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The Bench-to-Bedside Transition

Familial C3 glomerulonephritis associated with mutations in the gene for complement factor B

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ABSTRACT

We report the first case of familial C3 glomerulonephritis (C3GN) associated with mutations in the gene for complement factor B (CFB). A 12-year-old girl was diagnosed with biopsy-proven C3GN. Her mother had a history of treatment for membranoproliferative glomerulonephritis, and her brother had hypocomplementemia without urinary abnormalities. DNA analysis revealed heterozygosity for CFB p.S367R in the patient, mother and brother. Evaluation of the structure–function relationship supports that this mutation has gain-of-function effects in CFB. The present case suggests that CFB has an important role in the etiology of C3GN and provides a new insight into anticomplement therapy approaches.

Keywords: C3 glomerulonephritis, complement alternative pathway, complement factor B, genetic mutation

BACKGROUND

C3 glomerulonephritis (C3GN) is a recently described disorder that results from dysregulation of the complement alternative pathway (AP) and is typically characterized by dominant C3 deposition with an absence or paucity of immunoglobulin deposition measured by immunofluorescence (IF) [1–6].

The continuous low-level activation of AP in plasma is tightly regulated by activating and regulatory complement proteins such as complement factor B (CFB), complement factor H (CFH), complement factor I (CFI) and membrane cofactor protein (MCP). CFB, a key molecule that activates the early stages of AP, is cleaved by complement factor D (CFD) into two fragments: Ba and Bb. Bb, a serine protease, then combines with C3b to generate C3 convertase, leading to the generation of a membrane attack complex. The causes of AP dysregulation in previous reported cases of C3GN include mutations in complement genes and autoantibodies that stabilize C3 convertase, or autoantibodies that affect pathway inhibition [4–6].

Here, we report a case of familial C3GN associated with mutations in the *CFB* genes. To the best of our knowledge, this is the first case report that demonstrates the involvement of CFB in the etiology of C3GN.

CASE

A 9-year-old girl was found to have proteinuria and hematuria during the Japanese school urinary screening system in May 2009. At the initial visit to a local hospital, her serum C3 level was low at 5 mg/dL (normal range, 65–135 mg/dL). In April 2012, at the age of 12 years, she developed massive proteinuria with gross hematuria and was referred to our hospital.

She had no symptoms at admission, and physical examination revealed no edema. Laboratory investigation on admission was as follows: serum total protein, 5.05 g/dL; serum albumin, 2.30 g/dL and serum creatinine, 0.43 mg/dL. By urinalysis, protein excretion was 2.1 g/day. Immunological evaluation was as follows: C3, 15 mg/dL; C4, 16 mg/dL (normal range, 13–35 mg/dL); hemolytic complement activity (CH50), 19 U/mL (normal range, 30–45 U/mL) and antinuclear antibody titer, <20-fold. Soluble C5b-9 was markedly elevated at 1.61 mg/L

compared with normal healthy controls (0.87 ± 0.22 mg/L, $n = 5$). Levels of soluble C5b-9 were determined by using a BD OptEIA™ human C5b-9 ELISA Set (BD Biosciences, San Diego, CA).

A kidney biopsy was performed and light microscopy showed membranoproliferative glomerulonephritis (MPGN); global mesangial proliferation and segmental endocapillary proliferation with lobular formation and remarkable double contour of glomerular basement membrane (Figure 1). IF analysis revealed dominant C3 deposition with an absence of immunoglobulin deposition, and electron microscopy demonstrated subendothelial, mesangial and intramembranous electron-dense deposits. She has been treated with drugs including steroid and cyclosporine for 2 years, and has now achieved partial remission with u-P/Cr of 0.2 g/g Cre and no hematuria despite persistent low C3 levels.

Regarding the family history, her mother was diagnosed with MPGN type I (IF findings were not available) in her teenage years, but she had discontinued periodic examination 18 years ago. Recent urinalysis of the mother showed moderate proteinuria with u-P/Cr of 0.5 g/g Cre without hematuria, and laboratory investigation showed hypocomplementemia (C3, 15 mg/dL) with normal kidney function. Her elder brother also had hypocomplementemia (C3, 24 mg/dL), but no urine abnormalities. Her father and younger sister had normal urinalysis and complement levels.

DNA ANALYSIS

DNA analysis was performed under written informed consent and approval of the ethics committee of Nara Prefectural Medical University, Nara, Japan, and the National Cerebral and Cardiovascular Center, Osaka, Japan. All exons of genes that encode the molecules regulating AP, *C3*, *CFB*, *CFH*, *CFI*, *MCP* and *THBD* were analyzed by direct sequencing of amplified genomic DNA obtained from a whole blood sample of the patient [7]. DNA analysis demonstrated heterozygous p.S367R in the *CFB* gene, p.R201S in the *CFI* gene and p.V916I in the *C3* gene (Figure 2A). polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the family members revealed CFB p.S367R in the patient's mother and elder brother, CFI p.R201S in her mother and younger sister and C3 p.V916I in her father (Figure 2B).

DISCUSSION

Here, we describe two cases of C3GN and one case of hypocomplementemia without urine abnormalities that occurred within a family, in which CFB p.S367R was considered to contribute to the dysregulation of AP.

It is highly likely that p.S367R causes a gain of function in CFB through a structure–function relationship. The p.S367R is located in a von Willebrand factor type A (vWfA) domain of the catalytic subunit Bb of CFB. *In vitro* experimental data demonstrated a strong association between mutations in the vWfA domain and gain of function in CFB through the promotion of high-affinity C3 binding [9]. In atypical hemolytic uremic syndrome, which also results from the dysregulation of AP, a recent report of functional analysis of *CFB* mutations revealed that six genetic changes which were concluded to have relevance to disease were all located in the vWfA domain of CFB, suggesting that this domain plays an important role in CFB function [8]. Additionally, structural evaluation demonstrates that p.S367R is located close to p.K323E/Q, one of the mutations that causes CFB gain of function as demonstrated by surface plasmon resonance analysis (Figure 2C) [8]. The p.K323E/Q showed increased resistance to inactivation of C3 convertase by CFH, and a similar functional consequence is considered for p.S367R [8].

In this study, CFB p.S367R was present concurrent with two other genetic changes, CFI p.R201S and C3 p.V916I, in the patient and in various combinations in family members; however, we speculate that these additional two genetic changes are not associated with potential disease relevance.

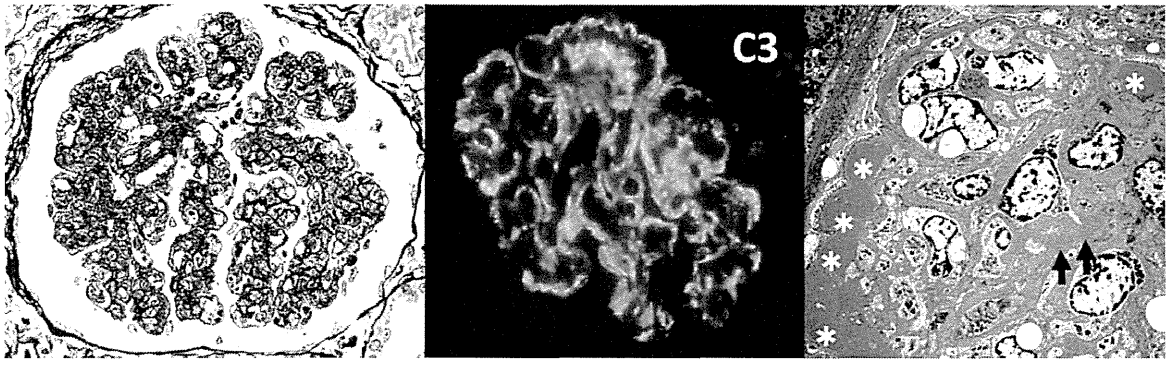

FIGURE 1: Histological findings of the patient. Light microscopy shows global mesangial proliferation and segmental endocapillary proliferation with lobular formation and remarkable double contour of glomerular basement (PAM, magnification ×400; left panel). IF shows dominant C3 deposition in the mesangium and along the capillary walls with negative immunoglobulin deposition (middle panel). Electron microscopy shows remarkable subendothelial electron-dense deposits (asterisk) and mesangial electron-dense deposits (black arrows). Mesangial interposition (white arrows), double contour of glomerular basement membrane and endocapillary hypercellularity are observed (right panel). These findings indicate C3GN rather than dense-deposit disease, because electron-dense deposits were mainly observed not within the glomerular basement membrane but in the subendothelial and mesangial areas, while ribbons of electron-dense transformation of glomerular basement membranes are not found.

