図 1：ウェスタンブロット法を用いた抗 H 因子抗体スクリーニング
精製 H 因子を泳動し、患者及び正常人の血漿を 1 次抗体として添加し、2 次抗体には抗ヒト IgG-HRP 抗体を用いた。抗 H 因子抗体強陽性（＞250 AU/mL）を示した aHUS1 症例では抗体陽性と考えられるバンドを認めたが、抗体弱陽性(10.5 AU/mL)を示した aHUS3 症例では抗体陽性と考えられるバンドを認めなかった。

3）遺伝子解析結果

3 例の患者に CFH 変異を、3 例に C3 変異を同定し、遺伝子変異が同定されなかった症例は 15 例であった（解析実施中の症例：10 例）。

CFH 変異について、3 例の患者に 4 変異 : p.F176L, p.D798N, p.R1215Q, p.R1215G を同定した。1 例の患者は p.F176L と p.R1215Q の 2 つの変異を有していた。 p.R1215G このうち R1215Q/G は欧米で既に aHUS の原因として報告されている変異であり、過去に本邦 aHUS 患者にも同定されている（Fan et al. 2013, Yoshida et al. 2015)。一方、F176L 変異は 1000 ゲノムプロジェクトのデータベースや日本人 1,208 人のエクソン・シークエンスの変異情報を掲載している Human Genetic Variation Database (HGVD)にも登録が無い新規変異であった。一方、D798N 変異は HGVD にのみ登録があり minor allele frequency (MAF) は 0.002 であった。

C3 変異について、p.S562L, p.R1042L , p.I1157T を同定した。R1042L と I1157T は aHUS の原因変異として既報のものであり、特に I1157T 変異は本邦で比較的好発に認められる変異である。S562L 変異は過去に、我々が有するコホート内の別症例にも同定されているが、HGVD (MAF=0.008) に登録があることから、aHUS 発症との関連性は明らかではなかった。

また次世代シークエンサーを用いた whole exome 解析を aHUS が疑われた 12 例で実施した。whole exome の生データである FASTQ ファイルから変異の検出、コピー数解析方法の立ち上げを行い、サンガー法で認められた変異が whole exome 解析によっても検出できることを確認した。

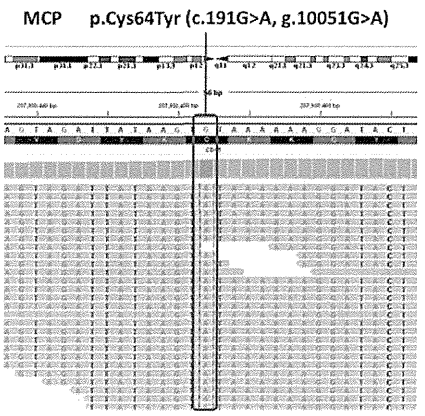

図 2: 次世代シークエンサーを用いた遺伝子診断の解析結果
24 歳男性、過去に aHUS を 5 回発症しており、兄も 0 歳時に aHUS を発症している。サンガー法を用いた解析結果と同様に whole exome 解析においても、MCP に p.Cys64Tyr 変異を認めた。

4）疫学的解析

今回、本邦で初めて妊娠を契機にしたaHUS症例が同定された。妊娠を契機としたaHUSは産後に発症する例が多いとされるが（Fakhouri F et al. JASN, 2010)、今回我々が同定した3例も全て産後にTMAを発症していた。

ACReSSを用いた臨床登録に関しては、昨年度解析した症例も含めて現在までに30例の正式な登録を終えた。今後は登録症例の詳細な解析を行うとともに、引き続き新規症例の登録を進めていく。

5）診療ガイドの作成

数回の会議を経て診療ガイド（案）の作成を行った。本診療ガイドと2013年に日本腎臓学会と日本小児科学会から公表されたaHUS診断基準の大きな変更点は“aHUSの定義”についてである。2013年に公表された診断基準ではaHUSをTMAから志賀毒素関連HUS及び血栓性血小板減少性紫斑病（TTP）を除いたTMA、と定義したのに対し、今回の診療ガイドでは除外診断項目として2次性TMAを追加して、aHUSの定義を“補体系の異常によるTMA”とした。このように2次性TMAとaHUSを明確に区別することで、個々のTMAに応じた適切な診断・治療の実施を促すように努めた。

