thrombopoietin receptor agonists (71.4 %, 5/7 patients). These lines of evidence suggest that rituximab can clinically useful for the treatment of Japanese patients with chronic refractory ITP.

The goal of treatment in ITP is to maintain the platelet count which reduces the risk of bleeding while minimizing treatment-related toxicity. To accomplish this goal, thrombopoietin receptor agonists are recently used. However, thrombocytopenia usually recurring shortly after the drug withdrawal is known as one of the drawbacks of these agonists. Thus, these agents are indefinitely used to maintain the platelet count to minimize bleeding [16]. In the sense, this study showed that rituximab's effect lasted longer after completion of treatment; the platelet counts at all the time points exceeded 30×10^9/L until week 24 after the last dose of the study drug at week 3.

Clinical significance of rituximab in patients with ITP is still being investigated extensively outside Japan to position the therapy at an alternative treatment for ITP prior to splenectomy [15–18]. Although the details of study design was varied from study to study in terms of target population (e.g., newly diagnosed or relapsed ITP), concomitant therapy (e.g., with or without steroids), dosage and administration of rituximab (e.g., 4-weekly 375 mg/m^2 or 2-times 1000 mg 2 weeks apart), and endpoints of efficacy analysis, rituximab commonly showed a clinically substantial efficacy and well tolerability in patients with ITP. Especially, several studies showed a clinically meaningful sustained response to have a chance for sparing splenectomy [12, 18–23], supporting our results in this study. Regarding the safety of rituximab, we observed severe adverse events in three patients. Two had viral infection of unknown etiology and one had hypermenorrhea. All three events were resolved by supportive treatment and all patients were discharged from hospital within a week of admission.

We, thus, conclude from the above-mentioned results that rituximab is clinically useful and involves no particular safety concerns in the treatment of Japanese patients with chronic refractory ITP.

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Compliance with ethical standards

Conflict of interest Dr. Miyakawa reports non-financial support from Zenyaku Kogyo, grants from Japan Medical Association Center for Clinical Trials (JMACCT), during the conduct of the study; grants and personal fees from Alexion pharmaceutical, personal fees from Daiichi Sankyo, personal fees from Fuji film, personal fees from GlaxoSmithKline, personal fees from Kyowa-Hakko Kirin, personal fees from Ono pharmaceuticals, outside the submitted work; Dr. Kanakura reports grants from Chugai Pharmaceutical Co. Ltd., personal fees from Zenyaku-Kogyo, during the conduct of the study; grants and personal fees from Kyowa-Hakkyo Kirin Co. Ltd., grants and personal fees from Otsuka Pharmaceutical Co. Ltd., grants from Takeda Pharmaceutical Co. Ltd., grants from Teijin Pharma, grants from Janssen Pharmaceutical Co. Ltd., grants and personal fees from Alexion Pharmaceutical Co. Ltd., personal fees from Shire Co. Ltd., grants from Bristol-Myers Squibb Co. Ltd., outside the submitted work; Dr. Yano has nothing to disclose. Dr. Shirasugi reports personal fees from Kyowa-Hakko Kirin, personal fees from Pfizer, personal fees from Ono pharmaceutical, personal fees from Bristol-Myers Squibb, personal fees from Celgene, personal fees from GlaxoSmithKline, outside the submitted work; Dr. Higashihara reports grants and personal fees from Chugai pharmaceutical, during the conduct of the study; grants and personal fees from Kyowa-Hakko Kirin, grants from MSD, grants from Jannsen pharmaceutical, grants from Takeda, personal fees from Sumitomo Dainippon pharma, grants from Teijin, grants from Astellas pharma, personal fees from GlaxoSmithKline, outside the submitted work; Dr. Nishiwaki reports grants from Zenyaku Kogyo Company, Limited, grants from Chugai Pharmaceutical, grants from Novartis Pharma K.K., outside the submitted work; Dr. Okamoto reports grants from Chugai pharmaceuticals, during the conduct of the study; personal fees from KyowaHakkoKirin, personal fees from Takeda, personal fees from Bristol-Myers Squibb, personal fees from Janssen pharmaceutical, personal fees from Celgene, personal fees from Alexion, personal fees from Eisai, grants from GlaxoSmithKline, outside the submitted work; Dr. Katsutani has nothing to disclose. Dr. Nomura has nothing to disclose. Dr. Kikuchi has nothing to disclose. Dr. Abe has nothing to disclose. Dr. Nishikawa has nothing to disclose. Dr. Ozaki reports grants from Japan Medical Association Center for Clinical Trials, during the conduct of the study; personal fees from Nippon Shinyaku, personal fees from Janssen pharmaceutical companies, personal fees from Chugai pharmaceutical, personal fees from Celgene, personal fees from Kyowa-Hakko Kirin pharmaceutical, outside the submitted work; Dr. Ikeda reports personal fees from Chugai pharmaceuticals, during the conduct of the study; personal fees from Novartis, personal fees from Sanofi, personal fees from Daichi Sankyo, outside the submitted work; Dr. Tomiyama reports grants from JMACCT, during the conduct of the study; personal fees from GSK, personal fees from Novartis, personal fees from Kyowa-Hokko Kirin, personal fees from Eisai, personal fees from Sysmex, outside the submitted work; Dr. Fujimura reports non-financial support from Kyowa-Hakko Kirin, during the conduct of the study; non-financial support from Medical support, outside the submitted work.

References

1. Kurata Y, Fujimura K, Kuwana M, Tomiyama Y, Murata M. Epidemiology of primary immune thrombocytopenia in children and adults in Japan: a population-based study and literature review. Int J Hematol. 2011;93:329–35.
2. Portielje JE, Westendorp RG, Kluin-Nelemans HC, Brand A. Morbidity and mortality in adults with idiopathic thrombocytopenic purpura. Blood. 2001;97:2549–54.
3. Fujimura K, Kuwana M, Kurata Y, Imamura M, Harada H, Ikeda Y, et al. Is eradication therapy useful as the first line of treatment in Helicobacter pylori-positive idiopathic thrombocytopenic purpura? Analysis of 207 eradicate chronic ITP cases in Japan. Int J Hematol. 2005;81:162–8.
4. Kojouri K, Vesely SK, Terrell DR, George JN. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a

systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. Blood. 2004;104:2623–34.
5. Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr, Crowther MA, American Society of Hematology. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood. 2011;117:4190–207.
6. Kashiwagi H, Tomiyama Y. Pathophysiology and management of primary immune thrombocytopenia. Int J Hematol. 2013;98:24–33.
7. Looney RJ. B-cell targeted therapy in diseases other than rheumatoid arthritis. J Rheumatol. 2005;32(Suppl 73):25–8.
8. Reddy V, Jayne D, Close D, Isenberg D. B-cell depletion in SLE: clinical and trial experience with rituximab and ocrelizumab and implications for study design. Arthritis Res Ther. 2013;15(Suppl 1):S2.
9. British Committee for Standards in Haematology General Haematology. Task force. Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. Br J Haematol. 2003;120:574–96.
10. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood. 2010;115:168–86.
11. Arnold DM, Dentali F, Crowther MA, Meyer RM, Cook RJ, Sigouin C, et al. Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. Ann Intern Med. 2007;146:25–33.
12. Godeau B, Porcher R, Fain O, Lefrère F, Fenaux P, Cheze S, et al. Rituximab efficacy and safety in adult splenectomy candidates with chronic immune thrombocytopenic purpura: results of a prospective multicenter phase 2 study. Blood. 2008;112:999–1004.
13. Kuter DJ, Bussel JB, Lyons RM, Pullarkat V, Gernsheimer TB, Senecal FM, et al. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. Lancet. 2008;371:395–403.
14. Cooper N, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, et al. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. Br J Haematol. 2004;125:232–9.
15. Zaja F, Vianelli N, Battista M, Sperotto A, Patriarca F, Tomadini V, et al. Earlier administration of rituximab allows higher rate of long-lasting response in adult patients with autoimmune thrombocytopenia. Exp Hematol. 2006;34:571–2.
16. Siegal D, Crowther M, Cuker A. Thrombopoietin receptor agonists in primary ITP. Semin Hematol. 2013;50:S18–21.
17. Khellaf M, Charles-Nelson A, Fain O, Terriou L, Viallard JF, Cheze S, et al. Safety and efficacy of rituximab in adult immune thrombocytopenia: results from a prospective registry including 248 patients. Blood. 2014;124:3228–36.
18. Zaja F, Baccarani M, Mazza P, Bocchia M, Gugliotta L, Zaccaria A, et al. Dexamethasone plus rituximab yields higher sustained response rates than dexamethasone monotherapy in adults with primary immune thrombocytopenia. Blood. 2010;115:2755–62.
19. Li Z, Mou W, Lu G, Cao J, He X, Pan X, et al. Low-dose rituximab combined with short-term glucocorticoids up-regulates Treg cell levels in patients with immune thrombocytopenia. Int J Hematol. 2011;93:91–8.
20. Arnold DM, Heddle NM, Carruthers J, Cook DJ, Crowther MA, Meyer RM, et al. A pilot randomized trial of adjuvant rituximab or placebo for nonsplenectomized patients with immune thrombocytopenia. Blood. 2012;119:1356–62.
21. Gudbrandsdottir S, Birgens HS, Frederiksen H, Jensen BA, Jensen MK, Kjeldsen L, et al. Rituximab and dexamethasone vs dexamethasone monotherapy in newly diagnosed patients with primary immune thrombocytopenia. Blood. 2013;121:1976–81.
22. Tran H, Brighton T, Grigg A, McRae S, Dixon J, Thurley D, et al. A multi-centre, single-arm, open-label study evaluating the safety and efficacy of fixed dose rituximab in patients with refractory, relapsed or chronic idiopathic thrombocytopenic purpura (R-ITP1000 study). Br J Haematol. 2014;167:243–51.
23. Ghanima W, Khelif A, Waage A, Michel M, Tjønnfjord GE, Romdhan NB, et al. Rituximab as second-line treatment for adult immune thrombocytopenia (the RITP trial): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet. 2015;385:1653–61.

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ORIGINAL ARTICLE

Genetic variations in complement factors in patients with congenital thrombotic thrombocytopenic purpura with renal insufficiency

Xinping Fan[1,6] · Johanna A. Kremer Hovinga[2] · Hiroko Shirotani-Ikejima[1] · Yuka Eura[1] · Hidenori Hirai[1] · Shigenori Honda[1] · Koichi Kokame[1] · Magnus Mansouri Taleghani[2] · Anne-Sophie von Krogh[3,4] · Yoko Yoshida[5] · Yoshihiro Fujimura[5] · Bernhard Lämmle[2,7] · Toshiyuki Miyata[1,8]

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Abstract The congenital form of thrombotic thrombocytopenic purpura (TTP) is caused by genetic mutations in ADAMTS13. Some, but not all, congenital TTP patients manifest renal insufficiency in addition to microangiopathic hemolysis and thrombocytopenia. We included 32 congenital TTP patients in the present study, which was designed to assess whether congenital TTP patients with renal insufficiency have predisposing mutations in complement regulatory genes, as found in many patients with atypical hemolytic uremic syndrome (aHUS). In 13 patients with severe renal insufficiency, six candidate complement or complement regulatory genes were sequenced and 11 missense mutations were identified. One of these missense mutations, C3:p.K155Q mutation, is a rare mutation located in the macroglobulin-like 2 domain of C3, where other mutations predisposing for aHUS cluster. Several of the common missense mutations identified in our study have been reported to increase disease-risk for aHUS, but were not more common in patients with as compared to those without renal insufficiency. Taken together, our results show that the majority of the congenital TTP patients with renal insufficiency studied do not carry rare genetic mutations in complement or complement regulatory genes.

Keywords Atypical hemolytic uremic syndrome · Complement factors · Renal insufficiency · Thrombotic thrombocytopenic purpura · Upshaw–Schulman syndrome

✉ Johanna A. Kremer Hovinga
johanna.kremer@insel.ch

✉ Toshiyuki Miyata
miyata@ncvc.go.jp

1 Department of Molecular Pathogenesis, National Cerebral and Cardiovascular Center, Suita, Japan

2 Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital and University of Bern, 3010 Bern, Switzerland

3 Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

4 Department of Hematology, St Olavs Hospital Trondheim University Hospital, Trondheim, Norway

5 Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Japan

6 Department of Clinical Laboratory, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China

7 Center for Thrombosis and Hemostasis (CTH), University Medical Center Mainz, Mainz, Germany

8 Department of Cerebrovascular Medicine, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

Introduction

Thrombotic thrombocytopenic purpura (TTP), one form of thrombotic microangiopathy, is characterized by microangiopathic hemolytic anemia and thrombocytopenia. The congenital form of TTP, also known as Upshaw–Schulman syndrome, is caused by a severe constitutional deficiency of ADAMTS13 due to homozygous or compound heterozygous *ADAMTS13* mutations [1, 2] and is assumed to represent less than 5 % of all TTP cases. The age at disease onset is variable. Some congenital TTP patients present with overt TTP soon after birth, others experience first signs only in adulthood, e.g., triggered by pregnancy, or even remain asymptomatic into their fifth or sixth decades of life [3, 4]. Clinical manifestations in congenital TTP are also heterogeneous. Besides the classical hematological

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findings, some patients show neurological symptoms, others kidney involvement up to renal failure. Both onset during pregnancy and kidney involvement are noteworthy as they are features also observed in atypical hemolytic uremic syndrome (aHUS), another type of thrombotic microangiopathy [5, 6]. Approximately 60 % of aHUS cases can be explained by dysregulation and/or excessive activation of the alternative pathway of the complement system due to mutations in complement regulatory genes (*CFH*, *MCP*, *CFI*, *THBD*), hyperfunctional mutations of complement factors (*C3*, *CFB*) or autoantibodies against complement factor H [5, 6]. Recently, mutations in the diacylglycerol kinase epsilon gene were reported to co-segregate with phenotypic aHUS [7, 8].

To explain the variable clinical phenotypes in congenital TTP, genetic mutations responsible for increased activation of the complement system may influence the severity of renal involvement and thus serve as disease modifiers. Of note in this context is the study of Noris et al. who reported on two sisters with congenital TTP who showed different clinical phenotypes [9]. One sister manifested exclusively with neurologic symptoms while the other sister had very severe renal insufficiency that required chronic dialysis. In the latter, a missense mutation in complement factor H was identified which was not present in the sister with neurologic symptoms only. These data suggest that increased activation of the complement system due to genetic mutations modifies the severity of renal involvement in congenital TTP, a scenario derived from a single-family and to be verified in a larger number of patients.

In the present study, we explored whether genetic mutations in complement or complement regulatory genes leading to increased activation of the complement system contribute to the clinical phenotype of congenital TTP patients with predominant renal involvement.

Patients and methods

Patients

From two congenital TTP cohorts, that of the Hemostasis Research Laboratory in Bern, Switzerland [10, 11] and the Japanese congenital TTP study [4], confirmed congenital TTP patients were selected based on the following two criteria: (1) the patient had not been extensively studied by other groups and (2) whole blood for DNA extraction was available. A total of 32 congenital TTP patients (30 from Europe and 2 from Japan) were included in this study, of which thirteen had severe renal insufficiency up to end-stage renal disease (Table 1). The definition for renal involvement was as follows: (1) acute renal insufficiency during one or more acute TTP bouts requiring dialysis, or (2) chronic kidney disease defined according to KDIGO (kidney disease: improving global outcomes) as persistence of a glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 or albuminuria for at least 3 months, with or without arterial hypertension; or (3) end-stage renal disease requiring renal replacement therapy (either dialysis or kidney transplant); or (4) documented tissue damage on renal biopsy; or (5) having a diagnosis of (atypical) hemolytic uremic syndrome established by a nephrologist based on the concomitant presence of thrombocytopenia, microangiopathic hemolytic anemia and renal insufficiency. All patients had severe ADAMTS13 deficiency (<10 % of the normal) in the absence of a functional inhibitor on at least two time points, at least two ADAMTS13 mutations and/or a plasma infusion trial demonstrating full recovery of infused ADAMTS13 and a plasma half-life of ADAMTS13 of 2–4 days. The plasma ADAMTS13 activity was measured as previously described [12–14]. The study was approved by the Institutional Review Board of each institution.