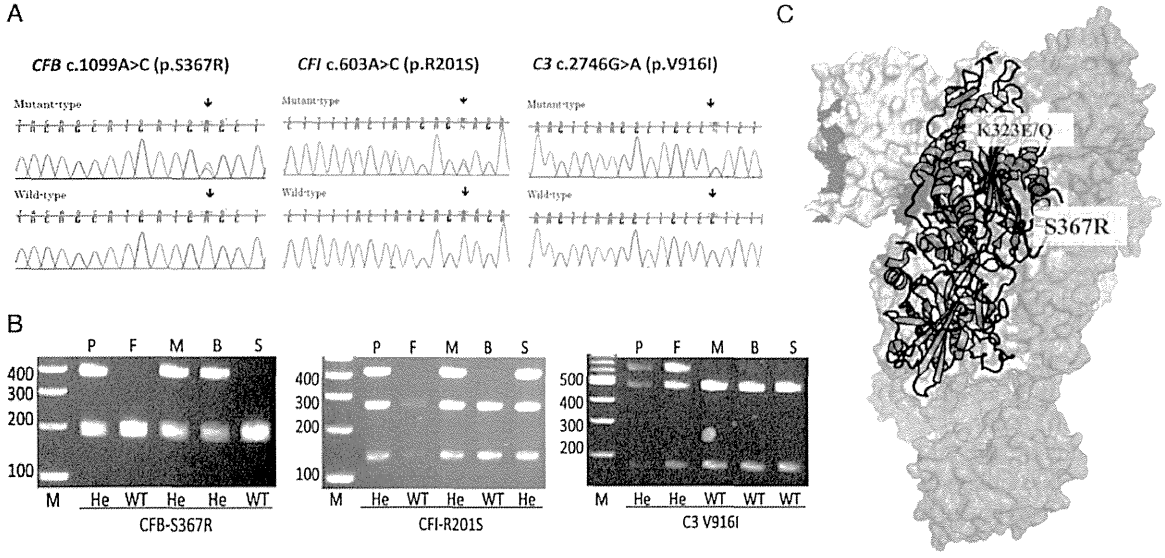

FIGURE 2: DNA analysis of the patient and family members and structural evaluation of CFB S367R. (**A**) Direct sequencing of patient genomic DNA shows heterozygous p.S367R (c.1099 A>C) in exon 8 of the *CFB* gene, p.R201S (c.603 A>C) in exon 4 of the *CFI* gene and p.V916I (c.2746 G>A) in exon 21 of the *C3* gene. The Met encoded by the translation initiation site (start codon) is numbered as residue 1. Upper row: mutant type, lower row: wild type. (**B**) PCR-RFLP analysis of family members shows CFB p.S367R in the mother and elder brother, CFI p.R201S in the mother and younger sister and C3 p.V916I in the father. P, patient; F, father; M, mother; B, elder brother; S, younger sister; WT, wild type; He, heterozygous. (**C**) Visualization of the complex of CFB, CFD and C3b. The figure was prepared using PyMOL (www.pymol.org). Each molecule is represented as follows: CFB, gray; CFD, yellow; C3b, cyan and p.S367R, red sphere. p.S367R is not located attached to CFD or C3b but rather on the surface of CFB and close to p.K323E/Q (blue sphere), of which the gain of function of CFB was proven by surface plasmon resonance analysis in a previous report [8]. p.K323E/Q was described as p.K298E/Q in the original article, with the notation using the nomenclature system of mature proteins.

First, the mutations of p.R201S and p.V916I had no impact on the phenotype of her sister and father, respectively. Second, although p.R201S was detected in the patient and her mother, both of whom had C3GN, p.R201S is a polymorphic allele found in Far East populations, with a frequency of about 0.03 in Japan [10]. However, functional assays of mutant CFB, CFI and C3 to assess their involvement in the activation of AP are required to prove a detailed etiological mechanism.

Some limitations exist in this study. C3NeF and other autoantibodies were not investigated; these autoantibodies may also be associated with our cases, concomitant with *CFB* mutations. Although low C3 levels and elevated soluble C5b-9 levels indicate continuous activity of the AP, detailed complement investigations (e.g. the measurement of Ba, Bb, C3a, C3d and C5a) are required to clarify the complement activation mechanisms more precisely.

In conclusion, this study suggests that CFB has a critical role in AP in the pathogenesis of C3GN and expands our understanding of the genetic factors conferring predisposition to C3GN and supports the development of anticomplement therapies, including those targeting CFB activation.

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CONFLICTS OF INTEREST STATEMENT

None declared.

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Clinical Case Reports

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CASE REPORT

STEC:O111-HUS complicated by acute encephalopathy in a young girl was successfully treated with a set of hemodiafiltration, steroid pulse, and soluble thrombomodulin under plasma exchange

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Key Clinical Message

We report a 14-year-old girl, who developed shigatoxin-producing *E. coli* (STEC)-HUS complicated by encephalopathy. She was successfully treated with hemodiafiltration, high-dose methylprednisolone pulse therapy, and soluble recombinant thrombomodulin under plasma exchange. von Willebrand factor multimers analysis provides potential insights into how the administered therapies might facilitate successful treatment of STEC-HUS.

Keywords

Encephalopathy, *Escherichia coli* O111, hemolytic uremic syndrome, plasma exchange, recombinant soluble thrombomodulin, von Willebrand factor.

Introduction

Hemolytic uremic syndrome (HUS) is a life-threatening disease, characterized by microangiopathic hemolytic anemia, destructive thrombocytopenia, and renal failure [1]. Most HUS occurs in association with Shiga toxin-producing *Escherichia coli* (STEC) infection [2]. Patients with STEC-HUS generally recover with fluid therapy and hemodialysis. Mortality is high among STEC-HUS patients with encephalopathy, despite treatments including plasma exchange, steroid pulse, and more recently eculizumab [3]. In recent STEC outbreaks in the United States (STEC-O111) and Germany (STEC-O104) in 2008 and 2011, respectively [4, 5], STEC-HUS incidence and mortality were 16.7% and 3.8% and 22% and 3.7%, respectively.

In 2011, an outbreak of STEC-O111 and/or -O157 infection in Toyama, Japan occurred following raw meat ingestion in a barbecue restaurant chain. Overall, 181 patients were infected, of whom 34 developed STEC-HUS (18.8%) including 21 with encephalopathy (61.8%) and five deaths (14.7%; all with encephalopathy) [6–8]. Ten STEC-HUS patients were aged 1–14 years, including eight with encephalopathy [7]. Seven children including five

with encephalopathy recovered and three died [7]. We report clinical and laboratory findings for a 14-year-old girl in the Toyama series with STEC-HUS and encephalopathy.

Case Report

In April 2011, a 14-year-old girl ingested raw meat in a barbecue restaurant in Toyama, and then traveled to Osaka. Bloody diarrhea developed 5 days later. At a local hospital, levofloxacin was prescribed without improvement. Six days later after raw meat ingestion, she was transferred to Yodogawa Christian hospital. Almost simultaneously, multiple outbreaks of hemorrhagic enterocolitis due to STEC: O111 (producing both shiga-toxin-1 and -2) were reported from several hospitals around Toyama. All affected patients had eaten raw meats in the same chain restaurants around Toyama. Admission laboratory findings included: white blood cell (WBC) [24,700/μl], red blood cell (RBC) [5.28 $\times$ 10^6/μL], hemoglobin (Hb) [16.7 g/dL], platelet [143 $\times$ 10^3/μL], C-reactive protein (CRP) [3.55 mg/dl], lactate dehydrogenase (LDH) [227 IU/L], blood urea nitrogen (BUN) [15.6 mg/dL], creatinine (Cr) [0.69 mg/dL], normal hemostatic tests, proteinuria, and no hematuria. Stool cultures showed normal flora, stool shigatoxin stool was negative, and both the antigens of STEC:O111 and O157 in stool were negative.

