

Genetics of Atypical Hemolytic Uremic Syndrome (aHUS)

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Abstract

Hemolytic uremic syndrome (HUS) is a rare, life-threatening disease characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure. The atypical form of HUS (aHUS), representing 5 to 10% of cases, lacks the association with infection by Shiga toxin producing *Escherichia coli* strains that characterizes the commonest clinical presentation of HUS. In the majority of aHUS cases, the disease results from the complement-mediated damage to the microvascular endothelium because of inherited defects in complement genes or autoantibodies against complement regulatory proteins. Incomplete penetrance of aHUS in carriers of mutations is common to all aHUS-associated complement genes and it is now established that the overall genetic predisposition to aHUS of an individual results from the combination of different inherited factors. Moreover, the patient's genotype influences the clinical evolution, the response to plasma therapies, and the recurrence after transplantation. Here, we describe the genetic component of aHUS, the lessons that we have learned from the functional characterization of the aHUS-associated mutations, and the benefits of a comprehensive genetic analysis of the patients.

Keywords

- ▶ complement
- ▶ aHUS
- ▶ thrombotic microangiopathy

Since the early 1980s, atypical hemolytic uremic syndrome (aHUS) has been associated with complement abnormalities and in particular with activation of the alternative pathway (AP). It was, however, the genome-wide linkage analysis performed in 1998 by Warwicker et al which formally established the relationship between aHUS and the RCA (regulator of complement activation) gene cluster at 1q32 encoding factor H and other complement regulatory proteins.¹ These findings triggered a series of decisive studies that delineated the genetic predisposition to aHUS and revealed its pathogenic mechanisms, changing the earlier perception that aHUS was a pathology related to hypocomplementemia (lack of complement) to the realization that aHUS is a disorder involving tissue damage caused by dysregulated complement activation.²⁻⁸

Most aHUS cases have a strong genetic component involving mutations (normally in heterozygosis) and polymorphisms in the genes encoding the complement regulatory proteins factor H (CFH),^{1-3,5,6,9} membrane cofactor protein (MCP),¹⁰⁻¹² and complement factor I (CFI),^{13,14} and in the genes encoding the complement components factor B (CFB)¹⁵ and C3 (C3).¹⁶ In addition, genes in the coagulation pathway such as thrombomodulin (*THBD*), which functions as a cofactor for thrombin to reduce blood coagulation and also regulates factor I-mediated C3b inactivation, and more recently plasminogen (*PLG*), a zymogen that is converted into plasmin and plays an important role in fibrinolysis, have also been implicated in aHUS.^{17,18} Finally, it has recently been found that a subgroup of aHUS patients with a very early onset (< 1 year of age) carry mutations in the gene encoding

