

## Translational Mini-Review Series on Complement Factor H: Genetics and disease associations of human complement factor H

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*Renal diseases associated with complement factor H: novel insights from humans and animals.* Clin Exp Immunol 2008; 151: doi:10.1111/j.1365-2249.2007.03574.x

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### Summary

Factor H is an abundant plasma glycoprotein that plays a critical role in the regulation of the complement system in plasma and in the protection of host cells and tissues from damage by complement activation. Several recent studies have described the association of genetic variations of the complement factor H gene (*CFH*) with atypical haemolytic uraemic syndrome (aHUS), age-related macular degeneration (AMD) and membranoproliferative glomerulonephritis (MPGN). This review summarizes our current knowledge of *CFH* genetics and examines the *CFH* genotype–phenotype correlations that are helping to understand the molecular basis underlying these renal and ocular pathologies.

**Keywords:** age-related macular degeneration (AMD), factor H, haemolytic–uraemic syndrome (HUS), membranoproliferative glomerulonephritis type II (MPGN2), RCA

### Complement activation and regulation

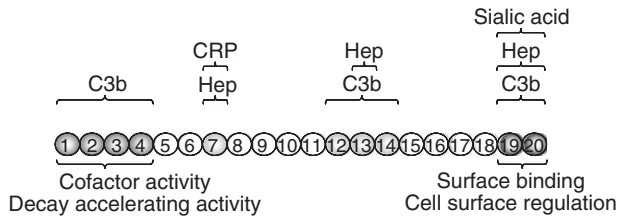
Complement is a major component of innate immunity, with crucial roles in microbial killing, apoptotic cell clearance and immune complex handling. Activation of complement by foreign surfaces (alternative pathway; AP), antibody (classical pathway; CP) or mannan (lectin pathway; LP) causes target opsonization, leucocyte recruitment and cell lysis. The critical steps in complement activation are the formation of unstable protease complexes, named C3-convertases (AP, C3bBb; CP/LP, C2aC4b) and the cleavage of C3 to generate C3b. Convertase-generated C3b can form more AP C3-convertase, providing exponential amplification to the initial activation. Binding of C3b to the C3-convertases generates the C5-convertases with the capacity to bind and cleave C5, initiating formation of the lytic membrane attack complex (MAC).

Nascent C3b binds indiscriminately to pathogens and adjacent host cells. To prevent damage to self and to avoid wasteful consumption of components, complement is under the control of multiple regulatory proteins that limit complement activation, either by inactivating C3b or C4b,

by dissociating the C3/C5 convertases or by inhibiting the MAC formation. In health, activation of C3 in the blood is kept at a low level and deposition of C3b and further activation of complement is limited to the surface of pathogens [1].

### Complement regulation by factor H

Factor H is a relatively abundant plasma protein that is essential to maintain complement homeostasis and to restrict the action of complement to activating surfaces. Factor H binds to C3b, accelerates the decay of the alternative pathway C3-convertase (C3bBb) and acts as a co-factor for the factor I-mediated proteolytic inactivation of C3b [2–4]. Factor H regulates complement both in fluid phase and on cellular surfaces. However, while factor H binds and inactivates C3b promptly in fluid phase, the inactivation of surface-bound C3b by factor H is dependent on the chemical composition of the surface to which C3b is bound. In the presence of polyanions such as sialic acids, glycosaminoglycans or sulphated polysaccharides (heparins), the affinity of factor H for surface-bound C3b increases as a consequence



**Fig. 1.** Functional domains in complement factor H. Factor H has three C3b-binding sites, short consensus repeats (SCR)1–4, SCR12–14 and SCR19–20, respectively [9–13]. Similarly, a total of three separate binding sites for heparin and sialic acid have been identified in SCR7, SCR13 and SCR19–20, respectively [14–17]. The critical sites for co-factor activity/decay accelerating activity and cell surface regulation at the N- and C-termini, respectively, are indicated. In addition to C3b and polyanion binding sites, there are other domains in factor H that have been shown to interact with plasma proteins or with microorganisms and that are interesting because of their potential relevance in pathology. In this regard, it has been shown that factor H binds to C-reactive protein (CRP) which may help to counteract and inhibit the CRP-dependent alternative pathway activation induced by damaged tissue [18,19]. The heparin- and CRP-binding sites in SCR7 are overlapping sites in which one substrate inhibits the binding of the others [20].

of the simultaneous recognition of both polyanionic molecules and bound C3b by the same factor H molecule [5–7].

### Factor H and factor H-related proteins

Factor H is a single polypeptide chain glycoprotein (155 kDa;  $\epsilon = 1.95 \text{ M}^{-1} \text{ cm}^{-1}$ ) composed of 20 repetitive units of 60 amino acids [8], named short consensus repeats (SCR) or complement control protein modules (CCP), arranged in a continuous fashion. The factor H molecule includes different interaction sites for C3b and polyanions which delineate distinct functional domains at the N- and C-termini (Fig. 1). The C3b binding site in SCR1–4 is the only site essential for the factor I co-factor activity of factor H. Similarly, the C3b/polyanions-binding site located within SCR19–20 is the most important site for preventing alternative pathway activation on host cells (reviewed in [21]). Factor H is produced constitutively by the liver [22,23] and is found in human plasma at concentrations of 116–562  $\mu\text{g/ml}$  [24]. Extrahepatic synthesis of factor H also occurs in a wide variety of cell types, such as retinal pigment epithelial cells, peripheral blood lymphocytes, myoblasts, rhabdomyosarcoma cells, fibroblasts, umbilical vein endothelial cells, glomerular mesangial cells, neurones, glia cells, etc. [25–27]. The extrahepatic production of factor H is interpreted as a mechanism to increase the local concentration, which could be advantageous for the protection of host cells from complement activation in sites of infection or inflammation.

