bjh research paper

Pregnancy-induced thrombocytopenia and TTP, and the risk of fetal death, in Upshaw–Schulman syndrome: a series of 15 pregnancies in 9 genotyped patients

Yoshihiro Fujimura,[1] Masanori Matsumoto,[1] Koichi Kokame,[2] Ayami Isonishi,[1] Kenji Soejima,[3] Nobu Akiyama,[4] Junji Tomiyama,[4] Kazuhiko Natori,[5] Yasunobu Kuranishi,[5] Yutaka Imamura,[6] Nobumasa Inoue,[7] Satoshi Higasa,[8] Masako Seike,[9] Teruhiko Kozuka,[9] Masamichi Hara,[9] Hideo Wada,[10] Mitsuru Murata,[11] Yasuo Ikeda,[12] Toshiyuki Miyata[2] and James N. George[13]

[1]*Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Nara, Japan,* [2]*National Cardiovascular Centre Research Institute, Suita, Osaka, Japan,* [3]*The First Research Department, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan,* [4]*Department of Haematology, Metropolitan Bokutoh Hospital, Tokyo, Japan,* [5]*Department of Haematology and Oncology, Toho University Omori Medical Centre, Tokyo, Japan,* [6]*Department of Haematology, Saint Mary's Hospital, Kurume, Fukuoka, Japan,* [7]*Department of Internal Medicine, National Hospital Organization Osaka National Hospital, Osaka, Japan,* [8]*Department of Haematology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan,* [9]*Department of Haematology, Ehime Prefectural Central Hospital, Matsuyama, Ehime, Japan,* [10]*Department of Clinical Laboratory, Mie University School of Medicine, Tsu, Mie, Japan,* [11]*Department of Laboratory Medicine, Keio University, Tokyo, Japan,* [12]*Department of Internal Medicine, Keio University, Tokyo, Japan, and* [13]*Departments of Medicine and Biostatistics & Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*

Received 31 July 2008; accepted for publication 16 October 2008

Correspondence: Prof Yoshihiro Fujimura. Department of Blood Transfusion Medicine, Nara Medical University, 840 Shijo-cho, Kashihara City, Nara, 634-8522, Japan.
E-mail: malon@naramed-u.ac.jp

First published online 25 November 2008
doi:10.1111/j.1365-2141.2008.07515.x

Summary

Upshaw–Schulman syndrome (USS) is a congenital thrombotic thrombocytopenic purpura (TTP) due to mutations in the gene that encodes for ADAMTS13 (*ADAMTS13*), but its clinical signs may be mild or absent during childhood. We have identified 37 patients with USS (24 females, 13 males) belonging to 32 families. The nine women from six families who were diagnosed during their first pregnancy are the focus of this report. Six of the nine women had episodes of thrombocytopenia during childhood misdiagnosed as idiopathic thrombocytopenic purpura. Thrombocytopenia occurred during the second–third trimesters in each of their 15 pregnancies, with 16 babies (one twin pregnancy), often followed by TTP. Of 15 pregnancies, eight babies were stillborn or died soon after birth, and the remaining seven were all premature except one, who was born naturally following plasma infusions to the mother that had started at 8 weeks' gestation. All nine USS women had severely deficient ADAMTS13 activity. *ADAMTS13* analyses demonstrated that eight women were compound heterozygotes of Y304C/G525D (2 siblings), R125VfsX6/Q1302X (2 siblings), R193W/R349C (2 siblings), I178T/Q929X, and R193W/A606P; one woman was homozygous for R193W. Only the R193W mutation has been previously reported. These observations emphasize the importance of measuring ADAMTS13 activity in the evaluation of thrombocytopenia during childhood and pregnancy.

Keywords: Upshaw–Schulman syndrome, pregnancy, *ADAMTS13* mutation, thrombocytopenia, haemolytic anaemia.

Upshaw–Schulman syndrome [USS, also described as congenital thrombotic thrombocytopenic purpura (TTP)] is a congenital deficiency of ADAMTS13 (A Disintegrin-like And Metalloprotease with ThromboSpondin type 1 motifs-13) activity due to *ADAMTS13* gene mutations (Kinoshita *et al*, 2001; Levy *et al*, 2001; Kokame *et al*, 2002). When ADAMTS13 activity is deficient, unusually large von Willebrand factor (VWF) multimers accumulate in the circulation that can cause platelet thrombi under high shear stress of the microcirculation (Yagi *et al*, 2001; Moake, 2002). In 2005, the presence of roughly 100 patients with USS in 80 families worldwide was estimated (Kremer-Hovinga *et al*, 2005). But still USS patients may often be overlooked in clinical practice. Therefore, the frequency of USS is presumably underestimated.

Acquired TTP, caused by neutralizing or non-neutralizing autoantibodies to ADAMTS13 that reduce plasma ADAMTS13 activity, is a disorder with an incidence of 1·7 per million population; 73% of the patients were women and most women were of child-bearing age (Terrell *et al*, 2005). Although acquired TTP and USS have some similarities, USS has important differences in clinical features from acquired TTP (Furlan & Lämmle, 2001; Fujimura *et al*, 2002).

During the past 10 years, we have diagnosed 37 patients with USS by assaying ADAMTS13 activity and its inhibitor titres in the laboratory of Nara Medical University. Further, analysis of the natural history and *ADAMTS13* mutations in these patients showed that severe neonatal jaundice requiring exchange blood transfusions, a classic hallmark of USS, was seen in only 16 (43%) of 37 patients. Twenty-nine (79%) of the 37 patients had a history of thrombocytopenia during childhood that was misdiagnosed as idiopathic thrombocytopenic purpura (ITP). Nine women from six families were first diagnosed during pregnancy. Further, we documented that thrombocytopenia inevitably developed during the second or third trimester of all 15 pregnancies in these nine women. Often, the initial isolated thrombocytopenia was followed by overt signs of microangiopathic haemolytic anaemia and TTP. Notably, of 15 pregnancies (one twin pregnancy), eight babies were stillborn or died soon after birth, and seven babies were all premature but survived. Only one mature baby was born at full term following plasma infusions to the mother that had started at 8 weeks gestation. Six of these nine USS-women had episodes of severe to mild thrombocytopenia during childhood that had been incorrectly diagnosed as ITP.

Because of the important association between USS and pregnancy we report the detailed natural history and clinical characterization of these nine women as well as their *ADAMTS13* mutations. Our experience emphasizes the importance of measuring ADAMTS13 activity as part of the evaluation of thrombocytopenia during childhood or pregnancy.

Patients, materials and methods

Patients with USS

Between July 1998 and April 2008, 37 patients with USS (24 females and 13 males) belonging to 32 different families were identified at Nara Medical University. Severe neonatal jaundice that required exchange blood transfusion, a hallmark of USS, was found in 16 (43%) of these 37 patients. Notably, nine USS patients belonging to 6 families (designated as K, L, M, O, R and Z) had been pregnant and they all developed thrombocytopenia, often followed by TTP, at the second or third trimester. Thus, these 9 patients were extensively studied here. None of these nine women had received contraceptive agents before their pregnancies. It is also important to note that the remaining 15 USS women had no pregnancies. These data will be published elsewhere.

Assays of ADAMTS13 activity and ADAMTS13 inhibitor

Plasma levels of ADAMTS13 activity and ADAMTS13 inhibitor were measured by both a classic VWF multimer assay (Furlan *et al*, 1998) and a chromogenic ADAMTS13-activity-enzyme-linked immunosorbent assay (ADAMTS13 act-ELISA) (Kainos Inc., Tokyo) (Kato *et al*, 2006). The ADAMTS13 activity of pooled normal plasma was defined as 100%, and the detection limits of classic VWF multimer assay and the act-ELISA were 3% and 0·5% of the normal controls respectively. The ADAMTS13 inhibitor titres were expressed in Bethesda units (BU), where one inhibitor unit was defined as the amount necessary to reduce ADAMTS13 activity to 50% of the control level. A titre of ≥0·5 BU/ml in both the assays was considered to be significant.

Assays of ADAMTS13 antigen

Plasma levels of ADAMTS13 antigen were determined by a quantitative sandwich antigen ELISA (ag-ELISA) by using two anti-ADAMTS13 monoclonal antibodies (mAbs); a neutralizing mAb A10 as the coating antibody and a non-neutralizing mAb C7 labeled with horseradish peroxidase (HRP) as the detection antibody (Yagi *et al*, 2007). The epitopes recognized by mAbs A10 and C7 were determined to reside in the disintegrin-like domain and the 7th and 8th thrombospondin type-1 domains respectively (Uemura *et al*, 2005). The ADAMTS13 antigen of pooled normal plasma was defined as 100%, and the detection limit was 0·1% of the normal controls.

Further, ADAMTS13 antigen was analysed by Western blot under reducing conditions, quantitatively and qualitatively (Ishizashi *et al*, 2007). Briefly, this was performed as the follows. An aliquot (2 μl) of diluted or undiluted plasma sample per lane were separated by sodium dodecyl sulfate-5% polyacrylamide gel electrophoresis (SDS-5%PAGE) under reducing conditions. After electrophoresis, the proteins were electrophoretically blotted onto polyvinylidene difluoride

(PVDF) microporous membranes. We probed the blots for ADAMTS13 antigen with WH2-11-1 as the primary mAb, followed by secondary staining with HRP-conjugated goat anti-mouse IgG (Kirkegaard & Perry Lab, Gaithersburg, MO, USA). The epitope of mAb WH2-11-1 resides in the fourth thrombospondin type-1 domain (Soejima *et al*, 2006). After incubation with Western Lighting Chemiluminescence Reagent (PerkinElmer Life Sciences, Shelton, CT), the blots were exposed to X-ray film. Densitomeric analysis of ADAMTS13 antigen was performed for the 190-kD band using the National Institutes of Health (NIH) image J (developed by NIH, http://rsb.Info.gov/noh-image/). The detection limit of plasma ADAMTS13 antigen by this method was 3% of the normal controls.

ADAMTS13 gene analysis

All DNA analyses were performed with the permission of Ethics Committees of both the sample-collecting hospitals and the institute that performed the gene analysis. Written informed consent was obtained from all patients. The nucleotide sequences of the entire 29 exons of *ADAMTS13*, including the intron–exon boundaries, were determined by direct sequencing of polymerase chain reaction (PCR) products, as previously described in detail (Kokame *et al*, 2002; Matsumoto *et al*, 2004). All the disease-causing *ADAMTS13* mutations reported here were excluded as common polymorphisms by screening 96 individuals in the Japanese general population.

Results

The natural history, clinical characterization, and ADAMTS13 activity of the 9 women with USS and their family members are summarized in Table I. Detailed descriptions of these patients are provided in the text.

Family K, clinical data

Proposita K-3, born in 1976, was the older of 2 siblings born to non-consanguineous parents (Fig 1, top). She had no history of severe neonatal jaundice. At the age of 6 years, she developed nasal bleeding with thrombocytopenia and was diagnosed with ITP. She then had repeated episodes of thrombocytopenia during childhood. Her first pregnancy was at the age of 27 years. The initial platelet count was normal (180×10^9/l), but at 24 weeks gestation the platelet count decreased to 17×10^9/l. She received steroid therapy and then high-dose immunoglobulin G (IVIg) infusions in a local hospital without clinical improvement. She subsequently developed microangiopathic haemolytic anaemia with neurological and renal abnormalities, was diagnosed with TTP, and treated with plasma exchange. Her fetus died at 25 weeks gestation and was removed by Caesarean section; necrotic lesions and intravascular thrombi were identified in a large area of the placenta and also the uterus, necessitating hysterectomy. Following hysterectomy and plasma exchange (twice during 2 d), a normal platelet count ($\geq 150 \times 10^9$/l) was achieved, but a few weeks after cessation of plasma exchange the platelet count dropped again requiring an additional plasma exchange treatment. Following recovery, her plasma ADAMTS13 activity and ADAMTS13 inhibitor were assayed by the VWF multimer method, demonstrating a severe deficiency of ADAMTS13 activity but without an inhibitor, suggesting a diagnosis of USS.