Genetic analysis

The coding exons and flanking intronic regions of *CFH*, *C3*, *MCP*, *CFI*, *CFB*, and *THBD* were sequenced as described previously [15, 16] in all 13 congenital TTP patients presenting with renal insufficiency. In the remaining 19 European congenital TTP patients without renal involvement, only a limited analysis of the 11 genetic missense mutations identified in the patients with renal insufficiency was performed. The allele frequencies of the found 11 missense mutations between the 11 European congenital TTP patients with renal involvement and the 19 European congenital TTP patients without renal involvement were compared by Chi square analysis. The nomenclature system of the amino acid and nucleotide numbers is given according to the recommendation of the Human Genome Variation Society. The A of the ATG of the initial Met codon is denoted as nucleotide +1, and the initial Met residue is denoted as amino acid +1. Multiplex ligation-dependent probe amplification analysis was used to screen for deletions of *CFH* and *CFHR*s using a commercially available kit (MLPA kit P236-A2; MRC-Holland, the Netherlands) [15]. The possible impact of the identified genetic mutations on structure and function of the respective proteins was examined by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html). The crystal structure of the complex of C3b and CCP 1-4 domains of CFH (ID: 2WII) [17] was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Molecular graphic imaging and analysis were generated using the PyMOL molecular visualization system (Schrödinger, Portland, OR).

Table 1 Clinical characteristics and genetic variations of 13 congenital TTP patients with renal insufficiency

Patient ID	Current age (y)	Sex	CFH			C3			CFI
			rs800292	rs1061170	rs1065489	rs2230199	rs147859257	rs1047286	rs145769028
			c.184G > A	c.1204T > C[a]	c.2808G > T	c.304C > G	c.463A > C	c.941C > T	c.603A > C
1	36	F		p.Y402H		p.R102G	**p.K155Q**[b]	p.P314L	
2	53	M		p.Y402H	p.E936D				
3	61	M	p.V62I						
4	33	M	p.V62I (homo)						
5	61	F		p.Y402H	p.E936D				
6	38	M	p.V62I			p.R102G		p.P314L	
7	29	F	p.V62I (homo)			p.R102G		p.P314L	
8	48	F	p.V62I	p.Y402H					
9	44	F		p.Y402H (homo)		p.R102G		p.P314L	
10	46	M							
11	29	F		p.Y402H	p.E936D	p.R102G		p.P314L	
12	43	M			p.E936D (homo)				
13	35	M	p.V62I		p.E936D (homo)				**p.R201S**[c]
NHLBI GO Exome Sequencing Project									
MAF			39 %	38 %	14 %	15 %	0.3 %	15 %	None
1000 Genomes Project									
MAF, EUR			26 %	36 %	18 %	22 %	0 %	21 %	0 %
MAF, JPT			41 %	7 %	46 %	0 %	0 %	0 %	2 %

Table 1 continued

Patient ID	CFB			THBD	Number of CFHR1-R3 allele	ADAMTS13			Clinical characteristics
	rs4151667 c.26T > A	rs641153 c.95G > A	rs4151651 c.754G > A	rs1042579 c.1418C > T		Amino acid and/or nucleotide change	Amino acid and/or nucleotide change	Activity (%)	
1	**p.L9H**[c]			p.A473V	2	p.D235H	p.W542G	3.5[d]	Adult onset during 1st pregnancy with renal complications and renal sequelae
2			**p.G252S**[c]		2	c.4143_4144 insA p.E1382Rfs*6	c.4143_4144 insA p.E1382Rfs*6	<1[d]	Acute bouts always with severe renal insufficiency
3			**p.G252S**[c]	p.A473V	0	c.4143_4144 insA p.E1382Rfs*6	c.4143_4144 insA p.E1382Rfs*6	<1[d]	Acute bouts with severe renal insufficiency requring dialysis; triggered by (mild/moderate) alcohol consumption
4		p.R32Q (homo)		p.A473V	2	c.4143_4144 insA p.E1382Rfs*6	c.4143_4144 insA p.E1382Rfs*6	<1[d]	Original diagnosis recurrent HUS
5				p.A473V	2	p.R507Q	c.4143_4144 insA p.E1382Rfs*6	<1[d]	End-stage renal disease and kidney transplantation
6		p.R32Q			1	p.G1239 V	p.G1239 V	<1[d]	In adulthood acute bouts always with severe renal insufficiency requring dialysis; triggered always by alcohol consumption
7					2	p.R692C	c.3044 + 2430_ 3568 + 81del3291	<3[e]	End-stage renal disease
8		p.R32Q			2	c.2000delA p.N667Tfs*31	p.R1219Q	3[d]	Renal sequelae
9					2	p.Y177C	p.R1060W	1.5 in acute bout, 5 in remission[d]	Original diagnosis aHUS/membranoproliferative glomerulonephritis
10		p.R32Q		p.A473V	0	p.L232Q	p.R1060W	1 in acute bout, 5.3 in remission[d]	Renal sequelae
11					2	p.R1095Q	not identified	<1[d]	Renal sequelae

Table 1 continued

Patient ID	CFB			THBD	Number of CFHR1-R3 allele	ADAMTS13			Clinical characteristics
	rs4151667 c.26T > A	rs641153 c.95G > A	rs4151651 c.754G > A	rs1042579 c.1418C > T		Amino acid and/or nucleotide change	Amino acid and/or nucleotide change	Activity (%)	
12				p.A473V	2	p.H234Q	p.R1206*	<0.5[f]	Acute bouts with renal insufficiency
13				p.A473V	2	c.1885delA p.R629Efs*69	p.C908Y	<0.5[f]	Original diagnosis aHUS; acute bouts with severe renal insufficiency requring dialysis; triggered by heavy alcohol consumption
NHLBI GO Exome Sequencing Project									
MAF	3 %	12 %	3 %	14 %					All NHLBI participants, 13,006 alleles
1000 Genomes Project									
MAF, EUR	5 %	9 %	3 %	19 %					European (CEU, FIN, GBR, IBS, TSI), 1006 alleles
MAF, JPT	4 %	7 %	0 %	26 %					Japanese, 208 alleles

Patient ID 1–11 are from Swiss registry and patient ID 12 and 13 are from Japan registry. Nonsynonymous mutations were not identified in the MCP gene

The A of the ATG of the initial Met codon is denoted as nucleotide +1, and the initial Met residue is denoted as amino acid +1

The MAF of the NHLBI GO Exome Sequencing Project was obtained from http://evs.gs.washington.edu/EVS/, and the MAF of the 1000 Genomes Project Phase 3 was obtained from http://www.1000genomes.org/analysis

TTP thrombotic thrombocytopenic purpura, *aHUS* atypical hemolytic uremic syndrome, *MAF* minor allele frequency

[a] Reference sequence of CFH (NM 000186.3) is c.1204C > T

[b] Bold and underlined, rare and potentially predisposing mutation

[c] Bold, low frequency mutation

[d] The ADAMTS13 activity was measured by FRETS-VWF73 assay

[e] Quantitative immunoblotting assay, or

[f] Act-ELISA assay

In two European patients (Table 1, patient ID 7 and 11), only a single causative *ADAMTS13* mutation had been identified. Therefore, we employed the newly developed genomic quantitative PCR method [18] to identify a second causative mutation.

Results

To identify genetic mutations in complement genes leading to increased activation of the alternative pathway of the complement system, we performed DNA sequencing of the 6 candidate genes, *CFH*, *C3*, *MCP*, *CFI*, *CFB*, and *THBD* and identified 11 missense mutations in 13 congenital TTP patients with renal insufficiency (Table 1). We retrieved the allele frequency of these missense mutations from population cohorts participating in the NHLBI GO Exome Sequencing Project and the 1000 Genomes project Phase 3 and found that C3:p.K155Q is a rare mutation with a minor allele frequency (MAF) of 0.3 % and the two CFB missense mutations, p.L9H and p.G252S, are low frequency mutations with a MAF of 3 % (Table 1). These three missense mutations were observed in the European patients. One Japanese patient (ID 13) with renal insufficiency carried the one Japanese-specific CFI missense mutation, p.R201S, which is a low frequency mutation with a MAF of 2 % in the Japanese population [19]. The remaining 7 missense mutations were classified as common mutations with a MAF of more than 5 %.