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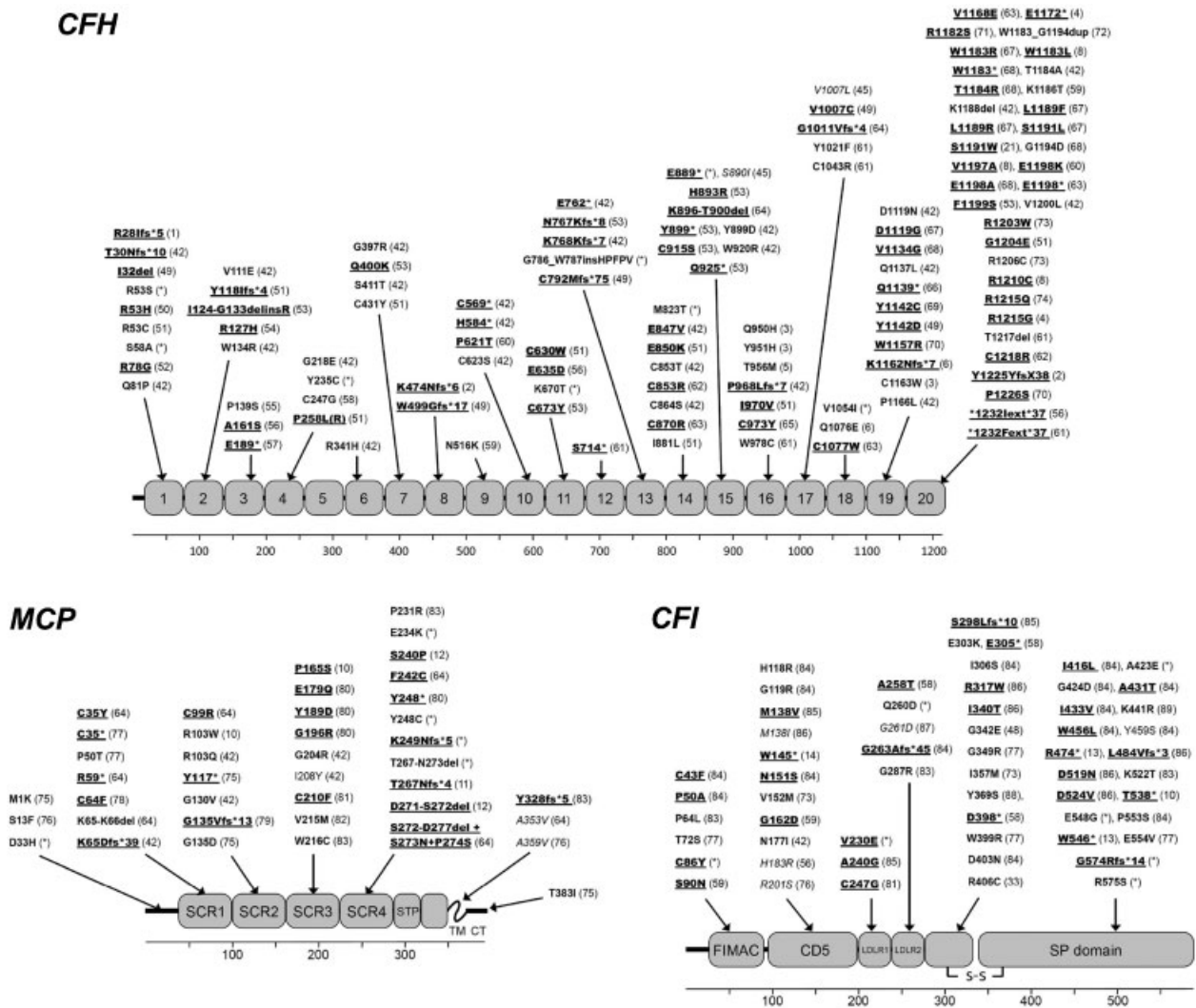


Fig. 1 *CFH*, *MCP*, and *CFI* mutations associated with aHUS. This figure shows a schematic representation with the location of the mutations in the protein domains of factor H, MCP, and factor I. Mutations that have been demonstrated to impair function or decrease expression levels are depicted in bold and underlined. Polymorphisms are shown in italics. References are in parenthesis. (*) indicates unpublished data by Rodríguez de Córdoba et al. aHUS, atypical hemolytic uremic syndrome; *CFH*, complement regulatory proteins factor H; *CFI*, complement factor I; *MCP*, membrane cofactor protein.

diacylglycerol kinase-ε (*DGKE*), a intracellular protein implicated in podocyte homeostasis and in modulating protein kinase C activity in endothelial cells and platelets.^{19,20} A summary of the mutations in all these aHUS-associated genes is depicted in ►Figs. 1 to 3 and ►Table 1.