作成した診療ガイドは日本腎臓学会、日本小児科学会を通じて公表し、パブリックコメントの募集を行うとともに、日本血栓止血学会及び日本血液学会にも査読を依頼した。得られたコメントを基に診療ガイドの訂正を終え、2016年2月に正式に公表された。

D. 考察

解析依頼症例の病歴を見ると、臨床の現場においては依然としてaHUSと2次性TMAの鑑別が困難であることが伺われた。早期診断・治療のためにも将来的には溶血試験に加え、遺伝子変異の種類、有無に関わらずaHUSと他のTMA疾患鑑別することができる試験の樹立が重要であると考える。

近年、遺伝子変異の解釈に関してはin vitroにおける発現実験等の機能的な解析による検証、データベースの活用など様々な側面からの解釈が勧められている。aHUS症例に見られる変異に関しても病的意義の解釈が困難である場合が多く、慎重に検討する必要があると考える。

E. 結論

前年度に引き続き蛋白質学的・遺伝学的解析を通して、本邦aHUS症例の蓄積を成し得た。また本研究班の成果をもとに診療ガイドの作成・公開を成し得たことから、本診療ガイドを通してaHUSの適切な診断・治療の向上が期待される。

F. 健康危険情報

なし

G. 研究発表

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28. 香美祥二. 教育講演 aHUS診療update、第37回日本小児腎不全学会学術集会、2015年11月26日、加賀市、石川県
29. 加藤秀樹. 非典型溶血性尿毒症症候群の診断・病態・治療、日本小児血液・がん学会学術集会、シンポジウム、2015年11月27日、甲府市、山梨県
30. 加藤秀樹. aHUSの診断 国内の現況、aHUS Forum2015、2015年9月12日、港区、東京都
31. 加藤秀樹. aHUS診療ガイドラインとregistryについて、第14回日本腎病理協会研究会、特別セミナー、2016年1月17日、新宿区、東京都

3. その他

・日本腎臓学会と日本小児科学会合同の非典型溶血性尿毒症症候群診断基準改訂委員会に参加（第二回：2015年4月23日、第三回：7月23日）

・非典型溶血性尿毒症症候群診療ガイドの作成を行い、日本腎臓学会、日本小児科学会を通じてパブリックコメントの募集を開始

・2016年2月に非典型溶血性尿毒症症候群診療ガイドが正式に公表

H. 知的財産権の出願・登録状況 （予定を含む。）

特になし。

Ⅲ．　研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Miyata T	GWA study for ADAMTS13 activity	Blood	125(25)	3833-3834	2015
Miyata T, Uchida Y, Ohta T, Urayama K, Yoshida Y, Fujimura Y	Atypical haemolytic uremic syndrome in a Japanese patient with *DGKE* genetic mutations.	Thromb Haemost	114(4)	862-863	2015
Ogawa Y, Matsumoto M, Sadakata H, Isonishi A, Kato S, Nojima Y, Fujimura Y	A unique case involving a female patient with Upshaw-Schulman syndrome: low titers of antibodies against ADAMTS13 prior to pregnancy disappeared after successful deliver.	Transfus Med and Hemotherapy	42(1)	59-63	2015
Yada N, Fujioka M, Bennett C, Hayakawa M, Matsumoto M, Inoki K, Miki T, Watanabe A, Yoshida T, Fujimura Y	STEC-HUS followed by acute encephalopathy in a young girl was favorably treated on a basis of hemodiafiltration, steroid pulse, and soluble thrombomodulin, under plasma exchange.	Clin Case Reports	3(4)	208-212	2015
Kato S, Tanaka M, Isonishi A, Matsumoto M, Samori T, Fujimura Y	A rapid, fully automated and highly sensitive ADAMTS13 gold particle immunoassay using a routine biochemistry analyser.	Br J Haematol	171(4)	655-658	2015

