(VWF:Ag) of all subjects using an immuno-turbidimetric assay, STA LIATEST VWF (Diagnostica Stago, Parsippany, NJ, USA). In both men and women, VWF:Ag increased with age (Fig. 1C), as reported previously [8]. The linear regression coefficient was 1.37 (95% CI, 1.21–1.52) in men and 1.30 (1.17–1.42) in women. Because of combined effects of the increase in VWF:Ag and the decrease in ADAMTS13 activity, the VWF:Ag-to-ADAMTS13 activity ratio was dramatically increased with age (Fig. 1D). This may partly explain the prothrombotic state of elderly men and women, because the imbalance between VWF and ADAMTS13 may be involved in thrombotic diseases such as acute myocardial infarction [9], advanced liver cirrhosis [10] and coronary artery disease [11]. As the FRETS-VWF73 assay itself was not affected by VWF concentration in plasma samples (0–160 μg mL^{-1}, data not shown), the reduced ADAMTS13 activity in the plasma of elderly subjects was not considered to be due to the assay-dependent artifactual phenomenon. In fact, when age-adjusted VWF:Ag was compared among quartiles of ADAMTS13 activity in the population using SPSS Statistics (IBM, Tokyo, Japan), no significant association between VWF:Ag and ADAMTS13 activity was observed in men (ANCOVA, $P = 0.153$) or in women ($P = 0.670$) (Fig. 1E).

ABO blood group has a significant influence on VWF:Ag; individuals with blood group O have lower VWF:Ag values than those of non-O groups [8]. We genotyped the ABO blood group by TaqMan assay (Applied Biosystems, Tokyo, Japan), which detects two polymorphisms, c.261Gdel and c.526C > G, on *ABO*. As expected, the subjects of blood group O exhibited a significantly lower VWF:Ag (men, 88 ± 43%; women, 80 ± 28%, mean ± SD) than those of the other blood groups (men A, 110 ± 35%; men B, 112 ± 36%; men AB, 115 ± 44%; women A, 103 ± 37%; women B, 105 ± 36%; women AB, 106 ± 31%) (Fig. 1F). In contrast, the plasma ADAMTS13 activity in men (A, 92 ± 24%; B, 94 ± 24%; AB, 96 ± 24%; O, 93 ± 25%) and women (A, 104 ± 26%; B, 109 ± 28; AB, 109 ± 28%; O, 106 ± 28) was not significantly associated with ABO blood group (Fig. 1G). This is consistent with the finding that ADAMTS13 antigen levels are not associated with ABO blood group in 387 male subjects [12]. The results are also consistent with the fact that VWF [13] but not ADAMTS13 [14] contains ABO blood group-related N-linked oligosaccharides.

In conclusion, this study demonstrated that, in the Japanese general population, the plasma ADAMTS13 activity is lower in men than in women, decreases with age, and is not significantly associated with ABO blood group. The VWF:Ag-to-ADAMTS13 activity ratio is increased with age in both men and women, and this increase may be involved in the prothrombotic state of elderly individuals.

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Disclosure of conflict of interests

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Polymorphisms and mutations of *ADAMTS13* in the Japanese population and estimation of the number of patients with Upshaw–Schulman syndrome

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Upshaw–Schulman syndrome (USS), also called hereditary thrombotic thrombocytopenic purpura, is an autosomal recessive disease characterized by thrombocytopenia and microangiopathic hemolytic anemia. USS is associated with hereditary severe deficiency of plasma ADAMTS13 activity; patients with USS have homozygous or compound heterozygous mutations in the *ADAMTS13* gene [1–5]. ADAMTS13 is a plasma metalloprotease that regulates platelet aggregation through the cleavage of von Willebrand factor (VWF) multimers. ADAMTS13-deficient plasma derived from patients with USS contains unusually large VWF multimers, which can induce unwanted hyperaggregation of platelets and microvascular thrombi. In this study, we analyzed the relationship between genetic variation of *ADAMTS13* and plasma ADAMTS13 activity in the Japanese general population. In addition, on the basis of the data obtained via our genetic analysis, we estimated the number of patients with USS in Japan.

The population examined is based on the Suita Study [6], an epidemiologic study consisting of randomly selected Japanese residents of Suita City, which is located in the second largest urban area in Japan. Our study protocol was approved by the ethical review committee of the National Cerebral and Cardiovascular Center, and only subjects who provided written informed consent for genetic analyses were included.