Complement Regulators in aHUS

Missense mutations in the C-terminus of factor H, a region that is critical to the capacity of the protein to bind cell surfaces and control local activation of complement, are prototypical of aHUS.²¹ *CFH* mutations are also the most prevalent genetic alteration, representing approximately 25% of the cases in all aHUS cohorts. Carriers of the C-terminal mutations express factor H molecules with a limited capacity to bind and protect cells from complement lysis.^{4,5,7,8} Interestingly, C-terminal factor H mutations do not alter complement regulation in plasma and carriers of these mutations have normal levels of C3 in plasma. These findings are in

agreement with the identification of aHUS-associated loss-of-function mutations in *MCP* and *CFI* for the reason that the *MCP* and *CFI* mutations also lead to an impaired protection of host cells from complement lysis without affecting significantly complement regulation in plasma.²² *CFH* mutations that lead to partial factor H deficiencies in aHUS patients also fit these ideas, as it is known for a long time that decreased levels of factor H affect primarily the complement regulation on surfaces.²³

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, carriers of aHUS predisposing factors cannot control deposition and amplification of C3b of the microvasculature cellular surfaces, which results in tissue damage and destruction. The

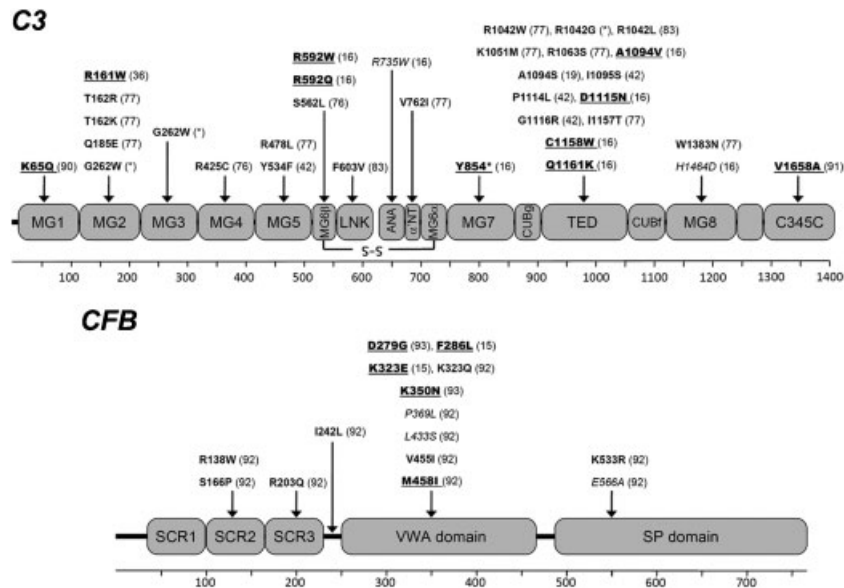


Fig. 2 *CFB* and *C3* mutations associated with aHUS. Figure shows a schematic representation with the location of the mutations in the protein domains of factor B and C3. Mutations that have been demonstrated to impair function or decrease expression levels are depicted in bold and underlined. Polymorphisms are shown in italics. References are in parenthesis. (*) indicates unpublished data by Rodríguez de Córdoba et al. aHUS, atypical hemolytic uremic syndrome; CFB, components factor B.

surface dysregulation that characterizes aHUS is clearly distinct from the lack of complement regulation in plasma, leading to complete C3 consumption and severe hypocomplementemia that is typical, for example, of dense deposit disease (DDD) patients. In this respect, it is now widely accepted that the differential association of mutations and polymorphisms in factor H and other complement proteins with aHUS and other glomerulopathies, depending on whether they cause surface or fluid phase dysregulation, illustrate a clear distinction between the pathogenic mechanisms underlying these pathologies.²⁴

aHUS-Associated *CFH*–*CFHRs* Genomic Rearrangements

The factor H-related proteins (FHRs) are relatively minor plasma components with concentrations in the range 5 to 50 mg/L, presenting a high degree of similarity with factor H. Of particular interest is the almost complete sequence conservation in FHR1 of the C-terminal region of factor H.²⁵ The genes *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5* encoding these FHR proteins are located downstream (in that order) and closely linked to the *CFH* gene within a region that shows significant genetic variability and it is characterized by the presence of large genomic duplications (ranging in size from 1.2 to 38 kb).⁵ These duplications make the region highly prone to genomic rearrangements through gene conversion and nonhomologous recombination, which are readily identified by MLPA (multiplex ligation-dependent probe amplification) technologies, CNV (copy number variation) microarrays, or western blots. Notably, several rearrangements have been identified in recent years associated with different pathologies involving complement dysregulation, including aHUS.²⁴

