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TABLE I (*Continued*)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
											From when		
16	M3	1969	F	–	–	< 0.5	C-Hetero	p.R193W/ p.R349C	33 y	–	–		[50]
17	M4	1971	F	–	–	< 0.5	C-Hetero	p.R193W/ p.R349C	30 y	–	–		[50]
18	N6	1986	F	+	+	< 0.5	C-Hetero	p.H234R/ c.3220delTACC	4 y	+	4 y		[17]
19	O4	1958	F	–	–	< 0.5	C-Hetero	p.I178T/ p.Q929X	26 y	+	26 y		[50]
20	P3	1971	M	-	+	< 0.5	C-Hetero	p.C908Y/ p.C322G, p.T323R, p.F324L	3 y	+	21 y		[42]
21	Q1	1983	M	+	+	< 0.5–0.7	C-Hetero	p.G227R/ p.C908Y	6 y	+	11 y		[56]
22	Q2	1988	M	+	+	< 0.5	C-Hetero	p.G227R/ p.C908Y	2 y	+	7 y		[56]
23	R5	1982	F	–	+	< 0.5	C-Hetero	p.R193W/ p.A606P	23 y	+	23 y		[50]
24	S3	1982	F	–	+	0.9	Not determined		4 y	+	*		
25	T4	1981	F	+	+	< 0.5	Homo	c.3220delTACC	1 m	+	*		[56]
26	U3	1990	F	+	+	< 0.5	Homo	c.2259delA	4 m	+	*		[56]
27	V3	1983	F	+	+	< 0.5	C-Hetero	p.W1081X/ p.R193W	6 y	+	6 y		[56]
28	W4	1990	F	–	+	< 0.5	C-Hetero	p.G550R/Not determined	15 y	+	15 y		[56]
29	X5	1963	F	–	–	< 0.5	Not determined		40 y	–	–		
30	Y3	1960	F	–	+	< 0.5	C-Hetero	p.G385E/ p.R1206X	45 y	+	45 y		[56]
31	Z3	1971	F	–	+	< 0.5	Homo	p.R193W	25 y	–	–		[50]
32	AA3	1987	F	–	–	< 0.5	Not determined		19 y	–	–		
33	BB3	1947	M	–	–	< 0.5	Homo	p.R193W	55 y	–	–		[56]
34	CC5	2004	M	+	+	< 0.5	C-Hetero	p.Q723K/ p.R398C	2 y	+	2 y		[56]

TABLE I (*Continued*)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
											From when		
35	DD5	2007	F	–	+	< 0.5	C-Hetero	p.R268P/ p.Y304C	1 m	–	–		[56]
36	EE4	2003	M	+	+	< 0.5	Homo	c.2259delA	4 y	–	–		[56]
37	FF3	1991	F	–	+	< 0.5	Homo	p.Q449X	6 y	+	6 y		[56]
38	GG2	1931	M	–	–	2.4–3.4	Homo	p.C1024R	63 y	+	63 y	Dead (stroke at the age of 79)	[56]
39	HH4	2003	F	+	+	< 0.5	C-Hetero	p.Q449X/ c.4119delG	1 y	–	–		[56]
40	II3	1977	F	+	+	< 0.5	Not determined		9 m	+	10 y		
41	JJ3	1980	M	–	+	< 0.5	C-Hetero	c.1885delT/ p.C908Y	12 y	+	25 y	Hemodialysis	[56]

C-Hetero: compound heterozygotes; Homo: homozygotes.

There have been two fatal USS cases, one is the above-mentioned USS-C3 and the other is patient USS-I4 (male, born in 1972), whose natural history was previously described in detail [51]. Briefly, patient USS-I4 was diagnosed with late-onset USS when he was 50 years old. The next year he received a cholecystectomy and then experienced a bout of TTP, which led to renal insufficiency. He received extensive treatment, including PE and hemodialysis but did not improve, and he died of renal insufficiency at the age of 51 years.

ADAMTS13 gene analysis

The parents of USS patients are usually asymptomatic carriers, and a major population of patients from unrelated parents is a compound heterozygote, while a minor population of patients from related parents is a homozygote [42,43,51–56].

We performed *ADAMTS13* gene analyses in 38 out of 41 USS patients and disease-causing mutations were identified in 37 patients: nine with homozygous and 28 with compound heterozygous *ADAMTS13* gene mutations. Furthermore, five of these 37 patients were siblings. Therefore, within 64 [$2 \times (37 - 5)$] allelic numbers (n) for *ADAMTS13* gene mutations, the three most frequently found mutations were in the following order: p.R193W ($n = 8$), p.Q449X ($n = 5$), and p.C908Y ($n = 4$). All these mutations were unique to Japanese individuals, perhaps to East-Asians, and were totally different from Europeans and white and black Americans. In addition, to date, we have not found an apparent association between specific *ADAMTS13* mutations and clinical phenotypes. However, Camilleri et al. [57] reported that some single nucleotide polymorphisms in the *ADAMTS13* gene could modulate ADAMTS13:AC and its secretion, indicating that further investigations are required.

Patient USS-GG2 (male, born in 1931) suddenly developed a bout of TTP when he was 63 years old. After this incident, he had repeated TTP bouts and required prophylactic FFP infusions under a clinical diagnosis of CR-TTP. Even under these circumstances, he developed a cerebellar infarction at 76 years of age. During the infusion intervals, ADAMTS13:AC was often measured and determined to be 2.4–3.4% of the normal levels but ADAMTS13:INH was not detected. Most recently, the patient was diagnosed with USS with the homozygous missense mutation C1024R based on an *ADAMTS13* gene analysis (unpublished).

Treatment

Except for exchange blood transfusions to treat jaundice in newborns, USS patients usually respond well to small FFP infusions. Therefore, the question arises; what is the best marker for deciding this indication? As suggested above, mild thrombocytopenia seems to occasionally occur in USS patients during childhood, but this condition might be overlooked

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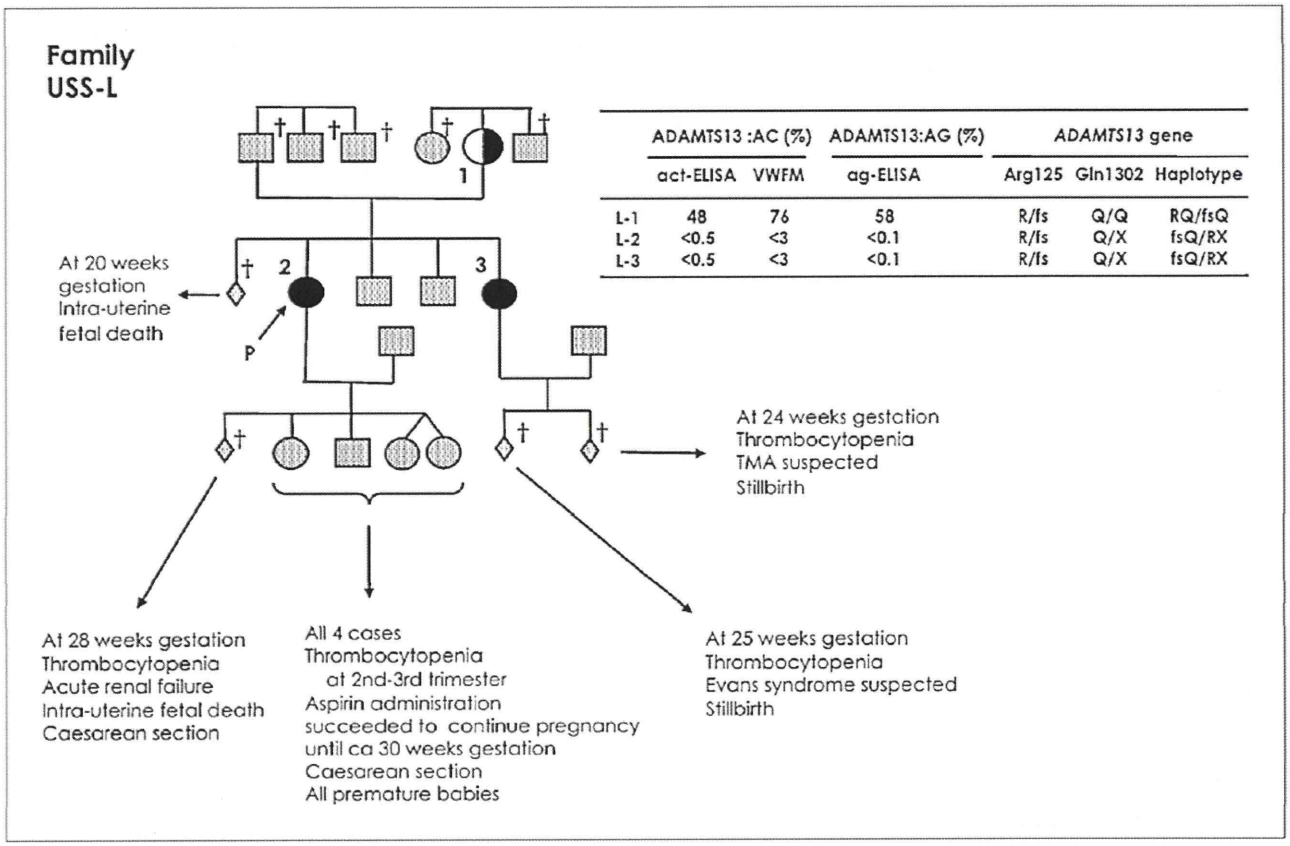