Isonishi A, Bennett CL, Plaimauer B, Scheiflinger F, Matsumoto M, Fujimura Y	Poor-responder to plasma exchange therapy in acquired TTP is associated with ADAMTS13 inhibitor boosting: Visualization of an ADAMTS13-inhibitor complex, and its proteolytic clearance from plasma.	Transfusion	55(10)	2321-2330	2015
Ito N, Hataya H, Saida K, Amano Y, Hidaka Y, Motoyoshi Y, Ohta T, Yoshida Y, Terano C, Iwasa T, Kubota W, Takada H, Hara T, Fujimura Y, Ito S	Efficacy and safety of eculizumab in childhood atypical hemolytic uremic syndrome in Japan.	Clin Exp Nephrol	in press		
Sei Y, Mizuno M, Suzuki Y, Imai M, Higashide K, Harris CL, Sakata F, Iguchi D, Fujiwara M, Kodera Y, Maruyama S, Matsuo S, Ito Y.	Expression of membrane complement regulators, CD46, CD55 and CD59, in mesothelial cells of patients on peritoneal dialysis therapy.	Mol Immunol	65(2)	302-309	2015
Nishigori N, Matsumoto M, Koyama F, Hayakawa M, Hatakeyayama K, Ko S, Fujimura Y, Nakajima Y	von Willebrand factor-rich platelet thrombi in the liver cause sinusoidal obstruction syndrome following oxaliplatin-based chemotherapy.	PLoS ONE	10(11)	e0143136	2015
Miyakawa Y, Katsutani S, Yano T, Nomura S, Nishiwaki K, Tomiyama Y, Higashihara M, Shirasugi Y, Nishikawa M, Ozaki K, Abe T, Kikuchi K, Kanakura Y, Fujimura K, Ikeda Y, Okamoto S.	Efficacy and safety of rituximab in Japanese patients with relapsed chronic immune thrombocytopenia refractory to conventional therapy.	Int J Hematol	102(6)	654-661	2015

Fan X, Kremer Hovinga JA, Shirotani-Ikejima H, Eura Y, Hirai H, Honda S, Kokame K, Taleghani MM, von Krogh AS, Yoshida Y, Fujimura Y, Lämmle B, Miyata T.	Genetic variations in complement factors in patients with congenital thrombotic thrombocytopenic purpura with renal insufficiency.	Int J Hematol	in press		

※その他、平成28年2月に日本腎臓学会と日本小児科学会より正式に公表された「非典型溶血性尿毒症症候群診療ガイド」を添付

Ⅳ．　研究成果の刊行物・別刷

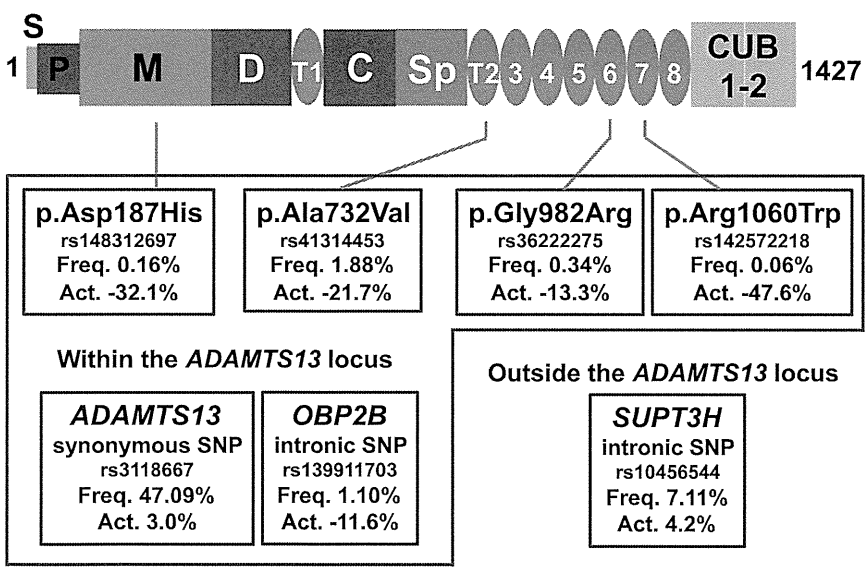
Comment on de Vries et al, page 3949

GWA study for ADAMTS13 activity

Toshiyuki Miyata NATIONAL CEREBRAL AND CARDIOVASCULAR CENTER

In this issue of *Blood*, de Vries et al report the contribution of genetic variants to plasma ADAMTS13 (disintegrin and metalloproteinase with thrombospondin motifs 13) activity with a hypothesis-free genome-wide association (GWA) approach using the Rotterdam Study, a large population-based cohort study.[1]