To identify common polymorphisms in the population, we first sequenced all 29 exons and exon–intron boundaries of *ADAMTS13* using 346 consecutive subjects, by means of previously described methods [2]. We identified 25 polymorphisms with allele frequencies of the respective minor allele > 0.01, including two in the promoter region, 10 in the exons, and 13 in the introns. Of these, six were missense single-nucleotide polymorphisms (SNPs): p.T339R (c.1016C > G), p.Q448E (c.1342C > G), p.P475S (c.1423C > T), p.P618A (c.1852C > G), p.S903L (c.2708C > T), and p.G1181R (c.3541G > A). Next, we performed TaqMan genotyping assays (Applied Biosystems, Tokyo, Japan) for the missense SNPs, using 3616 subjects whose plasma ADAMTS13 activities had been measured with the FRETS-VWF73 assay [7]. Allele frequencies for the minor alleles were 0.027 for p.T339R, 0.192 for p.Q448E, 0.050 for p.P475S, 0.027 for p.P618A, 0.048 for p.S903L, and 0.022 for p.G1181R. The observed genotypes did not deviate significantly from Hardy–Weinberg equilibrium. The p.T339R and p.P618A SNPs were in absolute linkage disequilibrium (r^2 = 0.97), whereas the other missense SNPs were not strongly linked (r^2 < 0.11).

The p.Q448E and p.P475S SNPs, but not the other missense SNPs, were significantly associated with plasma ADAMTS13 activity (Fig. 1A). The ADAMTS13 activity (97% ± 25% in men, 111% ± 28% in women, mean ± standard deviation) of p.Q448E heterozygotes (QE) and minor allele homozygotes (EE) was slightly but significantly higher than that of major allele homozygotes (QQ) (91% ± 24% in men, 104% ± 26% in women). In contrast, the ADAMTS13 activity (79% ± 20% in men, 92% ± 24% in women) of p.P475S heterozygotes (PS) and minor allele homozygotes (SS) was significantly lower than that of major allele homozygotes (PP) (94% ± 24% in men, 108% ± 27% in women). The difference in activity was consistent with the observation that the recombinant ADAMTS13-P475S mutant has approximately 70% of the activity of wild-type ADAMTS13 [8]. It is interesting that p.P618A was not associated with plasma ADAMTS13 activity in the present study, whereas the conditioned medium of HEK293 cells expressing the A618 variant showed lower levels of activity (27%) and antigen (14%) than the wild type [9]. PolyPhen-2, a program that predicts damaging missense mutations [10], identified p.T339R and p.P618A as 'possibly damaging' and 'probably damaging', respectively, whereas the other four SNPs were predicted to be 'benign'.

As the *ADAMTS13* locus is near (130–190 kb) the *ABO* locus on chromosome 9q34, we compared the frequencies of the SNPs among ABO blood group genotypes (Fig. 1B). The relative frequencies of p.T339R minor allele homozygotes and

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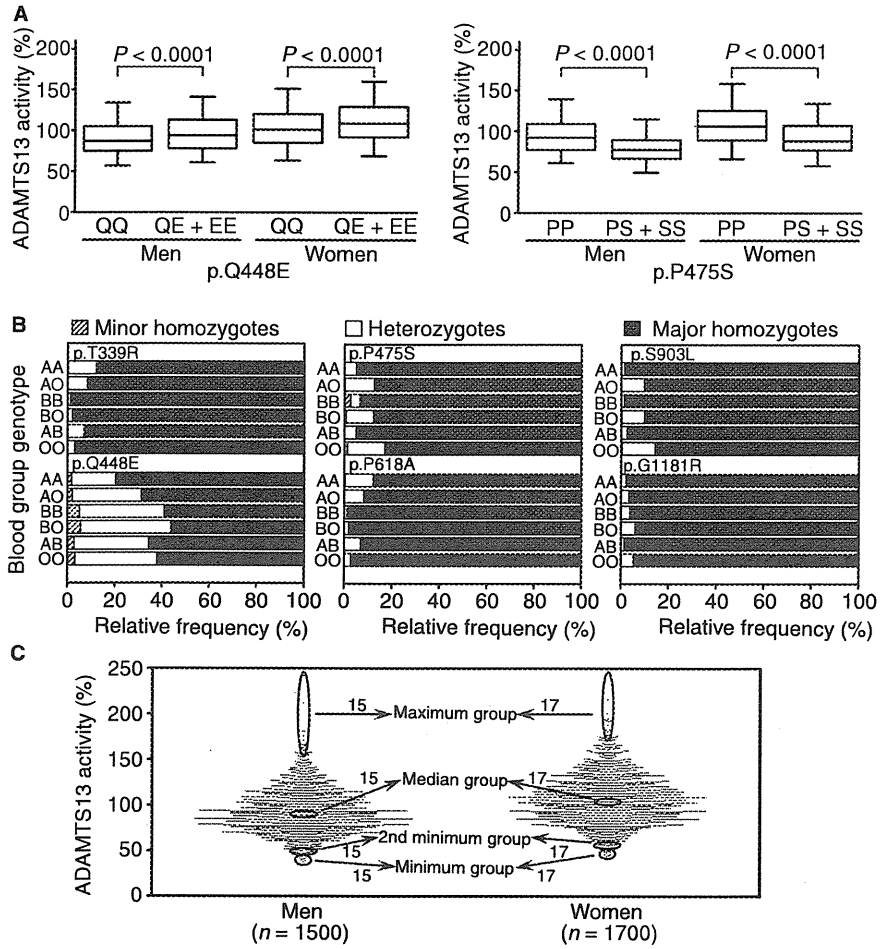