	ADAMTS13 :AC (%)		ADAMTS13:AG (%)	*ADAMTS13* gene		
	act-ELISA	VWFM	ag-ELISA	Arg125	Gln1302	Haplotype
L-1	48	76	58	R/fs	Q/Q	RQ/fsQ
L-2	<0.5	<3	<0.1	R/fs	Q/X	fsQ/RX
L-3	<0.5	<3	<0.1	R/fs	Q/X	fsQ/RX

FIGURE 3

Family pedigree and ADAMTS13 analyses in family USS-L

The propositus is L2, and L3 is her younger sister. Both siblings had an abortion along with thrombocytopenia of an unknown etiology. When propositus L2 became pregnant the second time, she had mild thrombocytopenia and her physician recommended low-dose aspirin, which enabled her to maintain the pregnancy until 30 weeks of gestation. However, it was uncertain why and how aspirin worked in this occasion. She delivered a live but premature baby by caesarean section. Then, she successfully bore three more children with the same treatment. All of the babies were premature and alive. During childhood, L2 had no episodes of thrombocytopenia, but L3 was diagnosed with ITP at 3 years of age. At 25 years of age, propositus L2 was diagnosed with USS based on an analysis of ADAMTS13:AC and ADAMTS13:INH. In addition, an *ADAMTS13* gene analysis provided solid evidence that the two siblings are compound heterozygotes for *ADAMTS13* gene mutations (p.R125fsx6/p.Q1302X). Squares and circles indicate males and females, respectively, and an arrow with P indicates the proposita. Closed circles and closed squares represent USS patients. The half-closed circles and squares represent asymptomatic carriers. The cross indicates deceased (cited from [50] with a slight modification).

because of the paucity of clinical signs. Thus, mild thrombocytopenia alone may not be a good marker. However, in clinical practice, some USS patients receive prophylactic FFP infusions (5–10 mL/kg BW) every 2–3 weeks because the half-life of ADAMTS13:AC in the plasma is thought to be 2–3 days, while other patients receive FFP infusions only when acute TTP bouts develop. In our registry, 26 of 41 (63%) USS patients received prophylactic FFP infusions. Currently, USS patients receive FFP infusions based on the physician's observations and the frequency of TTP bouts. However, the efficacy of prophylactic FFP infusions needs to be more precisely evaluated over a long observation period because our two patients who developed renal insufficiency had been receiving FFP infusions since they were clinically diagnosed with CR-TTP.

One serious adverse effect of repeated plasma infusions is that nine out of 41 (22%) USS patients were infected with hepatitis C virus. In this regard, virus-free rADAMTS13 preparations would be a promising product for USS patients.

Acquired thrombotic thrombocytopenic purpura/ADAMTS13:AC deficiency

Figure 4 shows age and gender distribution of acquired TTP. Of 195 patients with acquired TTP and a severe ADAMTS13:AC deficiency due to ADAMTS13:INH, 17 (6%) were childhood patients, including 14 with acquired idiopathic TTP (ai-TTP)

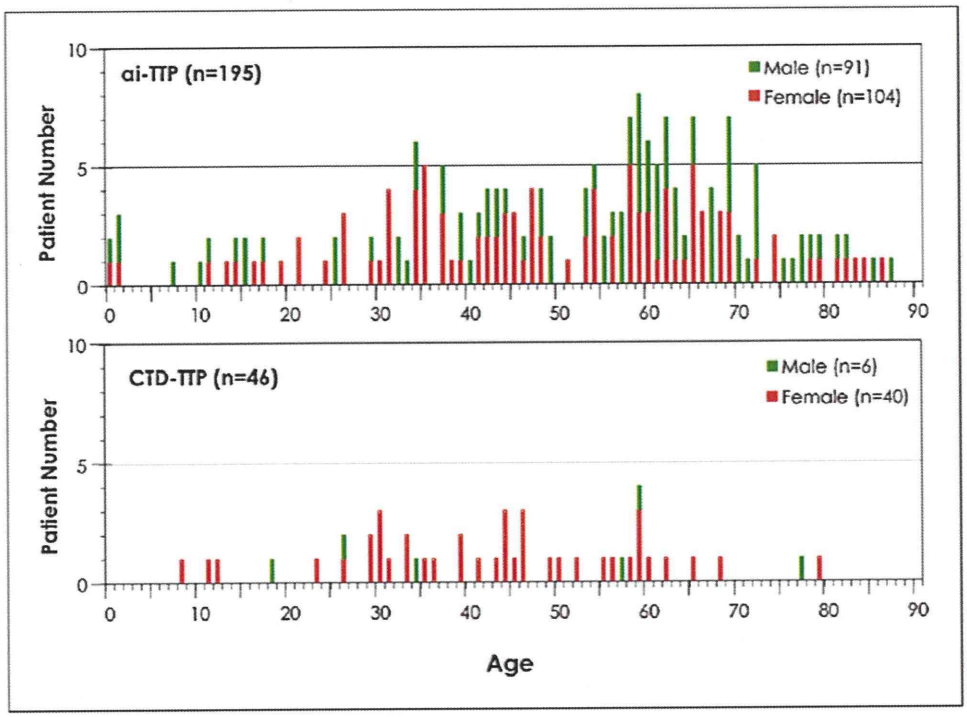

FIGURE 4

Age and gender distribution of patients with acquired idiopathic (ai)-thrombotic thrombocytopenic purpura (TTP) and connective tissue disease (CTD)-associated TTP

The upper panel shows the age and gender distribution of 195 ai-TTP patients who were registered at Nara Medical University during 1998–2008 and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. The largest population peak is found at approximately 60 years old, and we identified 14 patients (14/195, 7.2%) aged less than 15 years. Of note, five were very young children below 2 years of age.

The lower panel shows the age and gender distribution of 46 CTD-TTP patients who were registered at our institution during the same time period and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. This CTD-TTP patient population is widely distributed across ages, but is more common in patients between 30–60 years old. There is an apparent female predominance (41/46, 89%) in CTD-TTP patients in this registry, and three childhood patients were identified.

and three with connective tissue disease (CTD)-associated TTP. Of note, the former group included five patients who were less than 2 years of age and were initially misdiagnosed with other thrombocytopenic disorders, such as ITP, HUS, and hemophagocytic syndrome (HPS). It is important to examine and compare the detailed clinical features of these TTP patients to adulthood patients in order for physicians to ascertain that TTP is not a rare childhood disease. Therefore, we herein describe the clinico-laboratory features of these five young infants with ai-TTP and three childhood patients with CTD-associated TTP.