A very prevalent rearrangement in this region, a true common polymorphism in humans, is the deletion of the *CFHR1* and *CFHR3* genes resulting from a nonhomologous recombination event between a duplicated region downstream of the *CFH* and *CFHR1* genes. The deletion of the *CFHR1* and *CFHR3* genes is included in a single extended *CFH*–*CFHRs* haplotype, H4, that associates with lower risk of age-related macular degeneration (AMD)²⁶ and IgA nephropathy²⁷ and increased risk of systemic lupus erythematosus.²⁸ Although it is currently unclear whether the deletion of the *CFHR1* and *CFHR3* genes by itself predisposes or protects from aHUS,^{29,30} the frequency of homozygosity for the

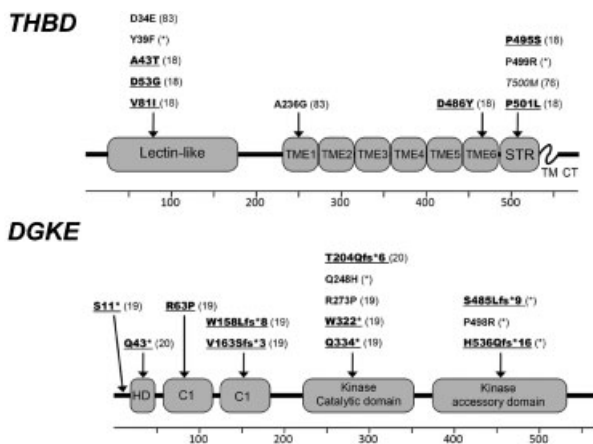


Fig. 3 *THBD* and *DGKE* mutations associated with aHUS. Figure shows a schematic representation with the location of the mutations in the protein domains of thrombomodulin and DGK-ε. Mutations that have been demonstrated to impair function or decrease expression levels are depicted in bold and underlined. References are in parenthesis. (*) indicates unpublished data by Rodríguez de Córdoba et al. aHUS, atypical hemolytic uremic syndrome; DGKE, diacylglycerol kinase-ε; THBD, thrombomodulin.

Table 1 Splice-site and intronic mutations

GENE	Nucleotide	Location	Consequences	References
<i>CFH</i>				
	c.2956 + 1G > A	Intron 19	ND	Westra et al (2010) ⁷³
	c.2957-1G > A	Intron 19	ND	Rodríguez de Córdoba et al unpublished data
	c.3493 + 1G > A	Intron 22	ND	Neumann et al (2003) ⁶¹
<i>CFI</i>				
	c.884-7T > C	Intron 6	ND	Bresin et al (2013) ⁴²
	c.1149-2 A > G	Intron 10	Decreased levels	Rodríguez de Córdoba et al unpublished data
	c.1429 + 1G > T	Intron 11	ND	Bresin et al (2013) ⁴²
	c.1534 + 5G > T	Intron 12	ND	Caprioli et al (2006) ⁶⁴
<i>DGKE</i>				
	c. 889-1G > A	Intron 4	ND	Lemaire et al (2013) ¹⁹
<i>MCP</i>				
	c.98-1G > C	Intron 1	Decreased levels	Caprioli et al (2006) ⁶⁴
	c.286 + 1G > C	Intron 2	Decreased levels	Esparza-Gordillo et al (2005) ¹⁰
	c.286 + 2T > G	Intron 2	Decreased levels	Fremaux-Bacchi et al (2006) ⁸⁰
	c.287-2A > G	Intron 2	Decreased levels	Caprioli et al (2006) ⁶⁴
	c.390-1G > C	Intron 3	Decreased levels	Saunders et al (2007) ⁵¹
	c.902-2A > G	Intron 7	ND	Fremaux-Bacchi et al (2006) ⁸⁰
	c.1027 + 2T > C	Intron 10	Decreased levels	Fremaux-Bacchi et al (2007) ⁹⁴