On day 3, the patient developed anemia (RBC [2.63 $\times$ 10^6/μL], Hb [8.2 g/dL], LDH [1148 IU/L], haptoglobin [8 mg/dl], and thrombocytopenia [12,000/μl], with an increase in BUN [26.6 mg/dL] and Cr [1.06 mg/dL] as shown in Figure 1). Schistocytes were seen in the peripheral blood smear. Plasma ADAMTS13 activity levels were 43% of normal. The patient became anuric and comatose (Glasgow Coma Scale [GCS] 14). Continuous hemodiafiltration was initiated with plasma exchange. On day 5, pleural effusions developed, respiratory function worsened, and consciousness deteriorated further. Intubation was performed. Brain magnetic resonance imaging showed high intensity areas in the bilateral thalamus and basal ganglia, and part of the pontine tegmentum on T2 FLAIR images (Fig. 1 Inset). Acute encephalopathy developed. STEC-HUS was diagnosed. High-dose methylprednisolone pulse therapy [500 mg/day] for days 5–7 was administered. On day 6, serum antibodies to STEC:O111 antigen were noted. On day 9, hemolysis worsened, whereas severe thrombocytopenia persisted. Plasma exchange was increased to twice daily. A second 3-day course of a high-dose methylprednisolone pulse therapy was administered. Gabexate mesilate, a synthetic anticoagulant was administered. Serum levels of fibrin/fibrinogen degradation product (FDP) and thrombin–antithrombin complex (TAT) increased to 120 μg/mL and 24.3 ng/mL, respectively. Soluble recombinant thrombomodulin (130 units/kg/day) was infused during days 9–14. Clinical and laboratory findings subsequently improved, including thrombocytopenia, hemolysis, and renal function (Fig. 1). Extubation occurred on day 22. Plasma exchange was tapered, and discontinued on day 24. After rehabilitation, the patient was discharged without appreciable sequelae on day 64.

Retrospective analyses of stored plasma samples were performed. Plasma samples from admission showed that levels of the following cytokines were not elevated: interleukin (IL)-6 [4 pg/mL (normal: <4)], IL-8 [59 pg/mL (normal: <2)], and tumor necrosis factor (TNF)α [12 pg/mL (normal: <15)]. In contrast, plasma samples from admission identified elevated levels of neopterin [98 nmol/L (normal: <5)], soluble form TNF receptor type I (sTNF-RI) [13,200 pg/mL (normal: 484–1407)], sTNF-RII [18,300 pg/mL (normal: 829–2262)], and tau protein [344 pg/mL (normal: undetectable)]. Plasma samples from day 3 identified reduced plasma ADAMTS13 activity (43%) levels and high levels of plasma VWF antigen levels (605% of normal).

Retrospective analysis of plasma VWF multimer patterns using citrated plasma samples (frozen at −80°C) was also performed (Fig. 2). During the acute phase, no high-to-intermediate sized VWF multimers were identified in samples taken three and 13 days prior to initiation of plasma exchange. After each plasma exchange, VWF multimer patterns were present, although high-sized VWF multimers continued to be absent. Plamsa exchange was performed once or twice daily until day 20, then tapered, and discontinued on day 24. UL-VWF multimers appeared in plasma at days 21 and 24, and disappeared at day 61 just before discharge. At discharge, plasma levels of VWF and ADAMTS13 had returned to almost normal ranges.

Discussion

We report a patient with STEC-HUS, mild-to-moderate reduction of plasma ADAMTS13 activity, and increased plasma levels of VWF antigen. Despite persistent thrombocytopenia in the acute phase, VWF multimers were degraded on one occasion and highly multimerized on a different occasion. Therapy with continuous hemodiafiltration, high-dose methylprednisolone pulse therapy and soluble recombinant thrombomodulin was successful and the patient was discharged without any deficits. In explaining our findings, several factors should be considered.

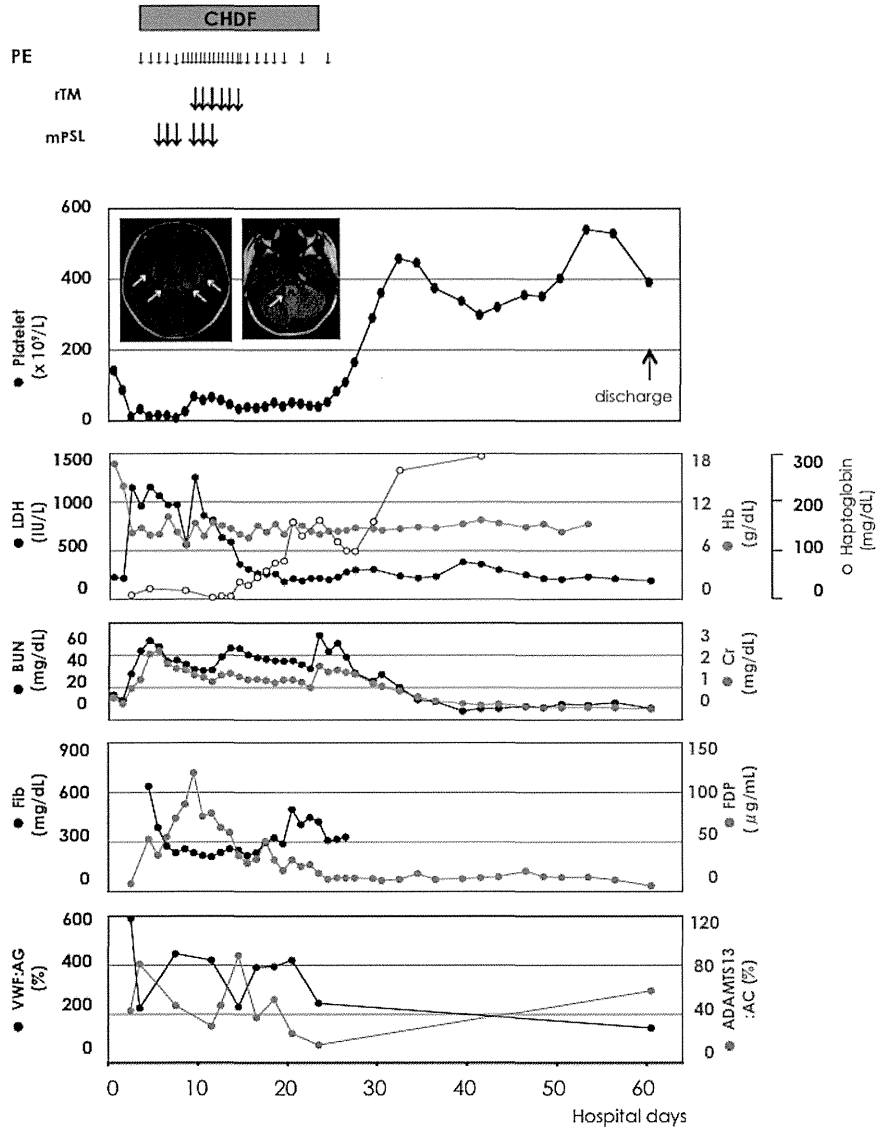
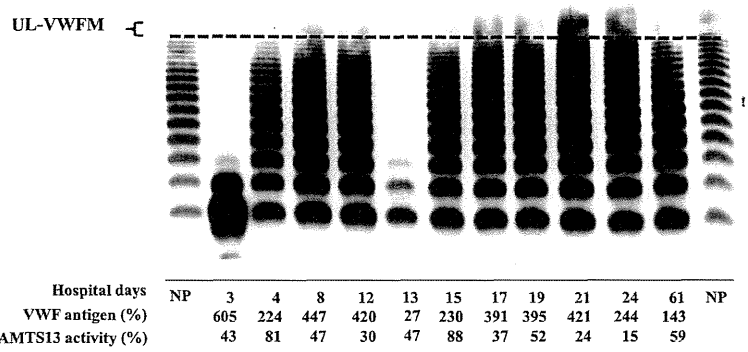


Figure 1. Clinical course in a 14-year-old girl with STEC-HUS complicated by acute encephalopathy after admission.

First, identification of UL-VWF multimers in this patient differs from the VWF pattern usually seen with STEC-HUS where the multimers are usually depleted. UL-VWFMs, stored in Weibel–Palade bodies (WPBs) of vascular endothelial cells, are released upon stimulation by inflammatory cytokines, such as IL-6, IL-8, and TNFα [9]. Likewise, UL-VWFMs are released into the circulation by injured vascular endothelial cells. On admission, plasma levels of cytokines including IL-8, neopterin, TNF-RI and RII, and tau protein were high, indicating vascular injury, inflammation, and neurological cell damages [6]. Also, the B-subunit of shigatoxin-1 and -2, both AB5-holotoxins, binds to globotrialosyl ceramide (Gb3) by which UL-VWFMs are released from Weibel–Palade bodies [10]. Shigatoxin binds to Gb3, internalizes, and blocks protein synthesis by attachment to ribosomal RNA. Shigatoxin also directly enhances platelet aggregation under high and low shear stress at very low concentrations [11]. Thus, in our patient, UL-VWFM, may have been released excessively from activated vascular endothelial cells, was involved in platelet thrombi formation, and then was consumed by proteases released from platelets and/or leucocytes.