At the same time, her younger sister (K-4), born in 1978 (age 25 years), was a primigravida at 22 weeks gestation, and she also had mild thrombocytopenia (59×10^9/l) and anaemia (Hb 91 g/l). She had a history of severe neonatal jaundice that required exchange blood transfusion. At the age of 4 years, she had an episode of severe thrombocytopenia with bleeding (site unknown) that required blood transfusion, and was diagnosed as ITP. Because of her elder sister's diagnosis of USS, this patient was tested for ADAMTS13 activity and ADAMTS13 inhibitor, demonstrating the same results as her elder sister. Thus, prophylactic FFP infusions (320 ml every 2 weeks) were instituted to maintain pregnancy. Under these circumstances, at 29 weeks gestation the patient had a premature boy by Caesarean section.

After the diagnosis of USS, both the siblings had receiving 320 ml of FFP infusions every 3 weeks. In 2005, the plasma levels of ADAMTS13 activity in both K-3 and K-4 were examined by the act-ELISA, and indeed they were shown to be severely deficient (<0·5% of the normal) but without its inhibitors (<0·5 BU/ml).

ADAMTS13 analysis (heterozygous Y304C/G525D mutation)

The *ADAMTS13* mutations of both the affected siblings (K-3 and K-4) were shown to be the compound heterozygotes of Y304C (*c.* 911A > G, exon 8) and G525D (*c.* 1574G > A, exon 13), and their parents were heterozygous carriers of either of the mutations. Five common single nucleotide polymorphisms (SNPs) found in the two affected siblings are listed in Table II.

Plasma levels of ADAMTS13 antigen by ag-ELISA were 0·4% of normal in both the affected siblings, and 31% and 36% in their father and mother respectively. Further, plasma levels of the respective ADAMTS13 antigen by Western blot were shown to be 5·1%, 4·8%, 39% and 34%. Thus, the Y304C/G525D mutation was apparently secreted into the plasma, but with a markedly reduced level (Fig 1, top right and Fig 2).

Family L, clinical data

Proposita L-2, born in 1967, was the second of five siblings to non-consanguineous parents. Of her five siblings, the first child died at 20 weeks gestation with a diagnosis of intrauterine fetal death of unknown aetiology, and two brothers (the third and fourth siblings) were apparently healthy. She had no

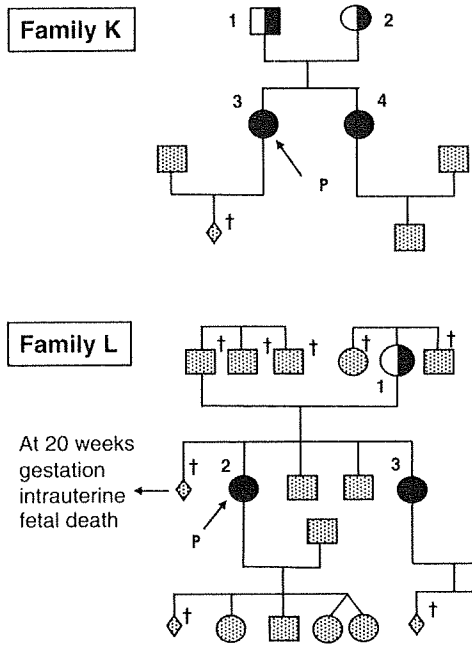
Table I. Clinical characterization of 9 female patients with Upshaw–Schulman Syndrome who developed TTP-bouts during pregnancy.

			Newborn period	Childhood	Adulthood (pregnancy)							
					Before	After				Therapy and outcome		
Number	USS-patients	Year of birth	Exchange blood transfusion for severe jaundice	Clinical diagnosis (age)	Clinical diagnosis	Age (years)	Gestation (weeks)	Therapy	Gestation (weeks)	Diagnosis	Babies	Mothers	Year of diagnosis for USS by ADAMTS13 assay
1	K-3	1976	No	ITP (6 years)	ITP	27	24	ST + IVIg	25	TTP	IUFD at 25 weeks gestation	PE (2 times), CS, necrosis of placenta and uterus, hysterectomy, remission	2003
2	K-4	1978	Yes	ITP (4 years)	ITP	25	20	FFP infusions	29	ITP	Live-birth at 29 weeks gestation, premature baby	CS under FFP infusions, remission	2003
3	L-2	1967	No	None	None	27	27	Conservative	28	HELLP syndrome or APS	IUFD at 27 weeks gestation	CS, remission	–
					APS	28	3rd trim.	Aspirin	37	APS	Live-birth at 37 weeks gestation under aspirin intake, Premature baby	CS, remission	–
					APS	30	3rd trim.	Aspirin	32	APS	Live-birth at 37 weeks gestation under aspirin intake, Premature baby	CS, remission	–
					APS	33	3rd trim.	Aspirin	32	APS	Live-birth at 32 weeks gestation under aspirin intake, Premature twins	CS, remission	2003
4	L-3	1972	No	ITP (3 years)	ITP	25	24	ST	25	Evans syndrome	Stillbirth at 25 weeks gestation	ST, remission after vaginal delivery	–
					ITP	27	23	PE, Haemodialysis	24	HUS	Live-birth of premature baby at 24 weeks gestation, but it died soon after delivery.	PE (2 times) and haemodialysis, Remission after vaginal delivery	2003
5	M-3	1969	No	None	None	33	17	PE	20	TTP	Miscarriage at 20 weeks gestation	PE (2 times), remission	2003

Table I. (Continued).

			Newborn period	Childhood	Adulthood (pregnancy)							
					Before	After				Therapy and outcome		
Number	USS-patients	Year of birth	Exchange blood transfusion for severe jaundice	Clinical diagnosis (age)	Clinical diagnosis	Age (years)	Gestation (weeks)	Therapy	Gestation (weeks)	Diagnosis	Babies	Mothers	Year of diagnosis for USS by ADAMTS13 assay
6	M-4	1971	No	None	None	30	28	CS		HELLP syndrome	Stillbirth at 28 weeks gestation	CS, remission	–
						31	33	Conservative	36	ITP	Live-birth at 36 weeks gestation	CS, remission	2003
7	O-4	1958	No	ITP (5 years)	ITP	26	23	Conservative	25	ITP	Live-birth of premature baby at 25 weeks gestation, but it died soon after birth	TTP-bout after vaginal delivery, PE (2 times), remission	–
					Chronic relapsing TTP	31	8	FFP infusions	39	Chronic relapsing TTP	Live-birth weighed 2·984 kg at 39 weeks gestation	Natural delivery	2004
8	R-5	1982	No	ITP (8 months)	ITP	23	23	Conservative	30	TTP (after delivery)	IUFD at 30 weeks gestation	TTP-bout after CS, PE (8 times) and ST, remission	2005
9	Z-3	1971	No	ITP (7 years)	ITP	25	12	Conservative	32	TTP (after CS)	Live-birth at 32 weeks gestation. Premature baby	TTP-bout after CS, PE (times unclear), remission	1998

USS, Upshaw–Schulman Syndrome; ITP, idiopathic thrombocytopenic purpura; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; APS, anti-phospholipid syndrome; ST, steroid; FFP, fresh frozen plasma; IVIg, high dose immunoglobulin G; HELLP, haemolysis, elevated liver enzyme and low platelet count; CS, Caesarean section; IUFD, interuterine fetal death.



	ADAMTS13 activity (%)		ADAMTS13 antigen (%)	*ADAMTS13* gene		
	act-ELISA	VWFM	ag-ELISA	Tyr304	Gly525	Haplotype
K-1	22	31	62	Y/C	G/G	YG/CG
K-2	26	36	58	Y/Y	G/D	YG/YD
K-3	<0·5	<3	0·4	Y/C	G/D	CG/YD
K-4	<0·5	<3	0·4	Y/C	G/D	CG/YD

	ADAMTS13 activity (%)		ADAMTS13 antigen (%)	*ADAMTS13* gene		
	act-ELISA	VWFM	ag-ELISA	Arg125	Gln1302	Haplotype
L-1	48	76	58	R/fs	Q/Q	RQ/fsQ
L-2	<0·5	<3	<0·1	R/fs	Q/X	fsQ/RX
L-3	<0·5	<3	<0·1	R/fs	Q/X	fsQ/RX

Fig 1. Pedigree and ADAMTS13 analyses of USS-Family K and L. Squares and circles indicate males and females, respectively, and arrows with P indicate the proposita. Filled symbols represent USS-patients. The half-filled symbols represent asymptomatic carriers. The cross indicates deceased. The ADAMTS13 activity by both act-ELISA and VWF multimers are shown as a percentage of the normal control. The ADAMTS13 antigen by ag-ELISA is shown as a percentage of the normal control. Mutations found in *ADAMTS13* are shown as one-letter amino acid abbreviations.

history of severe neonatal jaundice or childhood thrombocytopenia. She was a primigravida at the age of 27 years, and her platelet count was 180×10^9/l at 6 weeks gestation, which dropped to 38×10^9/l at 27 weeks, complicated with acute renal failure, followed by intrauterine fetal death. She was suspected to have HELLP (Hemolysis, Elevated Liver enzyme, and Low Platelet count) syndrome or anti-phospholipid antibody syndrome. Interestingly, soon after Caesarean section her platelet count returned to normal. Because of this episode, her physician recommended that she took a low-dose aspirin (81 mg/d) whenever she had thrombocytopenia in association with pregnancy. At the age of 28 years, she had her second pregnancy, and again developed thrombocytopenia (21×10^9/l) and proteinuria during the third trimester, and low-dose aspirin was administered. She delivered by Caesarean section at 37 weeks gestation. After delivery, her platelet count soon returned to normal (150×10^9/l). At the age of 30 years, she had her third pregnancy and was treated similarly as above, and was delivered by Caesarean section at 37 weeks gestation. Finally, at the age of 33 years, she had her fourth pregnancy with twins. Even with the administration of low-dose aspirin, she developed haemolytic anaemia with thrombocytopenia (53×10^9/l) at 30 weeks gestation, which was suspected to be Coombs'-negative Evans' syndrome and therefore she was treated with oral steroids and IVIg therapy. Her platelet count did not increase, she developed generalized oedema and pleural effusions at 32 weeks gestation, and her premature twins were delivered by Caesarean section. One week after delivery, her platelet count spontaneously increased to 200×10^9/l. Since then, she has never taken steroid or aspirin.