Idiopathic (ai-) thrombotic thrombocytopenic purpura

Table II summarizes the features of the 14 childhood patients with ai-TTP, including five infantile patients. Case 1 was previously reported [58] and case 5 was more recently described [59]. Interestingly, in contrast to the adulthood ai-TTP patients with a severe ADAMTS13:AC deficiency, the childhood patients had a slightly male predominance (female:male = 5:9). Five patients (5/14, 33%) had apparent prodromal illnesses, such as an upper respiratory tract infection (n = 3), Rotavirus infection (n = 1), or urinary tract infection (n = 1). Ten patients (10/14, 67%) had neurological findings, including headache (n = 5), altered mental status (n = 4), hemiparesis (n = 1), seizures (n = 1), and vision disturbance (n = 1). These patients exclusively presented with renal involvement (11/14, 73%) and fever (13/14, 93%). All the patients had hemolytic anemia (Hb, 4.5–11.3 g/dL) and thrombocytopenia (platelet, 7–38 $\times$ 10^9/L), but their serum creatinine levels (Cr, 0.19–1.0 mg/dL) remained within the normal range. Most of the childhood patients had five clinical signs that are characteristic of classic TTP ('pentad'), but six patients, including five young infants aged below 2 years, were initially misdiagnosed with other thrombocytopenic disorders, such as ITP (n = 2), HUS (n = 2), HPS (n = 1), and paroxysmal

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TABLE II

Clinical features in childhood patients with acquired TTP with severe ADAMTS13 deficiency

Case	Age	Sex	Prodromal illness	Initial diagnosis	Clinical findings on admission					
					Neurological symptom	Renal involvement	Cr (mg/dL)	Fever	Hb (g/dL)	Platelet (×109/L)
1	9 m	F	Rotavirus	HUS	Altered mental status	Yes	0.3	Yes	4.5	2
2	19 m	F	URI	ITP	No	Yes	0.19	Yes	11.3	38
3	19 m	M	URI	HUS	Altered mental status	Yes	1	Yes	5.4	9
4	12 m	M	No	HPS	Hemiparesis	Yes	0.31	Yes	8.5	38
5	8 m	M	No	ITP	No	No	0.19	Yes	8.7	25
6	7 y	M	No	TTP	Altered mental status	No	0.32	Yes	5.4	7
7	10 y	M	UTI	TTP	No	Yes	0.4	No	10.5	19
8	11 y	M	No	TTP	Headache	Yes	0.7	Yes	8.8	3
9	11 y	F	URI	TTP	No	Yes	0.6	Yes	5.5	6
10	13 y	F	No	TTP	Headache	No	0.58	Yes	5.7	12
11	14 y	F	No	TTP	Altered mental status, convulsion	No	0.4	Yes	7.8	6
12	15 y	M	No	PNH	Headache	Yes	1	Yes	10.5	17
13	15 y	M	No	TTP	Headache	Yes	0.91	Yes	8.1	11
14	14 y	M	No	TTP	Headache, visual disturbance	Yes	1.05	Yes	7	28
15	8 y	F	SLE	DIC	Altered mental status, headache	Yes	0.44	Yes	8.8	38
16	11 y	F	MCTD	TTP	Headache	Yes	0.6	Yes	11.9	43
17	12 y	F	SLE	TTP	No	No	0.61	Yes	5.6	1

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
1	< 0.5	> 100	19	No	SP	Yes	No	Alive	Cerebral infarction	[58]
2	< 0.5	4.3	3	Yes	SP, PSL	Yes	Yes	Dead		
3	< 0.5	2.3	3	Yes	PSL	Yes	No	Alive		
4	< 0.5	1.7	2	No	PSL	Yes	No	Alive	Cerebral infarction	
5	< 0.5	4.8	6	No	SP, PSL	Yes	No	Alive		[59]
6	< 0.5	2.1	3	No	Rituximab	No	No	Alive		
7	< 0.5	0.5	No	No	SP, PSL, MZR	Yes	No	Alive		
8	< 0.5	1.3	No	Yes	SP	No	No	Alive		
9	< 0.5	5.6	5	No	PSL	Yes	No	Alive		
10	< 0.5	2	9	No	SP, PSL	No	No	Alive		

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TABLE II (*Continued*)

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
11	< 0.5	3.2	17	No	SP, PSL, VCR	No	No	Alive		
12	< 0.5	34	39	No	PSL, VCR, CSA	No	Yes	Alive		
13	< 0.5	2.3	3	No	SP, PSL, AZT	No	No	Alive		
14	< 0.5	6.8	30	Yes	PSL	No	No	Alive		
15	< 0.5	1.2	5	Yes	SP, PSL, CY	No	No	Alive		
16	< 0.5	1.8	3	No	SP, PSL	No	No	Alive		
17	< 0.5	0.7	9	Yes	SP, PSL	No	No	Alive		

Cr: creatinine; Hb: hemoglobin; ADAMTS13:AC: ADAMTS13 activity; ADAMTS13:INH: ADAMTS13 inhibitor; PE: plasma exchange; FFP: fresh frozen plasma; URI: upper respiratory infection; UTI: urinary tract infection; SLE: systemic lupus erythematosus; MCTD: mixed connective tissue disease; TTP: thombotic thrombocytopenic purpura; ITP: idiopathic thrombocytopenic purpura; HUS: hemolytic uremic syndrome; HPS: hemophagocytic syndrome; PNH: paroxysmal noctural hemoglobinuria; SP: steroid pulse; PSL: predonosolone; MZR: mizoribine; VCR: vincristine; CSA: cyclosporine A; AZT: azathioprine; CY: cyclophosphamide.

nocturnal hemoglobinuria (PNH, $n = 1$). After analyzing ADAMTS13, they were all correctly diagnosed with ai-TTP.

Of these 14 childhood patients with ai-TTP, 13 received plasma exchange (PE, 2–39 times, median 5 times), including four patients who subsequently received a FFP infusion. They also received immunosupressive therapy, including steroid pulse ($n = 7$), predonisolone ($n = 12$), vincristine ($n = 2$), cyclosporin ($n = 1$), azathioprine ($n = 1$), mizoribine ($n = 1$), and rituximab ($n = 1$). As an adjunctive therapy, the patients were given intravenous immune globulin ($n = 3$) or an antiplatelet agent ($n = 2$). Of note, seven patients received platelet transfusions before or after they were diagnosed with acquired TTP. In five of the seven patients who received platelet transfusions, there were no apparent serious complications. However, case 1 developed general convulsions soon after the platelet transfusion, and case 2 died from bleeding without appreciable hemostatic effects from the platelet transfusion. As consequence, 13 out of 14 childhood patients with ai-TTP achieved one clinical remission, but two patients relapsed, including one who died. We think that clinicians should be aware of the existence of ai-TTP during very early childhood, and herein we present a short summary for each of these five infants with ai-TTP.

Case 1

In March 2000, a 9-month-old girl presented with a fever. She subsequently showed loss of appetite, a drop in physical activity, a pale complexion, and vomiting followed by diarrhea related to a Rotavirus infection. On the following day, these symptoms continued and generalized petechiae appeared. She was taken to a family doctor who determined that she had severe anemia and thrombocytopenia. As a result, she was admitted to a nearby hospital for treatment. Upon admission, she was drowsy and her laboratory findings showed severe anemia (Hb, 4.5 g/dL), thrombocytopenia (platelet, 2.0×10^9/L), hyperbilirubinemia (total bilirubin, 2.6 mg/dL), and an elevated LDH level (2,925 IU/L). Both direct and indirect Coombs' tests were negative, and fragmented RBCs were detected in the blood film. The hemostatic tests showed the following: prothrombin time (PT, 14.0 sec), activated PTT (35.9 sec), fibrinogen (268 mg/dL), thrombin-antithrombin complex (TAT, 31.7 µg/L), D-dimer (7.14 µg/mL), and fibrin degradation product (FDP, 82.3 µg/mL). The levels of blood urea nitrogen (BUN) and creatinine were within the normal ranges (25 mg/dL and 0.3 mg/dL, respectively). Neither Shiga-like toxin nor *E. coli* O157:H7 was detected in her stool. However, she had proteinuria, hematuria, and marked petechiae on her body due to thrombocytopenia. She was tentatively diagnosed with HUS, and she received five units of platelet transfusion. Soon after completing the platelet transfusion, she developed generalized convulsions followed by right hemiplegia, and therefore, PE therapy was immediately instituted. On the following day, both CT and MRI examinations of her brain revealed a diffuse hemorrhagic infarction in the left posterior region. The PE therapy was continued for the next 37 days on a total of 19 occasions, along with steroid pulse therapy and high-dose intravenous immunogloburin (IVIG) infusions until clinical improvements were noted. The ADAMTS13:AC and ADAMTS13:INH titers measured by the VWFM assay were less than 3% and greater than 100 BU/mL, respectively (later, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasmas, and the respective data were

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less than 0.5% and greater than 100 BU/mL [58]). Now, almost 10 years have passed, and the patient is apparently healthy with a minimal sequela of the right hemiplegia.