ADAMTS13 is a plasma metalloprotease that specifically cleaves the plasma adhesive protein von Willebrand factor (VWF). VWF is a large plasma glycoprotein and mediates platelet adhesion at sites of vascular injury. VWF is synthesized in an ultralarge multimeric form that has a very strong platelet aggregation activity. VWF-induced platelet aggregation depends on its multimeric size, which is controlled by ADAMTS13.[2] ADAMTS13 has a discrete domain structure, and the gene consists of 29 exons located on chromosome 9. Severe deficiency of ADAMTS13 activity due to *ADAMTS13* variants or acquired autoantibodies that inhibit ADAMTS13 activity leads to thrombotic thrombocytopenic purpura (TTP), a disease caused by platelet aggregation by ultralarge VWF multimers. Rare causative loss-of-function genetic variants have been identified in patients with congenital TTP.[3] In addition to TTP, ADAMTS13 may contribute to other thrombotic disorders. Low plasma levels of ADAMTS13 are associated with an increased risk for myocardial infarction and stroke. In addition, low plasma level is observed in severe sepsis, disseminated intravascular coagulation, and complicated malarial infection.[4] These findings suggest a contribution of VWF-dependent platelet aggregation in some thrombotic disease states. If so, the identification of genetic and acquired factors that affect plasma ADAMTS13 activity is very important.



ADAMTS13 domain structure and functional genetic variants. Variants boxed with green are located within the *ADAMTS13* locus. Variants boxed with red are significantly associated common variants among genome-wide SNPs. Variants boxed with blue are significantly associated rare variants among exome-wide SNPs. Act., percentage of increased or decreased plasma ADAMTS13 activity; C, Cys-rich domain; CUB, complement C1r/C1s; D, disintegrin-like domain; Freq., allele frequency; M, metalloprotease domain; P, propeptide; S, signal peptide sequence; Sp, spacer domain; T, thrombospondin type 1 repeat.

So far, rare variants of the *ADAMTS13* gene causing TTP have been identified. In addition, a few common genetic variants with modest effects on ADAMTS13 are reported. However, it is not clear whether the genetic variants exhibit strong associations at the locus. Furthermore, genetic variations outside the *ADAMTS13* locus remain unknown.

In this issue, de Vries et al adopted a systematic hypothesis-free GWA study approach to identify genetic variants that affect plasma ADAMTS13 activity by using a large, prospective, population-based cohort study, the Rotterdam Study.[1] ADAMTS13 activity was measured using a fluorogenic peptidyl substrate (fluorescence resonance energy transfer substrate [FRETS]-VWF73)[5] in >6000 individuals. Genome-wide single nucleotide polymorphisms (SNPs) for common genetic variants and exome-wide SNPs for rare genetic variants were genotyped. After careful examination of these genotyped data, de Vries et al identified p.Ala732Val (rs41314453) in *ADAMTS13* as the strongest genetic determinant of ADAMTS13 activity; the minor allele was associated with a decrease of >20% (see figure). Furthermore, they identified independent associations with a common variant in *SUPT3H* outside the *ADAMTS13* locus and 5 genetic variants at the *ADAMTS13* locus. The variant p.Ala732Val in *ADAMTS13* explained 3.6% to 6.5% of the variance in ADAMTS13 activity, which was comparable to the variance explained by age (3.9%-6.5%) or by sex (4.5%-6.7%). The 4 independently significant common SNPs (boxed with red in the figure) explained 5.8% to 8.2% of the variance.

The genetic variants influencing plasma ADAMTS13 activity have been mostly restricted to the *ADAMTS13* locus (see figure). The only exception was *SUPT3H* outside the *ADAMTS13* locus, and its effect was relatively small. This restriction is in sharp contrast to genetic factors influencing VWF. Genome-wide analysis for plasma VWF levels showed that multiple loci are involved.[6] Various steps, including VWF synthesis, packaging into Weibel-Palade bodies, secretion, and removal from the circulation, are involved in determining VWF levels,[7] and genetic variants of proteins involved in these steps could affect plasma VWF levels. ADAMTS13 activity may be

regulated in a much simpler manner than VWF.

Because rare genetic variants have generally emerged relatively recently, they show greater geographic clustering than common variants. A previous study done in the Japanese population showed that p.Pro475Ser in the *ADAMTS13* gene, restricted to the East Asian population and having an allele frequency of 5%, was associated with 14% decreased activity.[8] In Northern and Central European countries, the E1382Rfs*6 mutation due to the 4143insA mutation is frequent among patients with TTP.[9] The present study did not identify these variants, probably because all the participants were from a small geographic area.