		Min. (*n* = 32)	2nd min. (*n* = 32)	Med (*n* = 32)	Max. (*n* = 32)
Average activity		47.1%	53.1%	97.6%	183%
Common SNP	p.T339R	2	1	3	0
	p.Q448E	8 (1)	8	8	16
	p.P475S	9 (2)	6	2	1
	p.P618A	2	1	3	0
	p.S903L	2	3	5	3
	p.G1181R	2	4	1	1
Rare mutation		7	3	2	2

Fig. 1. *ADAMTS13* variation in a Japanese general population. (A) Box-and-whisker plot (5th–95th percentiles) of plasma ADAMTS13 activity in each genotype of p.Q448E and p.P475S. *P*, Kruskal–Wallis test. (B) The relative frequency of minor homozygotes, heterozygotes and major homozygotes for each genetic polymorphism. (C) Scatter dot plot of plasma ADAMTS13 activity for men and women. On the basis of these activity measurements, 128 subjects were selected for sequencing of *ADAMTS13*. (D) The numbers of minor allele carriers in each group. One in eight p.Q448E carriers and two in nine p.P475S carriers in the minimum group were homozygotes for the respective minor alleles.

heterozygotes were higher for AA, AO and AB than for BB, BO and OO, suggesting that p.T339R is associated with the blood group A allele. The p.P618A SNP, which is tightly associated with p.T339R, exhibited the same pattern. The p.P475S and p.S903L SNPs tended to be associated with the blood group O allele.

We then utilized the plasma ADAMTS13 activity data to estimate the frequency of hereditary ADAMTS13 deficiency. In the population, 3200 DNA samples (1500 men and 1700 women) were available, from a quantitative standpoint, for sequencing of *ADAMTS13*. We selected 128 subjects according to their plasma ADAMTS13 activity (Fig. 1C): 32 subjects of the 'minimum' group (average activity, 47.1%), consisting of 15 men and 17 women with the lowest activities in each gender; 32 subjects of the 'second minimum' group (53.1%), consisting of 15 men and 17 women with the second lowest activities; 32 subjects of the 'median' group (97.6%), consisting of 15 men and 17 women with median activities; and 32 subjects of the 'maximum' group (183%), consisting of 15 men and 17 women with the highest activities. Each group corresponds to 1% of the population examined. All DNA samples from the four groups were subjected to *ADAMTS13* sequencing, which

revealed that 70 individuals had at least one of the six missense SNPs described above (Fig. 1D). Of these, only p.P475S showed a significant difference in minor allele frequency among four groups (P = 0.028, chi-square test). In addition, 14 individuals had rare non-synonymous mutations: seven (p.F324L, p.F418L, p.I673F, p.Q773X, p.Y1074AfsX46, p.R1095Q, and p.S1314L) in the 'minimum' group; three (p.I380T, p.Y1074AfsX46, and p.R1274C) in the 'second minimum' group; two (p.Q723K and p.N1321S) in the 'median' group; and two (p.L19F and p.R268Q) in the 'maximum' group. Of these, p.I673F (c.2017A > T) and p.Y1074AfsX46 (c.3220delTACC) had been identified as causative mutations in patients with USS [11,12]. All of the others were newly identified mutations.

To estimate the number of individuals with a hereditary ADAMTS13 deficiency, we generated several hypotheses: (i) as two individuals in each of the 'median' and 'maximum' groups had rare mutations, two of every 32 people should have a mutation that does not cause a functional defect of ADAMTS13; (ii) thus, five (= 7 – 2) individuals in the 'minimum' group and one (= 3 – 2) individual in the 'second minimum' group should be the heterozygotes carrying a mutation with a functional defect; (iii) other than these six (= 5 + 1) individuals in the 'minimum' and 'second minimum' groups, no individual should have any mutations that confer a functional defect. These hypotheses were consistent with a prediction based on PolyPhen-2: the p.S1314L, p.I380T, p.Q723K, p.N1321S, p.L19F and p.R268Q mutations are 'benign', p.I673F and p.R1274C are 'possibly damaging', and the others are 'probably damaging'. According to the hypotheses, we estimated that six of 3200 individuals were heterozygotes for ADAMTS13 deficiency. This estimation suggested that $\sim$ 1 individual in 1.1×10^6 (= 6/3200 × 6/3200 × 1/4) should be a homozygote or a compound heterozygote for ADAMTS13 deficiency. In Japan, which has a population of approximately 1.3×10^8, $\sim$ 110 individuals may have hereditary ADAMTS13 deficiency or USS. If we adjusted our estimate of ADAMTS13 deficiency from 6/3200 to 7/3200 or 5/3200, the number of patients would be 160 or 80, respectively. The validity of these calculation procedures was confirmed by StaGen Co., Ltd (Chiba, Japan), a company specializing in genetics, statistics, and data analysis.

