Genetics of hemolytic uremic syndromes

Michal Malina¹,², Lubka T. Roumenina³, Tomáš Seeman², Moglie Le Quintrec³, Marie-Agnes Dragon-Durey⁴, Franz Schaefer¹, Véronique Fremeaux-Bacchi⁴

1. Center for Pediatrics and Adolescent Medicine Heidelberg, Division of Paediatric Nephrology, 69120 Heidelberg, Germany
2. Charles University in Prague-2nd Faculty of Medicine and Teaching Hospital Motol, Department of Paediatrics, 150 06, Prague, Czech Republic
3. Cordeliers Research Center, Inserm UMR5 872, 75006 Paris, France
4. Assistance publique–Hôpitaux de Paris, hôpital Européen Georges-Pompidou, service d’immunologie biologique, 75908 Paris cedex 15, France

Correspondence: Véronique Fremeaux-Bacchi, Hôpital Européen Georges-Pompidou, service d’immunologie biologique, 20-40, rue Leblanc, 75908 Paris cedex 15, France.
veronique.fremeaux-bacchi@egp.aphp.fr

Summary

Hemolytic uremic syndrome (HUS) is a very rare disease (two cases per year per 1 million population) but represents the most common cause of acute renal failure in young children that require dialysis. The majority of cases in childhood (90%) is caused by Shiga toxin producing Escherichia coli infection. This typical form of the disease does not relapse and has a good prognosis if the acute status can be managed successfully. Atypical HUS (aHUS) is a severe and frequently relapsing disorder with the same triad of thrombocytopenia, hemolysis and acute renal failure in the absence of Shiga toxin E. coli infection. More than 50% of patients with atypical HUS progress to chronic renal dysfunction and 10% die due to complications of the disease. Atypical HUS appears to have a genetic basis. Mutations in genes coding for components of the alternative complement pathway are found in about 60% of cases. The clinical presentation of aHUS overlaps with that of other thrombotic microangiopathies, rendering the diagnosis on clinical grounds alone extremely difficult. In recent years, genetic testing has opened the way for molecular diagnostics and helped establishing therapeutically and prognostically useful genotype-phenotype correlations. This review summarizes recent findings regarding the genetic basis of the HUS. The pathophysiology of the disease and the implication of genetic abnormalities in the complement system for the different types of HUS are discussed.

Hemolytic uremic syndrome (HUS) is a severe life-threatening disease, characterized by non-immune hemolytic anemia, thrombocytopenia and acute loss of renal function. In 1955, Gasser et al. first described five small children with this association of symptoms [1]. Interestingly, as if
they had known that the pathophysiology of the disorder would be anything but uniform, they put the title in plural—Hemolytic uremic syndromes. The HUS belongs to a group of diseases called thrombotic microangiopathies (TMA) together with thrombotic thrombocytopenic purpura (TTP) [2] and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. Characteristic histological lesions are common to this group of disorders. A hallmark feature is the destruction of endothelium and subsequent intravascular formation of microthrombi. Standard histology shows intravascular fibrin and platelet thrombi obliterating arterioles and capillaries, and endothelial swelling with accumulation of subendothelial debris. These lesions have been known for a long time, even before the Gasser’s HUS defining paper [3].

In patients with HUS, the kidney glomeruli and arterioles are most severely affected, leading to typical clinical signs. In TTP, the main clinical symptoms are hematological and neurological manifestations due to thrombi in the central nervous system microvasculature. In HELLP syndrome, the hepatic circulation is most severely affected. The clinical symptoms frequently overlap (neurological signs in HUS, renal impairment in TTP in long term follow-up) making the diagnosis on clinical grounds difficult. The mechanisms underlying the target organ predisposition of the individual disorders are still unknown.