As for the relation to congenital TTP or other thrombotic disorders, 2 rare genetic variants, p.Asp187His and p.Arg1060Trp, have previously been reported in patients with TTP. The present study revealed that their allele frequencies are 0.16% and 0.06%, respectively, indicating that a substantial number of individuals carry these variants in a homozygous or compound heterozygous state and have a genetic risk for TTP. Furthermore, it may be important to prospectively follow these variant heterozygous carriers to determine an increased risk for myocardial infarction or stroke. Thus, the results obtained from the GWA study for ADAMTS13 activity have a big impact for not only TTP but also other thrombotic disorders.

Conflict-of-interest disclosure: T.M. is employed by the National Cerebral and Cardiovascular Center, which has an awarded patent on the use of the reagent FRETS-VWF73. ■

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Atypical haemolytic uraemic syndrome in a Japanese patient with *DGKE* genetic mutations

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Dear Sirs,

Atypical haemolytic uraemic syndrome (aHUS) is characterised by thrombosis in the microvasculature and is caused by dysregulation of the complement alternative pathway via mutations or autoantibodies. Recessive mutations in the diacylglycerol kinase ε gene (*DGKE*) were recently identified in aHUS patients under two years old (1, 2) as well as in patients with membranoproliferative glomerulonephritis, membranoproliferative-like glomerular microangiopathy, or thrombotic microangiopathy (TMA) (3, 4). A clinical feature of patients with homozygous or compound heterozygous *DGKE* mutations is initial acute kidney injury, typically in children less than one year old. The aHUS caused by *DGKE* mutations is independent of complement dysregulation (5) and the exact mechanism is not known. Loss of DGKE expression in endothelial cells showed a proinflammatory and prothrombotic phenotype, with increased expressions of ICAM-1 and tissue factor (6).

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To obtain additional clinical information for aHUS patients with *DGKE* mutations, we performed a genetic analysis of *DGKE* in Japanese patients with an aHUS onset in the first two years of life. Japan's Nara Medical University has functioned as a TMA referral centre since 1988 (7), and has collected 1,122 Japanese TMA patients until the end of 2013. The database includes 77 patients with aHUS, which is defined by acute renal failure, thrombocytopaenia, and microangiopathic haemolytic anaemia, with no severe ADAMTS13 activity deficiency or Shiga toxin-producing *Escherichia coli* infection. Patients with organ or haematopoietic stem cell transplantation were excluded from aHUS. Some of genetic analyses of aHUS have been previously reported (8, 9). From the database, we selected 14 aHUS patients with a disease onset in the first two years of life. Direct sequencing of the polymerase amplification reaction products was performed using the 3730xl DNA Analyzer (Applied Biosystems Japan, Tokyo, Japan) (8). The study protocol was approved by the Ethical Committee of the National Cerebral and Cardiovascular Center, the Hiroshima Prefectural Hospital, and Nara Medical University, and written informed consents for genetic analysis were obtained.

Among the 14 selected patients, we identified one patient who had a splice site mutation c.1213–2A>G derived from his father and a frameshift mutation c.71delT encoded with p.Leu24Cysfs*145 derived from his mother (▶Figure 1). Both mutations are likely deleterious for the DGKE function and pathogenic loss-of-function mutations. Neither genetic mutation was found in the NCBI database. A DNA sequence analysis in six complement-related genes (*CFH, C3, MCP, CFI, CFB* and *THBD*) showed that the patient had the missense polymorphisms *CFH*: p.Val62Ile, p.Glu936Asp, *CFB*: p.Leu9His, p.Arg32Gln, and *THBD*: p.Ala473Val, but did not have mutations predisposing for aHUS. The patient did not have autoantibodies against complement factor H.

Another aHUS patient who presented aHUS at eight months of age had a missense mutation, p.Ile195Met, in the *DGKE* gene. *In silico* analyses for functional prediction of the missense mutation suggested that the p.Ile195Met mutation in *DGKE* was "tolerated" by the SIFT algorithm (10) and "benign" by the Polyphen-2 algorithm (11). To date, all of the aHUS patients with *DGKE* mutations are in the homozygous or compound heterozygous state, and parents with heterozygous *DGKE* mutation had no clinical abnormalities (5). We thus did not regard a *DGKE* p.Ile195Met mutation as a disease-causing mutation. The remaining 12 patients did not carry the nonsynonymous *DGKE* mutations.