The younger sister (L-3), born in 1972, had no history of severe neonatal jaundice. At the age of 3 years, she had mild thrombocytopenia (70×10^9/l) at the time of a respiratory infection, and was diagnosed with ITP. At the age of 25 years, she was a primigravida, and at 24 weeks gestation developed proteinuria and thrombocytopenia (20×10^9/l) that was suspected to be Evans' syndrome and was treated with steroids. Then she had a stillbirth at 25 weeks gestation. After delivery, her platelet count soon recovered to normal. She had her second pregnancy when aged 27 years, and at 23 weeks gestation she developed thrombocytopenia (14×10^9/l) and gross hematuria accompanied by renal failure. She was suspected to have haemolytic-uremic syndrome (HUS) and was treated with plasma exchange for 2 d (once per day) and haemodialysis. Despite this intensive care, she showed no clinical improvement, and at 24 weeks gestation she delivered a premature baby transvaginally but it died soon after birth. Her platelet count recovered within 1 week after delivery. These two sisters were referred to Nara Medical University in 2003, and plasma levels of ADAMTS13 activity and ADAMTS13 inhibitor were assayed by the VWF multimer method, demonstrating a severe deficiency of ADAMTS13 activity but without an inhibitor, consistent with a diagnosis of USS. In 2005, the act-ELISA revealed that the plasma levels of ADAMTS13 activity in both L-2 and L-3 were severely deficient (<0·5% of the normal control), but without an inhibitors (<0·5 BU/ml).

Table II. Disease-causing mutations and common SNPs of *ADAMTS13 gene* in 9 patients with Upshaw–Schulman syndrome.

Patients with USS	Exon/ Intron	Nucleotides	Amino acid	Remarks
K-3	exon 8	911 A > G	Y304C	disease causing
K-4				mutation
	exon 9	1016 C > G	T339R	common SNP
	exon 12	1342 C > G	Q448E	common SNP
	exon 13	1574 G > A	G525D	disease causing mutation
	exon 15	1716 G > A	silent	common SNP
	exon 16	1852 C > G	P618A	common SNP
	exon 19	2280 C > T	silent	common SNP
L-2	exon 4	3712 ins GT	R125VfsX6	disease causing
L-3				mutation
	exon 9	1016 C > G	T339R	common SNP
	exon 12	1342 C > G	Q448E	common SNP
	exon 15	1716 G > A	silent	common SNP
	exon 16	1852 C > G	P618A	common SNP
	exon 19	2280 C > T	silent	common SNP
	exon 28	3904 C > T	Q1302X	disease causing mutation
M-3	exon 6	577 C > T	R193W	disease causing
M-4				mutation
	exon 9	1045 C > T	R349C	disease causing mutation
	exon 19	2280 C > T	silent	common SNP
O-4	exon 5	533T > C	I178T	disease causing mutation
	exon 19	2280 C > T	silent	common SNP
	exon 22	2785 C > T	Q929X	disease causing mutation
R-5	exon 6	577 C > T	R193W	disease causing mutation
	exon 9	1016 C > G	T339R	common SNP
	exon 12	1342 C > G	Q448E	common SNP
	exon 15	1716 G > A	silent	common SNP
	exon 16	1816 G > C	A606P	disease causing mutation
	exon 16	1852 C > G	P618A	common SNP
	exon 19	2280 C > T	silent	common SNP
Z-3	exon 6	577 C > T	R193W	disease causing mutation
	exon 19	2280 C > T	silent	common SNP

ADAMTS13 analysis (heterozygous R125VfsX6/Q1302X mutation)

The *ADAMTS13* mutations were the compound heterozygotes of R125VfsX6 (c.372insGT, exon 4) and Q1302X (*c.* 3904C > T, exon 28) in both the affected siblings (L-2 and L-3). Their parents were heterozygous carriers of either of the mutations. Seven common SNPs found in the two affected siblings are listed in Table II.

Plasma levels of ADAMTS13 antigen by the ag-ELISA were severely deficient in both patients, <0·1% of the normal, and 58% in their mother. The ADAMTS13 antigen analysis by Western blot confirmed these results: <3% of normal in both the patients, 79% in their mother, and no other bands with a molecular weight less than 190 kDa. Thus, neither mutant protein, R125VfsX6 or Q1302X, was present in plasma (Fig 1, bottom right and Fig 2).

Family M, clinical data

Proposita M-3, born in 1969, was the second of four siblings to non-consanguineous parents (Fig 3, top). She had no history of severe neonatal jaundice or thrombocytopenia during childhood. The 1st child in this family died of utero-placental thrombosis at 32 weeks gestation, and the 4th child (M-5) has never been pregnant and is apparently healthy. At the age of 33 years, M-3 was a primigravida and at 17 weeks gestation she developed petechiae and oedema in both the lower extremities, and was diagnosed as TTP at 19 weeks gestation. She was treated with plasma exchange twice for 2 d but eventually miscarried at 20 weeks gestation. Thereafter, her platelet count quickly recovered, but 1 month later her platelet count again dropped to 24×10^9/l. She received plasma infusion, which increased her platelet count to normal.

Her younger sister (M-4), born in 1971, also had no history of severe neonatal jaundice or thrombocytopenia during childhood. At the age of 30 years, she had her first pregnancy and at 28 weeks gestation she developed thrombocytopenia, hypertension, and proteinuria, suggesting a diagnosis of HELLP syndrome. A Caesarean section was performed, but the child was stillborn. The next year, she had her second pregnancy, and at 33 weeks gestation she developed thrombocytopenia, and Caesarean section was performed at 36 weeks gestation, delivering a live female infant.

Both the affected siblings (M-3 and M-4) in this family were diagnosed with USS in 2003 after determination of ADAMTS13 activity and ADAMTS13 inhibitor by VWF multimer assay. In 2005, the act-ELISA revealed that the plasma levels of ADAMTS13 activity in both M-3 and M-4 were indeed severely deficient (<0·5% of the normal), without inhibitors (<0·5 BU/ml).

ADAMTS13 analysis (heterozygous R193W/R349C mutation)

The *ADAMTS13* mutations were compound heterozygotes of R193W (*c.* 577C > T, exon 6) and R349C (*c.* 1045C > T, exon 9) in both affected siblings (M-3 and M-4); their parents were heterozygous carriers of either of the mutations. One common SNP was found in these two affected siblings (Table II).

Plasma levels of ADAMTS13 antigen by the ag-ELISA were 8·6% and 7·7% of the normal in the patients, 57%, and 63% in their parents. Further, plasma levels of ADAMTS13 antigen by Western blot were 24% and 23% in two patients, and 45% and 43% in their parents. Thus, the R193W/R349C mutation is apparently secreted into plasma, but at a significantly reduced level (Figs 2 and 3, top right).

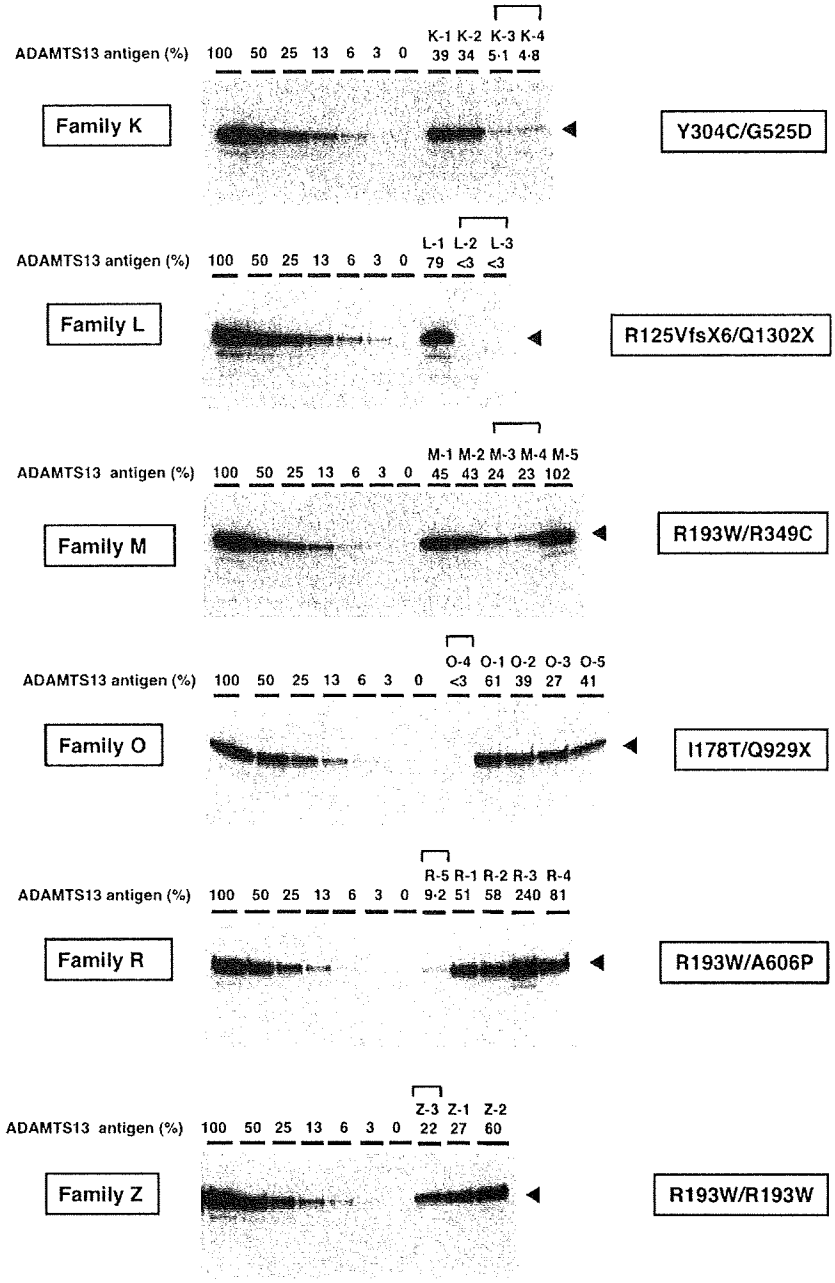
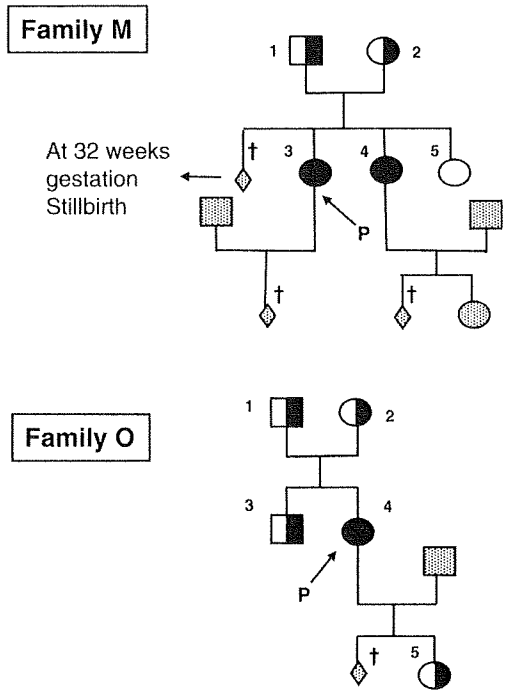


Fig 2. Western blot analyses of ADAMTS13 antigen in plasma milieu of members in USS-Family K~Z. Plasma ADAMTS13 antigen was qualitatively and quantitatively analyzed by Western blot, as described in Materials and methods. Briefly, each 2 μl of diluted or undiluted plasma sample per lane were separated by SDS-5%PAGE under reducing conditions. After electrophoresis, the proteins were blotted onto polyvinylidene difluoride (PVDF) membranes. The ADAMTS13 antigen was incubated with WH2-11-1 as the primary mAb, followed by secondary staining with HRP-conjugated goat anti-mouse IgG, and finally detected by luminography. Densitomeric analyses of ADAMTS13 antigen were performed for the 190-kD band using NIH image J.

