Local or systemic CFH concentration is another factor that might influence complement activation. Systemic CFH plasma concentrations increase with age [62,63]. In the case of AMD, studies have found slightly reduced [56], similar [57], or slightly higher levels of CFH [63] in patients compared to control. Locally, CFH deposits seem to be invariably stronger in AMD patients compared to controls [5,26,45,64].

In spite of extensive research, it remains difficult to pinpoint which of the CFH functions is altered in AMD and how polymorphisms affect CFH functions. The Y402H polymorphism in SCR7 [7] and the mutation in SCR20 [47] change CFH affinity with polyanionic surfaces [29,65], such as glycosaminoglycans. CFH is believed to protect cells by recognizing cell surface glycosaminoglycans, such as sialic acid and the glycosaminoglycan chains of proteoglycans and thus, inhibiting complement activation on the cell surfaces. It has been proposed that SCR7 has a particular affinity to polyanionic structures in the eye, while SCR20 is necessary for fixation in the kidney [66]. This might explain the importance of the Y402H polymorphism in AMD. However, the recent discovery of an association of SCR20 mutations in AMD patients [47] brings the significance of these findings into question. Furthermore, CFH immunoreactivity is invariably stronger in the choroid and in Drusen of AMD patients [5,26,45,64], not weaker as might have been expected if disease-associated CFH variants display a lower affinity to cell surfaces. Indeed, alterations in terms of affinity to glycosaminoglycans induced by the Y402H polymorphism are more complex and differ depending on the polyanionic structure. Clark et al. have showed that a SCR6–8 protein carrying the disease-associated tyrosine (Y) binds more strongly to certain heparan sulfates (HS) and dermatan sulfates (DS) [29]. The authors propose that the enhanced binding of factor H to developing Drusen bodies (which contain glycosaminoglycans [67]) could lead to reduced complement-mediated opsonization, giving rise to impaired phagocytosis and debris accumulation [29]. The notion of increased CFH binding in AMD fits the observation of increased CFH immunoreactivity in AMD donor tissues [5,26,64]. However, the observed increase in CFH-labelling has been shown to be independent of the CFH Y402H polymorphism [64].

Weismann et al. proposed that differences in malondialdehyde (MDA) affinity of CFH might be the link between the CFH Y402H polymorphism and AMD [26]. They reported that CFH SCR7 and SCR20 bind cellular debris via malondialdehyde (MDA), and that the "protective" CFH Y402 inhibits MDA-induced IL-8 transcription in RPE cells *in vivo* [26]. They also showed that the disease-associated CFH H402 markedly reduces CFH MDA binding biochemically, but the authors did not present evidence that this difference affects MDA-induced inflammatory mediators *in vivo* or *in vitro* [26]. The authors propose by extension of their *in vitro* results, that the decreased MDA affinity of CFH H402 increases an MDA-induced inflammatory reaction in AMD

patients. They show diffuse MDA staining in Drusen and Bruch's membranes of AMD subjects. However, the MDA adducts strongly co-localized with CFH, even in an AMD subject homozygous for the H402 SNP, which should have strongly reduced MDA affinity [26] and it is not clear how this observation supports the proposed mechanism.

Shaw et al. proposed that differences in oxLDL affinity in Y402H polymorphic CFH are implicated in AMD pathogenesis [45]. They show that CFH binds oxidized oxLDL and antagonizes oxLDL-induced inflammatory mediators in RPE and macrophages. The reduced affinity of the "risk" CFH H402 increased oxLDL-induced inflammatory mediators compared to CFH Y402 [45]. The authors also show that subretinal oxLDL induces choroidal neovascularization and conclude by extension that the decreased oxLDL binding capacity of the risk associated CFH H402 impacts the risk of AMD [45].

To evaluate the role of CFH *in vivo*, several groups have turned to transgenic animals: $CFH^{-/-}$ mice develop C3 accumulation at the RPE level and mild retinal dysfunction, but show no significant histological photoreceptor or RPE degeneration even at advanced age (24 months) [68]. They do however develop exaggerated laser-induced choroidal neovascularization [69]. The use of the $CFH^{-/-}$ mice to evaluate CFH function in AMD is further hampered by the fact that CFH deficiency results in systemic uncontrolled C3 activation and secondary C3 deficiency [32]. The $CFH^{-/-}$ mice (absent CFH and systemically low C3) therefore differ significantly from AMD patients that present increased C3a and CFH levels, which might explain the weak mouse phenotype. To more accurately study the influence of the Y402H polymorphism, Ufret-Vincenty et al. created a $CFH^{-/-}$ mouse that expresses a transgene containing the human SCR6–8 under an ApoE promoter [70]. The mice have normal systemic C3 levels and show signs of subretinal inflammation, but no differences were observed between the risk and protective SCR6–8 bearing proteins [70].

As described above, the deletion of *CFHR1* and *CFHR3* were found in a protective haplotype [9,50,51] independent of the CFH polymorphism [10]. *CFHR1* and *CFHR3* have been shown to be partial competitive CFH antagonists in an *in vitro* complement-induced hemolysis assay [10]. It is however unclear to what extent *CFHR1* and *CFHR3* are present in AMD lesions to compete with CFH in AMD patients.

7. Conclusions

The past decade of AMD research has produced abundant evidence that CFH and the alternative complement cascade play an important role in the pathogenesis of ARM and AMD. CFH is a complex protein that interacts with many ligands and has multiple functions. We are only beginning to understand CFH and how its modifications are involved in AMD pathogenesis. It is still unclear how AMD-associated CFH alterations contribute to inflammation or phagocytosis in AMD. Several convincing studies have shown how the risk associated CFH H402 biochemically affects the affinity to one of CFH's ligands and changes *in vitro* experimental results. However, it may be too soon to extrapolate these findings in complex *in vivo* interactions. Clinical trials of complement pathway inhibitors have so far not fulfilled their expectations. We need to better understand the implications of CFH in AMD pathogenesis to be able to design efficient complement-based therapies.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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