Second, our findings may explain how plasma exchange may have had therapeutic benefit in this patient. In par-



Hospital days	NP	3	4	8	12	13	15	17	19	21	24	61	NP
VWF antigen (%)		605	224	447	420	27	230	391	395	421	244	143	
ADAMTS13 activity (%)		43	81	47	30	47	88	37	52	24	15	59	

Figure 2. Change of VWF multimer patterns during the acute phase.

ticular, plasma exchange might work bifunctionally: one effect was to reduce concentrations of various cytokines, UL-VWFM, and shigatoxin, and the other effect was to supply normal VWFM (for hemostasis). During the acute phase of STEC-HUS, the STEC vigorously produces shigatoxin, which consistently activates platelets, even at low concentrations (pg/ml). So, plasma exchange alone for STEC-HUS is likely to be inefficient, unless shigatoxin function is blocked. Hence, in addition to basic supportive therapy for STEC-HUS such as dialysis and fluid therapy, cytokine adsorption is favorable, and high-dose methylprednisolone pulse therapy might suppress cytokine production [12].

Third, in comparison to previous reports, the occurrence of acute encephalopathy associated with STEC-HUS in Toyama was high, and the deceased cases had encephalopathy. This toxicity is attributable to brain edema, presumably due to increased vascular permeability and/or severe vascular endothelial cell injuries mediated by shigatoxin itself and cytokines, yet the mechanism is not fully understood [13]. Strains of STEC:O111 isolated in Toyama predominantly produced shigatoxin-2, which is more toxic than shigatoxin-1. However, a peculiar MRI finding on high intensity areas, often symmetrical in thalamus, basal ganglia, and pontine tegmentum, has not been favorably addressed [14].

Fourth, common therapeutic features on seven survived childhood patients in Toyama included continuous hemodiafiltration, high-dose methylprednisolone pulse therapy, and recombinant thrombomodulin. High-dose intravenous immunoglobulin infusion was administered to six of the seven survivors. Administration of recombinant thrombomodulin may have been particularly important, as this drug has been available in Japan as treatment for disseminated intravascular coagulation (DIC) since 2008 [15]. Recombinant thrombomodulin is a multifunctional protein. A lectin-like domain directly absorbs and neutralizes high mobility group box1 (HMGB1), which is a pro-inflammatory cytokine that acts as a lethality factor when endotoxin shock occurs [16]. Also, EGF-like domains 4–6 of the recombinant thrombomodulin can bind thrombin and inactive the catalytic activity of thrombin. The thombin–recombinant thrombomodulin complex can accelerate activation of protein C and thrombin activatable fibrinolytic inhibitor (TAFI) to activated protein C and TAFIa, respectively. In turn, activated protein C generates anticoagulant action via inactivation of Va and VIIIa and TAFIa suppresses complement activation via inactivation of C3a and C5a [15]. As the action of recombinant thrombomodulin on platelets remains unclear, we are unable to directly address how recombinant thrombomodulin can resolve STEC-HUS. There are at least two possibilities: one is direct inhibitory activity to platelet aggregation, and the second is to block fibrin clot formation over platelet thrombi, as suggested by significant increases of FDP and TAT during the clinical course before recombinant thrombomodulin is administered.

In conclusion, we report a novel therapy for STEC-HUS. VWF-dependent hemostatic defect that is generated in STEC-HUS appears to have been restored by plasma exchange. Hypercoagulability, presumably induced by shigatoxin or cytokine storms, appears to have been suppressed with high-dose methylprednisolone pulse therapy and recombinant thromobomodulin.

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Conflict of Interest

Nothing to declare.

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特集：TTP/HUS/aHUS

補体・凝固関連 aHUS の病態

Pathogenesis of complement-mediated and coagulation-mediated atypical hemolytic uremic syndrome

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Hideki KATO, Yoko YOSHIDA, and Masaomi NANGAKU

要　旨

非典型溶血性尿毒症症候群は，近年急速に原因遺伝子が判明し，病態が解明されてきた稀少疾患である。病原性大腸菌感染を伴わないHUSのなかから，補体経路の遺伝子異常が次々と見つかり，本邦においても2013年にaHUSの診断基準が発表された。また，さらに最近では凝固系の遺伝子異常もaHUSの原因として同定されてきており，aHUSの病態解明が急速に進んでいる。

本稿では，補体関連，凝固関連のaHUS分類，また病態について概説する。

補体・凝固関連 aHUS の歴史

血栓性微小血管症(thrombotic microangiopathy：TMA)は，微小血管症性溶血性貧血，消耗性血小板減少，毛細血管内血小板血栓を3主徴とする症候群である。代表的疾患として溶血性尿毒症症候群(hemolytic uremic syndrome：HUS)と血栓性血小板減少性紫斑病(thrombotic thrombocytopenic purpura：TTP)とがある。以前は，臨床的に消耗性の血小板減少症，微小血管での溶血性貧血，急性腎障害の3徴を呈する疾患をHUS，さらに発熱，動揺性精神神経障害の5徴を示す疾患をTTPと臨床的に鑑別したが，両者は臨床症状のみでは鑑別しえない場合が多かった。近年その病因が解明されつつあり，志賀毒素を産生する病原性大腸菌によるものを典型HUS，ADAMTS13活性が低下したものをTTPと称している。HUSの約90％は血性下痢を伴う志賀毒素産生性大腸菌感染によるものであるが，残りの約10％は，下痢を伴わず，志賀毒素も検出されないことから，かつてはD(－)HUSと呼ばれ，原因が不明であった。志賀毒素産生性大腸菌感染によるHUSが典型HUSであることに対し，頻度が少ないながらも志賀毒素産生性大腸菌感染を伴わないHUSは非典型溶血性尿毒症症候群と呼ばれていた。また家族性のHUSも1975年に報告されていたが，原因は不明であった[1]。1981年に兄弟でcomplement factor H(CFH)の蛋白量の減少を示しHUSを呈する例が報告され，この疾患が劣性遺伝を示すことから遺伝性のHUSの存在が示唆されていた[2]。その後，1998年にWarwickerらの連鎖解析により，CFHの遺伝子異常が示され，これが遺伝性HUSの最初の遺伝子異常の報告である[3]。その後，C3やcomplement factor B(CFB)，complement factor I(CFI)，membrane cofactor protein(MCP)，CFHR1/3などの補体関連遺伝子異常によるHUSが次々と見つかってきたため，遺伝性のaHUSは補体制御の遺伝子異常と捉えられてきた。

しかし2009年には，thrombomodulin遺伝子の変異によるaHUSがNEJMに報告され[4]，2013年にはdiacylglycerol kinase ε(DGKE)[5,6]の変異が報告され，さらに2014年にはplasminogenといった明らかに補体系ではなく，凝固系制御因子と考えられる原因遺伝子も報告された[7]。歴史的にaHUSは志賀毒素によるHUSではないatypicalなHUSとして，補体関連因子の異常から発見されてきており，またthrombomodulinも補体系への異常が*in vitro*で示されており，凝固関連因子の異常も補体関連aHUSと呼ばれてきたが，下記のように，これまでの補体関連aHUSを補体系と凝固系の異常に分ける分類も提唱されている。

aHUS の分類

本邦においても2008年に，信州大学からCFH missense変異をヘテロ接合体で持つaHUS症例が報告され[8]，以後

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表　aHUSの遺伝子異常と予後

遺伝子	蛋白名	変異の影響	頻度 欧米(本邦)	血漿交換の 短期的効果	血漿交換の 長期的効果	腎移植後の腎予後
CFH	Factor H	内皮に結合できない	20～30％ (10％)	寛解率60％	死亡または腎死 70～80％	再発率80～90％
CFHR1/3	Factor HR1，R3	抗H因子抗体の出現	6％ (6％)	寛解率70％	腎死30～40％	再発率20％
CD46(MCP)	Membrane cofactor protein	内皮表面の発現低下，補体制御機能低下	10～15％ (13％)	一般的に軽症	死亡または腎死 20％以下	再発率15～20％
CFI	Factor I	Cofactor機能低下	4～10％ (0％)	寛解率30～40％	死亡または腎死 60～70％	再発率70～80％
CFB	Factor B	C3 convertase活性化	1～2％ (3.3％)	寛解率30％	死亡または腎死 70％	1例再発の報告
C3	Complement C3	C3b不活化低下	5～10％ (43％)	寛解率40～50％	死亡または腎死 60％	再発率40～50％
THBD	Thrombomodulin	C3b不活化低下	5％ (3.3％)	寛解率60％	死亡または腎死 60％	1例再発の報告
DGKE[6]	Diacylglycerol kinase epsilon	DAGシグナルによる血栓形成	不明，2013年に13例の報告 (報告なし)	不明	20歳までの腎死が多い。	3例中1例が移植後腎死
PLG[7]	Plasminogen	血栓形成	5％？ (報告なし)	不明	不明	不明