Case 2

In July 2000, a 19-month-old girl presented with a fever and cough. The next day, she was taken to a family doctor and then transferred to a nearby hospital because of thrombocytopenia (platelet, 21×10^9/L). Upon admission, her laboratory data revealed slight anemia (Hb, 11.3 g/dL), thrombocytopenia (platelet, 38×10^9/L), and an elevated LDH level (994 IU/L). An analysis of a bone marrow aspiration showed no abnormalities, and therefore she was suspected to have ITP. She was administered high-dose IVIG with steroid therapy but her platelet count did not increase. Her platelet count slightly increased soon after the platelet transfusions, while the number of schistocytes in the blood films gradually increased. This patient never had renal dysfunction or neurological signs. Thus, her physician suspected that the patient had USS but did not measure ADAMTS13:AC. The patient was given an infusion of 80 mL of FFP, but her platelet count did not increase. During this period, she was alert and no clinical deterioration was noted. Three months after admission, plasma samples from this patient were sent to our laboratory for ADAMTS13:AC and ADAMTS13:INH testing. Based on the results of the VWFM assays, the patient was diagnosed with a severe ADAMTS13:AC deficiency (< 3%) with ADAMTS13:INH (4.0 BU/mL). However, in those days we were unable to clearly determine whether this patient had USS and developed alloantibodies or acquired TTP with autoantibodies to ADAMTS13. The patient was not given PE therapy because she did not show any clinical deterioration during the subsequent 3 months. Therefore, she was discharged and then carefully observed at the outpatient clinic. However, 1 month after discharge, she was re-admitted to the hospital and received PE therapy because of exacerbated anemia and thrombocytopenia. However, her clinical signs did not improve, even after whole blood exchange therapy. Thus, she was treated with RBC and platelet transfusions, but 2 weeks later she fell into coma and died of tracheal bleeding, which was 8 months after her first hospital admission (plasma ADAMTS13:AC and ADMTS13:INH in this patient were measured only on one occasion. In recent years, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasma samples, and the data were less than 0.5% and 4.3 BU/mL, respectively).

Case 3

In July 2002, a 19-month-old boy developed a low-grade fever and cough followed by petechiae. He was taken to a family doctor because his nasal bleeding did not stop. The doctor noted thrombocytopenia and anemia and suspected HUS, and the patient was transferred to a local hospital. He had mild thrombocytopenia (platelet, 70×10^9/L) soon after birth, which spontaneously improved. Upon admission, he was drowsy and his laboratory data showed anemia (Hb, 5.4 g/dL), thrombocytopenia (platelet, 9×10^9/L), elevated LDH (1991 IU/L), and proteinuria. He was administered FFP infusions, steroid therapy, and IVIG. A platelet transfusion was performed but his platelet counts did not significantly increase. Since he was negative for DIC markers, the patient was clinically diagnosed with TTP and then administered PE therapy. After three consecutive PE therapies, he became alert, recovered, and his laboratory markers returned to normal levels. The ADAMTS13:AC and ADAMTS13:INH titers were measured by classic VWFM assays using frozen plasma that was obtained before the PE therapy was administered, and the results were less than 3% and 2.3 BU/mL, respectively (later, chromogenic act-ELISA gave values of less than 0.5% and 2.3 BU/mL, respectively). His plasma ADAMTS13:AC deficiency with ADAMTS13:INH continued for more than 6 months but without appreciable clinical manifestations. After 4 years, ADAMTS13:AC (77%) had normalized and ADAMTS13:INH (< 0.5 BU/ml) was absent.

Case 4

In June 2002, a 13-month-old boy developed a fever followed by dark urine and diarrhea. He was taken to a nearby clinic, where he was determined to have leukocytosis (WBC, 12,000/μL), anemia (Hb, 7.2 g/dL), and thrombocytopenia (platelet, 46×10^9/L). In addition, a peripheral blood film showed phagocytosis and therefore the patient was diagnosed with suspected HPS. He was transferred to a university hospital where platelet transfusions were performed twice for two consecutive days, but his platelet counts only transiently increased. Soon after the second platelet transfusion, a bone marrow aspiration was performed, but the HPS diagnosis was not confirmed. On the other hand, there was a transient increase in his platelet count (platelet, 40×10^9/L) after he was infused with a small amount of FFP, and therefore the physician suspected a diagnosis of USS. Therefore, the patient received a daily plasma infusion therapy for the next 5 days. However, hematuria developed followed by right hemiplegia. An MRI revealed a hemorrhagic infarction (3 × 4 cm) in the left parieto-occipital region. Based on the clinical course, he was eventually diagnosed with ai-TTP. After he received PE therapy for two consecutive days with orally administered prednisolone, his clinical conditions rapidly improved and his laboratory findings recovered. After his recovery, the ADAMTS13:AC and ADAMTS13:INH levels were tested using the classic VWFM assay and deep-frozen plasma samples that were obtained before the PE therapy, and the results were less than 3% and 1.9 BU/mL, respectively (later, these values were re-examined with the chromogenic act-ELISA, and the results were less than 0.5% and 1.9 BU/mL, respectively). He subsequently improved and was discharged. Three years later, his plasma

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TABLE III

Comparison of clinical features and outcomes between childhood and adulthood patients with acquired idiopathic (ai)-TTP

Ai-TTP (n = 195)	Childhood patients (n = 14)	Adulthood patients (n = 181)
Age at the onset of TTP bouts (years old), Median (25, 75 percentile)	11 (1.6, 14)	57 (41, 65)
Female (%)	35.7	55.2
"Pentad"		
(1) Platelet count ($\times 10^9$/L), Median (25, 75 percentile)	15 (8, 24)	10 (7, 17)
(2) Hemoglobin (g/dL), Median (25, 75 pecentile)	8.0 (5.6, 8.8)	7.5 (6.3, 8.8)
(3) Renal involvement (%)	71.4	75.7
Serum creatinine (mg/dL), Median (25, 75 percntile)	0.5 (0.3, 0.9)	1.0 (0.7, 1.3)
(4) Central nervous system involvements (%)	71.4	79.6
(5) Fever ($\geq$ 37.0 °C) (%)	92.9	69.6
Times of plasma exchange	5.5 (3.0-17.5)	ND
Mortality in the current episode of TTP bouts (%)	7.1	15.5

ND: not determined.

ADAMTS13:AC had normalized. At present, he has fully recovered and has no residual right hemiplegia.

Case 5

In January 2005, a 9-month-old boy with generalized petechiae and a fever was referred to a local hospital, where he was determined to have thrombocytopenia (platelet, 9×10^9/L). He was admitted to a university hospital and diagnosed with acquired TTP based on ADAMTS13:AC (< 3%) and ADAMTS13:INH titers (2.8 BU/mL) that were determined using the classic VWFM assays. (Later, these values were re-examined by the chromogenic act-ELISA, and the results were less than 0.5% and 4.8 BU/mL, respectively). After he was diagnosed with ai-TTP, he was administered PE therapy for six consecutive days at a different hospital. His clinical symptoms rapidly improved, but the increase in platelet counts was only transient and his platelet count was consistently lower than 10×10^9/L. To prevent serious bleeding complications, the physician administered oral prednisolone, together with continuous low-dose platelet transfusions. Two months later, he was discharged, despite having an ADAMTS13:AC deficiency with ADAMTS13:INH that lasted for at least 8 months. Two years later, we were able to examine the plasma ADAMTS13:AC and ADAMTS13:INH in this patient, and determine that ADAMTS13 had fully normalized [59].