The classical clinical classification of HUS is given by the presence or absence of disease prodromi. HUS with preceding diarrhea (typically bloody) is known as D+ HUS (diarrhea positive) or typical HUS. Typical HUS is mainly caused by infection with enterohaemorrhagic types of Escherichia coli (EHEC) producing Shiga-like toxin (serotypes O157:H7, O26, O123, etc.) [4]. Shiga-like toxin (STX) producing bacterial infection accounts for almost 90% of HUS cases in childhood. These cases are mostly benign if acute kidney failure is managed successfully. Typical HUS does not relapse and kidney function usually recovers completely. This form is most frequent in children under 5 years of age [5]. However, late sequelae can occur even after more than 10 years after the primary event [6]. HUS may occur secondary to other infectious triggers like Streptococcus pneumoniae invasive infection [7], certain viral infections [8] or non-infectious triggers like malignant tumors (especially adenocarcinomas) [9] as well as after administration of certain drugs like mitomycin-C or calcineurin inhibitors [10]. Defective cobalamin metabolism also may lead to HUS presentation in young infants [11].

In about 10% of the children and in almost all adult cases of HUS, no shiga-like toxin-producing bacteria are detected and also any known cause of secondary HUS can be ruled out [12]. Since these cases have insidious abrupt onset without the typical gastrointestinal prodromi, they are termed “D−” (diarrhea negative) or “atypical” HUS (aHUS). Their clinical course is far more severe, with more than 50% affected progressing to chronic renal insufficiency and up to 10% dying from disease complications [13].

The clinical criteria for HUS classification as recently proposed by an international expert panel [14] are summarized in table 1 (see figure 1).

As seen from the classification, the etiological spectrum of atypical idiopathic HUS is very broad. The large overlap of clinical symptoms renders the diagnosis on clinical grounds extremely difficult. Early histological findings and biochemical studies pointed to involvement of the complement cascade in the pathophysiology of atypical HUS. Low C3 but normal C4 concentrations suggested alternative complement pathway activation [15]. Biopsy and post mortem studies confirmed local granular C3 deposition [16] and surface activation in the renal vasculature [17]. Finally, genetic studies have provided evidence for a causative role of complement abnormalities in the molecular pathogenesis of aHUS. Recent studies suggest a permissive function of complement hyperactivation also in the pathogenesis of shiga toxin associated typical HUS [18–20].

**Complement function in health**

The complement system is a part of innate immunity and exerts a broad spectrum of functions [21]. It comprises a cascade of about 30 plasma and membrane bound proteins, which are activated sequentially. Three activation pathways lead to formation of enzyme complexes with C3 convertase activity. These C3 convertases cleave and activate the central complement component C3 to a big fragment C3b, which binds covalently to any bystander surface and a small fragment C3a, which is an anaphylatoxin. Subsequent activation of the common terminal pathway leads to release of potent anaphylatoxins such as C5a and formation of a membrane attack complex (MAC) composed of C5b-9. The main functions of complement are clearing of immune complexes and apoptotic cells, opsonisation of pathogens and attraction of pro-inflammatory cells at sites of damage. In order to avoid damage of intact autologous tissue, the complement cascade is very tightly regulated by fluid phase (factor H, factor I) and membrane bound factors (MCP/CD46, DAF/CD55, CD59 and recently
## Table I

Clinical classification of thrombotic microangiopathies (adapted from classification by European pediatric research group [14])

<table>
<thead>
<tr>
<th></th>
<th>Typical HUS (STX)</th>
<th>Atypical HUS (idiopathic)</th>
<th>Secondary HUS (triggered)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical description</strong></td>
<td>Mostly children between 2–5 years of age</td>
<td>No age preference</td>
<td>No age preference</td>
</tr>
<tr>
<td></td>
<td>Complete recovery</td>
<td>Insidious onset without gastroenteritis</td>
<td>Onset after triggering disease</td>
</tr>
<tr>
<td></td>
<td>Non-recurrent course</td>
<td>High rate of late sequel including ESRD</td>
<td></td>
</tr>
<tr>
<td><strong>Etiology</strong></td>
<td>Shiga-like toxin producing bacterial infection: <em>Escherichia coli</em>, mainly serotype 0157</td>
<td>Mutations in complement proteins (around 60%) Factor H Factor I Factor F MCP/CD46 Factor B C3 Thrombomodulin Factor H antibodies</td>
<td><em>Streptococcus pneumoniae</em> invasive infection Defective cobalamin metabolism Transplantation (solid organs and bone marrow) Calcineurin inhibitor administration (ciclosporin A, tacrolimus) Malignant tumor (adenocarcinomas) chemotherapy (mitomycin), ionizing radiation HIV Autoimmunity disorders – SLE Pregnancy (overlap with HELLP syndrome)</td>
</tr>
</tbody>
</table>

HUS: Hemolytic uremic syndrome; STX: Shiga-like toxin.