We previously reported the clinical phenotypes of the former patient with compound heterozygous *DGKE* mutations (12). He was a male baby who developed plasmapheresis-resistant aHUS at four months of age and showed extremely severe hypertension. His C3 level was undetectable (<20 mg/dl), and he had high lactate dehydrogenase and creatinine levels. He received repeated plasma infusions and nine sessions of plasmapheresis. However, no treatment was effective for his haemolysis and renal failure. His severe hypertension did not initially respond to fluid removal by haemodiafiltration and was also refractory to treatment with a large intravenous dose of nicardipine chloride, peroral enarapril, and losartan. Finally, treatment with the complement C5 blockage drug eculizumab every three weeks for 17 months resulted in the control of severe hypertension and the cessation of peritoneal dialysis (12). After the administration of eculizumab, the platelet counts and C3 level increased and the lactate dehydrogenase levels decreased.

The number of aHUS patients with *DGKE* mutations who were treated with eculizumab at the acute phase and as maintenance therapy is limited. At the acute phase, one patient showed a negative

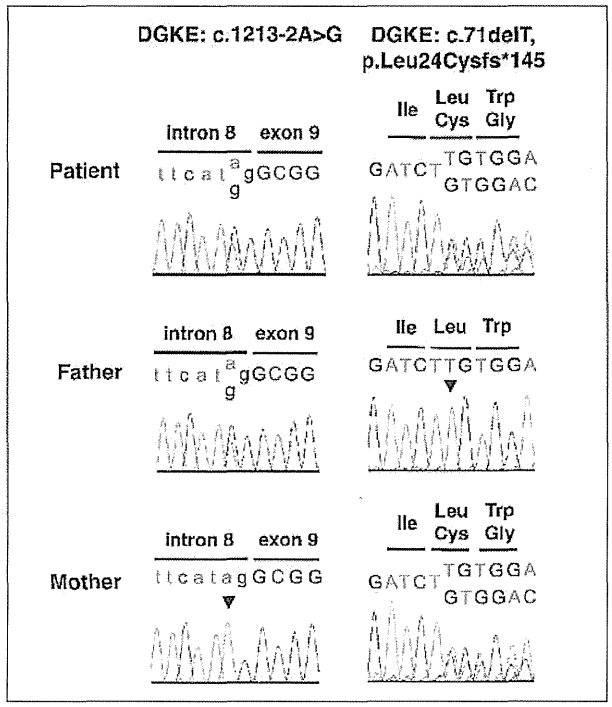

Figure 1: Chromatograms of two mutations in the *DGKE* gene in an affected patient and his parents. Small letters and capital letters are intron and exon, respectively. The A of the ATG translation initiation start site was designated as position +1, and the initial Met was denoted as +1. The c.1213–2A>G mutation disturbs the invariant splicing consensus sequence AG at the end of the intron. The c71delT mutation produces the frameshift, leading to the stop codon downstream at 145 amino acid residues.

response and another showed a positive response (1, 2). Lemaire et al. reported seven aHUS patients with *DGKE* mutations treated with eculizumab (1). None of the patients showed an abnormality in the complement system. In that study, one patient with *DGKE* mutations had aHUS recurrences even after eculizumab treatment. The genetic study of a Spanish aHUS registry reported an aHUS patient with concurrent *DGKE* and *C3* mutations who was treated with eculizumab (2). After presenting with aHUS at eight months of age, she had several aHUS recurrences, and biweekly plasma infusions were effective in normalising blood parameters; subsequent eculizumab treatment resolved the infection-associated edemas that were typical in this patient. Sanchez Chinchilla et al. suggested that the association of *DGKE* mutations concomitant with a *C3* gene mutation in this particular patient possibly contributed to more severe disease with chronic activation of TMA and a positive response to eculizumab treatment.

We report here a patient with *DGKE* mutations who presented plasmapheresis-resistant aHUS and severe hypertension in the first year of life. We did not identify mutations predisposing for aHUS in 6 complement genes in the patient, however he was successfully treated with eculizumab. The treatment strategy for aHUS patients with *DGKE* mutations is not yet settled (1, 2, 5). Further studies are needed to identify the appropriate therapeutic strategies for aHUS patients with *DGKE* mutations.