Family O, clinical data

The proposita O-4, born in 1958, was the second child of two siblings to non-consanguineous parents (Fig 3, bottom). She had no history of severe neonatal jaundice or thrombocytopenia during childhood. At the age of 26 years, she had her first pregnancy, and at 23 weeks gestation she developed thrombocytopenia (20×10^9/l) and a diagnosis of ITP was made. At 25 weeks gestation she delivered a premature infant, but it died soon after birth. After delivery, she developed overt TTP, and received plasma exchange for 2 d (one each day). She was diagnosed with chronic relapsing TTP and received



	ADAMTS13 activity (%)		ADAMTS13 antigen (%)	*ADAMTS13* gene		
	act-ELISA	VWFM	ag-ELISA	Arg193	Arg349	Haplotype
M-1	63	44	57	R/W	R/R	RR/WR
M-2	43	49	63	R/R	R/C	RR/RC
M-3	<0·5	<3	8·6	R/W	R/C	WR/RC
M-4	<0·5	<3	7·7	R/W	R/C	WR/RC
M-5	127	142	115	R/R	R/R	RR/RR

	ADAMTS13 activity (%)		ADAMTS13 antigen (%)	*ADAMTS13* gene		
	act-ELISA	VWFM	ag-ELISA	Ile178	Gln929	Haplotype
O-1	27	18	44	I/T	Q/Q	IQ/TQ
O-2	26	16	51	I/I	Q/X	IQ/IX
O-3	28	26	57	I/I	Q/X	IQ/IX
O-4	<0·5	<3	<0·1	I/T	Q/X	TQ/IX
O-5	29	26	65	I/T	Q/Q	IQ/TQ

Fig 3. Pedigree and ADAMTS13 analyses of USS-Family M and O. Symbols are the same shown in Fig 1. Mutations found in *ADAMTS13* are shown as one-letter amino acid abbreviations.

prophylactic plasma infusions (400 ml) every 2–4 weeks. At the age of 31 years, she had her second pregnancy and was managed with prophylactic plasma infusions every 1–2 weeks, but at 8 weeks gestation she developed proteinuria and thrombocytopenia (93×10^9/l), and therefore she received more frequent plasma infusions (fresh frozen plasma, about 10 ml/kg; 400–480 ml per week) beginning at this time. Under these circumstances, at 39 weeks gestation, she naturally delivered a mature baby who weighed 2·984 kg. The platelet count of this patient decreased to 73×10^9/l at 7 d after delivery and plasma was administered every 3–4 weeks. In this patient, plasma levels of ADAMTS13 activity and ADAMTS13 inhibitor were assayed in 2004 by the VWF multimer method, showing a severe deficiency of enzyme activity but without an inhibitor, suggesting a diagnosis of USS. In 2005, the act-ELISA revealed that the plasma level of ADAMTS13 activity in this patient was severely deficient (<0·5% of the normal), without an inhibitor (<0·5 BU/ml).

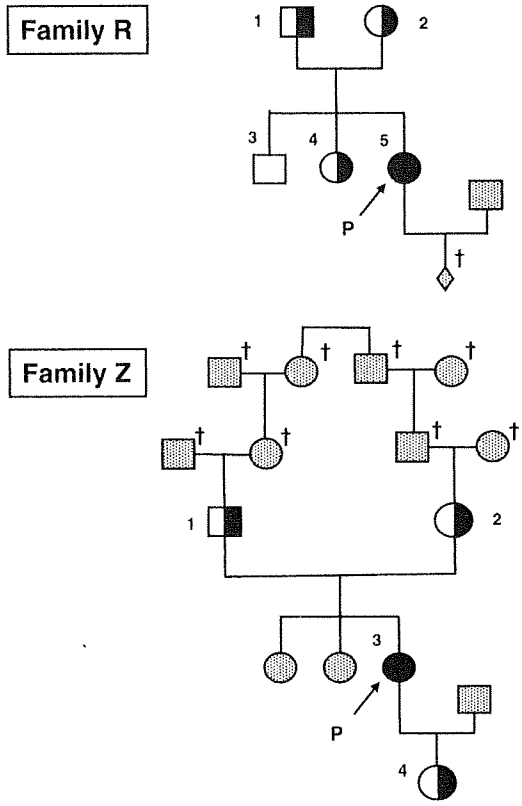
ADAMTS13 analysis (heterozygous I178T/Q929X mutation)

The *ADAMTS13* mutation was the compound heterozygote of I178T (*c.* 533T > C, exon 5) and Q929X (*c.*2785C > T, exon 22) in the proposita (0–4), and her parents, elder brother, and 2nd child (girl) were heterozygote carriers of either of the mutations. One common SNP was found in the patient (Table II).

Plasma level of ADAMTS13 antigen by the ag-ELISA was <0·1% of the normal in the proposita, and ADAMTS13 antigen levels of her 4 family members were 44~65%. These results were also confirmed by Western blot studies (Fig 2). Further, no other band with a molecular weight less than 190 kDa was found. Thus, neither the mutant protein of I178T nor Q929X was present in plasma (Fig 2).

Family R, clinical data

The proposita R-5, born in 1982, was the last child of 3 siblings to non-consanguineous parents (Fig 4, top). She had no history of severe neonatal jaundice. At the age of 8 months she developed generalized petechiae with thrombocytopenia (20×10^9/l) and was diagnosed with ITP. Until aged 5 years she had repeated episodes of purpura accompanied by fever that improved spontaneously within a few days. Her two elder siblings were asymptomatic with no history suggesting TTP. At the age of 23 years, she had her first pregnancy. At 23 weeks gestation, she developed mild thrombocytopenia (82×10^9/l), which further decreased to 47×10^9/l at 29 weeks gestation, and was diagnosed with ITP exacerbated by pregnancy. At 30 weeks gestation she had headache and nausea, and a week later she had sudden intrauterine fetal death. After Caesarean section, she developed overt TTP, treated with steroids and plasma exchange, which was performed eight times during the following 23 d. On this occasion in 2005, she was diagnosed with USS after determinations of ADAMTS13 activity (<0·5% of the normal) and ADAMTS13 inhibitor (<0·5 BU/ml) by the act-ELISA. After the diagnosis was confirmed, she has received prophylactic plasma infusions (320 ml every 2 weeks).



	ADAMTS13 activity (%)		ADAMTS13 antigen (%)	*ADAMTS13* gene		
	act-ELISA	VWFM	ag-ELISA	Arg193	Ala606	Haplotype
R-1	22	10	25	R/W	A/A	RA/WA
R-2	25	36	50	R/R	A/P	RA/RP
R-3	117	96	126	-	-	-
R-4	22	35	66	R/W	A/A	RA/WA
R-5	<0·5	<3	1·6	R/W	A/P	WA/RP

	ADAMTS13 activity (%)		ADAMTS13 antigen (%)	*ADAMTS13* gene
	act-ELISA	VWFM	ag-ELISA	Arg193
Z-1	32	29	43	R/W
Z-2	53	58	83	R/W
Z-3	<0·5	<3	11	W/W
Z-4	27	19	17	R/W

Fig 4. Pedigree and ADAMTS13 analyses of USS-Family R and Z. Symbols are the same shown in Fig 1. Mutations found in *ADAMTS13* are shown as one-letter amino acid abbreviations.

ADAMTS13 analysis (heterozygous R193W/A606P mutation)

The *ADAMTS13* mutation was the compound heterozygote of R193W (exon 6) and A606P (c.1816G>C, exon 16) in the proposita (R-5), and her parents and elder sister were both heterozygote carriers of either of the mutations. Five common SNPs were found in this patient (Table II).

Plasma level of ADAMTS13 antigen by the ag-ELISA was 1·6% of normal in the proposita; the levels in her three family members were 25~65%. These results were also confirmed by Western blot studies (Fig 2). Thus, the R193W/A606P mutant protein is secreted into plasma, but at a markedly reduced level (Fig 2).

Family Z, clinical data

The proposita Z-3, born in 1971, was the last of three siblings of consanguineous parents; the patient's grandparents were cousins (Fig 4, bottom). She had had neonatal jaundice of moderate severity. She was diagnosed of ITP at the age of 7 years, and had been treated with steroids until 15 years of age. She had her first pregnancy at age 25 years, and at 12 weeks gestation she developed thrombocytopenia (15×10^9/l) and was diagnosed with pregnancy-associated ITP. At 32 weeks gestation, she had a live birth by Caesarean section, after which she developed overt TTP, which was treated effectively with daily plasma exchange. This patient was referred to our laboratory in 1998, and plasma levels of ADAMTS13 activity and ADAMTS13 inhibitor were assayed by the VWF multimer method, demonstrating a severe deficiency of enzyme activity but without an inhibitor, suggesting a diagnosis of USS. Prophylactic plasma infusions were not initiated in this patient, and she had more than 5 episodes of TTP during 1998–2005. Each episode was treated with a single plasma infusion (320 ml). In 2006, when she had TTP triggered by a respiratory infection, infusion of plasma induced a severe anaphylactic reaction requiring steroid treatment. The act-ELISA confirmed her plasma level of ADAMTS13 activity was severely deficient (<0·5% of the normal) and ADAMTS13 inhibitor was negative (<0·5 BU/ml).

ADAMTS13 analysis (homozygous R193W mutation)

The *ADAMTS13* mutation was homozygous R193W (exon 6) in the proposita (Z-3), and her parents and child were heterozygote carriers of this mutation. One common SNP was found in this patient (Table II).

The plasma level of ADAMTS13 antigen by the ag-ELISA was 11% of normal in the proposita; the levels in her three

family members were 17~83%. These results were also confirmed by Western blot studies (Fig 2). Thus, the R193W mutation is apparently secreted into plasma, but at a reduced level.

Discussion

Coombs'-negative haemolysis with neonatal jaundice requiring exchange blood transfusions and thrombocytopenia have been considered to be a clinical hallmark of USS (Fujimura *et al*, 2002). However, only 16 (43%) of our 37 USS patients had episodes of exchange blood transfusion for severe jaundice in this period. Since USS is an autosomal recessive order, the ratio of female-to-male patients should be 1-to-1, but there was female predominance (24-to-13) among our 37 patients. The reason for this apparent gender disparity is probably because many USS patients have a mild disorder and thrombocytopenia is misdiagnosed as ITP. Physicians may overlook this important sign, as in fact 29 (78%) of our 37 USS patients had a history of thrombocytopenia; the remaining 8 were unclear as to whether their platelet count had been checked during their childhood.

Episodes of pregnancy and infections are well known precipitating factors for overt TTP in USS patients, but it is not well understood how often and why TTP episodes are induced by these factors (Weiner, 1987; Vesely *et al*, 2004; Terrell *et al*, 2005; Scully *et al*, 2006). In contrast, a chemical compound, DDAVP (1-deamino-8-D-arginine vasopressin), is well documented to induce TTP in USS patients (Hara *et al*, 1986; Veyradier *et al*, 2006), presumably through a rapid increase of plasma VWF by releasing ultra large(UL)-VWF multimers from vascular endothelial cells, leading to platelet thrombi formation under high shear stress (Yagi *et al*, 2001).