We also genotyped the found 10 missense mutations, with exclusion of one Japanese-specific mutation, in the 19 European congenital TTP patients without renal involvement (data not shown). C3:p.K155Q was not identified in this group. None of the remaining 9 missense mutations found in the European congenital TTP patients was significantly more common among patients with compared to those without renal involvement.

Since the *ADAMTS13* mutation, 4143_4144 insA, is frequent among patients with congenital ADAMTS13 deficiency in Northern and Central European countries [20], it was frequent in our cohort of European origin. Three of 11 (27.3 %) European congenital TTP patients with renal involvement were homozygous carriers for the frequent *ADAMTS13* mutation 4143_4144insA, as were 6/19 (31.6 %) European congenital TTP patients without renal involvement. Though no difference in plasma ADAMTS13 activity between congenital TTP patients with and without renal involvement was observed, patients without renal involvement were younger (median 33 years, range 14–75 years) than patients with renal involvement (median 43 years, range 29–61 years).

We have recently developed a new genomic quantitative PCR method to identify large gene deletions in the *ADAMTS13* gene [18]. Employing this method, we identified a second causative mutation in patient ID 7 *ADAMTS13* c.3044 + 2430_3568 + 81del3291, a 3291-bp deletion including exons 24 and 25. In patient ID 11, no large deletion was observed and though the obligatory second mutation still remains unknown, the plasma infusion trial confirmed the congenital TTP diagnosis in this patient.

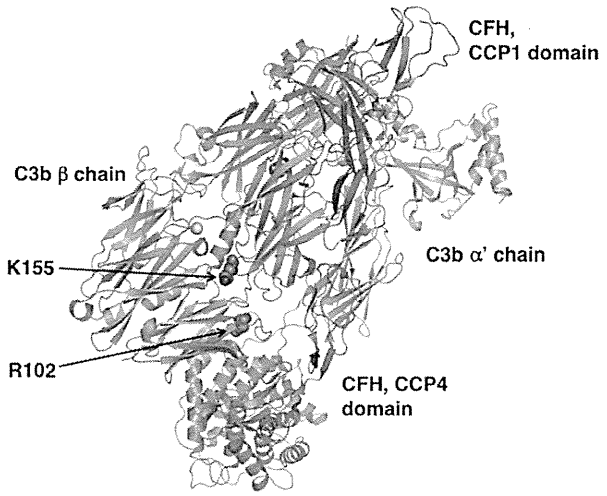

Fig. 1 Location of the C3:p.K155Q and C3:p.R102G mutations in the C3b-CFH CCP1-4 complex. C3b β-chain containing macroglobulin-like domains (MG) 1-5 is shown in *blue* and C3b α'-chain is shown in *green*. Complement control protein (CCP) domains 1-4 in CFH are depicted in *orange*. A calcium ion is shown as *gray sphere*. The p.K155 and p.R102 residues in C3b are depicted by *magenta spheres*. Both mutations, p.K155Q and p.R102G, are not positioned at the contact interface of the two proteins. Diagram was generated with the PyMOL molecular visualization system

Discussion

In the present study, we identified 11 missense mutations in six candidate complement (*C3*, *CFB*) and complement regulatory (*CFH*, *MCP*, *CFI*, *THBD*) genes in 13 congenital TTP patients with renal insufficiency and classified them into rare, low frequency, and common mutations.

The rare missense mutation, C3:p.K155Q found in patient ID 1 is located in the macroglobulin-like (MG) 2 domain of C3, where other mutations predisposing to increased complement activation were previously identified. Figure 1 depicts the location of the C3:p.K155 residue in the crystal structure of the complex of C3b and CFH CCP1-4 domains [17]. The p.K155Q mutation is positioned slightly away from the interface between C3b and CFH CCP1-4, making it unlikely that their interactions are directly affected. Prediction of the impact of this mutation by PolyPhen-2 and SIFT showed "benign" and "tolerated"

effects, respectively. For a definite verdict, however, functional studies of the C3:p.K155Q mutation would be needed. In case of common mutation C3:p.R102G, which is also positioned away from the interface between C3b and CFH CCP1-4 (Fig. 1), experimental data indicate that this C3 variant weakly binds to CFH, resulting in reduced CFH cofactor activity thereby favoring alternative complement pathway amplification [21].

The low frequency mutation, CFB:p.L9H within the CFB signal peptide sequence has been reported to be protective for age-related macular degeneration [22]. The low frequency mutation CFB:p.G252S is located in the CFB linker region between the CCP3 domain and the von Willebrand factor A domain. Functional consequences of this mutation are unknown. On the basis of the CFI crystal structure, the low frequency mutation CFI:p.R201S resides on the surface region of the protein away from the proposed cofactor and/or substrate interaction sites, indicating a non-dysfunctional mutation [23]. This mutation is found only in Far East populations including Japanese [19].

The remaining seven mutations found in our cohort are commonly present in the general population. In the study of a rare renal affection, dense deposit disease, both p.R102G and p.P314L mutations in C3 were identified as genetic risk factors for developing this disease [24]. C3:p.R102G is also strongly associated with age-related macular degeneration with an estimated population attributable risk of 22 % [25, 26]. In our cohort, both C3:p.R102G and C3:p.P314L were in perfect linkage disequilibrium and five patients with renal insufficiency carried both mutations (Table 1), pointing to susceptibility for renal involvement through probable hyperactivation of the complement cascade.

Previous functional analyses indicated that the common mutations, CFH:p.V62, C3:p.G102, and CFB:p.R32, are disease-risk mutations [21, 27–29]. The combination of these three mutations yielded sixfold higher hemolytic activity compared to the protective mutations CFH:p.I62, C3:p.R102, and CFB:p.Q32 [21]. The patient with the rare C3:p.K155Q mutation (ID 1) and two other congenital TTP patients with renal involvement (ID 9 and 11) are carriers of the combined disease-risk mutations (Table 1). The CFH:p.E936D mutation in the CCP16 domain of CFH found in congenital TTP patients ID 2, ID 5, ID 11, ID 12 (in homozygous state) and ID 13 (in homozygous state) has been associated with aHUS [30, 31]. Although these common mutations are not extremely destructive, the combined effects of disease-risk mutations as well as the rare missense mutation C3:p.K155Q may influence susceptibility to renal involvement in congenital TTP patients with hereditary ADAMTS13 deficiency.

Two of 13 congenital TTP patients with renal insufficiency carried homozygous deletions of *CFHR1/CFHR3* genes (Table 1). The complete absence as well as barely detectable levels of CFHR1/CFHR3 are related to the occurrence of autoantibodies to CFH [32] that account for 5–10 % of aHUS cases [5]. Therefore, in addition to the above-mentioned missense mutations in complement genes, the deletion of *CFHR1/CFHR3* may contribute to renal affection in congenital TTP patients.

Taken together, our study demonstrates that most of the congenital TTP patients with renal insufficiency do not carry genetic mutations in complement or complement regulatory genes known to predispose to renal insufficiency. Second, although some congenital TTP patients with renal insufficiency were found to be carriers of common aHUS-risk mutations, such as CFH:p.V62, CFH:p.D936, C3:p.G102, CFB:p.R32, and homozygous deletions of *CFHR1/CFHR3* genes, these mutations were not more common than in the general population or in our 19 congenital TTP patients without renal involvement.