Conflicts of interest

T. Miyata has received lecturing fees from Bayer, Daiichi Sankyo, Boehringer Ingelheim, Shino-Test, Kyowa Kirin and Bristol-Myers. T. Ohta has received lecturing fees from Asahikasei Pharma, Pfaizer, Alexion Pharma, Daiichi Sankyo, Kyowa Kirin and Kyorin. Y. Fujimura is a recipient of research grant from Alexion Pharmaceuticals. None of the other authors declares a conflict of interest.

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Transfusion Medicine and Hemotherapy

Case Report

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A Unique Case Involving a Female Patient with Upshaw-Schulman Syndrome: Low Titers of Antibodies against ADAMTS13 prior to Pregnancy Disappeared after Successful Delivery

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Summary
Background: Upshaw-Schulman syndrome (USS) is usually suspected based on severe deficiency of ADAMTS13 activity without ADAMTS13 antibody, but the definitive diagnosis is made by *ADAMTS13* gene analysis. We present a unique case of USS with low titers of ADAMTS13 antibodies before pregnancy. Interestingly, titers of ADAMTS13 antibodies decreased to almost undetectable levels after delivery. **Case Report**: In patient LL4, the diagnosis of USS was confirmed at age 27 by *ADAMTS13* gene analysis. She became pregnant at age 30. During the pregnancy, she received regular fresh frozen plasma (FFP) infusion. Plasma von Willebrand factor levels increase as pregnancy progresses. To prevent platelet thrombi, much more ADAMTS13 supplementation is necessary during late gestation in patients with USS. Therefore, we shortened the interval between and increased the volume of FFP infusions as pregnancy progressed. At 39 weeks, she delivered a healthy baby girl. Before pregnancy, she had low titers of both neutralizing and binding anti-ADAMTS13 antibodies. Despite frequent FFP infusions, titers of the antibodies did not increase, but rather decreased to almost undetectable levels during pregnancy. **Conclusion**: Both the neutralizing and binding antibodies against ADAMTS13 decreased to almost undetectable levels after delivery in this patient, which can be caused by an immunological reset.

Introduction

Upshaw-Schulman syndrome (USS) is caused by a deficiency of ADAMTS13 activity due to a mutation in its gene [1]. ADAMTS13 specifically cleaves unusually large von Willebrand factor (VWF) multimers (UL-VWFMs) released from vascular endothelial cells. When ADAMTS13 activity is deficient, UL-VWFMs are not cleaved, which induces platelet thrombi formation in the microcirculation under high shear stress. Deficiency of ADAMTS13 activity is also caused by autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura (TTP) [2]. There are two types of ADAMTS13 autoantibodies. One type acts as an inhibitor of ADAMTS13 function, and the other type binds to ADAMTS13, accelerating its clearance from the circulation. USS is usually suspected to be based on severe deficiency of ADAMTS13 activity without the presence of autoantibodies, but the definitive diagnosis is usually made by *ADAMTS13* gene analysis.

USS patients often experience episodes of severe neonatal jaundice with a negative Coombs test requiring an exchange blood transfusion as well as repeated episodes of thrombocytopenia and

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Table 1. Plasma levels of anti-ADAMTS13 autoantibodies

Age, years	Gestational weeks	FFP infusion	ADAMTS13 activity, %	ADAMTS13 inhibitor, BU/ml	ADAMTS13 IgG type antibody, units/ml	Clinical status
21	*	–	<0.5	1.4	42.9	TTP bout
22	*	–	<0.5	1.7	35.0	remission
27	*	–	3.7	0.8	48.9	remission
27	*	–	1.9	1.6	33.3	remission
30	8	–	<0.5	<0.5	28.2	pregnancy
30	10	+	6.5	0.5	34.4	pregnancy
30	11	+	4.5	0.5	31.2	pregnancy
30	13	+	3.4	0.5	19.9	pregnancy
30	15	+	3.3	0.8	30.2	pregnancy
30	20	+	3.2	0.9	23.7	pregnancy
30	24	+	2.9	<0.5	21.6	pregnancy
30	29	+	2.3	0.6	13.9	pregnancy
30	33	+	3	<0.5	19.7	pregnancy
30	38	+	2.9	<0.5	14.9	pregnancy
30	39	+	6.9	0.5	16.1	pregnancy
30	*	–	1.9	0.6	13.9	1 month after delivery
32	*	–	5.2	<0.5	15.4	1.5 years after delivery
32	*	–	1.8	<0.5	9.8	2 years after delivery

microangiopathic hemolytic anemia in childhood that are reversible by infusions of fresh frozen plasma (FFP) (early-onset phenotype) [3]. On the other hand, patients with the 'late-onset phenotype' are diagnosed with USS in adulthood, usually during episodes of infectious disease or pregnancy [3]. Moatti-Cohen et al. [4] reported that the rate of USS is much higher in pregnancy-onset TTP patients than in all adulthood-onset TTP patients.