In human plasma there are six proteins that are structurally related and cross-react immunochemically with factor H. FHL-1 is the product of the alternative splicing of the

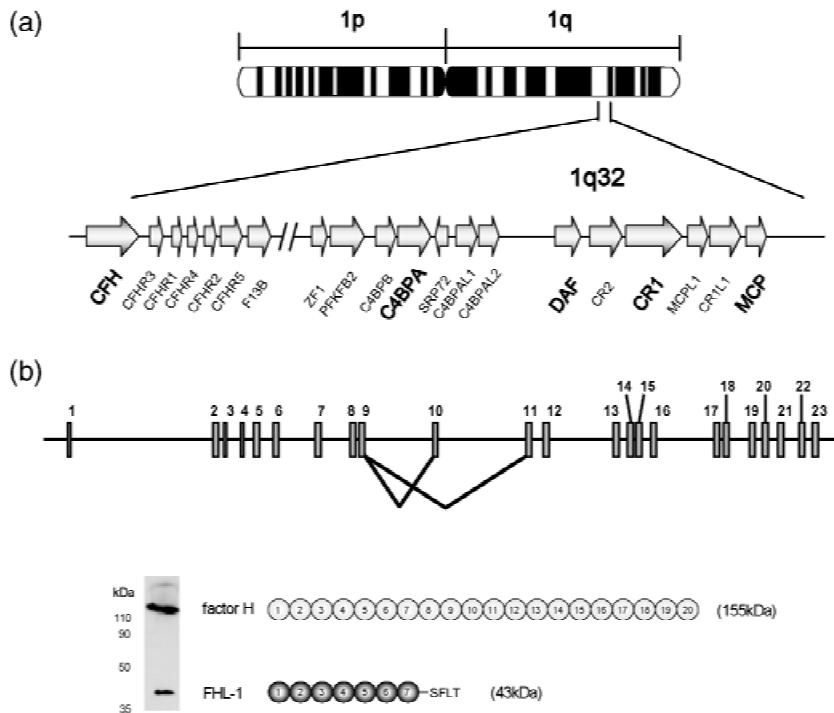
gene encoding factor H (*CFH*) [9,23,28–30]. In addition, five proteins related to factor H are encoded by the five genes *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* linked closely to *CFH* [31–34]. These proteins are also probably synthesized in the liver, but their concentrations are much lower than that of factor H. The functional properties of *CFHR1*, -2, -3, -4 and -5 are not defined fully. They are all composed of SCRs with different degrees of identity with SCRs in factor H [33–37].

### The *CFH* gene

*CFH* is a member of the regulator of complement activation (RCA) gene cluster on chromosome 1q32 (Fig. 2) [21,39]. *CFH* comprises 23 exons and spans over 94 kb of genomic DNA [37,40]. The first exon encodes the 5' untranslated region of the mRNA and the N-terminal 18 amino acids that organize the signal peptide. Each SCR in factor H is encoded by a single exon except for SCR2, that is encoded by exons 3 and 4. Exon 10 does not contribute to the factor H transcript. It is used exclusively in the alternative transcript that codes for the FHL-1 molecule. Exon 10 encodes the last four amino acids (Ser-Phe-Leu-Thr) and the 3' untranslated region of FHL-1 [28].

The single nucleotide polymorphism (SNP) database at the National Center for Biotechnology Information (NCBI) lists a total of 569 SNPs in the human *CFH* gene region (locus ID: 3075). Of these, roughly a dozen are located in the *CFH* proximal promoter region or result in an amino acid substitution in the *CFH* coding sequence. The potential functional implications of some of these *CFH* polymorphisms will be discussed later in the context of the *CFH*-disease associations. Of interest, however, is the observation that there is very strong linkage disequilibrium (LD) in the *CFH* genomic region, which reduces the genetic variability within this region to the combination of four SNP-haplotype blocks spanning the *CFH* and *CFHR1–5* genes [41].

Levels of factor H in human plasma vary widely (116–562  $\mu\text{g/ml}$ ) in the population. This variation is not a consequence of *CFH* null alleles, which are extremely rare, but the result of the combined effect of genetic and environmental factors. Using variance-component methods [42] it was determined that factor H plasma levels show an age-dependent increase and are decreased in smokers [24]. Most important, these studies showed that 63% of the variation in plasma levels of factor H is determined genetically [heritability ( $h^2$ ) = 0.63  $\pm$  0.07;  $P < 0.0001$ ]. A genome-wide screen in order to identify genes regulating the factor H trait provided suggestive evidence of linkage to three genomic regions (1q32, 2p21–24 and 15q22–24) [24] and more recently we have obtained evidence that demonstrates the existence of low expression *CFH* alleles [43]. It is therefore likely that genetic variations in both *cis*- and *trans*-regulatory elements contribute to the variation in the levels of expression of factor H.