(文献1より引用，改変)

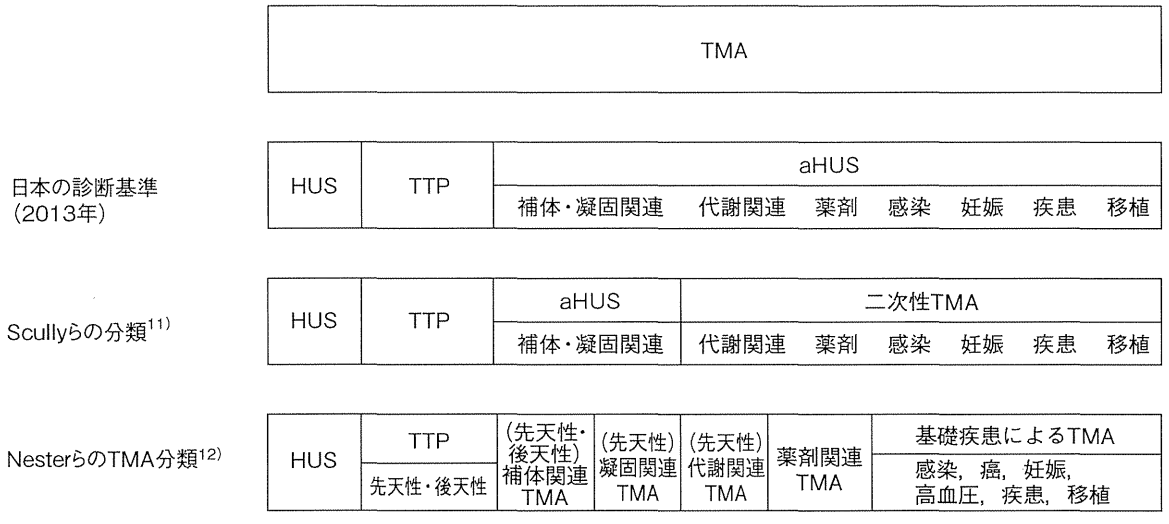

図1　TMAやaHUSの各分類のまとめ

は本邦においても次々とaHUS症例が報告されてきた。このような背景から，2013年には日本腎臓学会と日本小児科学会から「非典型溶血性尿毒症症候群(aHUS)診断基準」が報告された[9,10]。日本の診断基準では，TMAは典型HUS，TTP，それ以外はaHUSと分類し(広義のaHUS)，aHUSのなかに補体系・凝固系制御異常のaHUS(狭義のaHUS)，代謝異常，感染症，薬剤性，移植後，他の疾患のある二次性TMAを含めている(図1)。Scullyらによる分類では，TMA

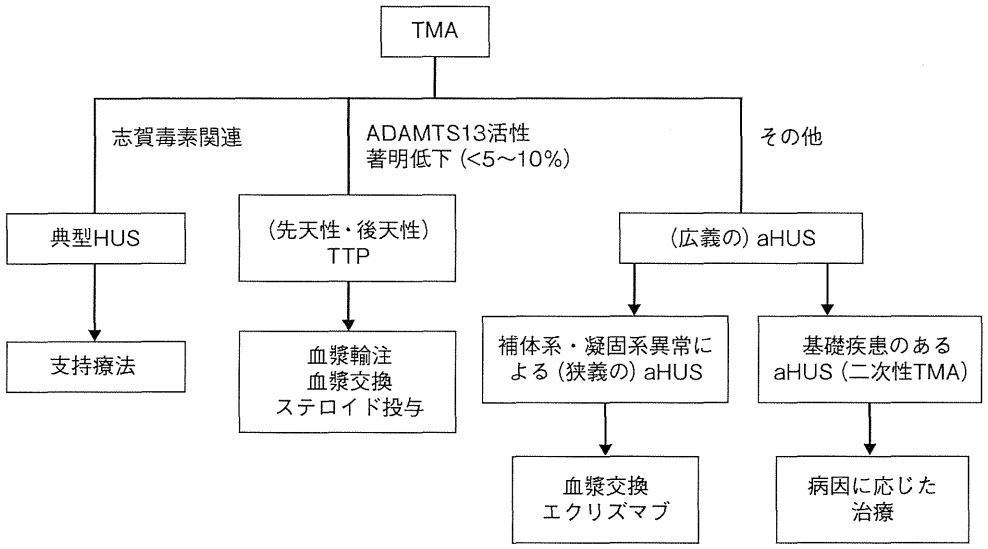

図 2　典型 HUS，TTP，aHUS の診断フローチャートと典型的な治療

全体から HUS，TTP を診断し，また基礎疾患がある TMA を二次性 TMA として除外し，それ以外を aHUS としている[11]。

また，2014 年の米国の Nester らによる NEJM の TMA の総説では，aHUS の atypical という用語は歴史的に HUS や TTP に対して使われてきただけであり，aHUS の原因もはっきりしてきたので，aHUS という用語は使用せず，すべてを TMA と呼び，TMA 全体を 9 つの原因に分類した。彼らは TMA 全体を先天性と後天性に分け，先天性の TMA を，①ADAMTS13 欠損 TMA（先天性 TTP，Upshaw-Schulman syndrome），②補体関連 TMA（現在までに判明している遺伝子異常では CFH，CFI，CFB，C3，CD46，CFHR など），③代謝関連 TMA，④凝固関連 TMA（現在判明しているもので thrombomodulin，DGKE，plasminogen）と分類し，後天性 TMA を⑤ADAMTS13 欠損関連 TMA（後天性 TTP），⑥志賀毒素関連 TMA（志賀毒素関連 HUS），⑦薬剤関連 TMA（免疫反応によるもの），⑧薬剤関連 TMA（毒性によるもの），⑨補体関連 TMA（②とは異なり factor H 抗体出現によるもの）と分類している[12]。各分類の違いを**図 1** にまとめた。

Nester らの分類は最終診断がついたうえでの病態学的な分類である。遺伝子異常や特殊な血液検査（CFH 抗体の有無など）の結果が判明し，最終診断には時間がかかるため，臨床的には**図 2** のように本邦の aHUS の診断基準に沿った形のフローチャートに従って鑑別を進めておくのがよいと思われる。また Nester らの分類では，二次性 TMA のうち基礎疾患による TMA（例えば移植後，強皮症，悪性高血圧

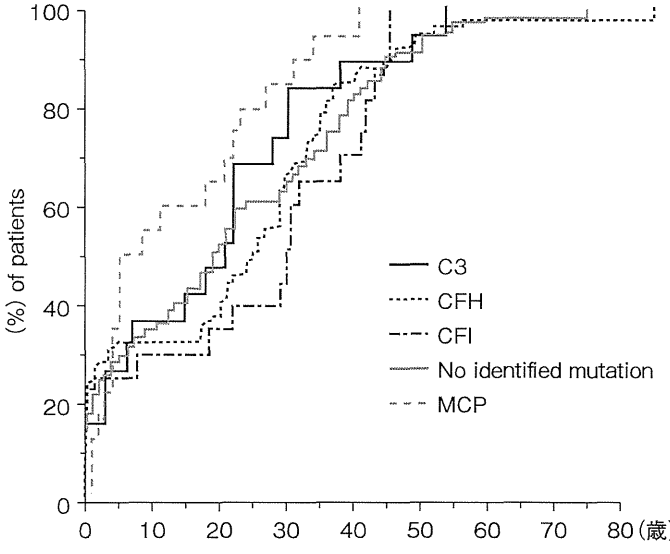

図 3　aHUS の発症年齢（文献 14 より引用）

による TMA など）は 9 つの分類には入っていない。今後，国際的な分類や名称の統一が待たれるところである。

補体・凝固関連 aHUS の疫学

補体・凝固関連 aHUS の正確な発症数は不明であるが，大人では 100 万人当たり年間 2 人程度，小児では 100 万人当たり年間 3.3 人との報告がある[13]。本邦での最初の報告が2008年であり，診断体制も近年になり整いつつある段階であるため，正確な本邦での発症数は不明であるが，これ

【非典型溶血性尿毒症症候群の治療】

Treatment of atypical hemolytic-uremic syndrome

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Hideki Kato　　Masaomi Nangaku