CFHR3-CFHR1 deletion is increased among aHUS patients as a consequence of the association between complete deficiency of the FHR1 protein and the generation of antifactor H autoantibodies.^{25,29} This is an intriguing association for which there is no clear explanation. Importantly, the antifactor H autoantibodies recognize the C-terminus of factor H³¹ and functionally mimic the prototypical factor H mutations that are frequently associated with the development of aHUS.³² Another relatively frequent rearrangement in the *CFH-CFHR* region involves an unequal crossover between homologous regions in the 3' ends of *CFHR3* and *CFHR4* genes that specifically removes the *CFHR1* and *CFHR4* genes. This deletion is also found in aHUS patients in association with antifactor H autoantibodies.^{29,33}

Most interesting are the various genomic rearrangements between the 3' end exons of *CFH* and the homologous regions in *CFHR1* or *CFHR3*, which have been associated with aHUS.^{9,34} These rearrangements result in the generation of *CFH::CFHR1* or *CFH::CFHR3* hybrid genes that alter the C-terminal region of factor H, further illustrating the remarkable correlation between a dysfunctional C-terminal region in factor H and aHUS. Similarly, the association of a *CFHR1::CFH* hybrid gene in which the C-terminal exons of FHR1 have been replaced by those in factor H (reversed *CFH::CFHR1* hybrid gene) suggests that competing the binding of the C-terminal region of factor H to substrates that are relevant in aHUS with a protein devoid of complement regulatory activity has the same consequences than the C-terminal factor H mutations characteristic of aHUS.³⁵

Gain-of-Function Mutations in Factor B and C3

Factor B and C3 mutations are characteristic of a subgroup of aHUS patients showing persistent activation of the AP in plasma.^{15,16,36} Importantly, while mutations in the complement regulators factor H, MCP, and factor I are loss-of-function mutations, mutations in factor B or C3 are gain-of-function mutations in the sense that they increase formation or stability of the C3 convertase or render it resistant to inactivation by the complement regulators.^{15,16} These data unequivocally establish the critical role that dysregulation of the complement AP plays 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 components of the C3 convertase of the AP.

From a pathogenic point of view, it is intriguing that these factor B and C3 gain-of-function mutations, decreasing C3 plasma levels and causing different degrees of hypocomplementemia, are nevertheless associated with aHUS. One possible explanation is that increased complement activation caused by gain-of-function mutations, a situation that may be similar to that occurring during infection, coincides with an additional aHUS risk factor impairing surface protection. In support of this possibility, it has been noted that carriers of *CFB* or *C3* gain-of-function mutations that develop aHUS are also carriers of a *MCP* risk haplotype (see later).^{15,36} In the case of the mutations in C3 associated with aHUS, different

experimental approaches have shown that these mutations alter the sensitivity of C3b to inactivation by factor H and MCP and/or change the susceptibility of the AP C3 convertase to accelerated decay by factor H and decay accelerating factor (DAF, CD55).^{16,36} This data again illustrate that aHUS associated mutations affect preferentially complement regulation on surfaces, which is in contrast with the fluid phase dysregulation caused by C3 mutations associated with other glomerulopathies.^{36,37}