This study has clearly shown that pregnancy consistently induces thrombocytopenia during the second–third trimester, often followed by overt TTP. In normal pregnancies, plasma VWF increases steadily during gestation and reaches a maximum level (200~500% of the normal control) at term with appearance of UL-VWF multimers. The elevated plasma levels of VWF return to normal within one week after delivery (Stirling *et al*, 1984). Thus, the plasma milieu during the third trimester and at delivery appears to be similar to that seen after DDAVP administration. Thus, it is conceivable that the rapidly increased plasma level of UL-VWF multimers plays a critical role in precipitating overt TTP in pregnant women with USS.

More interestingly, however, the onset of clinically overt TTP observed in our USS patients during pregnancy is in contrast to mice with *ADAMTS13* knock-out (Motto *et al*, 2005; Banno *et al*, 2006). As previously shown, the *ADAMTS13* knock-out mice have UL-VWF multimers in their plasma and were potentially thrombogenic in *in vitro* studies using a parallel plate flow-chamber system under high shear stress. However, no thrombotic complications were found in the mice after pregnancy. We assume that it is important to note that haemodynamics formed by anatomically different vasculature networks in these two species significantly differs.

It has been proposed that there may be clinically distinct phenotypes of USS, with early-onset and late-onset types (Furlan & Lämmle, 2001; Camilleri *et al*, 2008). Our experience supports this hypothesis. In fact, three out of the nine women who were diagnosed with USS during pregnancy appeared to lack any clinical signs of TTP during their childhood. The distinction of USS patients into two phenotypes by their plasma levels of ADAMTS13 activity is less likely, because all nine of our USS patients had undetectable ADAMTS13 activity (<0·5% of the normal control) by the sensitive act-ELISA. In the nine women, eight of the *ADAMTS13* mutations were compound heterozygotes of Y304C/G525D (2 siblings), R125VfsX6/Q1302X (2 siblings), R193W/R349C (2 siblings), I178T/Q929X, and R193W/A6060P, and one was homozygous for R193W/R193W. Except for R193W, these mutations were all novel, and distributed from exons 4–28. The location of the *ADAMTS13* mutations in our nine USS patients, seven in the metalloprotease domain and the remaining two on the disintegrin/cysteine-rich domains, may be relevant to the clinical presentation. Camilleri *et al* (2008) recently reported that prevalence of R1060W missense mutation at the seventh thrombospondin-1 domain was associated with late-onset adult TTP, but we have not found the R1060W mutation in our 37 Japanese patients with USS (unpublished). Thus, presently there is no clear evidence dividing USS into two phenotypes on a basis of ADAMTS13 activity levels or *ADAMTS13* mutations. Our observations below also support this concept; two siblings of family M (M-3 and M-4) were the late-onset type, whereas other two siblings of family L (L-2 andL-3) shared two different onset types.

Veyradier *et al* (2003) described that prophylactic plasma infusions (fresh frozen plasma at a single dose of 10 ml/kg) every 3–4 weeks were efficiently performed to prevent periods of TTP in their USS-patients, but more plasma infusions (10 ml/kg during 2–5 consecutive days) were required during each relapse as early as possible. However, a standard therapeutic protocol for plasma infusions for pregnant women with USS has not been established. Our experience here clearly demonstrates that women with USS during pregnancy expose their infants to high risk without prophylactic plasma infusions. The risks for fetal loss are presumably caused by the disturbance of utero-placental circulation with platelet thrombi formed, as shown in Patient K-3 with extensive necrotic lesions in both the placenta and uterus. Among 15 pregnancies (one twin pregnancy), eight infants were stillborn or died soon after birth, and the remaining eight survived but they were premature except for one mature baby (O-5 in Fig 3). Further, seven of eight surviving infants were born by Caesarean section; one with prophylactic plasma infusions, two without plasma infusions, and four with aspirin. Notably, one mature baby (O-5) was born at 39 weeks gestation by natural delivery following plasma infusions to the mother that had started in the very early stage of pregnancy (8 weeks

gestation). This experience clearly indicated that prophylactic plasma infusions (fresh frozen plasma, about 10 ml/kg; 400–480 ml per week) at this stage of pregnancy allowed an uneventful delivery. But the best protocol for plasma infusions to women with USS during pregnancy remains to be determined in future studies.

It is clear that deficient ADAMTS13 activity in maternal plasma is harmful to both the mother and fetus. Retrospectively, we suspect that the first two children from asymptomatic carriers of L-1 (Fig 1, bottom) and M-1/-2 (Fig 3, top) might have had USS, because the former died of intrauterine fetal death at 20 weeks gestation and the latter was a stillbirth at 32 weeks gestation. This speculation is based on the observation of UL-VWF multimers in normal newborn cord blood, despite of subnormal levels of ADAMTS13 activity (Johnson *et al*, 1981; Tsai *et al*, 2002).

Because of these clinical observations, here we strongly recommend that ADAMTS13 activity, and ADAMTS13 inhibitor should be measured in patients with thrombocytopenia during childhood and in association with pregnancy.

Conflict-of-interest disclosure

YF is a member of clinical advisory boards for Baxter BioScience.

Acknowledgements

This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan and from the Ministry of Health, Labor, and Welfare of Japan.

References

Banno, F., Kokame, K., Okuda, T., Honda, S., Miyata, S., Kato, H., Tomiyama, Y. & Miyata, T. (2006) Complete deficiency in ADAMTS13 is prothrombotic, but it alone is not sufficient to cause thrombotic thrombocytopenic purpura. *Blood*, **107,** 3161–3166.

Camilleri, R.S., Cohen, H., Mackie, I.J., Scully, M., Starke, R.D., Crawley, J.T., Lane, D.A. & Machin, S.J. (2008) Prevalence of the ADAMTS-13 missense mutation R1060W in late onset adult thrombotic thrombocytopenic purpura. *Journal of Thrombosis and Haemostasis*, **6,** 331–338.

Fujimura, Y., Matsumoto, M., Yoshioka, A., Matsui, T. & Titani, K. (2002) von Willebrand factor-cleaving protease and Upshaw–Schulman syndrome. In: Progress in Hematology. *International Journal of Hematology*, **75,** 25–34.

Furlan, M. & Lämmle, B. (2001) Aetiology and pathogenesis of thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome: the role of von Willebrand factor-cleaving protease. *Best Practice & Research. Clinical Haematology*, **14,** 437–454.

Furlan, M., Robles, R., Galbusera, M., Remuzzi, G., Kyrle, P.A., Brenner, B., Krause, M., Scharrer, I., Aumann, V., Mittler, U., Solenthaler, M. & Lämmle, B. (1998) von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *New England Journal of Medicine*, **339,** 1578–1584.

Hara, T., Kitano, A., Kajiwara, T., Kondo, T., Sakai, K. & Hamasaki, Y. (1986) Factor VIII concentrate-responsive thrombocytopenia, hemolytic anemia, and nephropathy. Evidence that factor VIII: von Willebrand factor is involved in its pathogenesis. *The American Journal of Pediatric Hematology/Oncology*, **8,** 324–328.

Ishizashi, H., Yagi, H., Matsumoto, M., Soejima, K., Nakagaki, T. & Fujimura, Y. (2007) Quantitative Western blot analysis of plasma ADAMTS13 antigen in patients with Upshaw–Schulman syndrome. *Thrombosis Research*, **120,** 381–386.

Johnson, S.S., Montgomery, R.R. & Hathaway, W.E. (1981) Newborn factor VIII complex: elevated activities in term infants and alterations in electrophoretic mobility related to illness and activated coagulation. *British Journal of Haematology*, **47,** 597–606.

Kato, S., Matsumoto, M., Matsuyama, T., Isonishi, A., Hiura, H. & Fujimura, Y. (2006) Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity. *Transfusion*, **46,** 1444–1452.

Kinoshita, S., Yoshioka, A., Park, Y.-D., Ishizashi, H., Konno, M., Funado, M., Matsui, T., Titani, K., Yagi, H., Matsumoto, M. & Fujimura, Y. (2001) Upshaw–Schulman syndrome revisited: a concept of congenital thrombotic thrombocytopenic purpura. *International Journal of Hematology*, **74,** 101–108.

Kokame, K., Matsumoto, M., Soejima, K., Yagi, H., Ishizashi, H., Funato, M., Tamai, H., Konno, M., Kamide, K., Kawano, Y., Miyata, T. & Fujimura, Y. (2002) Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. *Proceedings of the National Academy of Sciences of the United States of America*, **99,** 11902–11907.

Kremer-Hovinga, J.A., Mäder, G., Lovey, P.Y., Morris, M.A., Oppliger-Leibundgut, E., Kuchler, H., Wermuth, B. & Lämmle, B. (2005) A second Swiss family with Upshaw–Schulman Syndrome (hereditary thrombotic thrombocytopenic purpura, TTP): Clinical course and molecular findings. *Journal of Thrombosis and Haemostasis*, **3**(Suppl. 1), Abstract P1139.

Levy, G.G., Nichols, W.C., Lian, E.C., Foroud, T., McClintick, J.N., McGee, B.M., Yang, A.Y., Siemieniak, D.R., Stark, K.R., Gruppo, R., Sarode, R., Shurin, S.B., Chandrasekaran, V., Stabler, K.R., Sabio, H., Bouhassira, E.E., Upshaw, Jr, J.D., Ginburg, D. & Tsai, H.M. (2001) Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*, **413,** 488–494.

Matsumoto, M., Kokame, K., Soejima, K., Miura, M., Hayashi, S., Fujii, Y., Iwai, A., Ito, E., Tsuji, Y., Takeda-Shitaka, M., Iwadate, M., Umeyama, H., Yagi, H., Ishizashi, H., Banno, F., Nakagaki, T., Miyata, T. & Fujimura, Y. (2004) Molecular characterization of ADAMTS13 gene mutations in Japanese patients with Upshaw–Schulman syndrome. *Blood*, **103,** 1305–1310.

Moake, J.L. (2002) Thrombotic microangiopathies. *New England Journal of Medicine*, **347,** 589–600.

Motto, D.G., Chauhan, A.K., Zhu, G., Homeister, J., Lamb, C.B., Desch, K.C., Zhang, W., Tsai, H.M., Wagner, D.D. & Ginsburg, D. (2005) Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *Journal of Clinical Investigation*, **115,** 2752–2761.

Scully, M., Starke, R., Lee, R., Mackie, I., Machin, S. & Cohen, H. (2006) Successful management of pregnancy in women with a history of thrombotic thrombocytopaenic purpura. *Blood Coagulation and Fibrinolysis*, **17,** 459–463.

Soejima, K., Nakamura, H., Hirashima, M., Morikawa, W., Nozaki, C. & Nakagaki, T. (2006) Analysis on the molecular species and concentration of circulating ADAMTS13 in blood. *Journal of Biochemistry*, **139,** 147–154.

Stirling, Y., Woolf, L., North, W.R., Seghatchian, M.J. & Meade, T.W. (1984) Haemostasis in normal pregnancy. *Thrombosis and Haemostasis*, **52,** 176–182.

Terrell, D.R., Williams, L.A., Vesely, S.K., Lämmle, B., Hovinga, J.A. & George, J.N. (2005) The incidence of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: all patients, idiopathic patients, and patients with severe ADAMTS-13 deficiency. *Journal of Thrombosis and Haemostasis*, **3,** 1432–1436.