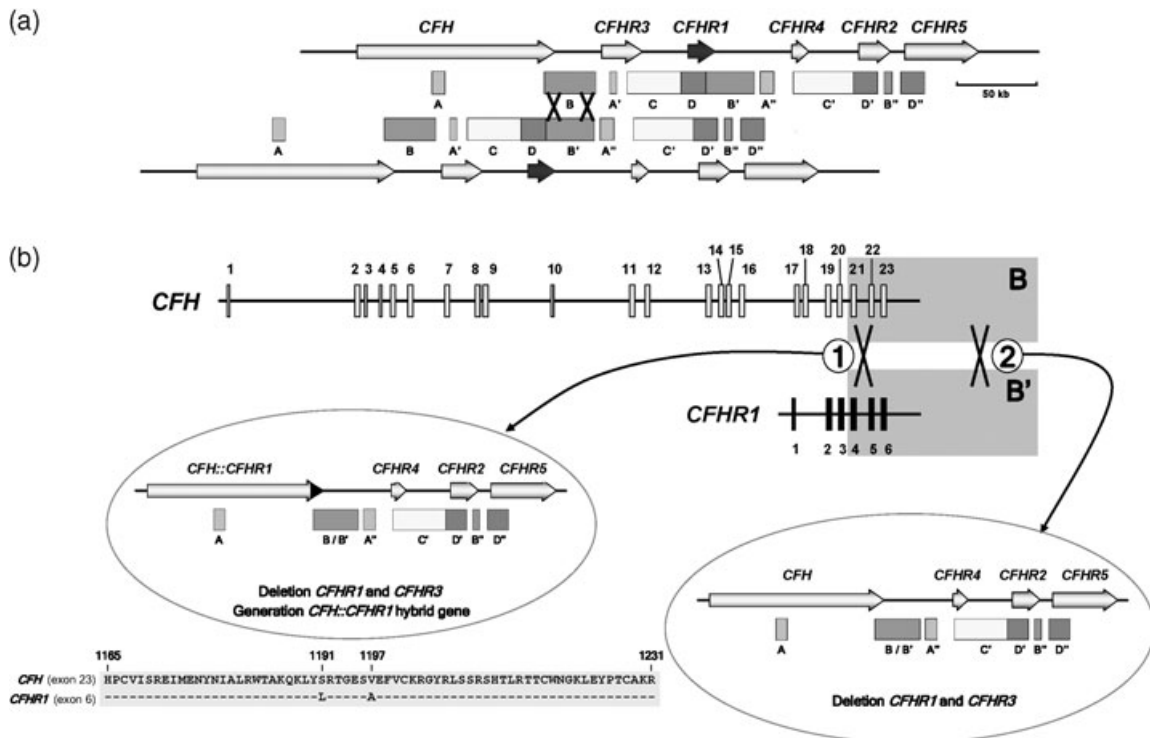
**Fig. 2.** Chromosomal location and structure of the factor H gene. (a) The human regulator of complement activation (RCA) gene cluster in 1q32. The human RCA gene cluster spans a total of 21.45 cM and includes more than 60 genes of which 15 are complement-related genes. All of the complement-related genes are arranged in tandem within two groups. The two groupings are a telomeric 900 kb-long DNA segment which contains the *CABPB*, *CABPA*, *CABPAL1*, *CABPAL2*, *DAF*, *CR2*, *CR1*, *MCPL1*, *CRIL1* and *MCP* genes and a centromeric 650 kb-long DNA segment that contains *CFH*, *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2* and *CFHR5*, as well as the gene coding for the B subunit of the coagulation factor XIII, *F13B*. These two gene groups are separated by 14.59 cM, a large amount of DNA-containing genes that are unrelated to complement and that have very diverse functions [38]. It is generally accepted that these complement regulatory genes share a common ancestor from which they originated by multiple events of gene duplication. (b) Structure of *CFH*, showing a diagram of the 23 exons and the two alternative splicing products of the *CFH* gene. Exon 10 does not contribute to the factor H transcript but it is utilized for the FHL-1 molecule. The figure also shows a Western blot, using a monoclonal antibody (35H9) that recognizes both factor H and FHL-1, to illustrate the relative amounts of these proteins in normal human plasma.

### Rearrangements in the *CFH* genomic region

In close proximity to the *CFH* gene there are the genes *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2* and *CFHR5* encoding the five factor H-related proteins (Fig. 2). Sequence analyses of the *CFH*–*CFHR1*–5 gene region demonstrated the existence of a number of large genomic duplications including different exons of the *CFH* and *CFHR1*–5 genes (Fig. 3a). These duplications range in size from 1.2 to 38 kb and present a pairwise nucleotide identity from 85% to 97% [37]. Low-copy repeats, or segmental duplications, such as these in the *CFH*–*CFHR1*–5 gene region, are highly dynamic regions in the genome and a potential source of additional genetic variation in the *CFH* and *CFHR1*–5 genes through mechanisms of gene conversion and non-homologous recombination.

Several examples of gene conversion events between exon 23 of *CFH* and the homologous exon 6 of *CFHR1* have been documented recently [37,45]. Similarly, there is also robust evidence of major rearrangements in the *CFH*–*CFHR1*–5 gene region that result in the deletion of the *CFHR1* and

*CFHR3* genes [41,46] and, occasionally, also in the generation of *CFH*::*CFHR1* hybrid genes [44] (see Fig. 3 for details). These *CFH*–*CFHR1*–5 rearrangements can be identified easily by MLPA (multiplex ligation-dependent probe amplification) technologies [44] or by Western blot in the case of homozygote carriers [46]. Chromosomes carrying both the deletion of the *CFHR1* and *CFHR3* genes and a *CFH*::*CFHR1* hybrid gene are rare and associated specifically with aHUS [44]. In contrast, the deletion of the *CFHR1* and *CFHR3* genes (without rearrangements in *CFH*) is a common genetic polymorphism included in a single extended *CFH* haplotype that associates with both lower risk to age-related macular degeneration (AMD) [41,47] and increased risk to aHUS [46]. Deletion of the *CFHR1* and *CFHR3* genes is not a recurrent phenomenon, but the result of a single rearrangement event that became fixed in the human population a long time ago [47]. Additional rearrangements are likely to occur in the *CFH*–*CFHR1*–5 region. For instance, there is one involving an unequal cross-over between homologous regions in the 3' end of the *CFHR3* and *CFHR4* that specifically removes the *CFHR1* and *CFHR4* genes [48].



**Fig. 3.** The complement factor H gene (*CFH*)–*CFHR1*–5 gene subregion of the regulator of complement activation (RCA) gene cluster. (a) Genomic organization of the *CFH* and *CFHR1*–5 genes and location of low copy number repeats. Arrows represent the genes with their names above. The boxes underneath indicate the sequence repeats. Low copy repeats are named with the same letter (i.e. A, A', A''). A grey colour-code is used to identify the different repeats. The figure shows two chromosomes aligned by the B and B' repeats to illustrate the *CFH*–*CFHR1* genomic rearrangement that occurred through non-homologous recombination between a 23 kb-long repeat region in the 3' end of the *CFH* and *CFHR1* genes, labelled B and B', respectively. (b) Deletion of the *CFHR1* and *CFHR3* genes and generation of a *CFH::CFHR1* hybrid gene. This rearrangement involves non-homologous recombination between the B and B' homologous regions downstream of the *CFH* and *CFHR1* genes and results in no sequence-modification of the *CFH* gene. A second rearrangement (labelled 1) is much less frequent and found associated exclusively with atypical haemolytic uraemic syndrome (aHUS). Non-homologous cross-over in this case occurred between the B and B' homologous regions in intron 21 of *CFH* and intron 4 of *CFHR1* and results in generation of a hybrid *CFH::CFHR1* gene. The hybrid gene consists of the first 21 exons of *CFH* [encoding SCRs 1–18 of *CFH*] and the last two exons of *CFHR1* (encoding SCR4 and 5 of *CFHR1*) [44]. Amino acid sequences of *CFH* exon 23 and *CFHR1* exon 6 are aligned to illustrate two amino acid differences between them (S1191L/V1197A) that are present in the protein product of the hybrid gene. These amino acid changes in SCR20 are associated with aHUS and are identical to those present in the *CFH* mutant protein, also associated with aHUS, that generates by gene conversion [45].