Table III compares the clinical features and outcomes of the childhood patients (n = 14) and adulthood patients (n = 181) with ai-TTP in our registry [13].

Connective tissue disease-associated thrombotic thrombocytopenic purpura

In 1939, Gitlow and Goldmark [60] first reported a close relationship between 'classic' TTP and systemic lupus erythematosus (SLE). In 1999, Brenner et al. [61] described five patients with childhood-onset 'classic' TTP and reviewed 30 other patients who were previously described in the literature. According to their analysis, nine (9/35, 26%) fulfilled four or more ACR criteria for SLE and eight (8/35, 23%) had incipient SLE. Interestingly, of the five patients who were initially diagnosed with 'classic' idiopathic TTP in their laboratory, three were diagnosed with SLE within 3 years, and the remaining two patients fulfilled the ACR classification criteria for SLE within 4 years of disease onset. However, at that time, ADAMTS13:AC assays were not generally available, and therefore no data on ADAMTS13 was provided in their report.

In our registry of 919 patients with TMAs, 221 had CTD-associated TMA, of which 92 had SLE-associated TMA [13]. For the 221 CTD-TMA and/or 92 SLE-TMA patients, the number of patients with a severe ADAMTS13:AC deficiency with ADAMTS13:INH was 46 and 24, respectively. Furthermore, within the 221 patients with CTD-TMA, 11 developed the disease in childhood (less than 15 years of age), including eight patients with SLE, 1 with RA (rheumatoid arthritis), and two with MCTD (mixed connective tissue disease), in whom three had a severe ADAMTS13:AC deficiency. These three patients included two SLE- and one MCTD-associated TTP patients, and they uniformly had relatively low titers of ADAMTS13:INH (0.7–1.8 BU/mL) at

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the onset of TTP, which slightly differed from those with ai-TTP (table II). Here we briefly describe these three childhood patients with CTD-associated TTP (cases 15–17) due to their relevance in clinical practice.

Case 15

In April 2005, a 7-year-old girl was determined to have proteinuria and occult blood in her urine based on a school health examination. The patient was admitted to a nearby university hospital for further examination in June 2005, where she was diagnosed with SLE (Lupus nephritis) based on her clinical manifestations and the following laboratory findings: proteinuria, positive for anti-nuclear antibodies and anti-double stranded DNA antibodies, and low complementemia. She was treated with prednisolone, mizoribine, and azathioprine. Because her clinical signs significantly improved with these treatments, she was discharged in September 2005.

In April 2006 (8 years of age), this patient noticed proteinuria and hematuria based on a self-examination at home. The next day, she was admitted to the same hospital. Her laboratory data at the second admission were as follows: Hb (8.8 g/dL), LDH (1608 IU/L), platelet (2×10^9/L), PT (11.0 sec), PTT (31.1 sec), fibrinogen (355 mg/dL), antithrombin (140%), TAT (9.06 µg/L), D-dimer (5.25 ng/mL), FDP (7.9 µg/mL), and schistocytes in a peripheral blood smear. She had a DIC score of 6 according to the DIC diagnostic criteria from the International Society of Thrombosis and Haemostasis [15]. As a result, she was initially treated with nafamostat, but there were no clinical improvements. ADAMTS13:AC and ADAMTS13:INH assays were performed for a differential diagnosis of TTP, and the results were less than 0.5% and 1.2 BU/mL, respectively. Thus, she was diagnosed with SLE-associated TTP and treated with PE (five times), steroid pulse therapy, and cyclophosphamide. These treatments saved her life, and to date, she has not had a relapse of TTP.

Case 16

In July 2006, an 11-year-old girl developed general fatigue, headache, and vomiting. Two days later, she was admitted to a local hospital where laboratory tests indicated the following: Hb (11.9 g/dL), LDH (1636 IU/L), total bilirubin (9.3 mg/dL), platelet (33×10^9/L), PT (12.7 sec), PTT (34.0 sec), fibrinogen (290 mg/dL), antithrombin (> 75%), D-dimer (< 2 ng/mL), FDP (< 5 µg/mL), BUN (18.0 mg/dL), and schistocytes in a peripheral blood smear. For a differential diagnosis, her plasma ADAMTS13:AC and ADAMTS13:INH levels were determined to be less than 0.5% and 1.8 BU/mL, respectively. In addition, upon admission she simultaneously had Raynaud's phenomenon and was positive for anti-nuclear antibodies and anti-RNP antibodies. Thus, she was diagnosed with MCTD-associated TTP, and was administered PE with steroid pulse therapy starting on the third day of hospitalization. During the third PE, she had anaphylactic shock, perhaps related to the infused plasma. Thus, she stopped the PE therapy and continued the steroid pulse therapy alone. As a result of these treatments, she recovered and on the hospital day 14 her ADAMTS13:AC increased to 67% of normal and ADAMTS13:INH became negative. To date, she has had no episodes of TTP.

Case 17

In October 2007, a 12-year-old girl suddenly developed jaundice with a fever. She was admitted to a nearby university hospital, and her routine laboratory data provided the following: Hb (5.6 g/dL), platelet (1×10^9/L), PT (14.0 sec), PTT (40.1 sec), fibrinogen (333 mg/dL), FDP (20.5 µg/mL), total bilirubin (5.5 mg/dL), and schistocytes in a peripheral blood smear. Upon admission, she had low levels of complement, and was positive for anti-nuclear antibodies, anti-double stranded DNA antibodies, and anti-SS-A antibodies. Plasma ADAMTS13:AC and ADAMTS13:INH were simultaneously measured by the act-ELISA and were less than 0.5% and 0.7 BU/mL, respectively. Based on these results, she was diagnosed with SLE-associated TTP and PE therapy was initiated. After three consecutive PE treatments with steroid pulse therapy, her platelet count increased. However, on hospital day 8, her platelet count decreased again, and her ADAMTS13:INH titer increased to 2.2 BU/mL. The PE therapy was re-initiated with steroid pulse therapy. A total of nine rounds of PE therapy and two courses of steroid pulse therapy resulted in remission on hospital day 23. At this time, ADAMTS13:AC and ADAMTS13:INH were 86% and less than 0.5 BU/mL, respectively. To date, she has had no TTP relapses.

Treatment of acquired thrombotic thrombocytopenic purpura

Plasma exchange (PE) is the first line therapy that was demonstrated to be effective in randomized clinical trials for acquired TTP [62]. PE removes ADAMTS13:INH, UL-VWFM, and hazardous cytokines from the circulation in TTP patients, and replenishes ADAMTS13 without circulatory overload. Corticosteroids are often used as an adjunctive treatment. In relapsing or refractory cases, other immunosuppressive drugs such as cyclosporine, cyclophosphamide, vincristine, and rituximab are empirically used. PE therapy should be initiated as soon as possible after TTP is diagnosed, but the onset of therapy tends to be delayed in childhood patients with acquired TTP because of difficult differential diagnoses, especially with HUS, unless ADAMTS13:AC is measured.

In regard to platelet transfusions in TTP patients with a severe ADAMTS13:AC deficiency, these transfusions have consistently been viewed as a contraindication because they may enhance thrombotic complications due to platelet aggregation and thrombus formation under high shear stress generated in the microvasculature. Our experience also partially supports this concept, but such adverse effects happened only on very few occasions. We believe that prophylactic platelet

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transfusions should be avoided in TTP patients with a severe ADAMTS13:AC deficiency, but that platelet transfusions must be done if patients experience overt bleeding.

In our 17 childhood patients with acquired TTP, 15 patients were promptly treated with PE and corticosteroid therapy, and 16 children (94%) achieved a first remission. Recently, McDonald et al. [63] reported that the number of PE courses to first remission was higher in children (median, 22.5; range, 10–30) than in adults (median, 15.5; range, 3–93) [64], suggesting that childhood TTP may be more resistant to treatment. By contrast, our results indicated that patients with acquired TTP and a severe ADAMTS13:AC deficiency responded well to PE (median number of PE courses, 5.5; range, 2–39), but two patients (2/17, 11.8%) relapsed and one (1/17, 5.9%) died. Furthermore, in this study, we observed that the children with a high ADAMTS13:INH titer (> 5 BU) tended to require more frequent PE courses to achieve remission.