Therapeutic Implications

The realization that aHUS is a disorder involving tissue damage caused by dysregulated AP complement activation provided strong support for the implementation of aHUS therapies based in the inhibition of the complement terminal pathway. Eculizumab, a blocking monoclonal antibody against human C5 that inhibits C5a release and terminal complement complex (TCC) formation in general, and membrane attack complex (MAC) generation on a cellular target, was first successfully used for treatment of paroxysmal nocturnal hemoglobinuria, a hemolytic and thrombotic disorder caused by deficiency of glycosylphosphatidylinositol-anchored complement regulators CD55 and CD59 on blood cells, which are therefore lysed by MAC formation. Eculizumab was successfully tested in aHUS patients, on a compassionate basis, to prevent relapses of the disease and recurrences after transplantation.^{38,39} Later on, based on the excellent results obtained in phase II clinical trials during 2009 to 2010, eculizumab was approved by the US Food and Drugs Administration and the European Medicines Agency and has rapidly become the accepted therapy in patients with aHUS,⁴⁰ both as a rescue therapy in acute episodes and as prophylaxis in labile patients and following renal transplant.⁴¹

Incomplete Penetrance of aHUS in Mutation Carriers

The penetrance of disease in carriers of mutations in any of the aHUS-associated genes is approximately 50%, indicating that additional genetic and environmental factors contribute to disease development in these individuals. In this respect, it is now well documented that concurrence of different genetic risk factors, either combined mutations in more than one gene or the combination of mutations and risk polymorphisms, greatly influences predisposition to aHUS.^{10,15,42,43}

The aHUS-associated polymorphisms are basically limited to three relatively frequent *CFH* and *MCP* haplotypes, which include both risk and protection factors.⁴⁴ The common *CFH* haplotype H2 including the common polymorphism Val62Ile has been associated with lower risk to aHUS, AMD, and DDD. Val62Ile lies within the N-terminal region that is essential for factor H regulatory activities. Consistent with the role of complement dysregulation in these pathologies it has been shown that the substitution of Val for Ile at position 62 increases the complement regulatory of factor H reducing activation of the complement AP. In fact, functional analyses

have shown that the Val62Ile substitution in factor H increases the affinity for C3b, competing more efficiently with factor B for C3b binding in the proconvertase formation and acquiring enhanced cofactor activity for the factor I-mediated proteolysis of C3b.⁴⁵

The *CFH-H3* and *MCPggaac* haplotypes are the most relevant aHUS risk polymorphisms described thus far. Both 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.^{3,10} Although additional studies are needed to fully characterize these haplotypes functionally, the association of *CFH-H3* and *MCPggaac* with aHUS is important because it may help to explain why some individuals are predisposed to aHUS in the absence of mutations in the known aHUS associated genes. It is also increasingly recognized that in carriers of mutations in the aHUS-associated genes, the *CFH* and *MCP* risk haplotypes may be needed for full manifestation of the disease.^{10,15,36,42}

A recent collaborative study by the European Working Party on Complement Genetics in Renal Diseases in 795 patients with aHUS has identified that 3% of these patients carry combined mutations, being combinations involving *MCP* or *CFI* mutations (25%) more frequent than those with *CFH*, *C3*, or *CFB* mutations (range, 8–10%). Furthermore, this large study also illustrated that the concomitant presence of *CFH* and *MCP* risk haplotypes significantly increased disease penetrance in combined mutation carriers, further suggesting that genotyping for the *CFH* and *MCP* risk haplotypes may help to predict risk of developing aHUS in affected carriers of mutations.⁴²

Apart from the *CFH* and *MCP* polymorphisms, other aHUS-associated polymorphisms in FHR proteins include the FHR-1**A*/**B* variant; possession of the **B* variant is risk for aHUS.^{29,30} Polymorphisms in the *CFHR5* gene have also been described and associated with risk of aHUS.⁴⁶

Taken together, genetic and functional analyses have established that aHUS involves complement AP dysregulation and develops as a consequence of defective protection of cellular surfaces from complement activation. Multiple hits, involving plasma and membrane-associated complement regulatory proteins as well as complement components, are likely required to cause dysregulation and significantly impair protection to host tissues. Environmental factors that activate complement likely modulate genetic predisposition and are also very important in aHUS. Infection, immunosuppressive drugs, cancer therapies, oral contraceptives, pregnancy, and childbirth are important factors that trigger attacks of aHUS in some patients. In carriers of multiple strong aHUS genetic risk factors, the contribution of the environment is probably minor. On the contrary, in those with a low-genetic predisposition, strong environmental factors may still precipitate disease.