In conclusion, this study demonstrated that, in the Japanese general population, there are six common missense SNPs: p.T339R, p.Q448E, p.P475S, p.P618A, p.S903L, and p.G1181R. Of these, p.Q448E and p.P475S are significantly associated with plasma ADAMTS13 activity. Allele frequencies of these SNPs correlate with ABO blood group. Finally, we estimated the number of patients with USS in Japan, yielding a figure that corresponds to approximately three times the number of patients already diagnosed as having this condition. Because of insufficient sample sizes, we may have underestimated the prevalence of USS. Further studies are needed to obtain more reliable conclusions.

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Disclosure of Conflict of Interests

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INVITED REVIEW

Natural history of Upshaw–Schulman syndrome based on *ADAMTS13* gene analysis in Japan

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Summary. Upshaw–Schulman syndrome (USS) is an extremely rare hereditary deficiency of ADAMTS13 activity, termed congenital TTP. The clinical signs are usually mild during childhood, often with isolated thrombocytopenia. But their symptoms become more evident when patients have infections or get pregnant. We identified 43 USS-patients in Japan, who ranged in age from early childhood to 79 years of age. Analysing the natural history of these USS patients based on *ADAMTS13* gene mutations may help characterise their clinical phenotypes. Severe neonatal jaundice that requires exchange blood transfusion, a hallmark of USS, was found in 18 of 43 patients (42%). During childhood, 25 of 43 patients were correctly diagnosed with USS without gender disparity. These 25 patients were categorised as having 'the early-onset phenotype'. Between 15 and 45 years of age, 15 were correctly diagnosed, and, interestingly, they were all female. The remaining three patients were male and were diagnosed when they were older than 45 years of age, suggesting that they were 'the late-onset phenotype'. Two of these three males developed sudden overt TTP when they were 55 and 63 years old, respectively. These two men had two different homozygous *ADAMTS13* gene mutations, p.R193W/p.R193W and p.C1024R/p.C1024R, respectively. Both of which were not discovered in the US or Western countries. *In vitro* expression studies showed that these two proteins were consistently secreted into the culture medium but to a lesser extent and with reduced activity compared to the wild-type protein. Our results indicate that 'the late-onset phenotype' of USS is formed with ethnic specificity.

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Introduction

Upshaw–Schulman syndrome (USS) is an hereditary deficiency in the activity of von Willebrand factor-cleaving protease (VWF-CP) [1], termed ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motifs 13) [2–4]. In the absence of ADAMTS13, unusually large VWF multimers (UL-VWFMs) released from vascular endothelial cells are not cleaved appropriately, which induces platelet hyperagglutination under high shear stress [5]. Thus, USS is alternatively called congenital thrombotic thrombocytopenic purpura (TTP). On the other hand, approximately half of congenital atypical haemolytic uremic syndrome (aHUS) cases are caused by genetic mutations in complement regulatory factors, such as factor H, I, and B, and membrane cofactor protein, or thrombomodulin [6]. However, the majority of both TTP and HUS occur in the acquired form, and it has been said that TTP and HUS patients predominantly exhibit neurotropic and nephrotropic signs, respectively. Further, it is often difficult to discriminate between these two diseases in clinical practice [7–9] because both diseases are identified based on common pathological features termed thrombotic microangiopathies (TMAs), which are characterised by organ dysfunction due to platelet thrombi in the microvasculature, consumptive thrombocytopenia, and microangiopathic haemolytic anaemia (MAHA) [10].

The classic hallmarks of USS are severe neonatal jaundice with a negative Coombs test that requires an exchange blood transfusion and repeated childhood episodes of thrombocytopenia and MAHA that are reversed by infusions of fresh frozen plasma (FFP) [11]. However, recent studies indicated that the clinical signs of USS during childhood may be much milder than expected, and often only an isolated thrombocytopenia occurs, causing physicians to sometimes overlook this important disease [12].

Despite a lengthy history of clinical diagnoses of USS, only 10 years have passed since the disease-related enzyme, ADAMTS13, was discovered [3,4,13,14]. Furthermore, USS is an extremely rare disease, and to date, it is estimated that there have been approximately 100 patients worldwide [15]. In this regard, Nara Medical University has functioned as a TMA referral centre in Japan since 1998 and collected a large dataset of 919 patients with TMA between 1998 and 2008 [16]. In this registry, we identified 41 USS patients in 36 different families who ranged in age from early childhood to 79 years old. Subsequently, until the end of March 2011, we have identified two new USS-patients belonged to different families in Japan. Analysing the natural history of these 43 USS-patients will further our understanding of the clinical significance of ADAMTS13, which functions to regulate the size of platelet-thrombi that form in the microvasculature under high shear stress.