Key words
atypical hemolytic-uremic syndrome, eculizumab

要　約

非典型溶血性尿毒症症候群（aHUS）は小児のみならず大人にも認められ，溶血性貧血，血小板減少症，急性腎障害を３徴とする希少疾患である。現在までにいくつかの遺伝子異常が報告され，また2012年には日本腎臓学会と日本小児科学会から合同でaHUSの診断基準が発表されており，本邦においてもこれまで見逃されていた本疾患の診断が増加すると考えられる。本稿では，aHUSの治療，また最近になり本邦でも保険適応となった抗C5モノクローナル抗体であるエクリズマブの治療効果について，最近の報告を概説する。

はじめに

非典型溶血性尿毒症症候群（aHUS）は1981年に遺伝性疾患であることが示唆され，その後多くの原因遺伝子が報告されてきた。また治療法も血漿療法，腎移植，肝・腎移植，抗C5モノクローナル抗体療法と進歩を遂げてきた。本稿ではaHUSの治療について概説する。aHUSの病態生理については，本号の藤村，吉田，宮田らの稿に譲る。

1．aHUSと予後

aHUSは半数以上が小児期に発症するが，成人発症も稀ではない。多くは重度の溶血性貧血，血小板減少症，急性腎障害を発症し，これ以外に中枢神経症状などの多臓器症状も約20%で認められる。血漿交換が施行されるようになり，予後は改善されたが，aHUS全体では約半数が末期腎不全に陥り，死亡率は約25%にも達する予後不良な疾患である。aHUSの中で，CFH，CFHR1/3，MCP，CFI，CFB，C3，THBD，DGKEの遺伝子変異で説明できる患者は約70%程度と，次々と原因遺伝子が見つかってきており，各遺伝子変異により，短期的，長期的な予後は異なる（表）[1]。欧米で最も頻度の高い変異はCFHであり，本邦で最も多いのはC3の変異であるが，いずれも予後は悪く，MCP変異の予後が最も良い。

2．aHUSの治療　血漿交換療法

aHUSの治療は1980年代から長らく血漿交換療法であった。血漿交換の効果としては，異常補体関連蛋白や，抗CFH抗体を除去し，正常補体関連蛋白を補充することにあると考えられる。The European Pediatric Study Group for HUSは，aHUSと診断されたら24時間以内に血漿交換を開始し，5日間連続で施行し，徐々に減量していく治療を勧めている。遺伝子変異や，血清蛋白質学的解析には時間がかかるため，aHUSと診断されたら確定診断を待たずに血漿交換を施行する。しかし，身体の小さい患者や状況に応じて血漿輸注が施行されることもある。通常は血小板数，LDH，ヘモグロビンの推移を見て，改善，または正常化したらtaperしていく。

aHUS全体としては，血漿輸注や血漿交換により，約70%が血液学的寛解に至り，それまでの死亡率50%から25%へと減少させたが，長期的には再発，腎不全の進行が認められる（表）[1,2]。

長期血漿交換により，アレルギー反応や，ブラッド

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表　aHUSの遺伝子異常と予後（文献1より改変）

遺伝子	蛋白名	変異の影響	頻度欧米（本邦）	血漿交換の短期的効果	血漿交換の長期的効果	腎移植後の腎予後
CFH	Factor H	内皮に結合できない	20～30%（10%）	寛解率60%	死亡または腎死70～80%	再発率80～90%
CFHR1/3	Factor HR1, R3	抗H因子抗体の出現	6%（6%）	寛解率70%	腎死30～40%	再発率20%
MCP	Membrane cofactor protein	内皮表面の発現低下、補体制御機能低下	10～15%（13%）	一般的に血漿交換は行なわない	死亡または腎死20%以下	再発率15～20%
CFI	Factor I	Co-factor機能低下	4～10%（0%）	寛解率30～40%	死亡または腎死60～70%	再発率70～80%
CFB	Factor B	C3 convertase活性化	1～2%（3.3%）	寛解率30%	死亡または腎死70%	1例再発の報告
C3	Complement C3	C3b不活化低下	5～10%（13%）	寛解率40～50%	死亡または腎死60%	再発率40～50%
THBD	Thrombomodulin	C3b不活化低下	5%（3.3%）	寛解率60%	死亡または腎死60%	1例再発の報告
DGKE[16]	diacylglycerol kinase epsilon	DAGシグナルによる血栓形成	不明、2013年に13例の報告			

アクセス不全・感染などの合併症がある。また腎機能を改善させるほどには効果は期待できないことが多い。血漿療法を施行しても反応しない場合には，エクリズマブの治療を検討する。

3. aHUSの治療　エクリズマブによる治療

補体の最終経路の活性化が，aHUSの内皮細胞障害の発症に重要とされているが，エクリズマブ（商品名　ソリリス）はヒト型リコンビナント・モノクローナル抗体で，C5に結合することにより，C5からC5aとC5bへの分解を押さえ，C5aとmembrane attack complex C5b-9の産生を抑制する。元々は発作性夜間ヘモグロビン尿症の治療薬として開発され，2007年に欧米で，2010年には本邦でも承認された。2009年にNEJMにおいて，難治性aHUSに対してエクリズマブを使用し，劇的に改善した2例が報告され[3, 4]，これらの報告以降，著効例が多数報告され[5]，2011年には米国で，2013年9月には本邦でもaHUSに対してエクリズマブが承認された。

投与方法は，1A 300mg/30mLの本剤を生食または5%グルコース溶液などで，5mg/mLに溶解し，成人18歳以上では1回900mgを25から45分かけて週1回で4回投与し，導入期とする。以後の維持期は4週間目から1回1200mgを2週に1回投与するのが日本での標準的な投与法である。

2013年にNEJM誌に37名のaHUSに対してエクリズマブを使用したphase2の臨床試験の結果が報告された[6]。血小板が低く，腎障害がありTMA症状を呈している患者群Trial1（17名，罹病期間の中央値は9.7か月）と，血小板は正常であるが腎障害があり，長期血漿療法を受けている患者群のTrial2（20名，罹病期間の中央値は48.3か月）の患者について調べられた。Trial1の患者では，血小板数は治療後第26週で平均7.3万／μL上昇し，血小板数低値であった13人全員が正常化した。TMA症状に関しては17人中15人で26週にわたってTMA症状が認められず，全期間にわたって血漿交換を必要としなかった。腎障害に関しては，第26週でeGFRが平均で32mL/min／1.73m^{2}の増加が認められ，5人中4人の透析患者が透析を離脱で