Fakhouri et al. [65] recently reported that adulthood TTP patients with high-titer ADAMTS13:INH could be successfully treated with a combination of PE and rituximab, a chimeric monoclonal antibody to CD20. The efficacy of rituximab in such patients is apparently due to a reduction in anti-ADAMTS13 IgG antibodies by depleting the patient's B-lymphocytes [65,66]. Recently, there have been many successful cases [67–69], and to date, no significant adverse effects have been reported. In our registry, only one childhood TTP patient (7 years old) with acquired TTP with ADAMTS13:INH was successfully treated with PE followed by rituximab, as shown in table II. However, the best choice or combination in regard to immunosuppressants for treating children with acquired TTP and a severe ADAMTS13:AC deficiency needs to be carefully determined in future studies.

Conclusion

The discovery of ADAMTS13 provided a breakthrough in our understanding of the mechanism of platelet thrombus formation under high shear stress and directly linked this enzyme to TTP pathogenesis in humans. Subsequently, the recent development of rapid and sensitive ADAMTS13 assays and their utilization in clinical practice have shown that the early- and late-onset phenotypes of USS are not different diseases and are likely affected by both acquired endogenous and exogenous circumstances. Furthermore, we have presented a novel category of ai-TTP that occurs during very early childhood (less than 2 years of age), which was perhaps totally overlooked or misdiagnosed before 2002 [39]. Thus, TTP should be recognized as a life-threathening generalized disease that not only occurs in adulthood, but also in childhood, causing a paradigm shift in our clinical understanding of TTP since the first discovery by Moschcowitz in 1924.

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Acquired Idiopathic ADAMTS13 Activity Deficient Thrombotic Thrombocytopenic Purpura in a Population from Japan

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Abstract

Thrombotic thrombocytopenic purpura (TTP) is a type of thrombotic microangiopathy (TMA). Studies report that the majority of TTP patients present with a deficiency of ADAMTS13 activity. In a database of TMA patients in Japan identified between 1998 and 2008, 186 patients with first onset of acquired idiopathic (ai) ADAMTS13-deficient TTP (ADAMTS13 activity <5%) were diagnosed. The median age of onset of TTP in this group of patients was 54 years, 54.8% were female, 75.8% had renal involvement, 79.0% had neurologic symptoms, and 97.8% had detectable inhibitors to ADAMTS13 activity. Younger patients were less likely to present with renal or neurologic dysfunction ($p<0.01$), while older patients were more likely to die during the TTP hospitalization ($p<0.05$). Findings from this cohort in Japan differ from those reported previously from the United States, Europe, and Korea with respect to age at onset (two decades younger in the other cohort) and gender composition (60% to 100% female in the other cohort). We conclude that in one of the largest cohorts of ai-TTP with severe deficiency of ADAMTS13 activity reported to date, demographic characteristics differ in Japanese patients relative to those reported from a large Caucasian registry from Western societies. Additional studies exploring these findings are needed.

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Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disorder and originally defined by classic "pentad"; thrombocytopenia, microangiopthic hemolytic anemia (MAHA), renal impairment, neurological symptoms, and fever [1]. In 1998, two studies identified deficiency of plasma ADAMTS13 (a disintegrin-like and metalloprotease with thrombospndin type 1 motifs 13) activity (ADAMTS13:AC) among persons with TTP [2,3]. ADAMTS13 cleaves the peptide bond between Thy1605 and Met1606 in the A2 domain of von Willebrand factor (VWF) subunit. VWF is synthesized in vascular endothelial cells and megakaryocytes. Vascular endothelial cell-derived VWF is released into the plasma as unusually large VWF multimers (UL-VWFMs). UL-VWFMs are degraded into smaller size VWF multimers by ADAMTS13. Severe deficiency of ADAMTS13:AC, either congenital or acquired, results in accumulation of UL-VWFMs and formation of platelet thrombi in the microvasculatures. In congenital TTP (Upshaw-Schulman syndrome), ADAMTS13 deficiency is caused by mutations in the ADAMTS13 gene [4]. In contrast, acquired TTP is frequently caused by inhibitory autoantibodies against ADAMTS13 [2,3]. Most acquired TTP patients have IgG antibodies. In rare cases, IgA and/or IgM antibodies are associated with IgG antibodies [5,6]. Patients with severe ADAMTS13:AC deficiency present with a lower platelet count and a significantly increased risk of TTP relapse [7–10]. Only a few small cohort studies of acquired idiopathic TTP patients characterized by severe ADAMTS13:AC deficiency have been reported previously. These studies characterize TTP with a predilection for the young and female, high rates of renal and central nervous system (CNS) involvement, and a 15% to 20% mortality. The largest cohort of acquired idiopathic (ai)-severely ADAMTS13-deficient TTP patients previously reported is from the Oklahoma TTP Registry (n = 60) [10]. In this study we systematically analyzed the clinical and laboratory features of a large cohort of Japanese patients with acquired idiopathic TTP and who also have severe ADAMTS13:AC deficiency.

Results

The number of ai-TTP patients fit the above inclusion criteria and retained for the study was 186. Of these, 31 (16.7%) were diagnosed between 1998 and 2001, 84 (45.2%) between 2002 and

2005, and 71 (38.2%) since 2006. This included individuals who did not experience any exposure to drugs that cause TTP or TMA, organ transplantation, stem cell transplantation, immunologic disease and also did not have a prior history of TTP. The age distribution of disease onset ranged from 8 months to 87 years old, with peak incidence occurring at age 60 (Figure 1, upper panel). Patients under 20 years accounted for 9.1% (17/186) of this subgroup, while patients over age 80 years accounted for 3.8% (7/186). Females accounted for 54.8%. Laboratory studies revealed that 100% of these patients were thrombocytopenic, 75.8% had renal involvement, and 79.0% had neurologic involvement. Overall, 16.1% died from TTP. ADAMTS13 inhibitors (≥0.5 BU/ml) were identified in 182 patients (97.8%). As shown in Figure 1 lower panel, 8.1% of these patients had inhibitor titers of 0.5~<1.0 BU/ml, 35.5% had titers of 1.0~<2.0, 33.3% had inhibitor titers of 2.0~<5.0, 12.9% had inhibitor titers of 5.0~<10, and 8.1% had inhibitor titers of ≥10 BU/ml. We found four ai-TTP patients without ADAMTS13 inhibitor (<0.5 BU/ml), whose ADAMTS13:AC, however, was normalized after remission. Therefore, these patients were included in this study.

The ai-TTP patients were evaluated according to the age at diagnosis (Table 1); Group 1 (<20 years old: n = 17), Group 2 (20~<40 years old: n = 36), Group 3 (40~<60 years old: n = 63), and Group 4 (60 years old~: n = 70). Rates of renal and neurologic dysfunction at the time of TTP presentation were lowest in the youngest age-subgroup (52.9% versus 72.2% to 81.0% for renal involvement, and 47.0% versus 69.4% to 88.6% for neurologic involvement; $p<0.01$) while in-patient mortality was highest among the oldest sub-group (28.6% versus 5.9% to 11.1%, $p<0.01$). Overall, females accounted for 54.8% of the patients (with rates of female gender ranging from 45.7% to 69.4% in each of the four age-groups).

Discussion

We evaluated 186 patients with initial onset of severely deficient ADAMTS13:AC levels TTP in Japan, representing the largest cohort of ai-TTP patients with ADAMTS13:AC deficiency reported from Japan. These individuals had presented with TMA-findings to medical centers throughout Japan over a ten-year period. In interpreting our findings, several factors should be considered.

These individuals accounted for 71.5% of 260 patients with a first episode of ai-TTP, who were diagnosed in out registry. This rate is similar to that reported previously for smaller cohorts of TTP patients from Europe, the United States, Canada, the United Kingdom, and Korea [7–13].

Sociodemographic characteristics of these TTP patients were compared to those reported from cohorts in Oklahoma, Saint Louis, France, and Korea [7–10,13] (Table 2). The median age of TTP patients with severely deficient ADAMTS13:AC levels reported from the other cohorts except for Saint Louis is 15 to 20 years less than that reported for our cohort. Also, females accounted for 53.8% of patients in our cohort versus 60% to 100% in other cohorts. Since additional information on predisposing factors for TTP are not known currently, it is not possible to identify factors accounting for age- and gender-related differences noted between TTP patients in Japan with severe ADAMTS13-deficiency versus those reported from other geographic regions.