Microvascular platelet thrombosis and endothelial injury resulting from ADAMTS13 deficiency initiate the coagulation and fibrinolytic pathways, which in turn may contribute to complement activation in congenital TTP [33, 34]. Complement activation with consumption of complement factors has already been demonstrated during acute TTP episodes [34, 35]. Overactivation of the alternative complement pathway also leads to coagulation cascade activation. Our results suggest that rare predisposing complement genetic mutations do not contribute to a large extent to the phenotypic variability in congenital TTP patients. Further, the common aHUS-risk mutations in complement or complement regulatory genes observed in our small series of congenital TTP patients with renal insufficiency were equally frequent in congenital TTP patients without renal failure. Recruitment of larger number of congenital TTP patients with well-defined phenotypes will be necessary to obtain a full conclusion.

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Compliance with ethical standards

Conflict of interest Dr. Lämmle is a member of the Data Safety Monitoring committee of the BAX 930 Study testing rADAMTS13 in congenital TTP patients. Dr. Fujimura is a recipient of the research fund from Alexion Pharmaceuticals. Other authors have no conflict of interests.

References

1. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the *ADAMTS* gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413:488–94.
2. Kokame K, Matsumoto M, Soejima K, Yagi H, Ishizashi H, Funato M, et al. Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. Proc Natl Acad Sci. 2002;99:11902–7.
3. Furlan M, Lämmle B. Aetiology and pathogenesis of thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome: the role of von Willebrand factor-cleaving protease. Best Pract Res Clin Haematol. 2001;14:437–54.
4. Fujimura Y, Matsumoto M, Isonishi A, Yagi H, Kokame K, Soejima K, et al. Natural history of Upshaw–Schulman syndrome based on *ADAMTS13* gene analysis in Japan. J Thromb Haemost. 2011;9(Suppl 1):283–301.
5. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361:1676–87.
6. George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med. 2014;371:1847–8.
7. Lemaire M, Fremeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, et al. Recessive mutations in *DGKE* cause atypical hemolytic-uremic syndrome. Nat Genet. 2013;45:531–6.
8. Miyata T, Uchida Y, Ohta T, Urayama K, Yoshida Y, Fujimura Y. Atypical haemolytic uraemic syndrome in a Japanese patient with DGKE genetic mutations. Thromb Haemost; 2015:114:862–3.
9. Noris M, Bucchioni S, Galbusera M, Donadelli R, Bresin E, Castelletti F, et al. Complement factor H mutation in familial thrombotic thrombocytopenic purpura with ADAMTS13 deficiency and renal involvement. J Am Soc Nephrol. 2005;16:1177–83.
10. Kremer Hovinga JA, Lämmle B. Role of ADAMTS13 in the pathogenesis, diagnosis, and treatment of thrombotic thrombocytopenic purpura. Hematol Am Soc Hematol Educ Program. 2012;2012:610–6.
11. Mansouri Taleghani M, von Krogh AS, Fujimura Y, George JN, Hrachovinova I, Knobl PN, et al. Hereditary thrombotic thrombocytopenic purpura and the hereditary TTP registry. Hamostaseologie. 2013;33:138–43.
12. Kremer Hovinga JA, Vesely SK, Terrell DR, Lammle B, George JN. Survival and relapse in patients with thrombotic thrombocytopenic purpura. Blood. 2010;115:1500–11.
13. Froehlich-Zahnd R, George JN, Vesely SK, Terrell DR, Aboulfatova K, Dong JF, et al. Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura. Haematologica. 2012;97:297–303.
14. Kato S, Matsumoto M, Matsuyama T, Isonishi A, Hiura H, Fujimura Y. Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. Transfusion. 2006;46:1444–52.
15. Fan X, Yoshida Y, Honda S, Matsumoto M, Sawada Y, Hattori M, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol. 2013;54:238–46.
16. Matsumoto T, Fan X, Ishikawa E, Ito M, Amano K, Toyoda H, et al. Analysis of patients with atypical hemolytic uremic syndrome treated at the Mie University Hospital: concentration of C3 p. I1157T mutation. Int J Hematol. 2014;100:437–42.
17. Wu J, Wu YQ, Ricklin D, Janssen BJ, Lambris JD, Gros P. Structure of complement fragment C3b-factor H and implications for host protection by complement regulators. Nat Immunol. 2009;10:728–33.
18. Eura Y, Kokame K, Takafuta T, Tanaka R, Kobayashi H, Ishida F, et al. Candidate gene analysis using genomic quantitative PCR: identification of *ADAMTS13* large deletions in two patients with Upshaw–Schulman syndrome. Mol Genet Genomic Med. 2014;2:240–4.
19. Yuasa I, Nakagawa M, Umetsu K, Harihara S, Matsusue A, Nishimukai H, et al. Molecular basis of complement factor I (CFI) polymorphism: one of two polymorphic suballeles responsible for CFI A is Japanese-specific. J Hum Genet. 2008;53:1016–21.
20. Schneppenheim R, Kremer Hovinga JA, Becker T, Budde U, Karpman D, Brockhaus W, et al. A common origin of the 4143insA ADAMTS13 mutation. Thromb Haemost. 2006;96:3–6.
21. Heurich M, Martinez-Barricarte R, Francis NJ, Roberts DL, Rodriguez de Cordoba S, Morgan BP, et al. Common polymorphisms in C3, factor B, and factor H collaborate to determine systemic complement activity and disease risk. Proc Natl Acad Sci. 2011;108:8761–6.
22. Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, et al. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. Nat Genet. 2006;38:458–62.
23. Roversi P, Johnson S, Caesar JJ, McLean F, Leath KJ, Tsiftsoglou SA, et al. Structural basis for complement factor I control and its disease-associated sequence polymorphisms. Proc Natl Acad Sci. 2011;108:12839–44.
24. Abrera-Abeleda MA, Nishimura C, Frees K, Jones M, Maga T, Katz LM, et al. Allelic variants of complement genes associated with dense deposit disease. J Am Soc Nephrol. 2011;22:1551–9.
25. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat Genet. 2007;39:1200–1.
26. Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, et al. Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med. 2007;357:553–61.
27. Tortajada A, Montes T, Martinez-Barricarte R, Morgan BP, Harris CL, de Cordoba SR. The disease-protective complement factor H allotypic variant Ile62 shows increased binding affinity for C3b and enhanced cofactor activity. Hum Mol Genet. 2009;18:3452–61.
28. Montes T, Tortajada A, Morgan BP, de Cordoba SR, Harris CL. Functional basis of protection against age-related macular degeneration conferred by a common polymorphism in complement factor B. Proc Natl Acad Sci. 2009;106:4366–71.
29. Marinozzi MC, Vergoz L, Rybkine T, Ngo S, Bettoni S, Pashov A, et al. Complement factor B mutations in atypical hemolytic uremic syndrome-disease-relevant or benign? J Am Soc Nephrol. 2014;25:2053–65.
30. Caprioli J, Castelletti F, Bucchioni S, Bettinaglio P, Bresin E, Pianetti G, et al. Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. Hum Mol Genet. 2003;12:3385–95.
31. Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010;5:1844–59.
32. Jozsi M, Licht C, Strobel S, Zipfel SL, Richter H, Heinen S, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Blood. 2008;111:1512–4.
33. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. Nat Rev Nephrol. 2012;8:622–33.

34. Tati R, Kristoffersson AC, Stahl AL, Rebetz J, Wang L, Licht C, et al. Complement activation associated with ADAMTS13 deficiency in human and murine thrombotic microangiopathy. J Immunol. 2013;191:2184–93.

35. Reti M, Farkas P, Csuka D, Razso K, Schlammadinger A, Udvardy ML, et al. Complement activation in thrombotic thrombocytopenic purpura. J Thromb Haemost. 2012;10:791–8.