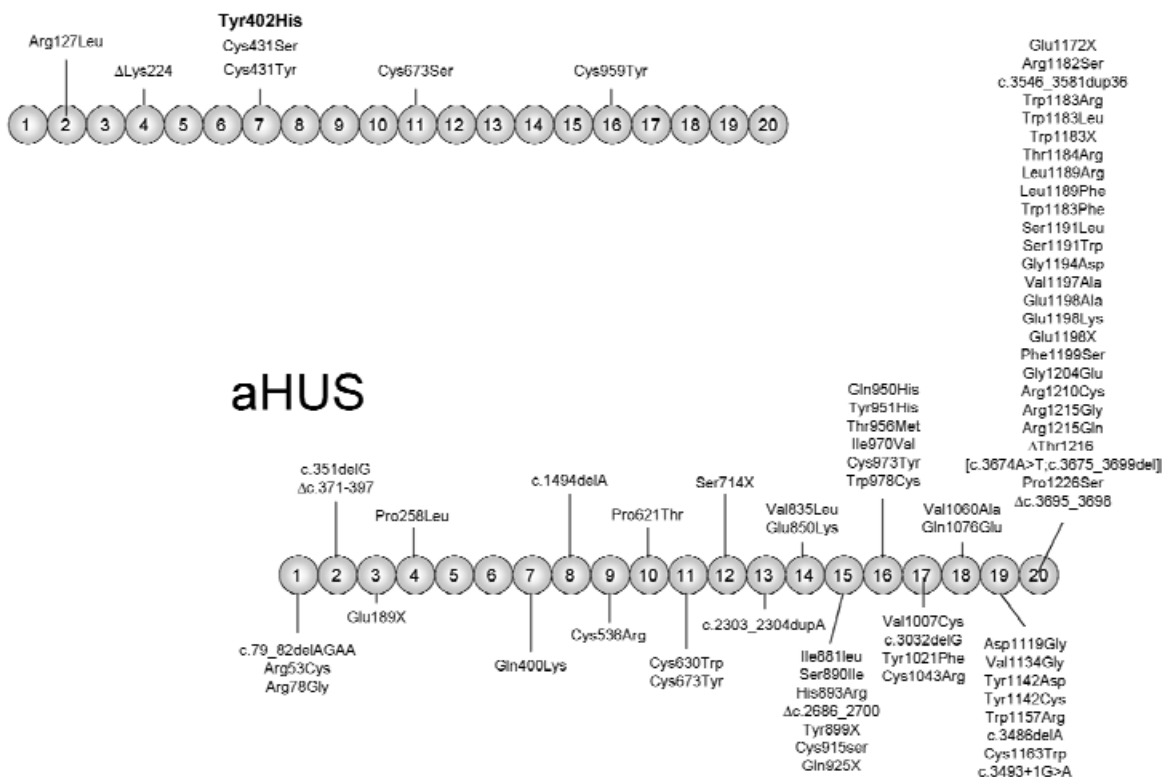
**Factor H disease associations**

Several reports in recent years have established that membranoproliferative glomerulonephritis type II (MPGN2) [49–52], atypical haemolytic uraemic syndrome (aHUS) [37,53–55] and AMD [27,56–58] are associated with mutations or polymorphisms in the *CFH* gene. The data available support the hypothesis that AP dysregulation is a unifying pathogenetic feature of these diverse conditions. They also illustrate a remarkable genotype–phenotype correlation in which distinct genetic variations at *CFH* predispose specifically to aHUS, AMD or MPGN2. As described below, functional characterization of these *CFH* genetic variations is helping to understand the molecular basis underlying these pathologies.

**Membranoproliferative glomerulonephritis**

Membranoproliferative glomerulonephritis (MPGN) is an uncommon cause of chronic nephritis characterized by proliferation of mesangial and endothelial cells and by thickening of the peripheral capillary walls (due to subendothelial immune and/or intramembranous dense deposits) that on light microscopy present a double-contour appearance. MPGN may be secondary to autoimmune diseases, chronic infections and malignancies or idiopathic, which accounts for approximately 5% of primary renal causes of nephrotic syndrome and affects predominantly children and young adults (6–30 years). Three distinct types of primary (idiopathic) MPGN have been described based on immunofluorescence staining, ultrastructural appearance and

## MPGN



**Fig. 4.** Complement factor H gene (*CFH*) mutations in MPGN and atypical haemolytic uraemic syndrome (aHUS) patients. The figure shows a diagram of the structure of human factor H with the 20 SCRs. The location of the missense mutations characterized thus far in membranoproliferative glomerulonephritis (MPGN) and aHUS patients is indicated. The position of the Tyr402His polymorphisms associated strongly with predisposition to AMD is highlighted in bold. Note that mutations associated with aHUS are clustered in the C-terminus, the region of factor H that is critical for the control of C3b deposited on cell surfaces.

complement profiles. The light microscopy features and clinical presentation are similar among the three types. Hypocomplementaemia is a characteristic finding with all types of MPGN, although the three types present different mechanisms of complement activation. Types I (MPGN1) and III (MPGN3) are variants of immune-complex-mediated disease, whereas type II (MPGN2) has no known association with immune complexes [59].