Thrombomodulin and Other Coagulation Genes

The anticoagulant protein thrombomodulin, which functions as a cofactor for thrombin to reduce blood coagulation and

also regulates factor I-mediated C3b inactivation, has been described associated with aHUS.¹⁸ Interestingly, functional analyses of these aHUS-associated *THBD* mutations supported a defect in the complement regulatory activities of this protein on cell surfaces, which is consistent with the complement dysregulation that characterize aHUS.¹⁸ However, it is currently unknown whether the anticoagulant activities of thrombomodulin are also disrupted by the aHUS-associated mutations and therefore may also be relevant in aHUS. In this regard, a very recent study has screened in 36 European-American sporadic aHUS patients, using targeted genomic enrichment and massive parallel sequencing, the coding sequences and splice sites in 85 genes, including all genes in both the complement and coagulation pathways.¹⁷ In addition to novel variants in various complement genes, the study found deleterious nonsynonymous rare variants in several coagulation genes. *PLG*, encoding plasminogen, a zymogen that is converted into plasmin and plays an important role in fibrinolysis, was the most frequently mutated gene.¹⁷ Although these data suggest that the coagulation pathway and *PLG* in particular also contribute to aHUS susceptibility, further studies are needed to confirm these associations and to determine the role of the coagulation pathway in aHUS.

Diacylglycerol Kinase- ϵ -aHUS

Using exome sequencing Lemaire et al¹⁹ have recently identified recessive mutations in *DGKE*, encoding *DGK- ϵ* associated with aHUS in 13 patients from 9 kindreds. Disease presentation in these patients occurred very early, typically before the age of 1 year, with multiple recurrences often progressing to end-stage renal disease in the second decade of life. It has been indicated that *DGKE* mutations may explain as much as 27% of cases presenting in the 1st year of life.¹⁹ An independent study also found recessive mutations in *DGKE* in nine patients from three consanguineous families with MPGN-like syndrome.²⁰ Onset of disease in this second set of patients was significantly delayed and none of the patients in the study presented acute episodes suggesting aHUS. However, their histology showed features of glomerular microangiopathy, supporting that all patients carrying *DGKE* mutations share a common pathogenic mechanism. Although the molecular basis is still unclear, one plausible explanation is that loss of *DGK- ϵ* increases signaling through arachidonic acid containing diacylglycerol substrates, enhancing protein kinase C activation in endothelial cells, platelets, and podocytes. As a consequence of this, prothrombotic factors such as von Willebrand factor, plasminogen-activator inhibitor-1, platelet-activating factor, and tissue factor would be upregulated, leading to a thrombotic phenotype.¹⁹ In addition, loss of *DGK- ϵ* may result in podocyte damage and impair slit diaphragm function, which could explain the heavy proteinuria observed in patients with *DGKE* mutations.²⁰

Importantly, it was indicated that these individuals do not carry mutations in any of the known aHUS candidate complement genes and show no alterations of complement in plasma, suggesting that *DGKE*-associated aHUS represents an

alternative mechanism leading to thrombotic microangiopathy.¹⁹ Although a role for complement dysregulation in the development of renal disease in carriers of *DGKE* mutations seems a priori excluded, we have recently identified three *DGKE*-associated early onset aHUS patients carrying additional mutations in the *THBD* and *C3* genes (Rodríguez de Córdoba et al., unpublished data; 2014). Further studies are therefore needed to determine whether complement dysregulation may influence the onset and severity of the disease phenotype in some carriers of *DGKE* mutations.