Historical backgrounds

In 1953, Dacie *et al.* [17] reviewed 12 patients with atypical congenital haemolytic anaemia and identified a 6-year-old girl who had experienced repeated episodes of severe jaundice, thrombocytopenia, haemolytic anaemia, and schistocytes since she was a newborn. Before first visiting Dacie, this patient had received a splenectomy but did not improve. She died of renal failure at 7 years of age. This patient was the third of four children, and both the first and second children were jaundiced at birth and died of haemorrhage at 2 years and 4 days old, respectively. The fourth child and the parents were asymptomatic. Thus, the authors concluded that these three patients must have a hitherto unrecognised hereditable blood disease.

In the absence of any known concept of TTP, in 1960, Schulman *et al.* [18] reported an 8-year-old girl who had no coagulation abnormalities but repeated bleeding episodes due to chronic thrombocytopenia and MAHA. These symptoms dramatically improved with fresh plasma infusions, suggested that the patient had a congenital deficiency in a 'platelet-stimulating factor' in her plasma. In 1978, Upshaw [19] reported the case of a 29-year-old female who had repeated episodes of thrombocytopenia and MAHA starting in childhood and was successfully treated with plasma infusions. Of note, both Schulman and Upshaw determined that plasma infusions successfully treated their patients. Rennard and Abe [20] reported a case that was originally identified by Upshaw with 'a slightly decreased level of plasma cold-insoluble globulin (fibronectin) during the acute phase', and proposed a nomenclature of USS for these types of patients. However, no correlation between the fibronectin levels and disease activity in USS patients was reported by Koizumi *et al.* [21] and Goodnough *et al.* [22], including Schulman's original case. Furthermore, after the thrombopoietin assay was established, Miura *et al.* [23] reported five Japanese USS patients with a normal plasma level of thrombopoietin. Thus, all the pathogenic features that were initially postulated for USS have been entirely excluded by subsequent investigations.

For this reason, the term USS was almost forgotten in 1997, when the assay for VWF-CP (now ADAMTS13) activity was established. Instead, the practical diagnostic term of 'chronic relapsing TTP (CR-TTP)' has been historically used. This term was coined by Moake *et al.* [24], who found that UL-VWFMs were present in the plasma of four CR-TTP patients including the Upshaw's case during the remission phase, but disappeared during the acute phase. In 1997, Furlan *et al.* [25] showed that four CR-TTP patients, who were distinct from the cases of Moake *et al.* [24], lacked VWF-CP activity, but did not examine the presence of ADAMTS13 inhibitors. However, it was retrospectively determined that two CR-TTP patients in both the case reports by Moake *et al.* [24] and Furlan *et al.* [25] had congenital TTP, while the remaining two cases in each report had acquired TTP. Under these circumstances, we re-visited the term USS [11], which included analysing three Japanese patients with USS, and found that they uniformly had a severe deficiency in VWF-CP activity (determined based on the VWFM assay in the presence of 1.5 mol L^{-1} urea) and no evidence of inhibitors. The parents of these patients were asymptomatic and had moderately decreased VWF-CP activity (17–60% of normal), except for one carrier who had very low VWF-CP activity (5.6% of normal). Later, this carrier was shown to have a unique single nucleotide polymorphism (SNP), a p.P475S mutation in the *ADAMTS13* gene in one allele, which is very common among Japanese people (9.6% of normal individuals are heterozygous for the p.P475S mutation) [26].

In 2001, Levy *et al.* [3] provided solid evidence that linked congenital TTP or USS and *ADAMTS13* gene mutations, and simultaneously other research groups successfully purified this enzyme and/or cloned the encoding cDNA [2,4,13,14].

Patients, materials and methods

USS patients

Forty-three USS patients (28 females and 15 males) belonging to 38 different families and their family members were enrolled in this study.

Assays for plasma ADAMTS13 activity and ADAMTS13 inhibitors

Between 1998 and 2004, our laboratory examined ADAMTS13 activity using a classic VWFM assay in the presence of 1.5 mol L^{-1} urea following the method of Furlan *et al.* [27]. The detection limit of this assay was 3% of the normal control [11]. In 2005, Kato *et al.* [28] developed a novel chromogenic ADAMTS13-act-ELISA using a recombinant VWF substrate (termed GST-VWF73-His). The detection limit of this assay was 0.5% of the normal control [28]. Both assays had a high correlation, and since then, the VWFM assay was completely replaced with the act-ELISA. In our patients with USS, the ADAMTS13 activity was determined at least two different occasions, using their plasmas obtained at more than 2 weeks

after the last plasma infusion therapy. Further, in some experiments with normal individuals as described below, FRETS-VWF73 assays [29] were used.

The ADAMTS13 inhibitor titers were evaluated using the Bethesda method, and the values < 0.5 Bethesda U (BU) mL^{-1} were negative, but those between 0.5 and 1.0 BU mL^{-1} were assumed to be marginal.