MPGN2 is very rare. Its morphological hallmark is the presence of dense deposits within the glomerular basement membrane (GBM), as resolved by electron microscopy. The chemical composition of these dense deposits is unknown. Notably, immunoglobulin (IgG) is absent from them and other regions of the glomerulus, which excludes a role for immune complexes in dense deposits formation. MPGN2 is associated with complement abnormalities that lead to intense deposition of C3c in GBM deposits and persistent reduction of C3 serum levels. Among the different factors associated with these complement abnormalities are factor H deficiencies due to mutations in the *CFH* gene. Approximately half a dozen MPGN patients are described in the

literature in which the deficiency of factor H, both heterozygous and homozygous, has been associated with the development of the disease. In all but one of these MPGN cases the factor H deficiency is caused by mutations in *CFH* that result in truncations or amino acid substitutions that impair secretion of factor H into the circulation [49,60,61] (Fig. 4). The exception is  $\Delta$ K224, a *CFH* mutation that results in the deletion of a lysine residue at position 224 [51]. K224 is located in SCR4 within the complement regulatory region of factor H (Fig. 1). Consistent with the location of the mutation, functional studies of factor H  $\Delta$ K224 have shown that binding to C3b is weak and that both factor I-mediated C3b co-factor activity and AP C3-convertase decay-accelerating activity of factor H  $\Delta$ K224 are reduced markedly. In contrast, and as expected from an intact C-terminal domain, the mutant factor H  $\Delta$ K224 protein shows normal binding to heparin, C3d and human umbilical vein endothelial cells [51]. The co-existence of a functionally inactive factor H (homozygous  $\Delta$ K224 mutation), or a factor H deficiency, with a complement activator such as C3NeF, probably exacerbates a situation of chronic complement activation and

results in the complete consumption of C3 in plasma that characterizes MPGN patients [51].

In addition to mutations in *CFH*, deficiencies of factor H due to inhibitory autoantibodies have also been reported in some MPGN patients, which also lead to accumulation of the AP C3-convertase, chronic C3 consumption and hypocomplementaemia [62].

Altogether, these genetic and functional data illustrate that those alterations that decrease factor H in plasma, or eliminate its complement regulatory activity, lead to unrestricted activation of the AP of complement, causing damage to glomerular cells and deposition of complement product in the GBM. The severe dysregulation of the AP of complement activation observed in MPGN2 is consistent with animal data that present this renal phenotype. In the pig, factor H deficiency results in a progressive glomerulonephritis, similar to human MPGN2, which leads to renal failure [63]. Similarly, the factor H knock-out mice develop spontaneously a glomerulonephritis that also resembles human MPGN2 [64]. These factor H-deficient animals have been very useful to demonstrate that the uncontrolled activation of C3 in plasma that results from the lack of factor H is essential for the development of MPGN2 [64]. Further studies are, however, necessary to unravel the precise molecular events that end up in MPGN2.

Although the majority of patients with MPGN2 do not have disease-causing mutations in *CFH*, some common alleles of *CFH* (and also of *CFHR5*) have been found to be increased significantly among these patients, supporting further the critical role of genetic variations in the *CFH* genomic region in the pathogenesis of MPGN2. One of these polymorphisms conferring predisposition to MPGN2 is the His402 allele of *CFH* [65,66], a major predisposing factor to AMD (see below).

### Haemolytic uraemic syndrome (HUS)

HUS is characterized by thrombocytopenia, Coomb's test negative microangiopathic haemolytic anaemia and acute renal failure. The typical form of HUS follows a diarrhoeal prodrome and is associated with 0157:H7 *Escherichia coli* infections. However, 5–10% of HUS patients lack an association with infection. This atypical form of HUS (aHUS) occurs in both adults and in very young children and has the poorest long-term prognosis. Recurrences in aHUS are common with a mortality rate that approaches 30%. aHUS has an incidence of about 2/10<sup>6</sup> per year and a prevalence of 1/10<sup>5</sup> children in the whole of the European Union.

Endothelial cell injury appears to be the primary event in the pathogenesis of HUS. The microvascular lesion of HUS consists of vessel wall thickening with endothelial swelling and detachment from the basement membrane. The endothelial damage triggers a cascade of events that result in the formation of platelet-fibrin hyaline microthrombi that occlude arterioles and capillaries. A hallmark of HUS is the

presence of schistocytes (fragmented cells) that generate as the red blood cells traverse these partially occluded microvessels [67].

aHUS is associated with mutations or polymorphisms in the genes encoding the complement regulatory proteins factor H (*CFH*) [37,44,53–55,68,69], membrane co-factor protein (*MCP*) [70–73] and factor I (*CFI*) [74,75] and with mutations in the complement activating components factor B (*CFB*) [76] and C3 genes [77]. Importantly, mutations in the complement regulators factor H, MCP and factor I are loss-of-function mutations [73,74,78] while mutations in the complement activator factor B are gain-of-function mutations [76]. These data establish unequivocally the critical role of complement AP dysregulation in the pathogenesis of aHUS and illustrate that complement dysregulation may result from either a defect in the regulatory proteins or an abnormally increased activity of the alternative complement pathway activators.

Missense mutations in the C-terminal region of factor H are the most prevalent genetic alterations among aHUS patients (Fig. 4). In a significant number of patients mutations in the C-terminal region of factor H are the result of gene conversion events between exon 23 of *CFH* and the homologous exon 6 of *CFHR1* [45] or of genomic rearrangements creating *CFH::CFHR1* hybrid genes [44]. An updated record of all mutations associated with aHUS can be found at the FH-HUS database (<http://www.fh-hus.org/>).

In contrast with *CFH* mutations associated with MPGN2, *CFH* mutations associated with aHUS rarely result in hypocomplementaemia or decreased factor H plasma levels. Most aHUS-associated factor H mutant proteins express normally and present normal co-factor activity for the factor I-mediated proteolytic inactivation of C3b in plasma [37,78]. As indicated, aHUS-associated *CFH* mutations cluster in the C-terminus of the protein, a region that is critical to control activation of complement on cell surfaces. Consistent with this location, carriers of these *CFH* mutations express factor H molecules that present normal regulatory activity in plasma but a limited capacity to protect cells from complement lysis [69,78,79]. These findings fit well with the identification of aHUS-associated loss-of-function mutations in *MCP* and *CFI* for the reason that the *MCP* and factor I mutations also lead to decreased protection of host cells from complement lysis without affecting significantly complement homeostasis in plasma [80]. The combination of both an active complement system in plasma and a defective protection of cellular surfaces portrays aHUS as a situation of 'autolesion' caused by the uncontrolled activation of complement on cell surfaces. By decreasing concentrations of factor H or factor I in plasma, or MCP on cell surfaces, aHUS-associated mutations predispose to disease. In a situation that triggers complement activation, deposition and amplification of C3b on the microvasculature cellular surfaces cannot be controlled and results in tissue damage and destruction. This is clearly distinct from the lack of complement regula-

tion in plasma, leading to complete C3 consumption and severe hypocomplementaemia, that characterizes MPGN2 patients with factor H deficiency due to mutations in *CFH*.