1 Pathogenesis questionable, association only.
suggested thrombomodulin). The schematic view on complement activation pathways and its regulation is seen on figure 2.

**Abnormalities of the complement cascade in hemolytic uremic syndrome**

In atypical HUS, complement consumption and lack of regulators has been known for a long time [22,23]. These observations were followed by genetic studies to prove the molecular basis of these abnormalities. Since atypical HUS is not associated with a particular infection, mutated protein culprits were thought to be either the regulators of complement or the complement cascade itself. The first direct link between aHUS and abnormalities in the complement cascade was reported in the late 1990s, when heterozygous factor H mutation was proven to be associated with aHUS [24]. Following these seminal findings, an array of genetic abnormalities in the complement system was found to be associated with aHUS. Six different genes have been identified to date (CFH, CFI, MCP, THBD, C3 and CFB). The first three genes are complement regulators. Complete protein deficiency or a loss of protein function lead to unregulated complement activation and hence promotion of the disease. The second group consists of the complement components themselves (factor B and C3). Upon mutation they form either a hyperactive C3 convertase or an enzyme resistant to regulation (review in [25]). A functional consequence has to be ascribed to each mutation, discovered in these genes in order to prove its association with the disease. An algorithm for alternative complement pathway abnormalities assessment in patients with atypical HUS was recently proposed [25].

The model of atypical HUS may not be strictly monogenic (i.e. a single gene mutation may not be enough to cause the disease). The aHUS should be classified as a complex oligogenic triggered disease. The mutations in complement regulatory proteins are considered susceptibility factors making the carriers prone to other, genetic or environmental factors that trigger the start of the disease. For most of the mutations identified to date, there are healthy carriers in the families. Atypical HUS usually follows an autosomal dominant inheritance pattern with about 50% penetrance. It is still unclear why some mutation carriers develop the disease and others do not. It has been demonstrated that a combination of mutations at risk haplotypes in

![Figure 2](image_url)

**Figure 2**

**Complement pathways, activation and regulation**

Complement cascade is activated via three pathways called classical, lectin and alternative. The classical pathway is triggered by immune complexes, the lectin pathway by molecules on bacterial surfaces and the alternative pathway starts by autoactivation of C3 which then reacts with activating surfaces (mainly bacterial cell wall). All pathways converge at the step of activation of C3 to C3b by enzyme complexes called C3 convertases. C3b then reacts with more C3b and other factors (factor B, D and properdin [FP]) to create so called C5 convertase, the enzyme complex that activates the late stage of complement cascade creating C5a (a powerful anaphylatoxin recruiting immune cells) and C5b, the latter recruits other complement proteins to form a multimeric complex creating pores in the cell surface (membrane attack complex per/terminal complement complex [MAC/Tcc]). This process is tightly regulated by many proteins, most importantly factor H, factor I and CD46/MCP.
CFH and/or in MCP genes or single nucleotide polymorphisms co-segregate with the disease. In a large family with three different genetic abnormalities, only the combination of all three factors led to disease manifestation [26]. In addition, a trigger event, as infection or pregnancy has been reported in more than 50% of reported cases of aHUS first manifested in adulthood, strongly suggesting the need for an additional environmental triggering factor. These environmental and/or epigenetic factors still remain elusive [12].

The genetic counseling remains difficult. Genetic testing can identify the carriers. In principle, carriers could be advised to avoid known trigger factors and could be carefully monitored when undergoing conditions with increased risk of developing aHUS (infections, pregnancy and certain drug treatment).

All the genetic abnormalities are leading to creation of mutated proteins that cause hyperactivation of the alternative complement pathway, liberation of potent anaphylatoxins and formation of the final lytic component of complement (TCC) in endothelial cells. All these factors participate in the destruction of the endothelium. When endothelial cells damage occurs, subendothelial matrix is exposed, leading to activation of the coagulation cascade and formation of intraluminal fibrin microthrombi.