補体反応

[1] 国立循環器病研究センター　分子病態部
[2] 同　高血圧・腎臓科
宮田敏行[1]　中村敏子[2]

point

- 補体は侵入した微生物などの異物に反応して殺菌反応や炎症反応を惹起する生体防御反応であり，3つの経路を通してC3が活性化する．
- 補体第二経路で活性化したC3は，微生物だけでなく自己細胞にも結合する．自己細胞上には補体の活性化を抑制する複数の蛋白質が存在し，C3を不活化している．
- 遺伝子異常や自己抗体によりC3の制御が不全に陥ると，C5がC5aとC5bに分解され白血球の動員や膜侵襲複合体の形成が起こり，血管内皮細胞が障害を受ける．これが非典型溶血性尿毒症症候群（atypical hemolytic uremic syndrome：aHUS）のメカニズムである．
- 最近，aHUSの治療薬として抗C5単クローン抗体であるエクリズマブが，本邦でも保険適応となり，aHUS患者の治療に効果を上げている．

Q 補体系はどのような機能をもっているのでしょうか？

A 補体は，侵入した微生物などの異物に反応して殺菌反応や炎症反応を惹起する生体防御反応です[1]．補体系には総計，30種以上の血漿蛋白質と細胞上の蛋白質が関わっています．生体に侵入した微生物などは，補体因子成分によって認識され，蛋白分解カスケードが作動し，これにより，炎症惹起物質が生成するとともに細胞溶解もしくは貪食によるクリアランスのための目印がつけられます．補体の活性化は3つの経路があります．すなわち，①抗原抗体反応により活性化される古典経路，②細菌やウイルス上の糖鎖に結合するレクチンによって活性化されるレクチン経路，および③認識分子がなくC3を活性化する第二経路です．3つの補体活性化経路はC3の段階で合流するので，C3は補体の中でもっとも重要な因子であり，血中量も極めて多いです（1.2g/L）（図1）．これらの経路から生成したC3bは，微生物などの表面に多数共有結合することによ

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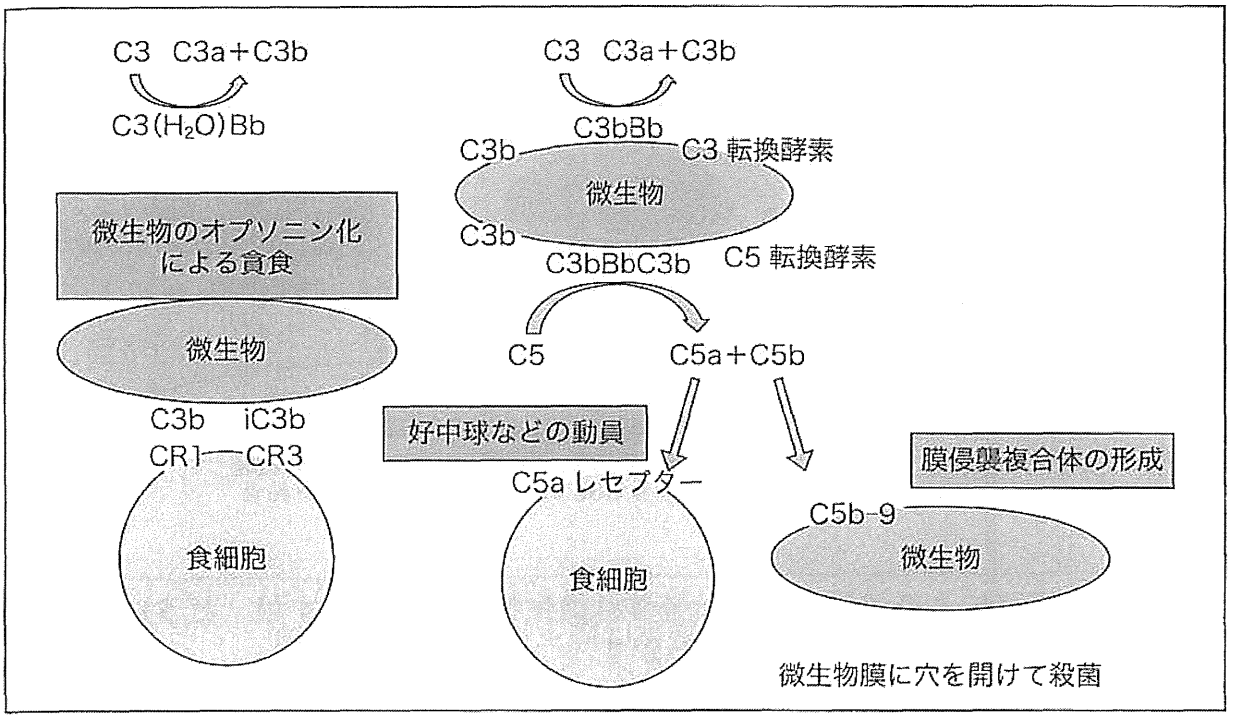

図1　C3を中心とした補体系による生体防御システム

り微生物をオプソニン化*します．オプソニン化された微生物は，食細胞により貪食されます．微生物上のC3bはC3転換酵素であるC3bBb複合体となり，さらにC5転換酵素であるC3bBbC3b複合体となります．C5転換酵素は，C5をC5aとC5bに分解します．C5aは強力なアナフィラトキシン活性をもち，炎症を惹起して好中球などを動員します．微生物上のC5bは，膜侵襲複合体であるC5b-9を形成し，微生物を溶解させ殺菌します．

*オプソニン化：微生物をはじめとする細胞が効率的に食細胞により貪食されやすいように変えられること．C3bや免疫グロブリンが結合すると，貪食されやすくなります．

補体第二経路は，どのようなメカニズムで活性化されるのですか？

A　第二経路は微生物などの水酸基をもつ表面で起こり，その開始には微生物に結合する蛋白質を必要としません．第二経路は，C3の自発的な加水分解によって始まります．すなわち，かなりの速度でC3が加水分解によりC3(H2O)になり，これにB因子が結合してC3(H2O)Bが形成されます．これにプロテアーゼであるD因子が作用し，液相C3転換酵素であるC3(H2O)Bbが形成されます．これは少量しかできませんが，多くのC3をC3aとC3bに分解します．生成したC3bの多くは加水分解により不活化されますが，一部のC3bは微生物や自己細胞の表面上の水酸基に共有結合して付着します（図2）．このC3bにB因子が結合してC3Bとなり，このB因子がD因子により活性型プロテアーゼBbへと分解され，第二経路のC3転換酵素であるC3bBbが形成されます．

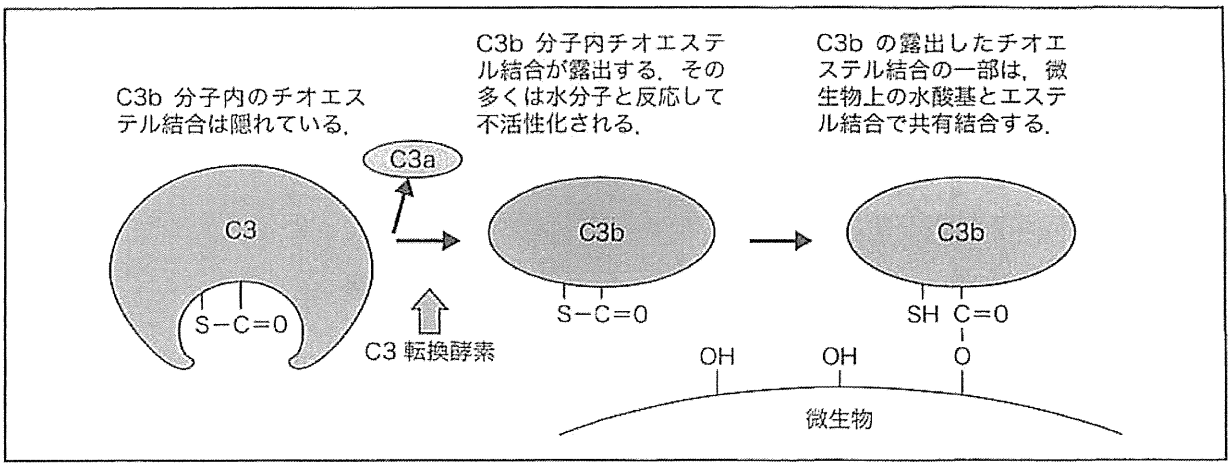

図 2　C3 の限定分解で生じる C3b の微生物表面への共有結合

第二経路により活性化された補体は微生物を攻撃しますが，どうして宿主細胞は攻撃されないのでしょうか？

第二経路の活性化で生じる C3b は水酸基に反応するので（図 2），微生物だけでなく自己細胞上にも結合します．自己細胞に結合した C3b は，有害なのですみやかに無毒化されます．このため，自己細胞は C3b を分解する仕組みをもっています（図 3）．血漿蛋白質 H 因子は自己細胞のシアル酸などの陰電荷をもつ糖鎖に結合し，細胞表面上の C3b に結合します．H 因子は血漿プロテアーゼ I 因子による C3b 分解のコファクターであり，C3b の iC3b への分解・不活化を促します．H 因子は，