Tsai, H.M., Sarode, R. & Downes, K.A. (2002) Ultralarge von Willebrand factor multimers and normal ADAMTS13 activity in the umbilical cord blood. *Thrombosis Research*, **108,** 121–125.

Uemura, M., Tatsumi, K., Matsumoto, M., Fujimoto, M., Matsuyama, T., Ishikawa, M., Iwamoto, T., Mori, T., Wanana, A., Fukui, H. & Fujimura, Y. (2005) Localization of ADAMTS13 to the stellate cells of human liver. *Blood*, **106,** 922–924.

Vesely, S.K., Li, X., McMinn, J.R., Terrell, D.R. & George, J.N. (2004) Pregnancy outcomes after recovery from thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Transfusion*, **44,** 1149–1158.

Veyradier, A., Bernadette, O., Haddad, E., Cloarec, S., Nivet, H., Foulard, M., Lesure, F., Delattre, P., Lakhdari, M., Meyer, D., Girma, J.-P. & Loriat, C. (2003) Severe deficiency of the specific von Willebrand factor-cleaving protease (ADAMTS13) activity in a subgroup of children with atypical hemolytic uremic syndrome. *Journal of Pediatrics*, **142,** 310–317.

Veyradier, A., Meyer, D. & Loriat, C. (2006) Desmopressin, an unexpected link between nocturnal enuresis and inherited thrombotic thrombocytopenic purpura (Upshaw–Schulman syndrome). *Journal of Thrombosis and Haemostasis*, **4,** 700–701.

Weiner, C.P. (1987) Thrombotic microangiopathy in pregnancy and the postpartum period. *Seminars in Hematology*, **24,** 119–129.

Yagi, H., Konno, M., Kinoshita, S., Matsumoto, M., Ishizashi, H., Matsui, T., Titani, K. & Fujimura, Y. (2001) Plasma of patients with Upshaw–Schulman syndrome, a congenital deficiency of von Willebrand factor-cleaving protease activity, enhances the aggregation of normal platelets under high shear stress. *British Journal of Haematology*, **115,** 991–997.

Yagi, H., Ito, S., Kato, S., Hiura, H., Matsumoto, M. & Fujimura, Y. (2007) Plasma levels of ADAMTS13 antigen determined with an enzyme immunoassay using a neutralizing monoclonal antibody parallel ADAMTS13 activity levels. *International Journal of Hematology*, **85,** 403–407.

Int J Hematol (2009) 90:328–335
DOI 10.1007/s12185-009-0421-3

ORIGINAL ARTICLE

National questionnaire survey of TMA

Naomi Ito · Hideo Wada · Masanori Matsumoto · Yoshihiro Fujimura · Mitsuru Murata · Takashi Izuno · Minoru Sugita · Yasuo Ikeda

Received: 17 June 2009 / Revised: 19 August 2009 / Accepted: 30 August 2009 / Published online: 18 September 2009

Abstract A questionnaire survey of Japanese patients with thrombotic microangiopathy (TMA) was carried out to investigate the frequency, laboratory abnormalities, and outcome in 2004. Out of 185 patients, there were 13 with familial TMA and 172 with acquired TMA. In acquired TMA, there were 66 with *Escherichia coli* O-157 infection (O-157)-related TMA, 35 with ADAMTS13-related TMA, and 22 with other types of TMA. The frequency of TMA in O-157-related TMA was high in patients from 0- to 15-year-old, and acquired TMA without O-157 was frequently observed in patients ranging from 31 to 65 years of age. In the treatment of acquired TMA, including plasma exchange (PE), steroid, antiplatelet agent, and anticoagulant, PE was carried out in 94.3% of ADAMTS13-related TMA, 77.3% of other TMA, and 7.6% of O-157-related TMA. The efficacy of PE and steroid therapy tended to be higher in ADAMTS13 TMA than in other types of TMA. The complete remission rate is the highest in O-157 TMA. The mortality rate was the lowest for O-157 TMA, and this rate also tended to be lower in ADAMTS13-related TMA than in other types of TMA. However, the determination of ADAMTS13 was not universal in Japan at the time of this questionnaire.

Keywords TMA · ADAMTS13 · Acquired TMA · Familial TMA · O-157

N. Ito
Department of Hematology and Oncology,
Mie University Graduate School of Medicine,
Mie, Japan

H. Wada (✉)
Department of Molecular and Laboratory Medicine,
Mie University Graduate School of Medicine,
Mie, Japan
e-mail: wadahide@clin.medic.mie-u.ac.jp

M. Matsumoto · Y. Fujimura
Department of Blood Transfusion Medicine,
Nara Medical University, Nara, Japan

M. Murata
Department of Laboratory Medicine,
Keio University School of Medicine,
Tokyo, Japan

T. Izuno
Bureau of Social Welfare and Public Health,
Tokyo Metropolitan Government, Tokyo, Japan

M. Sugita
Department of Environmental and Occupational Health,
Toho University School of Medicine, Tokyo, Japan

Y. Ikeda
Faculty of Science and Engineering,
Life Science and Medical Bioscience,
Waseda University, Tokyo, Japan

1 Introduction

Thrombotic thrombocytopenic purpura (TTP) [1–3], which is characterized by thrombocytopenia and microangiopathic hemolytic anemia, is often associated with neurological dysfunction, renal failure, and fever. Unusually large Von Willebrand factor (VWF) multimers, produced in and then quickly released from vascular endothelial cells, are found in patients plasma in both familial and non-familial thrombotic thrombocytopenic purpura (TTP) [4, 5]. These unusually large VWF multimers are thought to interact with circulating platelets, thus resulting in platelet clumping due to an elevated shear stress [5]. ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13), which was identified in 2001 [6–8], is a zinc

metalloprotease that specifically cleaves unusual VWF multimer at the Tyr (1605)-Met(1606) bound located in the A2 region of VWF [9, 10].

TTP was previously a life-threatening syndrome, and the survival rate has increased from 20 to 80% since the development of plasma exchange (PE) [11], and recently it has reached about 90% [12]. The mainstay of treatment is therapeutic PE, both to remove the causative antibody to ADAMTS13 and to replace ADAMTS13 [13]. The current guidelines [14] for thrombotic microangiopathy (TMA) recommended that PE should be initiated within 24 h of presentation [15]; however, considerable discussion is ongoing with regard to the optional schedule for therapy and the type of replacement fluids to be used [11].

Several questionnaires survey for Japanese patients with TMA were carried out in 2004 and 2005 [16]. This questionnaire survey was carried out to investigate the frequency, laboratory abnormalities, and outcome in 2004.

2 Materials and methods

One hundred eighty-five patients diagnosed with TMA from January 1, 1999 to December 31, 2003 were examined by a national questionnaire survey. The questionnaire was mainly a selective type, and the contents of the questions were about age, sex, underlying disease, acute symptoms, laboratory data, including ADAMTS13, treatment, outcome, and so on. The questionnaire was sent to 994 departments of hematology in Japanese hospitals or institutes: 429 hospitals replied and 73 had 185 cases of TMA. The list of replying hospitals was as follows: Anjo Kosei Hospital, Akashi City General Hospital, Chiba Children's Hospital, Cyukyo Hospital, Fukui Prefectural Hospital, Gunma University Hospital, Higashiosaka City General Hospital, Higashi Sapporo Hospital, Himeji Medical Center, Hiroshima-Nishi Medical Center, Hiroshima University Hospital, Hyogo College of Medicine Hospital, Hyogo Prefectural Cancer Center, Hyogo Prefectural Nishinomiya Hospital, Iizuka Hospital, Ikeda Municipal Hospital, Iseikai Hospital, Juntendo University Shizuoka Hospital, Kagoshima City Hospital, Kagoshima Rousai Hospital, Kawasaki Medical School Hospital, Kitano Hospital, Kokura Medical Center, Kokura Memorial Hospital, Komaki City Hospital, Kure Medical Center, Kyoto City Hospital, Mie University Hospital, Nagaoka Red Cross Hospital, Nagasaki University Hospital of Medicine and Dentistry, Nara Medical University Hospital, National Defense Medical College Hospital, Nihon University Itabashi Hospital, Niigata City General Hospital, Nippon Medical School Hospital, Osaka City General Medical Center, Osaka City University Hospital, Osaka General Medical Center, Osaka University Hospital, Research Hospital, The Institute of Medical Science, The University of Tokyo, Rinku General Medical Center, Saga University Hospital, Saiseikai Maebashi Hospital, Saitama Medical University Hospital, Sapporo Hokuyu Hospital, Sapporo-Kosei general Hospital, Sasebo City General Hospital, SHIN-KOKURA Hospital, Shinsyu University Hospital, Shizuoka General Hospital, Showa University Fujigaoka Hospital, Takasaki National Hospital, Tokai University Hospital, Tokyo Medical And Dental University Hospital Faculty of Medicine, Tokyo Metropolitan Bokutoh Hospital, Tokyo Metropolitan Geriatric Hospital, Tokyo Women's Medical University Hospital, Tottori Prefectural Chuou Hospital, Toyama University Hospital, Tsukuba University Hospital, University of Fukui Hospital, University of Miyazaki Hospital, University of Occupational and Environmental Health Hospital, Usui Hospital, Uwajima City Hospital, Yaizu City Hospital, Yamada Red Cross Hospital, Yamagata Prefectural Central Hospital, Yamaguchi University Hospital, Yodogawa Christian Hospital, Yokkaichi Municipal Hospital, Yokohama City University Hospital, and Yokohama Minami Kyousai Hospital.

Derangement, lethargy, behavior disorder, convulsion, stupor, coma, and other neurological abnormalities were considered as neurological symptoms. Creatinine levels >1.3 mg/dl indicated renal injury. A body temperature >37.5°C was considered as fever.

TMA was classified to 4 groups; ADAMTS13-related TMA (ADAMTS13 TMA), ADAMTS13 levels was less than 20% or positive for inhibitor for ADAMTS13; *Escherichia coli* O-157 infection (O-157)-related TMA (O-157 TMA), the cause of TMA was due to an O-157 infection; other TMA, the cause of TMA was not known; not measured TMA (NM TMA), the ADAMTS13 level was not measured and the cause was not due to an O-157 infection.

The study protocol was approved by the Human Ethics Review Committees of Keio University School of Medicine and Mie University School of Medicine.

2.1 Statistical analysis

The data are expressed as the median (25–75% tile). Differences between the groups were examined for significance using the Chi-square for independence test. A P value of less than 0.05 was considered to indicate a significant difference. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

3 Results

The patients include 13 with familial TMA and 172 with acquired TMA. In familial TMA, the ADAMTS 13 level

was markedly reduced in 8 (ADAMTS13 TMA), but not in 1 (other TMA), and it was not measured in 4 (NM TMA). In acquired TMA, the 66 cases of TMA were caused by O-157 infection (O-157 TMA), and 35 were ADAMTS13 TMA, 22 were other TMA, and 49 were NM TMA (Table 1). There tended to be more females than males among those with ADAMTS13 TMA and O-157 TMA of acquired TMA. The frequency of TMA in O-157 TMA was high in patients from 0- to 15-year-old and that in acquired TMA without O-157 tended to be high in patients from 31- to 65-year-old (Fig. 1). O-157 infection was the most frequent underlying disease in patients with TMA, collagen disease was the second, malignant tumor was the third, and transplantation- and drug-induced TMA were the forth, etc. (Table 2). In collagen diseases, the rate of acquired ADAMTS13 TMA (42.9%) tended to be higher than that of other TMA ($P = 0.09$). In Malignant tumor, drug-induced TMA and post-operation, the rate of other TMA tended to be higher than that of ADAMTS13 TMA. In underlying disease, the rate of ADAMTS13-related TMA was significantly higher in collagen diseases than in malignant tumor, transplantation, and drug induced TMA ($P < 0.05$, respectively). Icterus neonatorum was observed in most patients with familial TMA (70%). The acute symptoms are shown in Table 3. Neurological symptoms tended to be more frequent in acquired TMA than in familial TMA ($P = 0.12$), and it was significantly lower in O-157 TMA than in all other types of acquired TMA ($P < 0.001$). The frequency of renal dysfunction was significantly higher in other TMA than in ADAMTS13 TMA ($P < 0.001$), and it tended to high in O-157 TMA. Fever was observed in more than 50% of acquired TMA. Respiratory symptoms were not regularly associated with TMA.