Assay for IgG-type plasma ADAMTS13 binding antibody titers

Measurement of plasma anti-ADAMTS13 IgG antibody titers in USS-patients was performed as described by Ferrari *et al.* [30] with a slight modification. Briefly, the recombinant (r) ADAMTS13 was directly coated to micro-titer plates, and after blocking with Protein-Free Blocking Buffers (Pierce, Rockford, IL, USA), the coated plates were incubated with normal and patient plasma dilutions. The IgG-type antibody bound to rADAMTS13 was detected by using horseradish peroxidase-conjugated goat anti-human IgG (AbD Serotec, Kidlington, UK) with a TMB substrate kit (Thermo Scientific, Rockford, IL, USA) at absorbance 450 nm at room temperature for 15 min. The results were calculated as a ratio of sample OD at each dilution divided by normal plasma OD at each dilution. The IgG antibody titer of a sample corresponds to the last dilution at which the ratio is above the cut-off level. This assay kit was kindly provided from Drs Barbara Plaimauer and Friedrich Scheiflinger of Baxter BioScience. In our laboratory, 25 normal plasmas (15 males and 10 males, aged between 20 and 40 years) consistently showed the titer of IgG-type binding antibody with a < 25-fold dilution (shown as < 25 × in Table 1).

ADAMTS13 gene analysis

All DNA analyses of the *ADAMTS13* gene were performed as previously described [26], with permission from the Ethics Committees of both the sample-collecting hospitals and the institute that performed the gene analysis. Hereafter, the disease-causing mutations (DCMs) of ADAMTS13 are highlighted in bold.

Results

ADAMTS13 SNPs among Japanese individuals

The human *ADAMTS13* gene is located on chromosome 9q34. The gene consists of 29 exons, and the translated enzyme contains 1427 amino acid residues with a multi-domain structure [2,4]. To date, more than 10 SNPs in *ADAMTS13* have been identified worldwide [3,26]. Among these, Japanese people (n = 3616) had six SNPs with the following allele frequencies, respectively: p.T339R (exon 9) (2.7%), p.Q448E (exon 12) (19.2%), p.P475S (exon 12) (5.0%), p.P618A (exon 16) (2.7%), p.S903L (exon 21) (4.8%), and p.G1181R (exon 25). (2.2%) [31]. Both p.T339R and p.P618A are almost completely linked in the general Japanese population, but this linkage may not exist in the Caucasian population as some reports have described individuals carrying p.P618A but not p.T339R [32,33]. Plasma and rADAMTS13 with the Asian-specific SNP, p.P475S [26,34,35], has markedly reduced activity compared to the wild-type protein in both the VWFM assay in the presence of urea (1.5 mol L^{-1}) [26] and the FRETS-VWF73 assay in the absence of urea [36], although the contribution of this polymorphism to thrombotic diseases has not been determined.

Recently, we have analysed the nucleotide sequences of *ADAMTS13* in 128 individuals without a history of TTP and identified 14 rare nonsynonymous mutations. Interestingly, among these 14 mutations, three mutations of **p.I673F** (exon 17), **p.Q723K** (exon 18), and **c.3220del TACC**, were also found as DCMs for USS in this study (below). Thus, the remaining 11 mutations may or may not be associated with a reduced activity of plasma ADAMTS13 [31].

Natural history of 43 USS-patients in Japan

Until the end of March 2011, we identified 43 USS patients in 38 different families (Family USS-A~LL) who ranged in age from early childhood to 79 years old. Hence, we present an up-dated natural history of these 43 USS patients together with their family members (Tables 1 and 2).

Family USS-A

Patient

One male (USS-A4) born in 1999.

Brief clinical data

The history of USS-A4 (*ADAMTS13* genotype: **p.R268P**/p.Q448E-**p.C508Y**) before he reached 5 years of age was previously described [11,26,37]. He currently receives biweekly FFP infusions (10 mL kg BW^{-1}) that are prepared from several fixed donors to prevent allergic reactions. He contracted the seasonal influenza A virus in 2010 and became severely ill with a reduced platelet count but did not develop overt TTP. He is currently in good clinical condition and has not had signs of renal or hepatic dysfunction. Both of his parents are asymptomatic carriers, but his father, aged 45 years old, has a **p.R268P**/p.P475S genotype and very low plasma ADAMTS13 activity at 5.6% of normal by the VWFM assay in the presence of 1.5 mol L^{-1} urea [26] and 3.6% by the chromogenic ADAMTS13-act-ELISA in the absence of urea (unpublished data).

Family USS-B

Patient

One female (USS-B3) born in 1986.