Age-related differences in rates of neurologic and renal involvement among TTP patients who had severely deficient ADAMTS13:AC levels have not been reported previously. We found lower rates of renal and neurologic dysfunction amongst the youngest TTP patients, and the highest short-term mortality rates among the oldest TTP patients. While our study evaluated 186 patients with initial onset of severe ADAMTS13:AC activity

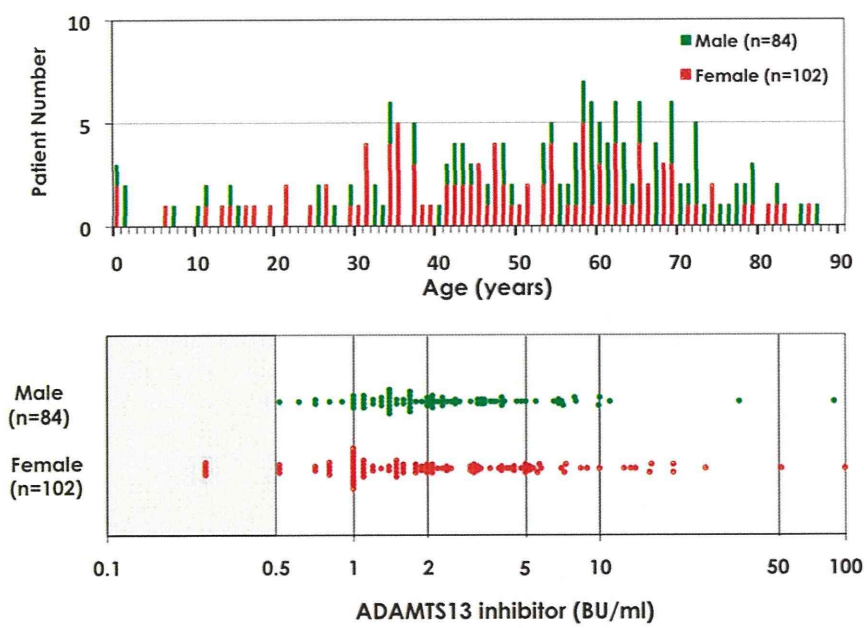

Figure 1. Age distribution and ADAMTS13 inhibitor levels in acquired idiopathic (ai−) TTP with severe deficiency of ADAMTS13 activity. Upper panel shows the age distribution of 186 patients with severe deficiency of ADAMTS13 activity under 5%. We found wide range of the age at TTP bouts from 8 months old to 87 years old. The highest incident peak was found around 60 years old. Lower panel shows the distribution of ADAMTS13 inhibitors in 186 ai-TTP patients with severe deficiency of ADAMTS13 activity. We found ADAMTS13 inhibitors (≥0.5 BU/ml) in 182 patients (97.8%). High titer inhibitors ≥2.0 BU/ml was seen in 101 patients (54.3%).
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Table 1. Clinical features in ai-TTP patients with severe deficiency of ADAMTS13:AC.

	All patients	Groups according to age				
		1	2	3	4	Overall p
Age (years)	54 (37, 65)	<20	20~<40	40~<60	60~	
	Median (25, 75 percentile)					
Patient Number	186	17	36	63	70	
Female (%)	54.8	52.9	69.4	57.1	45.7	NS
"Pentad"						
(1) Platelet count ($\times 10^9$/L), Median (25, 75 percentile)	10 (7, 16)	9 (7, 12)	10 (7, 20)	10 (6, 18)	10 (8, 15)	NS
(2) Hemoglobin (g/dL), Median (25, 75 pecentile)	7.3 (6.1, 8.7)	7.4 (5.4, 8.7)	6.7 (5.9, 7.8)	7.1 (6.0, 8.8)	7.8 (6.6, 8.8)	NS
(3) Renal involvement (%)	75.8	52.9	72.2	81.0	78.5	NS
Serum creatinine (mg/dL), Median (25, 75 percntile)	0.9 (0.7, 1.3)	0.58 (0.31, 0.80)	0.86 (0.70, 1.16)	0.95 (0.80, 1.50)	1.00 (0.80, 1.40)	<0.01[a]
Blood urea nitrogen (mg/dL), Median (25, 75 percntile)	24 (17, 37)	15 (12, 23)	19 (14, 26)	27 (17, 41)	27 (21, 43)	<0.01[b]
(4) CNS involvement (%)	79.0	47.0	69.4	82.5	88.6	<0.01[c]
(5) Fever (≥37.0°C) (%)	71.5	76.5	63.9	69.8	75.7	NS
Mortality in the current episode of TTP bouts (%)	16.1	5.9	5.6	11.1	28.6	<0.05[d]

NS: not significant difference (≥0.05).
Overall p values were caluculated using the Kruskal-Wallis H tests or chi-square tests with Yates' correction for 2×4 tables.
Significant differnces between 4 groups (overall p<0.05) were further analyzed by Mann-Whitney U-test or chi-squre test.
[a]p<0.01 between Group 1 and Groups 2, 3, 4.
[b]p<0.01 between Group 1 and Groups 3, 4, and between Group 2 and Groups 3, 4.
[c]p<0.01 between Group 1 and Groups 3, 4.
[d]p<0.05 between Group 2 and Group 4.
doi:10.1371/journal.pone.0033029.t001

Table 2. Comparison of our findings with those reported from Europe, Asia, and the United States for acquired idiopathic TTP patients with severely deficient ADAMTS13:AC levels.

	This study	Vesely et al[7]	Zheng et al[8]	Coppo et al[9]	Kremer-Hovinga et al[10]	Jang et al[13]
	(n = 186)	(n = 16)	(n = 16)	(n = 31)	(n = 60)	(n = 20)
Geographic region	Japan	Oklahoma (USA)	Saint Louis (USA)	France	Oklahoma (USA)	Korea
Ethnicity/race	Japanese 100%	White 50%,	White 32%,	White 52%,	African-American 35%	Korean 100%
		African-American 50%	African-American 68%	Afro-Caribbean 48%		
Idiopathic etiology	100%	100%	100%	100%	77%	70%
Prior TMA	0%	0%	38%	13%	0%	ND
ADAMTS13:AC	<5%	<5%	<5%	<5%	<10%	<10%
ADAMTS13:INH	98%	94%	44%	55%	83%	ND
Age (years)	54 (8 m–87)	39 (19–71)	51 (21–79)	36 (19–67)	41 (9–72)	40.5 (mean)
% female	55	75	100	65	82	60
Platelets (10^9/ul)	10 (1–88)	11(4–27)	17 (6–47)	12 (2–69)	11 (2–101)	24 (mean)
Hb (g/dl)	7.3 (4.3–11.9)	ND	ND	7.3 (4.6–13.7)	ND	7.7 (mean)
Ht (%)	ND	21 (15–30)	25 (13–33)	ND	21 (13–33)	ND
Creatinine (mg/dl)	0.9 (0.7–10.7)	1.2(0.9–5.5)	1.1 (0.7–3.1)	1.1 (0.67–5.2)	1.6 (0.7–6.6)	1.6 (mean)
BUN (mg/dl)	23.4 (2.5–154)	ND	ND	ND	ND	ND
Fever (%)	72	ND	31	36	ND	70
CNS involvement (%)	79	50	56	74	50	25
% Survival	84	81	81	87	78	81

ND: no data.
Median (minimum-maximum).
doi:10.1371/journal.pone.0033029.t002

deficiency, the other cohorts included smaller number of patients with idiopathic TTP and severe ADAMTS13:AC deficiency [7–9,11–13]. These age-related differences in clinical findings may account in part for higher short-term mortality rates observed among older patients with TTP in our cohort, as well as in the cohort reported from Canada [14].