While mutations at the C-terminus of factor H are distinctive of aHUS, there are some aHUS patients with partial factor H deficiency due to mutations in the *CFH* gene [81–84]. These individuals develop aHUS and not MPGN2 mainly because partial factor H deficiencies, like mutations in SCR19–20, affect primarily the control of complement activation on cellular surfaces [85]. In addition, genetic and environmental factors may provide a ‘context’ that influences the pathological outcome for some of these factor H deficiencies. Thus, concurrence of factor H deficiencies with other mutations that decrease protection to host cells have been described in aHUS [37,78], whereas the coincidence of factor H deficiencies with strong complement activators such as C3NeF may be critical in MPGN2 [51].

Mutations in *CFH*, *MCP*, *CFI*, *CFB* and *C3* reveal the molecular defect in approximately 50% of the aHUS patients. To identify additional aHUS susceptibility factors, the complement regulator genes have been analysed further in genetic association studies [53,70]. These and subsequent replication studies [66,71,86] unravelled two relatively frequent *CFH* and *MCP* alleles (*CFH-H3* and *MCPggaac* haplotypes) that were significantly more frequent in aHUS patients (either with or without *CFH*, *MCP*, *CFI* or *CFB* mutations) than in controls. Moreover, in a significant number of aHUS families where *CFH*, *MCP*, *CFI* or *CFB* mutations segregated with the phenotype aHUS, it could be shown that the proband had inherited the allele carrying the mutation from one parent and an allele carrying the disease-associated *CFH* and/or *MCP* haplotype from the other parent. Most interestingly, the healthy *CFH*, *MCP*, *CFI* or *CFB* mutation carriers in these families did not inherit the aHUS-associated *CFH* and *MCP* polymorphisms [53,76,87].

Both *CFH-H3* and *MCPggaac* haplotypes include SNPs located in the promoter region of *CFH* and *MCP* that have potential functional implications in the expression of factor H and MCP [53,70]. Although additional studies are needed to fully characterize functionally these *CFH* and *MCP* haplotypes, the association of *CFH-H3* and *MCPggaac* with aHUS is extremely important because it indicates that common variations at the *CFH* and *MCP* genes predispose to aHUS in the absence of mutations in *CFH*, *MCP*, *CFI* or *CFB* and that even in carriers of mutations in these genes, these *CFH* and *MCP* variations may be needed for full manifestation of the disease. In fact, it is now well established that concurrence of different susceptibility alleles greatly influences predisposition to aHUS and provides an explanation for the incomplete penetrance of aHUS (close to 50%) in carriers of mutations in *CFH*, *MCP*, *CFI* and *CFB* [70,76,87]. In the Spanish aHUS cohort ( $n = 98$  unrelated patients), seven patients (7%) carry more than one mutation in the complement genes (*CFH*, *MCP*, *CFI* and *CFB*). In addition, in the 28 patients with mutations in *CFH*, *MCP*, *CFI* and

*CFB*, the allele frequencies of *CFH-H3* and *MCPggaac* haplotypes is increased from 0.19 to 0.34 [controls *versus* aHUS;  $P = 0.012$ ; odds ratio (OR), 95% confidence interval (CI) = 2.27 (1.21–4.27)] and from 0.29 to 0.45 [controls *versus* aHUS;  $P = 0.027$ ; OR, 95% CI = 2.77 (1.54–4.95)], respectively (Goicoechea de Jorge *et al.*, unpublished).

Recently, two additional polymorphisms in the *CFH* genomic region have been reported to influence predisposition to aHUS. The *CFH-H1* haplotype was found to be associated with lower risk of aHUS [66]. Similarly, homozygosity for the deletion of the *CFHR1* and *CFHR3* genes associated strongly with increased risk of aHUS [46]. Moreover, it has been shown that approximately 10% of aHUS patients, mostly children, present auto-antibodies to factor H and that these antibodies have functional consequences similar to those caused by the mutations in the C-terminal region of factor H [88,89]. Interestingly, individuals presenting anti-factor H antibodies are, with very few exceptions, homozygous for the deletion of the *CFHR1* and *CFHR3* genes, which make unclear whether the deletion of these genes and the presence of auto-antibodies are independent risk factors for aHUS [48,90,91].

In conclusion, genetic and functional analyses have established that aHUS involves alternative complement dysregulation and probably develops as a consequence of defective protection of cellular surfaces from complement activation due to an improper function of complement regulatory proteins. Multiple hits, involving plasma and membrane-associated complement regulatory proteins, as well as complement activators, are probably required to cause dysregulation and impair protection to host tissues significantly. Environmental factors that activate complement probably modulate genetic predisposition and are also very important in aHUS. Infection, immunosuppressive drugs, cancer therapies, oral contraceptives, pregnancy or childbirth are important factors that trigger aHUS in a significant number of patients. In carriers of multiple strong aHUS risk factors the contribution of the environment is probably minor. On the other hand, strong environmental factors may compensate for low genetic predisposition, which perhaps helps to explain the severe or fatal outcome of a small percentage of individuals with the more common diarrhoea-associated typical HUS.

## AMD

AMD is one of the most common causes of visual disability in the elderly in developed countries. The hallmark of early-stage disease is the development of drusen, lipoproteinaceous deposits localized between the retinal pigment epithelium (RPE) and Bruch’s membrane. Later, an extensive atrophy of the RPE and overlying photoreceptor cells (geographical atrophy; GA) or aberrant choroidal angiogenesis is observed. This choroidal neovascularization (CNV) under the macular area is the leading cause for blindness. Although the pathogenesis of AMD is still unclear, it has

been proposed that inflammatory response play an important role in the development of AMD [92].