Table 1 Fourty-three Japanese patients with USS registered in Nara Medical University and their ADAMTS13 gene mutations

No.	Patient	Year of birth	Gender	ADAMTS13 Activity (%)	ADAMTS13 Inhibitor (BU mL^{-1})	ADAMTS13 Titer of IgG-type binding antibody (Year of examination)	*ADAMTS13* gene mutations Father's origin DCM	Father's origin Missense SNP	Mother's origin DCM	Mother's origin Missense SNP	Reference numbers and remarks
1	A4	1999	M	< 0.5	< 0.5	< 25 × (2001), < 25 × (2011)	p.R268P		p.C508Y	p.Q448E	11,26,37
2	B3	1986	F	< 0.5	< 0.5	< 25 × (2005), < 25 × (2008)	p.Q449X		p.Q449X		11,26,38
3	C3	1972	M	< 0.5	< 0.5	< 25 × (1999)	c.414+1G>A		c.414+1G>A		39,40
4	D4	1978	F	< 0.5	< 0.5	25 × (2001), < 25 × (2009)	p.I673F		c.414+1G>A		40,41
5	E4	1985	M	< 0.5	< 0.5	< 25 × (2001), < 25 × (2011)	p.I673F		p.C908Y		40
6	F3	1993	M	0.6	< 0.5	< 25 × (2002), < 25 × (2009)	p.R193W		c.1244+2T>G		40
7	G3	1987	F	< 0.5	< 0.5	< 25 × (2009)	p.R1123C		c.686+1G>A		40
8	H3	1951	M	0.6	< 0.5	*	p.A250V		c.330+1G>A		42
9	I4	1972	M	< 0.5	< 0.5	< 25 × (2003), < 25 × (2009)	p.H234Q		p.R1206X		43
10	J3	1977	F	< 0.5–0.8	< 0.5	< 25 × (2000)	p.R312C		c.3198delCT		†
11	J4	1979	M	< 0.5	< 0.5	< 25 × (2000)	p.R312C		c.3198delCT		†
12	K3	1976	F	< 0.5–0.7	< 0.5	200 × (2003), 400 × (2011)	p.Y304C		p.G525D	p.T339R, p.Q448E, p.P618A	12
13	K4	1978	F	< 0.5	< 0.5	25 × (2003), 100 × (2011)	p.Y304C		p.G525D	p.T339R, p.Q448E, p.P618A	12
14	L2	1967	F	< 0.5	< 0.5	< 25 × (2003)	p.Q1302X	p.P618A	p.R125VfsX6	p.T339R, p.Q448E	12
15	L3	1972	F	< 0.5	< 0.5	< 25 × (2003)	p.Q1302X	p.P618A	p.R125VfsX6	p.T339R, p.Q448E	12
16	M3	1969	F	< 0.5	< 0.5	< 25 × (2002)	p.R193W		p.R349C		12
17	M4	1971	F	< 0.5	< 0.5	< 25 × (2002)	p.R193W		p.R349C		12
18	N6	1986	F	< 0.5	< 0.5	< 25 × (1999), < 25 × (2005)	p.H234R	p.P475S	c.3220delTACC		11,37
19	O4	1958	F	< 0.5	< 0.5	< 25 × (2009)	p.I178T		p.Q929X		12
20	P3	1971	M	< 0.5	< 0.5	< 25 × (2003), < 25 × (2005)	p.C908Y				45 *de novo* mutation (p.C322G, p.T323R, p.F324L)
21	Q1	1983	M	< 0.5–0.7	< 0.5	25 × (2004), 25 × (2009)	p.G227R	p.G1181R	p.C908Y		46
22	Q2	1988	M	< 0.5	< 0.5	< 25 × (2007), < 25 × (2009)	p.G227R	p.G1181R	p.C908Y		46
23	R5	1982	F	< 0.5	< 0.5	25 × (2005)	p.R193W		p.A606P	p.T339R, p.Q448E, p.P618A	12
24	S3	1982	M	0.9	< 0.5	< 25 × (1998)	*		*		†
25	T4	1981	F	< 0.5	< 0.5	< 25 × (2009)	c.3220delTACC		c.3220delTACC		47
26	U3	1990	F	< 0.5	< 0.5	< 25 × (2009)	c.2259delA		c.2259delA		†
27	V3	1983	F	< 0.5	< 0.5	< 25 × (2009)	p.W1081X		p.R193W		†
28	W4	1990	F	< 0.5	< 0.5	< 25 × (2005), < 25 × (2009)	p.G550R	p.Q448E	*	p.P475S	†
29	X5	1963	F	< 0.5	< 0.5	200 × (2004)	*	p.G1181R	*	p.P475S	†
30	Y3	1960	F	< 0.5	< 0.5	< 25 × (2005)	p.G385E		p.R1206X		†
31	Z3	1971	F	< 0.5	< 0.5	< 25 × (2006), < 25 × (2009)	p.R193W		p.R193W		12
32	AA3	1987	F	< 0.5	< 0.5	200 × (2006)	*		*		†
33	BB3	1947	M	< 0.5	< 0.5	< 25 × (2006)	p.R193W		p.R193W		†
34	CC5	2004	M	< 0.5	< 0.5	< 25 × (2007)	p.Q723K		p.R398C		†
35	DD5	2007	F	< 0.5	< 0.5	25 × (2007), < 25 × (2009)	p.R268P		p.Y304C		†
36	EE4	2003	M	< 0.5	< 0.5	200 × (2008), 200 × (2011)	c.2259delA		c.2259delA		†
37	FF3	1991	F	< 0.5	< 0.5	< 25 × (2008)	p.Q449X		p.Q449X		48

Table 1 (Continued).