In typical HUS, the pathogenesis seemed clear when direct toxicity of shiga toxin (STX) to the endothelium was demonstrated [27]. However, complement has also a role in typical HUS. Aggravation of the cytotoxic effect of STX through complement activation was noted recently. In patients with STX-mediated HUS alternative pathway activation was observed [28]. Moreover, STX appears to affect the function of complement regulators (mostly factor H) on molecular level. The functional blockade of factor H by STX promotes the endothelial injury through alternative pathway activation [19].

**Genetic background in different hemolytic uremic syndrome etiology groups**

The summarized data from the cohorts suggest that different clinical presentations have different quotients of genetic background influence by HUS associated genes. The links seem to be the strongest in the group of patients with pregnancy triggered HUS where mutations were found in about 80% of cases. It is followed by the “idiopathic” aHUS (up to 60%). In the de novo TMA after renal transplantation mutations are found in about 25% of patients. In typical HUS only few cases (most of them devastating or fatal) were described to harbor mutation in one of the associated genes. In secondary HUS, no associated mutations in the cohorts were reported up to date.

**Pregnancy associated Hemolytic uremic syndrome**

Pregnancy associated HUS is a special entity of secondary HUS. This HUS variant has been demonstrated to be strongly associated with mutations in complement genes recently. In 18 of 21 affected women, genetic complement abnormalities were observed in the genes CFH, CFI, MCP and C3. In contrast to pregnancy associated TTP, which can manifest throughout pregnancy, aHUS manifests mostly after delivery or in the last trimester. The risk of HUS triggering rises with the number of pregnancies and the clinical course is usually severe with more than three quarters of patients progression to ESRD [29].

**Atypical hemolytic uremic syndrome “idiopathic”**

Within the last 10 years, knowledge regarding the factors predisposing to aHUS has increased dramatically. More than 60% of atypical idiopathic HUS has been found to be etiologically linked to mutations in six complement genes (table II). The frequencies of CFH, CFI, and MCP mutations from five large aHUS cohorts (US, Italian, German, Spain and French) were published during the last several years (review in [30] and [31]). The variation of the frequency distribution of mutations could be explained by the criteria of patient recruitment and according to the age of onset of the diseases. Of note, in all cohorts patients with more than one mutation (CFI-MCP, CFH-MCP, FB-FI) have been reported. In approximately 10% of patients, anti FH antibodies have been linked to the disease in association with a complete deletion of CFHR1-CFHR3 genes [32]. For the initial management of aHUS, it is not essential to know the genetic origin of the disorder. First line therapy consists of symptomatic treatment, replacement of kidney function and plasmapheresis/complement blockade irrespective of etiology. In the subsequent maintenance phase, and especially in the stage of planning kidney transplantation, knowledge of the genetic disease background becomes essential. The initial reaction to plasmapheresis and progression to end-stage renal disease (ESRD) varies among the different underlying mutations. Patients with mutations in FH, FI and C3 have some response to plasmapheresis (till 50%), but most of the cases (70%) progress to ESRD during the first disease episode. Cases with underlying MCP and THBD mutations are usually not responsive to plasmapheresis since MCP and thrombomodulin are membrane bound proteins. In patients with MCP mutations, spontaneous disease remission is common and ESRD occurs only in 15% of cases with or without plasma exchange. By contrast, thrombomodulin cases have a very bad prognosis with a high rate of progression to ESRD [33].

**Hemolytic uremic syndrome/thrombotic microangiopathies after kidney transplantation**

The genetic background clearly determines the risk of disease recurrence after kidney transplantation. In contrast to D+ HUS, where recurrence is almost never seen, atypical forms have a
high rate of recurrence (in a form of aHUS or isolated thrombotic microangiopathy in the graft), mandating genetic exploration of patients prior to transplantation. The type of underlying genetic abnormality is highly predictive of the recurrence risk. In patients with mutations in factor H, I, B, C3 the risk of recurrence is very high (till 90%). In contrast, MCP mutations have a favorable outcome with a very low rate of recurrence [34].

Atypical HUS can arise also in recipients of kidney grafts without a previous aHUS history. A pathogenic or facilitator role of calcineurin inhibitors, particularly ciclosporin A [10,35] has been hypothesized, but it is unclear why some patients respond to calcineurin inhibitors and/or ischemic-reperfusion trauma to the endothelium by developing HUS/TMA while the vast majority of transplant recipients exposed to the same conditions does not [34].