AMD is a multi-factorial disease, influenced by age, ethnicity and a combination of environmental and genetic risk factors. Genetic predisposition in AMD has been suggested based on familial segregation and twin studies, involving several candidate genes such as ABCA4, APOE, FBLN5, ELOVL4 and TLR4. However, the individual contribution of these genes to overall AMD prevalence appears relatively minor [93]. Two major AMD susceptibility loci (1q31, *CFH*, and 10q26, *LOC387715/HTRA1*) that contribute independently to AMD disease risk have been identified recently by candidate region linkage studies and whole genome association analyses [27,56–58,94].

The most studied SNP at the *CFH* locus is rs1061170, which causes a Tyr402His amino acid substitution in complement factor H. Several independent studies have shown that the allele 402His confers a significantly increased risk to AMD in many different populations with an OR between 2.1 and 7.4 [27,56–58]. Interestingly, the frequency of the 402His allele varies greatly between populations, which may contribute to the observed differences in the incidence of AMD among different ethnic groups [47].

The Tyr402His polymorphism lies in the SCR7 of factor H, within the cluster of positively charged amino acids implicated in the binding of heparin, C-reactive protein (CRP) and M protein [20] (Fig. 1). Structural studies have shown that the substitution occurs towards the centre of SCR7 and that the 3D structures of both allotypes are otherwise identical [95]. *In vitro* functional studies with factor H recombinant fragments indicate that the substitution of Tyr for His at position 402 alters the binding specificity of SCR7 for different glycosaminoglycans [96,97] and decreases its binding to retinal pigment epithelial cells [98], although the physiological relevance of these observations is still unclear. It has also been reported that the Tyr402His polymorphism influences the binding of factor H to C-reactive protein [98–100], but the difficulties in replicating these data question the implication of the Tyr402His polymorphism in C-reactive protein binding [101,102].

Within the *CFH* gene, downstream of the SNP-haplotype block including the Tyr402His polymorphism, there is a second SNP LD group containing three polymorphisms, a synonymous SNP in exon 11 (rs2274700) and two intronic SNPs (rs1410996 and rs7535263), showing a stronger association with disease susceptibility than the Tyr402His variant [103,104]. Although these polymorphisms and the His402 variant form part of an extended *CFH* haplotype, they were described as independent predisposition variants that may be important in regulating the expression of *CFH*, or other nearby complement genes or both [103].

In addition to these *CFH* variants conferring increased risk to AMD, two common extended haplotypes in *CFH* gene [27,41,104] and two common SNPs in complement factor B [105] have been described associated with lower risk to AMD.

*CFH* haplotype H2, decreased markedly in AMD [27], is also decreased in MPGN2 and aHUS [66] (Fig. 5). *CFH* haplotype H2 carries the Ile62 factor H variant within the C3b binding site in the N-terminal region (SCR1–4) that is essential for the factor I-mediated co-factor and decay-accelerating activities of factor H (Fig. 1). Substitution of Val for Ile at position 62 may increase the factor H regulatory activity [106] and thus confer lower risk to AMD, MPGN2 and aHUS by reducing AP activation.

*CFH* haplotype H4 is also associated with decreased risk of AMD [41,66], but not to MPGN2 or aHUS [66] (Fig. 5). Interestingly, this *CFH* haplotype is also unique because it carries a deletion of the *CFHR1* and *CFHR3* genes [41]. Although it has been indicated that *CFHR1* and *CFHR3* proteins have the potential to compete with factor H for C3b binding [41], the potential benefit of the absence of *CFHR1* and *CFHR3* proteins is puzzling, in particular because it has been reported recently that the deletion of *CFHR1* and *CFHR3* genes is associated with increased risk to aHUS [46].

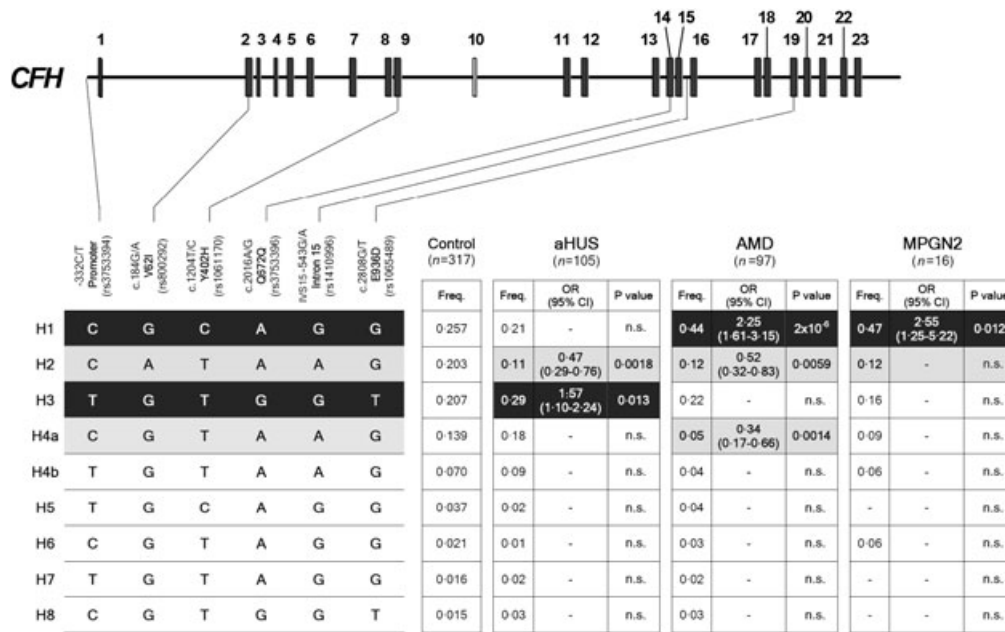
Two factor B polymorphisms were identified that were protective for AMD [105], one of which (32Q) had been reported previously to reduce the haemolytic activity of factor B [107] and the other, in the signal peptide, was suggested to modulate secretion of factor B. The observed protection in each case was ascribed to a reduced activity of the complement alternative pathway.