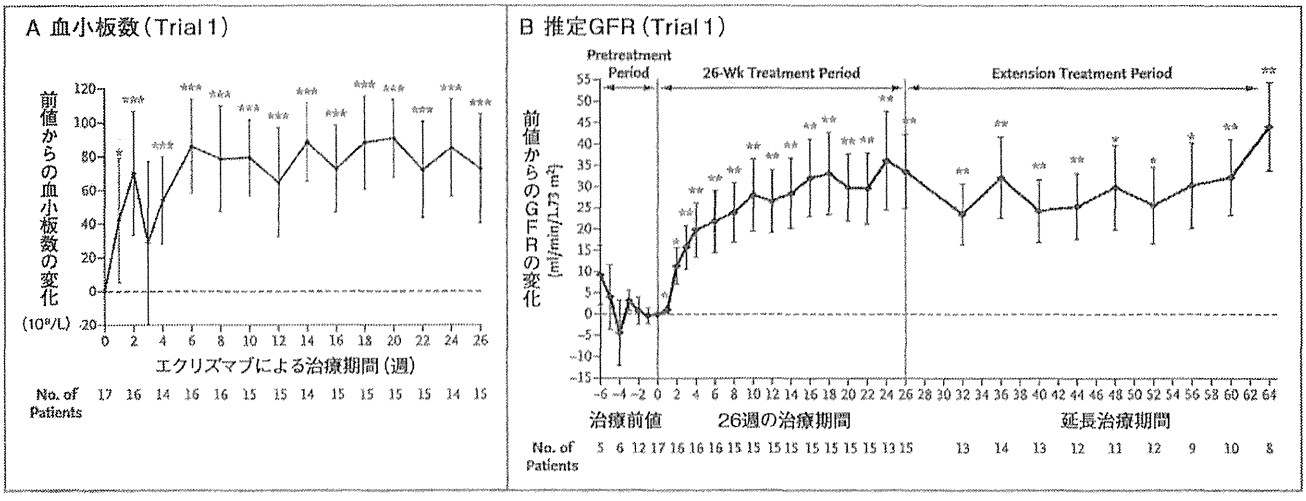

図1　Trial 1 患者のエクリズマブ使用後の血小板と eGFR の推移（文献 6 より改変）

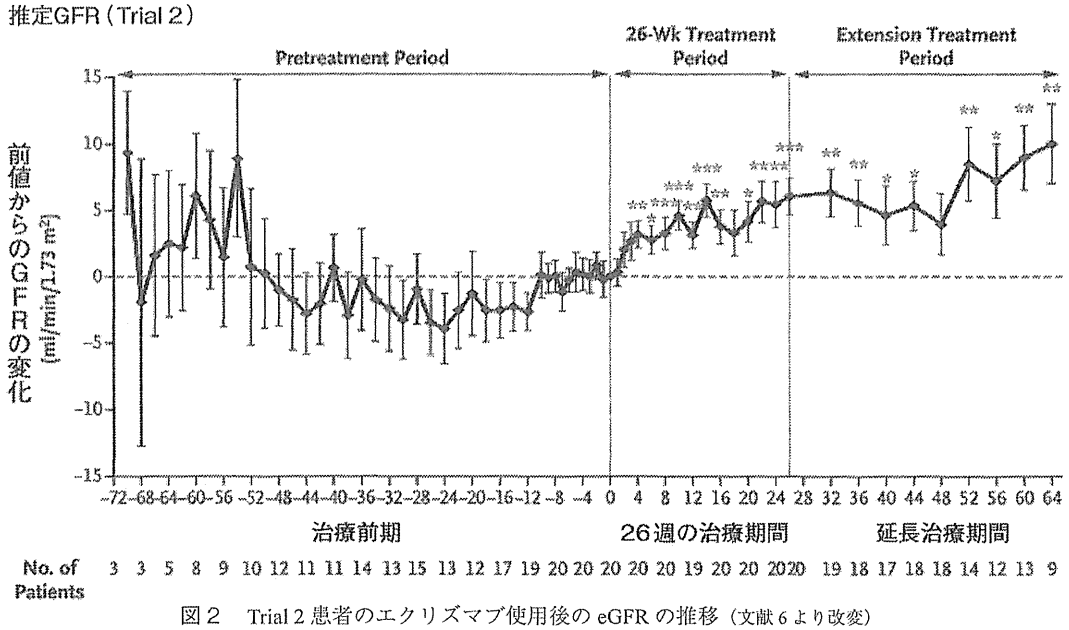

図2　Trial 2 患者のエクリズマブ使用後の eGFR の推移（文献 6 より改変）

きた（図1）。また発症から治療開始までの期間が短いほど，eGFR 改善傾向が認められた。

Trial2 では 20 人の内，80% が第 26 週まで血小板数が減少せず，TMA 症状が発症しなかった。腎障害に関しては，6mL/min ／ 1.73m² の増加が認められ，また蛋白尿の減少も認められた（図2）。

副作用としては，（全員が臨床試験の前に髄膜炎菌ワクチンを投与されていたが）髄膜炎菌の発症は 0 で，感染関連の重大な副作用も認められなかった。一部の患者に高血圧，腹膜炎，インフルエンザ，静脈疾患などが認められた。

今後はさらに遺伝子変異ごとの治療反応性，長期予後についての報告が待たれるところである。

4. aHUS の腎移植

aHUS 患者は，血漿療法に反応しない場合や，診断が遅れることにより，末期腎不全に進行し，エクリズマブを使用しない場合には，約 50% の患者は最終的に末期腎不全に至る。これまでの報告では，aHUS に対する腎移植後の 5 年腎生存率は全体として約 50% 程度であり，元々の遺伝子変異のタイプ，aHUS の再

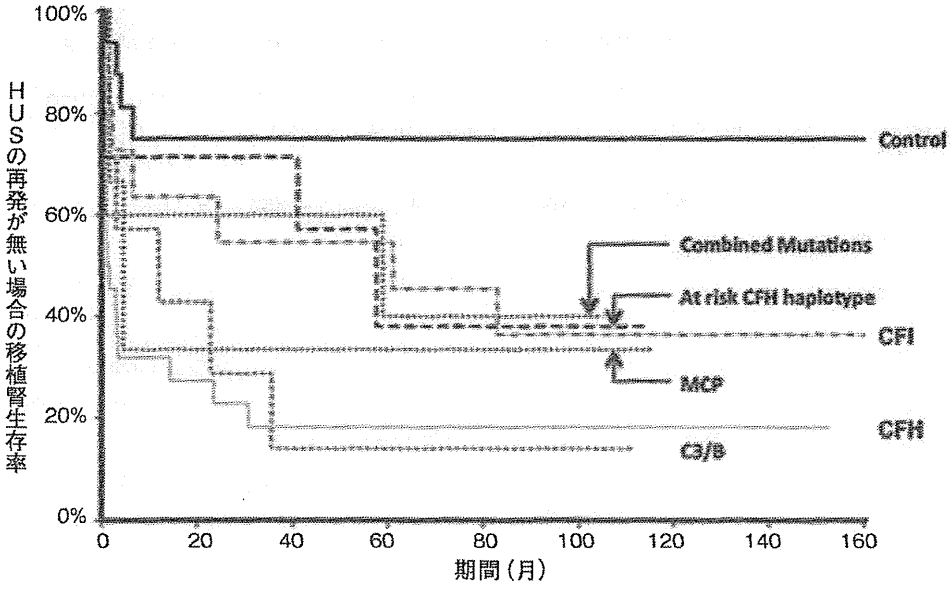

図３　各原因遺伝子別 aHUS 患者の，エクリズマブを使用しない場合における腎移植後の腎生存率（aHUS の再発が無い場合）（文献７より改変）

発の有無が，移植腎喪失の決定要因となっていると報告されているため[7]，腎移植の前には原因遺伝子の検索，血清蛋白質学的検査を施行しておくことが推奨されている（図3）。エクリズマブが使用可能となり，近年では腎移植を受ける患者は事前にエクリズマブを投与し，1から2回の血漿交換を施行してから腎移植を行うのが良いとされる。10人の患者に対して，移植前後からエクリズマブを使用した例では，1例の術後血栓例を除いて数ヶ月から3年のフォローアップ期間で全例が再発無く経過していることが報告されている[8]。また腎移植後の再発にもエクリズマブが良好であることが報告されている[9]。各遺伝子変異におけるエクリズマブ使用下における移植後腎生存率のデータは今後の解析が待たれるところである。

CFH，CHI，CFB，C3は肝臓から産生されるため，これらの遺伝子変異が原因の aHUS では，肝腎移植も検討され，いくつかの報告もあるが，合併症による死亡率も高く，今後はまずはエクリズマブの使用を第一選択とする方向に向かうと考えられる。

おわりに

aHUS の治療について概説した。今後はエクリズマブの各遺伝子変異での予後，長期予後，本邦での使用成績が待たれるところである。

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までの報告数は100例前後と思われる。奈良県立医科大学輸血部では2011年以降本格的に診断を開始したが，近年のaHUS疑い患者の問い合わせ件数が年間30例程度，累積aHUS診断患者が80例弱程度である。発症年齢は原因遺伝子により違うが，主な原因遺伝子別累積発症率は**図3**からはおよそ20歳で50％程度と考えられる。奈良県立医科大学での集計では，初発年齢の平均は16.3歳であった。

非典型溶血性尿毒症症候群(aHUS)の診断

上記のように，本邦ではaHUSは，「志賀毒素によるHUSとADAMTS13活性著減によるTTP以外の血栓性微小血管障害(TMA)で，微小血管症性溶血性貧血・血小板減少・急性腎障害を3主徴とする疾患」と定義される。臨床的には，補体・凝固関連の(狭義の)aHUS診断のためには，まずはHUS，TTP，基礎疾患のあるaHUS(二次性TMA)の除外診断を行うことが重要である。

まずTMAを疑った際に考慮する検査として，以下のものがあげられる。

1）溶血性貧血の確認と他疾患の除外
- 溶血性貧血であることの確認：血液像で破砕赤血球の有無の確認，ハプトグロビン，クームス試験など

2）急性腎障害をきたす他の疾患の鑑別

3）典型HUS，TTP鑑別
- 典型HUSの診断：便培養検査，志賀毒素直接検出法，抗LPS-IgM抗体など
- TTPの診断：ADAMTS13活性測定，ADAMTS13インヒビター測定