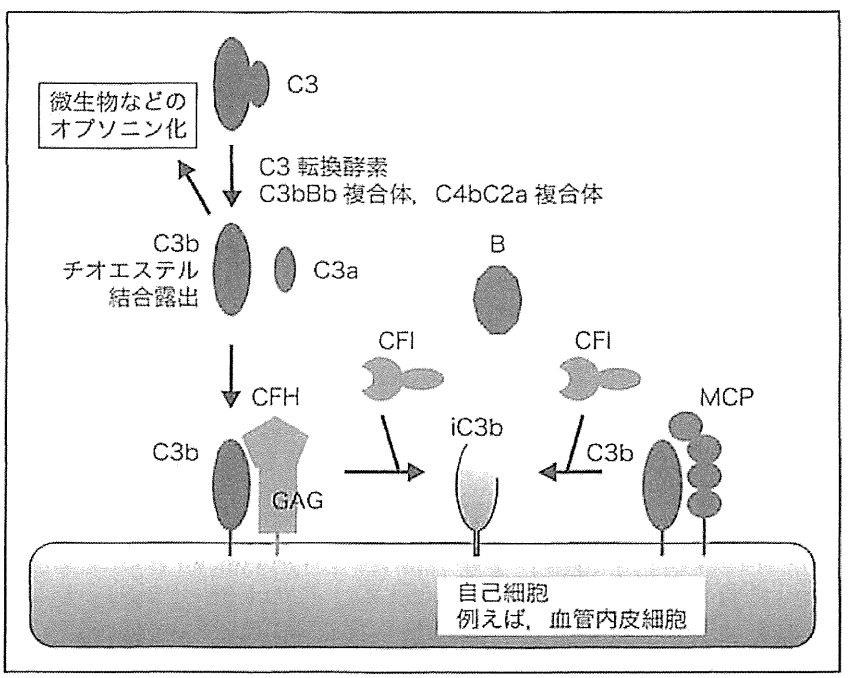

図 3　自己細胞上での C3b の分解による不活化

C3bBb から Bb を遊離させる能力ももちます．自己細胞上には Membrane cofactor protein（MCP）とよばれる膜蛋白質が存在し，I 因子による C3b の分解を促進します．血液凝固制御因子である膜蛋白質トロンボモジュリンは H 因子の存在下，I 因子の C3b の分解を促進します．このように，自己細胞に結合した C3b は，H 因子，I 因子，MCP，トロンボモジュリンの協調作用により分解・不活化されます．しかし，これらの因子に loss-of-function の遺伝子変異が生じたり，H 因子に対する自己抗体が生じると，C3b の分解が妨げられることになります．また，C3b が分解を受けにくい gain-of-function の遺伝子変異をもつ場合や，B 因子の gain-of-function の変異をもつ場合も，C3b の分解が障害されることとなり，分解が遅延して自己細胞が障害を受けます．

Q aHUS について教えてください

A aHUS は，破砕赤血球を伴う溶血性貧血，血小板減少，急性腎不全を三徴とする疾患です[2,3]．2012 年，本邦における aHUS 診断基準が日本腎臓学会と日本小児科学会の合同で作成されました．本診断基準では，aHUS は志賀毒素による HUS と ADAMTS13 の活性著減による血栓性血小板減少性紫斑病以外の血栓性微小血管障害（TMA）であり，溶血性貧血・血小板減少・急性腎障害を三主徴とする疾患と定義されています（http://www.jsn.or.jp/guideline/ahus.php）．したがって，本診断基準での aHUS には，補体系の遺伝子変異を有する HUS と二次性 TMA が含まれます．二次性の TMA を除外する aHUS の定義もあります[3]．

最近の欧米の研究により，aHUS の多くは補体活性化の制御に異常が生じ，糸球体などの血管内皮細胞が補体により攻撃を受けるというメカニズムが広く受け入れられるようになってきました[2]．aHUS の約半数の症例で，補体制御因子である H 因子，I 因子，MCP，トロンボモジュリンに

表 1　aHUS の遺伝子異常と予後

異常蛋白	異常遺伝子	aHUS での頻度（%）	血漿療法に対する短期反応
H 因子	CFH	20～30	寛解率 60%
CFHR1 と CFHR3＋抗 H 因子抗体	CFHR1/3	6	血漿交換に免疫療法を加え寛解率 70～80%
MCP	MCP	10～15	血漿療法の適応なし
I 因子	CFI	4～10	寛解率 30～40%
B 因子	CFB	1～2	寛解率 30%
C3	C3	5～10	寛解率 40～50%
トロンボモジュリン	THBD	5	寛解率 60%
diacylglycerol kinase epsilon	DGKE	不明，13 例の報告	不　明

（文献 2 を参照して作成）

loss-of-function の遺伝子変異，および補体因子の C3 と B 因子に gain-of-function の遺伝子変異が同定されています（図 3，表 1）．これらの遺伝子変異により，血管内皮細胞上で C5 転換酵素が形成され，C5 から C5a と C5b が形成され，内皮が障害されると考えられます．中でも腎の微小血管の障害が顕著にみられ，急性腎障害を示すと考えられています．

TOPICS

aHUS の治療薬としての抗 C5 抗体薬

aHUS は C3b が自己細胞に共有結合し，補体制御系が十分に働かなかった場合に発症します．最近，aHUS の治療薬として抗 C5 単クローン抗体であるエクリズマブ*（ソリリス®）が，本邦でも保険適応となりました．自己細胞の表面に C5 転換酵素が形成されると，C5 が C5a と C5b に限定分解されますが，抗 C5 抗体は C5 に結合し，この限定分解を阻止し，強力なアナフィラトキシン活性を有する C5a の産生や膜侵襲複合体の形成を阻害します（図 4）．これにより炎症反応が抑制され，加えて自己細胞は膜破壊から免れます．2009 年，難治性 aHUS 患者 2 名にエクリズマブが使用され，劇的に症状が改善し，その後，著効例が多数報告されました[4]．本薬剤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，承認されています．

*エクリズマブ

本邦の発作性夜間ヘモグロビン尿症患者への投与例では，約 3%の患者に症状の改善がみられず，これらの患者は C5 分子に p.Arg885Cys 変異を有していました[5]．

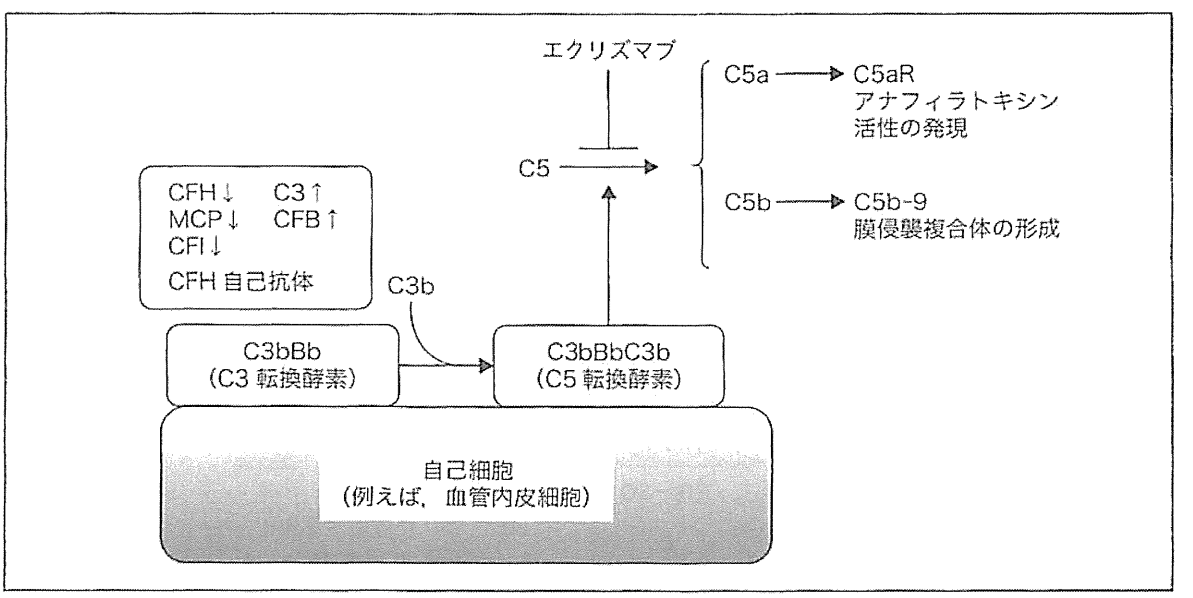

図 4　抗 C5 抗体薬の作用点

aHUS 患者の約半数に，H 因子自己抗体や，H 因子，I 因子，MCP，トロンボモジュリンの loss-of-function 変異，C3，B 因子の gain-of-function 変異が同定されています．