Post-transplant de novo thrombotic microangiopathy appears to be a distinct entity. It shares some features with idiopathic aHUS and is connected to the genes of the complement alternative pathway. In 29% of patients with de novo renal graft TMA confirmed by biopsy genetic abnormalities in the CFH and CFI genes were observed, in contrast to a control group of kidney graft recipients without TMA in whom no mutations in complement alternative pathway genes were found [36]. These findings suggest that for patients with less severe mutations in complement regulators, kidney transplantation could serve as a primary disease triggering factor [34].

### Typical Hemolytic uremic syndrome (Shiga-toxin caused)

The majority of the typical and secondary HUS cases are not associated with genetic abnormalities in complement and therefore genetic testing is generally not recommended. Nevertheless, complement mutations were found in patients with typical HUS manifesting with unusual severity. A case of STX caused HUS with lethal course has been described in a patient with an MCP mutation [37]. Recent studies also found a direct effect of STX on the complement pathway, suggesting that direct endothelial toxicity of STX via the GB3 receptor is not the only pathogenic mechanism in these patients [19]. A recent study demonstrated hyperactivation of the complement pathway up to its terminal lytic component in 17 D+ HUS patients [28]. Hence, the specific pathogenicity of STX might be originated via its direct toxic effect on endothelial cells and propagated via hyperactivation of the alternative complement pathway due to factor H incapacitation, thereby aggravating endothelial destruction. The clinical consequence from these novel insights might be to consider specific complement blocking therapy (i.e. eculizumab) in patients with STX mediated HUS taking a severe clinical course, particularly if complement hyperactivation is suggested by biochemical and/or genetic findings [20].

Other genetic factors might add to the risk of developing typical HUS. In a study of 150 children, strong associations were observed with numerous common gene variants, mainly in genes affecting the coagulation pathway and the
renin-angiotensin-aldosterone system [38]. The direct patho-
genetic links of these polymorphisms with disease pathology, and their potential significance also for atypical HUS awaits clarification.

Genes proofed to be associated with Hemolytic uremic syndrome

Molecular genetic testing should be performed in all patients with signs of HUS in a specialized laboratory capable of direct mutation analysis. It is recommended to directly sequence all six genes starting from the most common (i.e. for factor H and MCP) to the least common ones (i.e. thrombomodulin and factor B). The rationale of sequencing all genes even if a mutation in one gene is found is given by the fact that up to 5% of patients have combined mutations in several genes. In addition, deletions and structural gene changes (mostly in factor H) should be evaluated by MLPA.

A synopsis of the six genes involved in the pathogenesis of aHUS and the clinical consequences of mutations is given in Table II.

Loss of regulatory function

Factor H

Factor H is the most important complement regulator in the fluid phase and on endothelial surfaces. It is produced primarily by the liver. Two types of mutations are known. In case of quantitative deficiencies (type I mutations), the serum concentration of factor H is lowered. These can be homozygous or compound heterozygous (with virtually no factor H in serum), or heterozygous with 50% of the normal factor H concentration. Type I mutations are identified easily by measuring complement proteins. Type 2 mutations are more insidious. They show normal factor H levels in serum, but the protein is functionally flawed. These mutations are typically located in the C terminus of the protein, hampering proper binding and activity on the endothelial cell surface [25]. Predisposition to HUS may also be caused by polymorphic variants in the factor H gene: a particular haplotype defined by SNP localized in the promoter and in the SCR 2, 7, 11 and 16, CFH-H3 “tgtgt” has been observed at increased prevalence in aHUS patients compared to healthy controls [12].

Factor H autoantibodies

The autoimmune form of aHUS is due to autoantibodies against FH, leading to functional FH deficiency [39]. According to several European studies, the autoimmune form of aHUS accounts for 5 to 11% patients with aHUS. The anti-FH IgG molecules have been shown to interfere with the FH binding to the alternative pathway C3 convertase and associated with defective FH-dependent cell protection. In the few cases where the epitopes of these antibodies were mapped, they appear to be located in the C-terminus of the FH, which is also a hot spot for type II mutations. These antibodies occur mostly in patients with homozygous deletions of a gene, which exhibit a high degree of sequence homology with CFH called CFHR1 (complement factor H related gene). The frequency of this deletion among the anti-FH IgG positive patients is estimated to be 84% as compared to 2% of the normal European population [32]. However the direct etiological link between the absence of CFHR1 and the development of the autoantibodies is still elusive.