The identification of *CFH* as a major susceptibility locus for AMD and the characterization of multiple genetic variants in the *CFH*–*CFHR1*–5 and *CFB* genomic regions conferring risk or protection to AMD indicate that the complement system plays a significant role in AMD pathogenesis. Further studies are, however, needed to determine the functional consequences of the *CFH* variants associated with AMD and to identify the molecular events influenced by the complement system in the pathogenesis of AMD. In the meantime, definition of the AMD at-risk or protective factors associated with the *CFH*, *LOC387715/HTRA1* and *CFB* genes has allowed the development of risk models for AMD [87,94,104] that should be very helpful to delineate the individual risk to develop AMD, facilitating the implementation of preventive therapeutics.

### ***CFH* genotype–phenotype correlations**

AMD [27,56–58], aHUS [37,53–55] and MPGN2 [49,50,52,103] are distinct pathological entities that are all associated with mutations and polymorphisms in the gene (*CFH*), which support the hypothesis that AP dysregulation is a unifying pathogenic feature of these diverse conditions. However, there are differences in the *CFH* genetic variants conferring risk or protection to one or other disease, indicating the existence of a peculiar genotype–phenotype relationship. AMD and MPGN2 share pathological similarities with accumulation of complement-containing debris within the eye and kidney, respectively. Indeed, AMD-like





**Fig. 5.** Complement factor H gene (*CFH*) haplotypes and their association with disease. Schematic illustration of the *CFH* exon structure showing the location of the six single nucleotide polymorphisms (SNPs) included in these studies. These SNPs represent a minimal informative set for genetic variation within the *CFH* gene. Haplotype frequencies in the control and patient cohorts were estimated using the expectation maximization (EM) algorithm implemented by the SNPStats software (available on-line at: <http://bioinfo.iconcolgia.net/SNPstats>). *CFH* haplotypes with a frequency > 1% are shown. The frequency of each *CFH* haplotype was compared between the controls and the atypical haemolytic uraemic syndrome, age-related macular degeneration and membranoproliferative glomerulonephritis type II cohorts and the *P*-values and the odds ratios (OR) were calculated. Risk haplotypes are shaded black, while protective haplotypes are shaded in grey. *P*-values were derived using the two-sided Fisher's exact test. OR and 95% confidence intervals are shown. The nucleotide and amino acid numbering are referred to the translation start site (A in ATG is +1; Met is +1) as recommended by the Human Genome Variation Society. This figure is an updated version of that published in Pickering *et al.* [66].

pathology is well-recognized in patients with MPGN2 [108]. The hallmark of AMD is drusen, complement-containing material that accumulates beneath the retinal pigmented epithelium [92], while in MPGN2 accumulation of C3 and electron-dense material is seen along the GBM [59]. In contrast to these 'debris-associated' conditions, aHUS is characterized by renal endothelial injury and thrombosis (thrombotic microangiopathy) resulting in haemolytic anaemia, thrombocytopenia and renal failure.

Consistent with these pathological differences, we have discussed earlier in this review that the complete factor H deficiency in humans, pigs and mice is associated with MPGN2, while aHUS-associated *CFH* mutations cluster within the carboxy-terminal SCR of the protein. In addition, *CFH* association data derived from a comparative genetic analysis, using a minimal set of informative *CFH* SNPs, in subjects with aHUS, AMD and MPGN2 from a single population showed no overlapping between *CFH* at-risk polymorphisms for aHUS and AMD (or MPGN2) [66] (Fig. 5). Recently, this *CFH* genotype-phenotype correlation has been established formally in a murine model. Factor H-deficient mice (*Cfh*<sup>-/-</sup>) develops MPGN2 as a consequence of the massive activation of C3 [64]. These mice present very low levels of C3 and complement activities in plasma.

Introduction into *Cfh*<sup>-/-</sup> mice of a transgenic factor H molecule (FHA<sub>16-20</sub>) that mimics the human aHUS-associated mutations restored the C3 levels and the complement activity in the plasma of these factor H-deficient animals. As a result, *Cfh*<sup>-/-</sup>FHA<sub>16-20</sub> animals switch their disease phenotype from MPGN2 to aHUS [66]. These data validate the previous hypothesis [37,78], establishing definitively that the combination of active complement in plasma with a decreased protection of cell surfaces leads to aHUS.

The challenge now is to understand the functional consequences of all the genetic variations in the complement genes associated with high and low risk to aHUS, MPGN2 and AMD and to determine how they influence the complex interplay of regulators and activators in the homeostasis of the complement system, in the elimination of cellular debris and in the protection of the host cells.

**Concluding remarks**

We have reviewed recent advances in the genetics of factor H and summarized overwhelming evidence that associates different genetic variants of factor H with ocular and renal disease. The data available support a strong genotype-phenotype correlation between *CFH* and these conditions

suggesting that, despite a common link involving complement dysregulation, there are distinct functional alterations in factor H that are essential in the pathogenesis of these disorders. It is now well established that mutations or polymorphisms altering the C3b/polyanions binding site located at the C-terminal region of factor H are associated strongly with aHUS. Specifically, these mutations impair the capacity of factor H to protect host cells. Accordingly, aHUS is emerging as a paradigm of disease resulting from the inefficient protection of the host cellular surfaces from complement activation. On the other hand, mutations that disrupt the plasma activities of factor H so that it fails to control complement activation result in a massive activation of C3 that causes MPGN2. AMD associates strongly and specifically with a common extended *CFH* haplotype carrying the 402His polymorphism, but the molecular bases of this association are controversial and still unclear. There are also common *CFH* haplotypes that associate specifically with lower risk to AMD. Understanding the functional consequences of the different *CFH* genetic variants should help to determine the molecular events that are critical in the pathogenesis of AMD. These studies, and the generation of animal models for the different disease-associated *CFH* genetic variants, should guide the future development of effective aHUS, MPGN and AMD therapeutics.

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