Fourth, inhibitory autoantibodies against ADAMTS13 were identified in 97.8% of patients with ADAMTS13:AC deficient TTP. Other cohorts identify inhibitory antibodies in 44% to 94% of TTP patients with severely deficient ADAMTS13:AC levels [7–13]. These findings reflect variable sensitivity and specificity of ADAMTS13:AC inhibitor tests. In our study, ADAMTS13 inhibitor levels of 5 or more BU/ml were identified in 21.0% of TTP patients with severely deficient ADAMTS13:AC levels and inhibitor levels of 10 or more BU/ml were noted in 8.1%. These TTP patients with severely deficient ADAMTS13:AC activity levels and high titer inhibitors to ADAMTS13 might represent a subgroup of TTP patients for whom rituximab therapy might be particularly beneficial [12]. In general, the role of IgG antibody levels in ai-TTP is felt to be controversial. Some investigators report an association of higher titers with increased mortality, refractoriness, and more severe presentation [10,15], while others have not found similar results [7,16].

Our study has the limitation that follow-up ended at the time of hospital discharge, which prevented us from reporting on relapse rates and long-term survival rates. A second limitation is that TTP patients who were not severely deficient in ADAMTS13:AC levels were not included in this study. As noted by others, this is a heterogenous group of patients- many of whom have diseases other than TTP. Another limitation is that while our laboratory is a distinguished referral center for TMAs in Japan, it is not mandatory that information on all TMA patients is sent to our laboratory, and hence a number of patients with TMAs in Japan are not entered into our database. A final limitation is that cohorts in two of the five comparison studies (from Korea and Oklahoma) included a minority of individuals with TTP who did not have primary idiopathic TTP [10,13].

In summary, findings from this cohort of TTP patients in Japan with severe ADAMTS13:AC deficiency parallel those reported from TTP cohorts in Europe, the United States, Canada, the United Kingdom, and Korea in several ways, but also provide insights that have not been reported previously [7–14]. Novel findings in this cohort include females accounting for only 54.8% of incident cases, a higher median age at TTP onset of 54 years, and higher mortality rates amongst patients who were older than 60 years of age. Given the rarity of TTP in the general population, aggregation of findings from various TTP cohorts reported from Japan, Korea, France, England, Saint Louis, Oklahoma, and Canada might yield important findings that single registries would be unable to identify. A particularly important finding might be development and validation of a multivariate model predictive of mortality of persons with incident TTP characterized by severe ADAMTS13:AC deficiency.

Methods

Since 1998, our laboratory has been a nationwide referral center within Japan for TMAs, with 919 patients having been registered in this database [17]. During the first years of the study, samples from all TMA patients were evaluated by our referral center. In recent years, commercial laboratories now provide access to ADAMTS13:AC evaluation and some centers therefore do not submit samples to our group. We are not able to ascertain which centers are sending samples to commercial vendors at this time. Of these 919 patients, 186 patients were diagnosed with first onset of ai-TTP characterized by severe deficiency of ADAMTS13:AC (<5%) and no prior history of TTP. Exclusion criteria were exposure to drugs that cause TTP or TMA, organ transplantation, stem cell transplantation, immunologic disease, or ADAMTS13:AC levels 5% and more. All patients gave written informed consent to participate in this study. The study protocol was approved by the Ethics Committee of Nara Medical University Hospital.

Diagnostic criteria

The classic pentad for TTP was defined as follows (i) microangiopathic hemolytic anemia (hemoglobin ≤12 g/dL), Coombs test negative, undetectable serum haptoglobin (<10 mg/dL), more than 2 fragmented red cells (schistocytes) in a microscopic field with a magnification of 100, and concurrent increased serum lactate dehydrogenase (LDH) above institutional baseline, (ii) thrombocytopenia (platelet count $\leq 100\times10^9$/L), (iii) fever ≥37°C, (iv) CNS involvement: ranging from headache to coma, including neurological dysfunction, convulsion, clouding of consciousness, and (v) renal involvement (including abnormal urinalysis in addition to elevation of serum creatinine level). Patients were excluded if they reported a prior episode of ai-severely ADAMTS13-deficient TTP (n = 18 patients).

Blood Sampling

Before therapeutic approaches were initiated, whole blood samples (~5 ml) were phlebotomized from each patient and placed into plastic tubes containing 1/10 volume of 3.8% sodium citrate. The plasma was separated by centrifugation at 3000 g for 15 min at 4°C, kept in aliquots at −80°C until testing, and sent to our laboratory with clinical information.

Assays of plasma ADAMTS13:AC and ADAMTS13:INH

Until March 2005, ADAMTS13:AC was determined by classic von Willebrand factor multimer (VWFM) assay with a detection limit of 3% of the normal control [18,19]. Thereafter, a chromogenic ADAMTS13-act-ELISA [20] with a detection limit of 0.5% of the normal control was developed, and replaced the VWFM assay. Thus, most of the plasma samples stored at −80°C were re-evaluated with chromogenic act-ELISA, but 22 samples were unable to evaluate by the new method, because of a short of the stored sample volume. Basically, however, the results obtained by both the assays had a high correlation (r = 0.99) [20]. Thus, the results determined by VWFM alone were also included in this study. Further, to compare the results from other investigators and potentially with different assay methods for ADAMTS13:AC, we here categorized plasma levels of ADAMTS13:AC of severe (<5%), moderate (5%~<25%), mild (25%~<50%) deficiency and normal (≥50%) of ADAMTS13:AC. Plasma ADAMTS13:INH titers were analyzed either by classic VWFM assay or chromogenic ADAMTS13-act-ELISA using heat-inactivated plasmas at 56°C for 30 min. Briefly, the tested samples were mixed with an equal volume of the normal plasmas and incubated at 37°C for 2 hours. After incubation, the residual ADAMTS13:AC was measured. One Bethesda unit (BU) is defined as the amount necessary to reduce ADAMTS13:AC to 50% of control levels according to the Bethesda method, which was originally developed for the measurement of factor VIII inhibitor [21]. Titers ≥0.5 BU/ml were classified as inhibitor-positive.

Statistical analysis

All continuous variables were reported as median values (25, 75 percentile). Comparisons between two patient groups (severe

deficiency and detectable ADAMTS13 activity) were tested for statistical significance using the Mann-Whitney U-tests or chi-square tests. Comparisons between 4 patients groups (under 20 years old, 20 to under 40 years old, 40 to under 60 years old, and over 60 years old) were calculated using the Kruskal-Wallis H tests or chi-square tests with Yates' correction for 2×4 tables. Significant differences between 4 groups (overall $p<0.05$) were further analyzed by Mann-Whitney U-tests or chi-square tests. Correlation between ADAMTS13:AC and :INH was analyzed by Sperman's correlation. A two-tailed P value less than 0.05 was considered to be significant.

Author Contributions

Conceived and designed the experiments: YF. Performed the experiments: AI YH MH YY HY. Analyzed the data: MM. Wrote the paper: MM CB YF ZQ.

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V. その他

平成２３年度厚生労働科学研究費補助金（難治性疾患克服研究事業）
非定型HUSの診断法と治療法の確立　（23163801）
平成２３年度　第１回班会議　プログラム

研究課題名：非定型HUSの診断法と治療法の確立　（23163801）

日時　平成２４年３月２日（金）　15:00-17:30
場所　奈良県立医科大学　厳橿会館　特別会議室１

出席予定者

奈良県立医科大学	輸血部	藤村吉博、松本雅則、吉田瑶子
	眼科学	緒方奈保子
国立循環器病研究センター		宮田敏行、Xinping Fan
近畿大学奈良病院	血液内科	八木秀男
大阪医科大学	小児科	芦田明
兵庫医科大学	血液内科	日笠　聡
倉敷中央病院	血液内科	上田恭典
三重大学	臨床検査医学	和田英夫

班会議予定

15：00ー開会挨拶　研究代表者　藤村吉博

15：10ー

（１）**非定型 HUS 患者の診断法**　タンパク質レベルでの診断法の開発　(45 分)
　　奈良県立医科大学　輸血部　吉田瑶子先生

（２）**非定型 HUS 患者の遺伝子背景**　遺伝子レベルでの診断（45 分）
　　国立循環器病研究センター　研究所　宮田敏行先生

（３）**加齢黄斑変性症と Factor H**　(20 分)
　　奈良県立医科大学　眼科学　教授　緒方奈保子先生

（４）その他