Factor I

Factor I is a circulating serine protease. It cleaves and inactivates C3b once the latter is bound to co-factor molecules such as factor H or MCP. Most of the CFI mutations are heterozygous and lead to lower levels of factor I in serum, thus lowering its regulatory function [40]. Mutations in factor I are found at variable prevalence in different cohorts, reaching up to 10% [30]. Interestingly, these mutations are frequently associated with a second genetic abnormality or with a complete CFHR1 deletion [41]. Patients with a complete deletion of the CFHR1 gene have significantly higher risk of a bad prognosis as compared with patients with CFI mutation as a unique susceptibility factor.

MCP/CD46

MCP is a membrane-bound regulator, serving as a cofactor for factor I in the process of C3b inactivation [42]. Most of the mutations are heterozygous and lead to a lack of protein expression. MCP is present on the granulocytes and therefore flow cytometry of peripheral blood leukocytes labeled for MCP/CD46 can be used as a quick diagnostic tool. Mutations in MCP are responsible for up to 10% of aHUS cases. A specific MCP haplotype, “ggac” defined by the following SNPs: -652 A>G (rs2796267), −366 A>G (rs2796268), IVS9-78 G>A (rs1962149), IVS12 + 638 G>A (rs859705) and c.4070 T>C (rs7144) is observed at increased frequency in aHUS patients compared to healthy controls [43].

Resistance to regulation or more potent activation

Factor B

Factor B is an essential part of the alternative complement pathway. It complexes with C3 to form the C3 and C5 convertases. Factor B mutations enhance convertase activity by increased binding to C3. Convertases with mutated factor B are more resistant to regulation by factor H and MCP. This “super-convertase” promotes the unregulated activation of the alternative pathway and enhanced C3 deposition on quiescent endothelial cells [44,45]. Factor B mutations account for less than 2% of aHUS cases.
**C3 complement component**

C3 is the hub of the whole complement system targeted by each of the three activation pathways. C3 activation is the key process of the alternative way and it is tightly controlled by the complement regulators. When a mutation occurs in the binding site of C3 for these regulators, the C3 convertase remains uncontrolled and produces large amounts of C3b. This vicious circle of positive feedback results in excessive generation of

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**Figure 3**

Diagnostic procedures and therapy
anaphylatoxins C3a and C5a, as well as C5b-9. All C3 mutations published to date are heterozygous; they account for about 5 to 10% of aHUS cases. All patients with C3 mutations identified so far exhibited low C3 levels due to consumption by constitutive activation of the alternative pathway with deficient regulation by factor H and MCP [46].

**Thrombomodulin – first protein outside complement cascade mutated in atypical hemolytic uremic syndrome**

Thrombomodulin (THBD) is the first factor outside of the complement cascade to show association with aHUS [47]. Mutations in thrombomodulin seem to be very rare (less than 5% of aHUS patients) and are frequently associated with other genetic abnormalities [31]. The proposed mechanism of action is a reduction of regulatory function of thrombomodulin on activated C3b. This mechanism is probably also potentiated by changed function of thrombomodulin in coagulation cascade where it belongs [47].

**Conclusion**

Hemolytic uremic syndrome is still an enigmatic disease showing large heterogeneity. Despite the recent major progress in pathophysiological understanding, many questions remain opened, including the predictive susceptibility of the renal endothelium to complement-mediated damage, and the linkage of complement damage and the pro-coagulatory phenotype. The recent insights regarding the underlying molecular mechanisms and the novel diagnostic and therapeutic options raise hope that this rare but devastating disease will eventually become manageable and treatment can be tailored according to the individual disease etiology. All patients with severe case of HUS with atypical presentation (severe clinical picture, complement abnormalities, atypical age of presentation) should undergo immunological studies and molecular genetic testing in a specialized laboratory.

(See diagnostic procedures and therapy in figure 3).

**Disclosure of interest:** the authors declare that they have no conflicts of interest concerning this article.

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