Table 1 Subjects

	Number	Sex (F:M)		Number	Sex (F:M)
Familial	13	9:4	ADAMTS13 TMA	8	7:1
			Other TMA	1	0:1
			NM TMA	4	2:2
Acquired	172	92:79[a]	ADAMTS13 TMA	35	20:15
			O-157 TMA	66	40:25[a]
			Other TMA	22	11:11
			NM TMA	49	21:28

ADAMTS13 TMA ADAMTS13 activity markedly decreased; *Other TMA* ADAMTS13 activity did not markedly decrease; *NM TMA* ADAMTS13 activity was not measured; *O-157 TMA* O-157-related TMA

[a] One patient did not describe any symptom

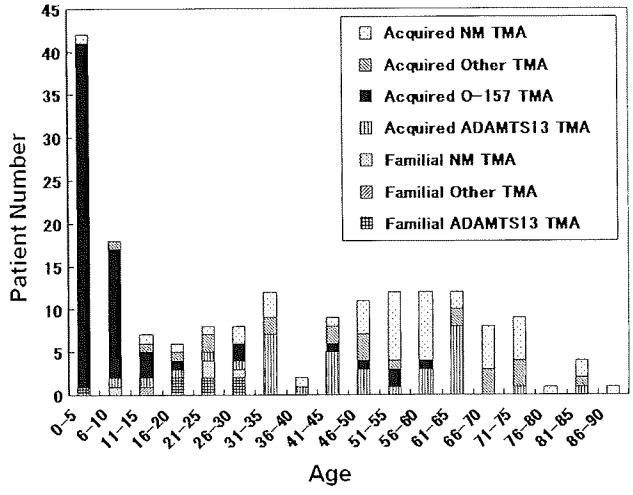


Fig. 1 Patient's age at the onset of TMA

The laboratory abnormalities are shown in Table 4. A decreased platelet count, red cell count, and hemoglobin and an increase of total bilirubin (T-bil) and lactate dehydrogenase (LDH) were frequently observed in each type of TMA. The platelet count was less than 110,000/μl in 98.9%, less than 50,000/μl in 85.4%, and 30,000/μl in 66.9% (Fig. 2). Hemoglobin was usually less than 13.0 g/dl and usually between 5.0 and 10.0 g/dl (Fig. 3). In acquired TMA, the frequency positive for antinuclear antibody was higher in ADAMTS13 TMA than in other TMA ($P < 0.05$). The frequency positive for PAIgG was higher in acquired TMA than in familiar TMA ($P < 0.01$). The Coombs test was negative in more than 90% of those with TMA. The haptoglobin level was reduced in most patients with TMA. Anticardiolipin antibodies (ACA) were not observed in most patients with TMA. Fibrin and fibrinogen degradation products (FDP) and D-dimer levels increased in most TMA patients, but fibrinogen was reduced in a few TMA patients.

Treatment of acquired TMA is summarized in Table 5. Plasma exchange (PE) was carried out in 94.3% of ADAMTS13 TMA, 77.3% of other TMA, and 7.6% of O-157 TMA. The efficacy of PE tended to be higher in ADAMTS13 TMA than in other TMA ($P = 0.052$). Transfusion of fresh frozen plasma (FFP) was frequently performed in familial TMA and ADAMTS13 TMA. The efficacy of FFP tended to be high in familial ADAMTS13 TMA (80.0%) but not high in acquired TMA. In acquired TMA, steroid treatment was carried out in 85.7% of ADAMTS13 TMA, in 72.7% of other TMA, and in 4.5% of O-157 TMA, and the efficacy of steroids tended to be higher in ADAMTS13 TMA than in other TMA ($P = 0.38$). Pulse therapy of methylprednisolone was frequently done in 65.7% of ADAMTS13 TMA and 68.2% of other TMA; but the efficacy was low. Antiplatelet therapy was carried out in 57.1% of ADAMTS13 TMA, 59.1% of other TMA, and 3.0% of O-157 TMA; but the efficacy was low. Hemodialysis was carried out in 37.9% of O-157 TMA and 31.8%

Table 2 Underlying disease

	Number	O-157 infection 66	Collagen diseases 14	Malignant tumor 11	Transplantation 7	Drug induced TMA 9	Pregnancy 4	Post-operation 2
Familial								
ADAMTS13 TMA	8	0/66	0/14	0/11	0/7	0/9	2/4 (50.0%)	0/2
Other TMA	1	0/66	0/14	0/11	0/7	0/9	0/4	0/2
NM TMA	4	0/66	0/14	0/11	0/7	1/9 (11.1%)	0/4	0/2
Acquired								
ADAMTS13 TMA	35	0/66	6/14*[1,+1] (42.9%)	1/11 (9.1%)	0/7	0/9	0/4	0/2
O-157 TMA	66	66/66	0/14	0/11	0/7	0/9	0/4	0/2
Other TMA	22	0/66	2/14 (14.3%)	5/11[+2] (45.5%)	2/7 (28.6%)	3/9[+2] (33.3%)	0/4	2/2[+2]
NM TMA	49	0/66	6/14 (42.9%)	5/11 (45.5%)	5/7 (71.4%)	5/9 (55.6%)	2/4 (50.0%)	0/2

*[1] $P < 0.05$ in comparison to acquired ADAMTS13 TMA in malignant tumor, transplantation, and drug-induced TMA

*[2] $P < 0.05$ in comparison to acquired ADAMTS13 TMA

[+1] $P = 0.09$ in comparison to acquired other TMA in collagen diseases

[+2] $P = 0.06$ in comparison to acquired ADAMTS13 TMA

Table 3 Acute symptoms

	Number	Neurological symptoms 82	Renal dysfunction 82	Fever (above 37.5°C) 113	Respiratory symptom 14
Familial					
ADAMTS13 TMA	8	2/6 (33.3%)	3/8 (37.5%)	3/8 (37.5%)	1/8 (12.5%)
Other TMA	1	0/1	0/1	1/1	0/1
NM TMA	4	0/2	0/2	2/2	0/2
Total	13	2/9 (22.2%)	3/11 (27.3%)	6/11 (54.6%)	1/11 (9.1%)
Acquired					
ADAMTS13 TMA	35	26/34 (76.5%)	9/34 (26.5%)	25/32 (78.1%)	1/20 (5.0%)
O-157 TMA	66	11/64 (17.2%)**[1]	33/64 (51.6%)	36/64 (56.3%)	3/56 (5.4%)
Other TMA	22	14/22 (63.6%)	19/22 (86.4%)**[2]	14/20 (70.0%)	0/7
NM TMA	49	29/46 (63.0%)	18/49 (36.7%)	32/49 (65.3%)	9/49 (18.4%)
Total	172	80/166 (48.2%)	79/169 (46.7%)	107/165 (64.8%)	13/132 (9.8%)

**[1] $P < 0.001$ in comparison to all other type of Acquired TMA

**[2] $P < 0.001$ in comparison to Acquired ADAMTS13 TMA

of other TMA; the efficacy was relatively high in O-157 TMA. Anticoagulant therapy such as heparin and synthetic protease inhibitor was carried out in about 30% of acquired TMA, and the efficacy was higher in O-157 TMA than in other types of TMA ($P < 0.01$). Platelet concentrate (PC) transfusion was carried out in 49.0% of NM TMA, 34.8% of O-157 TMA, 22.7% of other TMA, and 20.0% of ADAMTS13 TMA; however, the efficacy was markedly low in ADAMTS13 TMA.

The outcome of acquired TMA is summarized in Table 6. The complete remission (CR) rate was the highest in O-157 TMA ($P < 0.001$), and the mortality rate was the lowest in O-157 TMA ($P < 0.001$). The mortality rate tended to be lower in ADAMTS13 TMA than in other TMA ($P = 0.53$).

4 Discussion

This study registered 185 patients, including 13 with familial TMA and 172 acquired TMA. In acquired TMA, the frequency of O-157 TMA was highest, and with the exception of O-157, ADAMTS13 TMA was 35 patients (61.4%) in 57 patients measured ADAMTS13. No measurement of ADAMTS13 was made in 46.2% of the patients with acquired TMA without O-157. Although ADAMTS13 TMA may be among the most frequent types of TMA, this questionnaire survey may reflect the bias of the participating physicians. Widespread use of the ADAMTS13 assay is required before it is possible to determine the frequency of ADAMTS13 TMA. A fluorescence resonance energy transfer (FRET) assay [17] for ADAMTS13 activity and an

Table 4 Frequency of abnormal laboratory data

	Number	Plt 176	RBC 137	Hb 155	T-bil 130	LDH 173	ANA 34	PAIgG 31	C-test 4	Haptoglobin 103	ACA 1	Fib 19	FDP 72	D-dimer 67
Familial														
ADAMTS13 TMA	8	7/7	5/7 (71.4%)	7/8 (87.5%)	6/8 (75.0%)	7/8 (87.5%)	0/5	2/4 (50.0%)	0/7	7/7	0/3	2/7 (28.6%)	2/5 (40.0%)	0/4
Other TMA	1	1/1	0/1	0/1	1/1	1/1	0/0	0/0	0/1	1/1	0/0	0/1	1/1	0/1
NM TMA	4	2/2	2/2	2/2	2/2	2/2	1/2	0/0	0/2	1/1	0/0	0/2	1/1	0/0
Total	13	10/10	7/10 (70.0%)	9/11 (81.8%)	9/11 (81.8%)	10/11 (90.9%)	1/7 (14.3%)	2/4 (50.0%)	0/10	9/9	0/3	2/10 (20.0%)	4/7 (57.1%)	0/5
Acquired														
ADAMTS13 TMA	35	34/34	30/32 (93.8%)	23/25 (92.0%)	29/33 (87.9%)	33/34 (97.1%)	17/30 (56.7%)*[1]	12/14 (85.7%)	1/21 (4.8%)	16/20 (80.0%)	0/12	4/29 (13.8%)	13/24 (54.2%)	12/15 (80.0%)
O-157 TMA	66	64/65 (98.5%)	37/39 (94.9%)	64/65 (98.5%)	41/62 (66.1%)	64/64	0/10	4/4	0/16	34/35 (97.1%)	0/1	4/35 (11.4%)	20/27 (74.1%)	18/21 (85.7%)
Other TMA	22	22/22	16/21 (76.2%)	14/15 (93.3%)	16/22 (72.7%)	22/22	3/14 (21.4%)	3/3	0/5	6/8 (75.0%)	0/1	5/20 (25.0%)	13/15 (86.7%)*[2]	11/11
NM TMA	49	46/47 (97.9%)	47/47	45/48 (93.8%)	35/47 (74.5%)	44/47 (93.6%)	13/37 (35.1%)	10/10	3/37 (8.1%)	38/40 (95.0%)	1/17 (5.9%)	4/43 (9.3%)	22/37 (59.5%)	26/26
Total	172	166/168 (98.8%)	130/139 (93.5%)	146/153 (95.4%)	121/164 (73.8%)	163/167 (97.6%)	33/81 (40.7%)	29/31**[3] (93.5%)	4/79 (5.1%)	94/103 (91.3%)	1/31 (3.2%)	17/127 (13.4%)	68/103 (66.0%)	67/73 (91.8%)