We previously described 43 USS patients in Japan up to the end of March 2011 [3]. Among them, 9 patients developed bouts of TTP and were correctly diagnosed with USS in association with pregnancy [5]. These pregnancies often result in premature delivery or fetal loss. Recent papers have reported successful delivery with FFP infusion therapy in patients with USS diagnosed prior to pregnancy [6, 7]. However, a detailed therapeutic protocol including FFP infusions for pregnant women with USS has not yet been established.

Here, we report a USS patient with low titers of neutralizing (inhibitory) and non-neutralizing (binding) antibodies against ADAMTS13 who successfully underwent delivery with the use of gradually increasing FFP infusions as the pregnancy progressed. The intervals between and volumes of FFP infused were determined by close monitoring of levels of ADAMTS13 activity and its inhibitor.

Material and Methods

Until 2005, ADAMTS13 activity was analyzed by a VWF multimer assay with a detection limit of 3% of normal controls [2, 8]. Since 2005, a highly sensitive chromogenic ADAMTS13-act-ELISA [9] with a detection limit of 0.5% of normal was developed and replaced the VWF multimer assay. Thus, we re-examined ADAMTS13 activity in stored plasma samples using this act-ELISA and reported the results by the act-ELISA in this study. Plasma ADAMTS13 inhibitor titers were also re-examined using the chromogenic ADAMTS13-act-ELISA in heat-inactivated plasma at 56 °C for 30 min. One Bethesda unit (BU) of inhibitor was defined as the amount of inhibitor that reduces ADAMTS13 activity to 50% of control [10]. ADAMTS13 inhibitor titers were defined as: <0.5 BU/ml (negative), 0.5–1.0 BU/ml (marginal), and ≥1.0 BU/ml (positive). Plasma levels of ADAMTS13 antigen were determined using a quantitative sandwich ELISA assay [11]. Plasma ADAMTS13 antigen was also analyzed by quantitative and qualitative western blotting (WB) under reducing conditions [12]. Densitometric analysis of ADAMTS13 antigen was performed for the 190 kDa band using NIH imageJ (developed by the National Institutes of Health, *http://rsb.info.gov/nih-image/*). Plasma anti-ADAMTS13 IgG antibody titers (binding antibody) were determined by TECHNOZYM® ADAMTS-13 INH (Technoclone, Vienna, Austria) according to the manufacturer's instructions. In this assay, plasma IgG levels less than 12 units/ml were defined as negative, 12–15 units/ml were considered borderline, and levels greater than 15 units/ml were defined as positive. *ADAMTS13* gene analyses [13] were performed with the permission of the Ethics Committees. The pathogenicity of missense mutations was analyzed in silico using PolyPhen-2 (*http://genetics.bwh.harvard.edu/pph2/*) to predict the functional significance of missense mutations. Written informed consent for *ADAMTS13* gene analysis was obtained from the patient and her family.

Case Report

Proband LL4 is a female born in 1981. Her parents and elder sister are apparently healthy. She did not have any episodes of severe neonatal jaundice requiring exchange blood transfusion. At 14 years of age, she developed thrombocytopenia and acute renal failure requiring hemodialysis during an upper respiratory tract infection. She had similar episodes during upper respiratory tract infections at the ages of 15, 16, 17, and 20 years. These bouts were ameliorated by FFP infusion. At 21 years of age, she was admitted to a local hospital complaining of diarrhea and high-grade fever. She was diagnosed with TTP based on the pentad of hemolytic anemia, thrombocytopenia, acute renal failure, fever, and mild neurological symptoms. Her condition improved with FFP administration. Soon after this episode, she got married. When the patient was 27 years old, detailed investigation including *ADAMTS13* gene analysis was performed in all members of her family. At 28 years of age, she underwent an elective termination at 6 weeks of gestation after the risk of developing TTP was taken into consideration. She has never received prophylactic FFP infusions without the presence of thrombocytopenia.