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Factor H genotype–phenotype correlations: lessons from aHUS, MPGN II, and AMD

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Missense mutations in the C-terminal region of Factor H are associated with atypical hemolytic uremic syndrome, whereas homozygous Factor H deficiency is more frequently associated with membranoproliferative glomerulonephritis type II (MPGN II). The report of Licht *et al.* of a mutation in the complement-regulatory N-terminal region of Factor H in MPGN II provides additional insight into the pathogenesis of this condition.

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Factor H is a soluble complement regulator produced by the liver. It has a pivotal role in regulating the activity of the alternative pathway. The gene encoding Factor H (*CFH*) lies within the regulation of complement activation (RCA) gene cluster at 1q32. The secreted protein product of *CFH* consists of 20 repetitive units (each ~60 amino acids) named short consensus repeats (SCRs) or complement control protein modules. Within each SCR there are four conserved cysteine residues that form two disulfide bridges essential for structural integrity. The 20 SCRs of Factor H are encoded by 22 exons. Factor H downregulates the activity of the alternative complement pathway by increasing the rate of dissociation of the alternative pathway convertase C3bBb (decay-accelerating activity) and by acting as a cofactor for the serine protease Factor I, which cleaves C3b (cofactor activity). In addition, Factor H is able to inactivate membrane-bound C3b by binding to anionic residues on cell surfaces and basement membrane. The complement-regulatory activity of Factor H is localized in the N-terminal region (SCRs 1–4), and the cell-binding activity is localized in the C-terminal region (SCRs 19 and 20). There is also additional C3b- and heparin-binding activity located in SCRs 12–14.

In the late 1990s, a series of reports showed an association between *CFH* mutations and atypical hemolytic uremic syndrome (aHUS).[1] The majority of these mutations are heterozygous missense changes in which the Factor H concentration is normal. These mutations cluster in the exons encoding SCRs 19 and 20, and functional studies have shown impaired C3b and heparin binding. aHUS is also associated with deficiency of Factor H. In the majority of cases this is heterozygous, and a range of mutations throughout the gene have been reported. Many of these affect the invariant cysteine residues. In contrast, homozygous Factor H deficiency is usually associated with membranoproliferative glomerulonephritis type II (MPGN II).[2]

There are two animal models of Factor H deficiency. The Norwegian Yorkshire pig has a point mutation (T3610G) in the exon encoding SCR 20, which results in an isoleucine-to-arginine change (I1166R). This presumably results in impaired secretion. Homozygous animals die from renal failure within 11 days of birth. Histologically they have the characteristic features of MPGN II. These changes are already present *in utero*, with deposits of C3 and the terminal complement complex visible within the glomerular basement membrane. Through selective breeding, this disease has now been eradicated from the Norwegian Yorkshire pig. Targeted deletion of *Cfh* in the mouse also results in the development of MPGN II but with only a 25% mortality at 8 months.[3] That uncontrolled activation of the alternative pathway is essential for the development of MPGN in the $Cfh^{-/-}$ mouse was shown through the introduction of a second mutation in the Factor B gene. The double homozygote ($Cfh^{-/-}$; $Bf^{-/-}$) did not develop MPGN.

The report of Licht *et al.*[4] (this issue) significantly extends these observations. *CFH* mutation screening in two sibs (products of a consanguineous marriage) with MPGN II showed a homozygous 3-bp deletion (743–745del) leading to the deletion of a lysine residue (K224del) in SCR 4. Because Factor H was detectable in the serum of the two sibs, albeit at slightly lower levels than normal, one can assume that the mutant protein is secreted. Functional analyses showed defective C3b binding, cofactor activity, and decay-accelerating activity. To my knowledge this is the first published report of a secreted mutant Factor H with impaired regulatory activity in association with MPGN II.

It is of interest that both sibs and their mother were positive for C3NeF, which is found in over 80% of patients with MPGN II and to a lesser extent in patients with MPGN I and III. C3NeF is an immunoglobulin G autoantibody directed against C3bBb, the alternative pathway convertase. It slows both the spontaneous and the Factor H-mediated decay of the convertase, thus increasing the activity of the alternative pathway. There has been debate as to the role of C3NeF in the pathogenesis of MPGN II. Some consider it to be an epiphenomenon, whereas others believe it is nephritogenic.[5] The evidence from the *CFH* knockout mouse suggests that uncontrolled activation of the alternative pathway is pivotal in the development of MPGN II. C3NeF,

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through its action on the convertase, undoubtedly leads to loss of control of the alternative pathway. However, the origin of C3NeF is a conundrum.[6] It has been suggested that it is produced in everyone from the time of birth but is controlled by the idiotypic network. Loss of control of the network or inappropriate stimulation might lead to the continuing presence of C3NeF. That the patients in the study by Licht *et al.*[4] had both a functionally significant *CFH* mutation and C3NeF does raise the possibility that hereditary systemic dysregulation of the alternative pathway predisposes to the presence of C3NeF. *CFH* mutation screening of patients known to have C3NeF may provide an answer to this question.

Patients with MPGN II also develop ocular lesions similar to the drusen seen in age-related macular degeneration (AMD). A series of recent studies have shown that *CFH* alleles act as a susceptibility factor for AMD. In particular, a polymorphism in *CFH* exon 9 (rs1061170; 1277T>C) associated with a tyrosine-to-histidine change (Y402H) has been shown to be a susceptibility factor in all these studies. Functionally, SCR 7 is a binding site for heparin and C-reactive protein; whether Y402H is functionally significant remains to be established. One of these recent studies has also shown that MPGN II and AMD patients share several *CFH* risk alleles.[7] Thus, naturally occurring variability in *CFH* acts as a susceptibility factor for MPGN II. It has also been established in four independent cohorts that *CFH* alleles act as a susceptibility factor for the development of aHUS.[8] However, the risk alleles for AMD and MPGN II are not the same as for aHUS. For instance, Y402H is not a susceptibility allele in aHUS. Thus, different *CFH* alleles are associated with predisposition to a different phenotype. In aHUS, *CFH* has been found to be not only a susceptibility factor but also the gene encoding the transmembrane complement regulator membrane cofactor protein (MCP; CD46). MCP is widely expressed and has cofactor activity for both the classical and the alternative pathway. In addition, functionally significant *MCP* mutations have been described in aHUS.[9]

Complement genes within the regulation of complement activation (RCA) cluster are arranged in tandem in two groups. In a centromeric 360-kb segment lie *CFH* and the genes for five Factor H-related proteins — *CFHL1–CFHL5* (aliases *FHR1–FHR5*). There is a high degree of sequence identity between *CFH* and *CFHL1–CFHL5*, and it has been reported that allelic variants of *CFHL5* are associated with MPGN II.[10] The serum concentrations of the five Factor H-related proteins are significantly lower than that of Factor H, and their role in complement regulation is uncertain.

In conclusion, there have been important advances in the past decade in our understanding of the phenotype associated with mutations and polymorphisms in Factor H. There are undoubtedly more to come.

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