Plt platelet count; *RBC* red blood cell count; *Hb* hemoglobin; *T-bil* total bilirubin; *LDH* lactate dehydrogenase; *ANA* antinuclear antibody; *C-test* Coombs test; *ACA* anticardiolipin antibodies; *Fib* fibrinogen; *FDP* fibrin and fibrinogen degradation products

*[1] $P < 0.05$ in comparison to Acquired Other TMA

*[2] $P < 0.05$ in comparison to Acquired ADAMTS13 TMA

**[3] $P < 0.01$ in comparison to Familial TMA

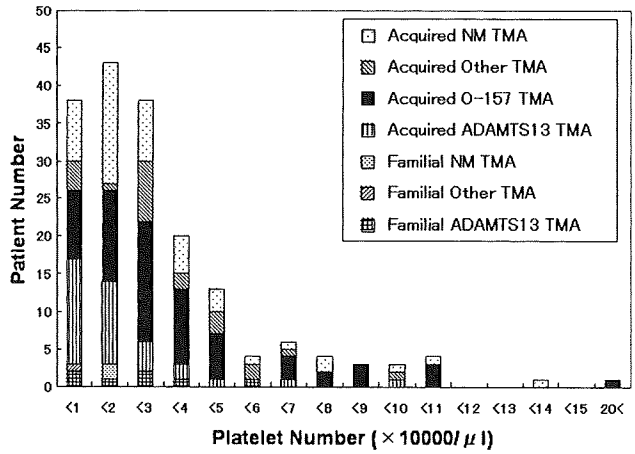


Fig. 2 Platelet number in patients with TMA

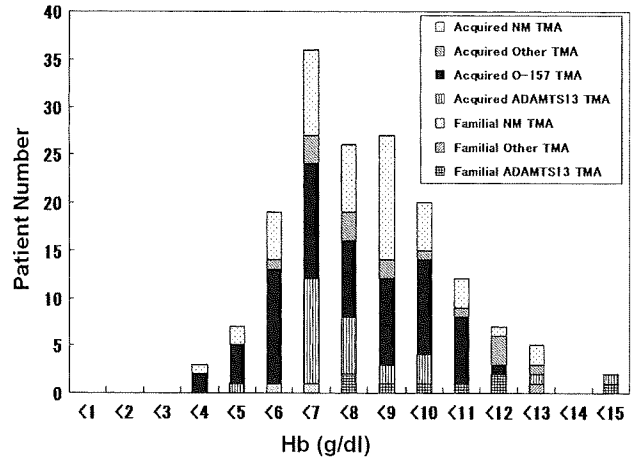


Fig. 3 Hemoglobin levels in patients with TMA

enzyme immunoassay (EIA) [18] of the ADAMTS13 activity have recently been developed, and is very easy to perform and not time-consuming. There was a high frequency of collagen disease, administration of ticlopidine, and the idiopathic type associated with ADAMTS13 TMA. Auto-antibodies for ADAMTS13 may be produced in collagen disease, administration of ticlopidine [19] or idiopathic state. Indeed, the frequency of positive antinuclear antibody was higher in ADAMTS13 TMA than in other TMA. While the detection of auto-antibodies for ADAMTS13 is rare in malignant diseases, post-operation, post-transplantation, infections etc., these conditions may cause TMA via vascular endothelial injuries and inflammation [20].

The frequency of several symptoms of TMA such as neurological symptoms, renal dysfunction, and fever were different in each type of TMA, but may depend on the sensitivity of diagnosis of TMA. In O-157 TMA, O-157 infection is initially diagnosed and then TMA is secondarily detected. The diagnosis of O-157 TMA is easily following the diagnosis of O-157 infection, and it is possible to diagnose the early state without other symptoms. With the exception of O-157 TMA, microangiopathic hemolytic anemia (MHA) with markedly decreased ADAMTS13 might be diagnosed as TMA, but MHA without markedly decreased ADAMTS13 requires a neurological symptom, renal dysfunction or fever for the diagnosis of TMA. Therefore, only about 25% of ADAMTS13 TMA has the symptoms of renal dysfunction.

The platelet count was decreased in 98.9% of TMA, but the frequency of a markedly decreased platelet count (less than 30,000/μl) was 66.9%. Abnormal red cell counts, hemoglobin, T-Bil, and LDH were frequently observed, but these abnormalities were not significantly dominant. Therefore, it is difficult to diagnose as TMA using only these laboratory abnormalities. FDP and D-dimer levels increased in most TMA patients, but fibrinogen was reduced in a few, suggesting that TMA includes thrombotic events but not marked secondary fibrinolysis. However, a few cases of TMA were either associated with disseminated intravascular coagulation (DIC) or were considered to have been caused by DIC.

PE is administered to treat most TMA patients without O-157 TMA, and the efficacy of PE tended to be higher in ADAMTS13 TMA than in other TMA, indicating that PE is usually applied as the standard therapy in Japan. It is clear that PE is effective by both removing the antibody to ADAMTS13 and to replacing ADAMTS13 in ADAMTS13 TMA [13], but it is not clear how PE affects other TMA. PE may remove platelet aggregation factors [21] and inflammatory cytokines [22]. Transfusion of FFP was frequently performed in familial TMA and ADAMTS13 TMA. The efficacy of FFP transfusion tends to be high in familial ADAMTS13 TMA but not high in acquired TMA. A Canadian Apheresis Study suggested that PE was more useful than FFP transfusion [23]. Steroid treatment was administered to most patients with acquired TMA without O-157 TMA, and the efficacy of steroids tends to be higher in ADAMTS13 TMA than in other TMA. Immunosuppressive therapy, including steroid therapy [24], is performed to inhibit of the synthesis of autoantibody against ADAMTS13. Anti-platelet and anti-coagulant therapies were administered to from 30 to 60% of the patients with acquired TMA without O-157 TMA, but these were not effective in this study. Although PC transfusion was not recommended in TMA, this therapy was still carried out in acquired TMA, and the efficacy was markedly low in ADAMTS13 TMA.

The mortality rate of TMA was the lowest in O-157 TMA and tended to be lower in patients with ADAMTS13 TMA in comparison to those with other TMA. The mortality of TMA in Japan was 26.8% in 1988 [25],

Table 5 Treatment of TMA

	Number		PE	FFP	Steroid	Pulse	Antiplatelet	Hemodialysis	Anticoagulant	PC
			90	78	87	57	58	43	53	63
Familial										
ADAMTS13 TMA	8	Enforcement	2 (25.0%)	5 (62.5%)	2 (25.0%)	0 (0.0%)	2 (25.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
		Efficacy	50.0%	80.0%	50.0%	0.0%	100.0%	0.0%	0.0%	0.0%
Other TMA	1	Enforcement	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)
		Efficacy	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	100.00%
NM TMA	4	Enforcement	1 (25.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	0 (0.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)
		Efficacy	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Total	13	Enforcement	3 (23.1%)	7 (53.8%)	3 (23.1%)	1 (7.7%)	3 (23.1%)	1 (7.7%)	1 (7.7%)	4 (30.8%)
		Efficacy	66.7%	57.1%	33.3%	0.0%	100.0%	0.0%	0.0%	25.0%
Acquired										
ADAMTS13 TMA	35	Enforcement	33 (94.3%)	23 (65.7%)	30 (85.7%)	23 (65.7%)	20 (57.1%)	1 (2.9%)	7 (20.0%)	7 (20.0%)
		Efficacy	45.5%+	21.7%	23.3%	13.0%	20.00%	100.0%	0.0%	0.00%
O-157 TMA	66	Enforcement	5 (7.6%)	12 (18.2%)	3 (4.5%)	2 (3.0%)	2 (3.0%)	25 (37.9%)	23 (34.8%)	23 (34.8%)
		Efficacy	60.0%	41.7%	33.3%	50.0%	50.0%	48.0%	47.8%**	43.4%
Other TMA	22	Enforcement	17 (77.3%)	7 (31.8%)	16 (72.7%)	15 (68.2%)	13 (59.1%)	7 (31.8%)	7 (31.8%)	5 (22.7%)
		Efficacy	17.7%	0.0%	12.5%	13.3%	7.7%	0.0%	0.0%	20.0%
NM TMA	49	Enforcement	32 (65.3%)	29 (59.2%)	35 (71.4%)	16 (32.7%)	20 (40.8%)	9 (18.4%)	15 (30.6%)	24 (49.0%)
		Efficacy	46.9%	34.5%	34.6%	37.5%	20.0%	66.7%	20.0%	16.7%
Total	172	Enforcement	87 (50.6%)	71 (41.3%)	84 (48.8%)	56 (32.6%)	55 (32.0%)	42 (24.4%)	52 (30.2%)	59 (34.3%)
		Efficacy	41.4%	28.2%	26.2%	21.4%	18.2%	45.2%	26.9%	25.4%

PE plasma exchange; *FFP* fresh frozen plasma; *PC* platelet concentrate

+ $P = 0.052$ in comparison to acquired other TMA

** $P < 0.01$ in comparison to all other types of Acquired TMA

Table 6 Outcome

	Number[a]	CR	Without remission	Mortality
		98	8	28
Familial				
ADAMTS13 TMA	4	0	4	0
Other TMA	1	1	0	0
NM TMA	2	1 (50.0%)	1 (50.0%)	0
Acquired				
ADAMTS13 TMA	19	13 (68.4%)	2 (10.5%)	4 (21.1%)
O-157 TMA	55	53 (96.4%)**	0	2 (3.6%)**
Other TMA	6	4 (66.7%)	0	2 (33.3%)
NM TMA	47	26 (55.3%)	1 (2.1%)	20 (42.6%)

** $P < 0.001$ in comparison to all other types of Acquired TMA

[a] Number of patients where the outcome was reported

26.0% in 1999 [26], and 22.0% in 2005, thus suggesting that the mortality of TMA is improving. An early diagnosis of TMA due to the development of an ADAMTS13 assay and the spread of PE treatment have all contributed to the improvement in the mortality of TMA patients.

Acknowledgment This work was supported in part by Grant-in-Aid for Blood Coagulation Abnormalities from Ministry Health, Labor and Welfare of Japan.

References

1. Bukowski RM. Thrombotic thrombocytopenic purpura: a review. Rev Prog Hemost Thromb. 1982;6:287–337.
2. Amorosi EL, Ultman JE. Thrombotic thrombocytopenic purpura: report of the 16 cases and review of the literature. Medicine. 1966;45:139–59.
3. Matsumoto M, Yagi H, Ishizashi H, Wada H, Fujimura Y. The Japansese experience with thrombotic thrombocytopenic purpura—hemolytic uremic syndrome. Seminar Hematol. 2004;41:68–74.