まとめ

補体第二経路は平素より常にわずかに活性化されており，侵入してくる微生物などの異物に即時に対応できるようになっています．この中心となるのはC3です．C3の活性化で生じるC3bは微生物などの水酸基に結合し，オプソニンとして働きます．さらに，C3bはC3転換酵素（C3bBb），C5転換酵素（C3bBbC3b）へと変化し，C5をC5aとC5bに分解し，アナフィラトキシン活性の発現と膜侵襲複合体の形成につながります．このC3bが自己細胞上に結合すると有害なので，無毒化するため，いくつかのメカニズムを備えています．この無毒化に関わる因子に対して，自己抗体が生じる場合，および遺伝子変異がある場合，補体系により自己細胞が障害を受けます．aHUS患者の半数に補体制御系の異常がみられます．

[文　献]

1）Java A, Atkinson J, Salmon J：Defective complement inhibitory function predisposes to renal disease. Annu Rev Med 64：307-324, 2013

2）Noris M, Remuzzi G：Atypical hemolytic-uremic syndrome. N Engl J Med 361：1676-1687, 2009

3）Campistol JM, Arias M, Ariceta G et al：An update for atypical haemolytic uraemic syndrome：diagnosis and treatment. A consensus document. Nefrologia 33：27-45, 2013

4）Nurnberger J, Philipp T, Witzke O et al：Eculizumab for atypical hemolytic-uremic syndrome. N Engl J Med 360：542-544, 2009

5）Nishimura J, Yamamoto M, Hayashi S et al：Genetic variants in C5 and poor response to eculizumab. N Engl J Med 370：632-639, 2014

日腎会誌 2014；56(7)：1058-1066.

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

加藤秀樹　吉田瑶子　南学正臣

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

東京大学医学部附属病院腎臓・内分泌内科

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)

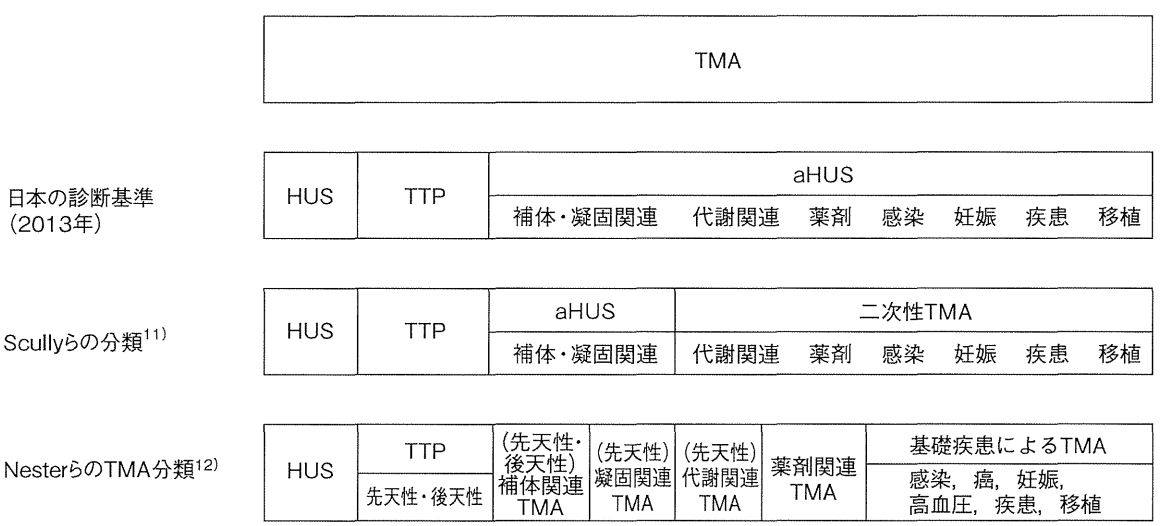

図1　TMAやaHUSの各分類のまとめ

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

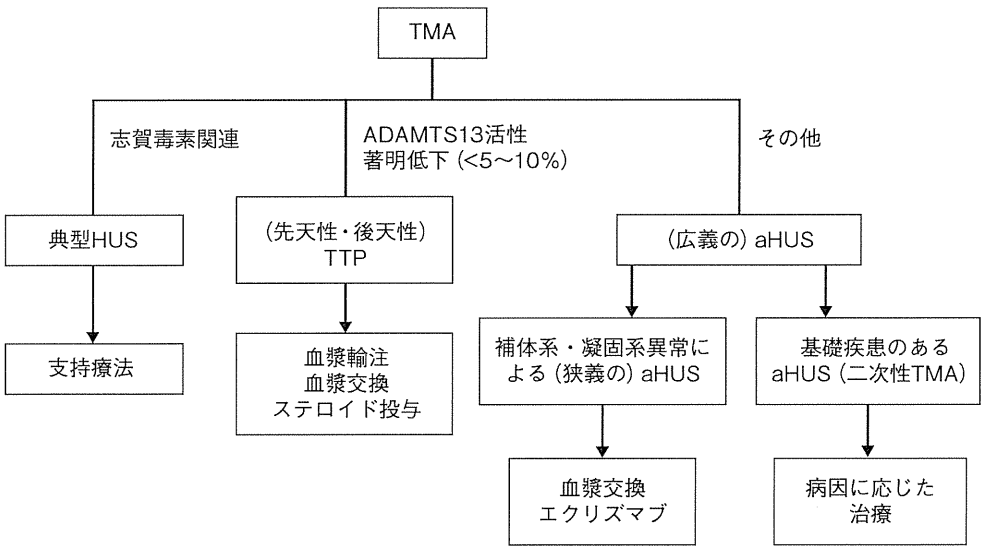

図 2 典型 HUS, TTP, aHUS の診断フローチャートと典型的な治療

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

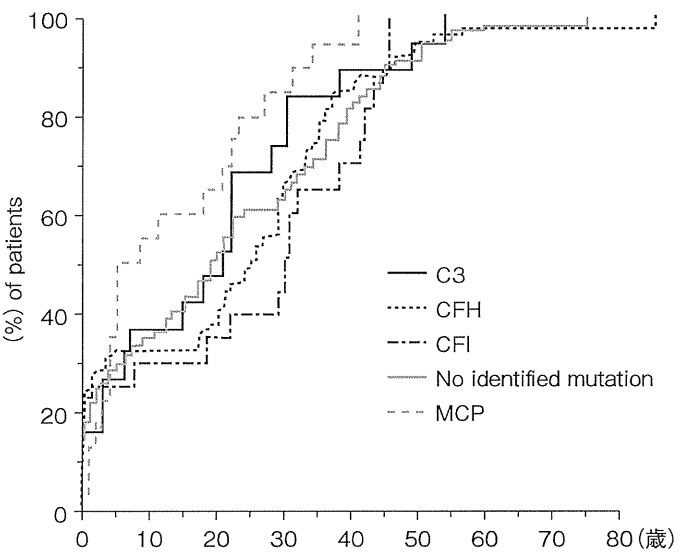

図 3 aHUS の発症年齢(文献 14 より引用)

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