Genotype–Phenotype Correlations

The clinical evolution of patients with aHUS, their response to plasma therapies, and the disease recurrence after kidney transplantation are influenced by the type of mutation involved. In general, patients with *CFH*, *CFI*, *CFB*, and *C3* mutations have a worse prognosis during the episode of aHUS and following months, with rates of mortality, and end-stage renal disease rising to approximately 50 to 70% and recurrences occurring in more than 50% of them. On the contrary, less than 20% of patients with *MCP* mutations die or develop end-stage renal disease, although the risk of recurrence is greater than 70%.⁴⁷

Historically, the success of kidney transplants in patients with end-stage renal disease caused by aHUS has been limited by the high percentage of posttransplant recurrence of disease (~50%; graft loss rate, 80–90%), although results vary based on the type of mutation present. *CFH* mutations are associated with a greater risk of recurrence or graft loss following renal transplantation (range, 75–90%), and high levels of risk are also associated with *C3* and *CFI* mutations (range, 40–80%).^{42,47,48} Until now, very few transplants have been attempted in patients with *CFB* mutations, but all cases that have been reported to date have involved recurrence of aHUS and graft loss.

Most complement factors involved in aHUS are plasma proteins primarily synthesized by the liver and thus patients with mutations of complement genes that code for these factors continue to be susceptible to aHUS after renal transplantation, as the dysfunctional factors continue to be produced. On the contrary, because *MCP* is a transmembrane protein expressed by all cells, a kidney transplant corrects the deficit by producing unaltered *MCP* in the new kidney. Consequently, more than 80% of patients with *MCP* mutations do not develop recurrent aHUS after transplantation, with a similar long-term survival rate to that of patients who receive transplants for other reasons. The risk of posttransplant recurrence in patients with *THBD* mutations or antifactor H antibody mutations is not well understood, although in the case of factor H antibodies, it appears that recurrence is related to persistently high-antibody titers.^{31,47,48}

Among patients with *CFH* or *CFI* mutations, the presence of mutations in other genes did not modify prognosis; in contrast, 50% of patients with combined *MCP* mutation developed end-stage renal disease within 3 years from onset compared with 19% of patients with isolated *MCP* mutation. In general, a similar situation was observed for kidney transplant outcomes.⁴²

DGKE-aHUS illustrates a very particular subgroup of aHUS patients. As indicated above, *DGKE*-associated aHUS may represent an alternative mechanism involving intracellular signaling leading to thrombotic microangiopathy, independent of complement dysregulation. Consistent with this idea, two patients with *DGKE* mutations who received plasma treatment or eculizumab, had aHUS recurrences. However, five other patients with *DGKE* mutations underwent eculizumab treatment without evidence of disease recurrence.¹⁹ On the other side, a total of six aHUS patients with *DGKE* mutations underwent renal transplantation without evidence of aHUS recurrence,¹⁹ but one transplanted patient with the MPGN-like form showed signs of disease recurrence in the transplanted kidney.²⁰

Concluding Remarks

We have summarized our current understanding of the genetics of aHUS and reviewed how the functional analysis of different aHUS-associated genetic variants has helped to determine the molecular events that are critical in aHUS pathogenesis. It is now well established that mutations or polymorphisms in complement components and regulators are strongly associated with aHUS because they specifically impair the capacity to protect host cells from complement activation. It is also increasingly appreciated that it is the combination of mutations, or mutations and common polymorphisms in *CFH* and *MCP*, what determines the individual predisposition to aHUS and that this genetic makeup influences disease progression, responses to therapies, and recurrences after transplantation. On the basis of these genotype-phenotype correlations, it is also widely recognized that a comprehensive understanding of the genetic component predisposing to the pathology and its functional consequences at the protein level is critical to guide appropriate diagnostics and effective treatment in aHUS.

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