4）補体・凝固関連以外のaHUSの除外に必要な検査
- コバラミン代謝異常症(生後6カ月未満で考慮)：血漿ホモシスチン，血漿メチルマロン酸，尿中メチルマロン酸
- 自己免疫疾患・膠原病：抗核抗体，抗リン脂質抗体，抗DNA抗体，抗セントロメア抗体，抗Scl-70抗体，C3，C4，CH50，IgG，IgA，IgMなど
- 悪性高血圧症の除外
- DICの除外：PT，APTT，FDP，Dダイマーなど
- 悪性腫瘍の除外
- 感染症によるaHUSの除外：肺炎球菌，HIV，インフルエンザ，百日咳，水痘など
- 妊娠関連aHUSの除外
- 薬剤性aHUSの除外：抗悪性腫瘍薬，抗血小板薬，免疫抑制薬
- 臓器移植・骨髄移植後aHUSの除外

これらを診断，除外した後に補体・凝固関連aHUSが疑われる。これまで奈良県立医科大学輸血部において補体・凝固関連aHUS診断のために行ってきた検査は，
- 患者血漿を用いて，ヒツジ赤血球を用いた溶血試験を行い，抗factor H抗体の有無，factor H蛋白異常のスクリーニング
- 患者血漿中の抗factor H抗体の有無の確認(ウエスタンブロット法，またはELISA法)
- 血漿中factor H蛋白の有無(Laurell法)
- 血漿中CFH関連蛋白質1/3(CFHR1/3)欠損の有無(ウエスタンブロット法)
- 最終診断は既知の遺伝子をシークエンスでの確認

しかし，これらは一般検査では行われていない。また，そのほかに血漿中factor B, I蛋白の有無，白血球上のCD46(MCP)の発現解析を行っているグループもある[15]。これまで奈良県立医科大学輸血部藤村吉博教授のもとで，TTPとaHUSの診断，研究が先駆的に行われていたが，aHUSに関しては最終的に腎死が問題となることから，2014年9月より東京大学病院腎臓・内分泌内科にて補体・凝固関連aHUS疑い患者の血液学的検査，また国立循環器病研究センター研究所の宮田敏行先生との共同での遺伝子診断を受け付けることとなった。上記の鑑別を行い，疑わしい患者がいた場合には，メールで相談，当科の外来を紹介受診していただければ幸いである(加藤，吉田，南学宛　ahus-office@umin.ac.jp)。

補体・凝固関連aHUSの病態

補体関連aHUSでは，第二経路の活性化異常により発症する。第二経路の活性化は，C3がC3aとC3bに分解されることで生じる。生じたC3bが微生物などの細胞膜表面に結合すると，B因子(complement factor B，CFB)やD因子などと反応してC3転換酵素(C3bBb)を形成する。このC3転換酵素は，さらにC3をC3aとC3bに分解し，生じたC3bと結合してC5転換酵素(C3bBbC3b)となる。C5転換酵素はC5をC5aとC5bに分解し，生じたC5bがC6～C9と順次反応することで膜侵襲複合体(membrane attack complex：MAC)となり，病原体の溶菌・細胞膜融解を引き起こす。C3の分解反応により生じたC3bはきわめて反応性の高いチオエステル結合を有するため，病原体だけでなく自己の細胞膜上にも結合しうる。C3bの自己細胞への結合は有害であるため，自己細胞上にはCFH，membrane cofactor protein(MCP)，thrombomodulinなどの制御因子が存在し，これ

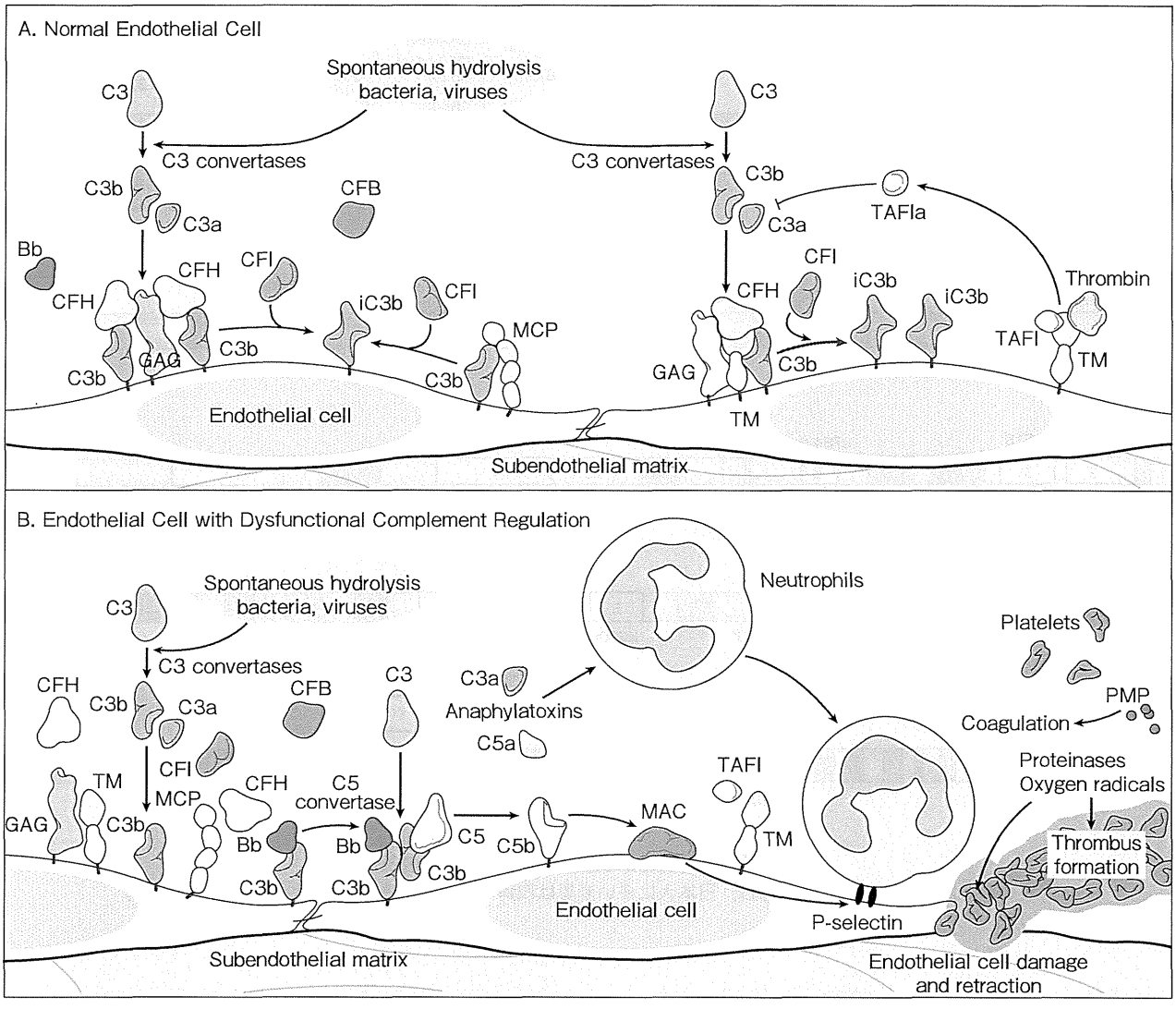

図 4 補体と血管内皮作用の模式図(文献 16 より引用，改変)

らの因子が proteinase である I 因子(CFI)による C3b の速やかな分解不活化を促し，補体による細胞傷害から自己細胞を保護している(図 4)。

補体関連制御因子の異常による aHUS は，抑制因子の loss-of-function と，活性化因子の gain-of-function に分けられる。抑制因子の loss-of-function の例として，CFH，CFI，CD46 の変異，または抗 factor H 抗体の出現による CFH の機能低下の場合があげられ，抑制機能の低下により補体系を活性化することによって aHUS が引き起こされると考えられる。活性化因子の gain-of-function の例としては，CFB，C3 の変異があげられ，いずれも第二経路の活性化により血管内皮細胞や血小板表面が活性化して発症すると考えられる。

一方，近年判明してきた thrombomodulin，DGKE，plasminogen などの凝固因子関連による aHUS の発症機序に関してはまだはっきりとわかっておらず，純粋に凝固系異常による aHUS 症状なのか，どこまで補体系を介した異常なのかははっきりしていない。

補体関連 aHUS 各論

1．補体系活性化を抑制する因子の異常による aHUS

1）Complement factor H(CFH)の異常

上記のように，最初に家族性の HUS の原因遺伝子として見つかった因子である。CFH は第二経路の制御因子として働き，欧米では aHUS の原因遺伝子として最も頻度が高い