No.	Patient	Year of birth	Gender	ADAMTS13 Activity (%)	ADAMTS13 Inhibitor (BU mL^{-1})	ADAMTS13 Titer of IgG-type binding antibody (Year of examination)	*ADAMTS13* gene mutations: Father's origin DCM	Father's origin Missence SNP	Mother's origin DCM	Mother's origin Missence SNP	Reference numbers and remarks
38	GG2	1931	M	2.4–3.4	< 0.5	< 25 × (2008)	p.C1024R		p.C1024R		†
39	HH4	2003	F	< 0.5	< 0.5	< 25 × (2008)	p.Q449X		c.4119delG		†
40	II3	1977	F	< 0.5	< 0.5	50 × (1998), 50 × (2009)	*		*		†
41	JJ3	1980	M	< 0.5	< 0.5	< 25 × (2009)	c.1885delT		p.C908Y		Parent's origin is unknown†
42	KK3	1976	F	< 0.5	< 0.5	< 25 × (2011)	*		*		†
43	LL4	1981	F	< 0.5–1.8	< 0.5–1.4	> 400 × (2002), 200 × (2011)	p.C438S		p.G909R	p.T339R, p.Q448E, p.P618A	†

DCM, disease causing mutation; SNP, single nucleotide polymorphism; USS, Upshaw–Schulman syndrome. *Undetermined. †Unpublished.

Brief clinical data

USS-B3 (*ADAMTS13* genotype: **p.Q449X/p.Q449X**) is an only child who was born in Hokkaido to non-consanguineous parents. Her history prior to reaching 5 years of age was previously described [11,26,38]. Since childhood, she has received prophylactic FFP infusions. As a consequence, she was infected with hepatitis C and has received interferon therapy on two different occasions. In both instances, accelerated thrombocytopenia was observed despite the regular prophylactic FFP infusions. Furthermore, during her early childhood, she received DDAVP (1-desamino-8-D-arginine vasopressin) infusion once that immediately aggravated her clinical signs, including haematuria and thrombocytopenia. Currently, her renal function is normal and her liver function is well preserved (communication with Dr Mutsuko Konno). Her parents initially stated that they had a non-consanguineous marriage. However, a subsequent ancestral analysis revealed that two great-grandparents of USS-B3 on the paternal and maternal sides migrated from the same area (a small fisherman's village) of Iwate to Hokkaido at the end of the 19th century when Hokkaido was an undeveloped island, and the pioneers settled from the Japanese mainland (Honshu). This fisherman's village is located in the northern part of Honshu (Tohoku) facing the Pacific ocean, an area severely damaged several times by earthquake and tsunami – most recently in March 11, 2011. In the old days, this small village was isolated from neighbours, and was surrounded by mountains, suggesting that there were many consanguineous marriages within this village.

Family USS-C

Patient

One male (USS-C3) born in 1972.

Brief clinical data

USS-C3 (*ADAMTS13* genotype: **c.414+1G>A/c.414+1G>A**) is the last of four children to consanguineous parents (first cousins). Notably, the patient's elder brother (third sibling) died of melena soon after birth. The history of this patient was previously described [39,40]. At 8 years of age, USS-C3 was clinically diagnosed with USS. Since then, he has received prophylactic FFP (160 mL) infusions every 2–4 weeks. However, his renal function due to chronic nephritis gradually deteriorated, and in 1995 he required continuous ambulatory peritoneal dialysis (CAPD). Because of repeated peritonitis associated with CAPD, his therapy for renal insufficiency was changed to haemodialysis (three times per week) in 1999. However, his cardiac function decreased, and he eventually died of chronic heart failure in 2010 at 38 years of age.

Family USS-D

Patient

One female (USS-D4) born in 1978.

Brief clinical data

USS-D4 (*ADAMTS13* genotype: **p.I673F/c.414+1G>A**) was born as the second of 2 children to non-consanguineous parents. Her history was previously described [40,41].

Family USS-E

Patient

One male (USS-E4) born in 1985.

Brief clinical data

USS-E4 (*ADAMTS13* genotype: **p.I673F/p.C908Y**) was born as the second of three children to non-consanguineous parents. His history was previously described [40]. The third sibling had Down's syndrome and died of an unknown cause soon after birth.

Family USS-F

Patient

One male (USS-F3) born in 1993.

Brief clinical data

USS-F3 (*ADAMTS13* genotype: **p.R193W/c.1244+2T>G**) was born as the first of three children to non-consanguineous parents. His history was previously described [40]. He currently receives 120 mL FFP infusions when he develops an occasional haemolytic crisis.

Family USS-G

Patient

One female (USS-G3) born in 1987.

Table 2 Clinical course of 43 Japanese patients with USS

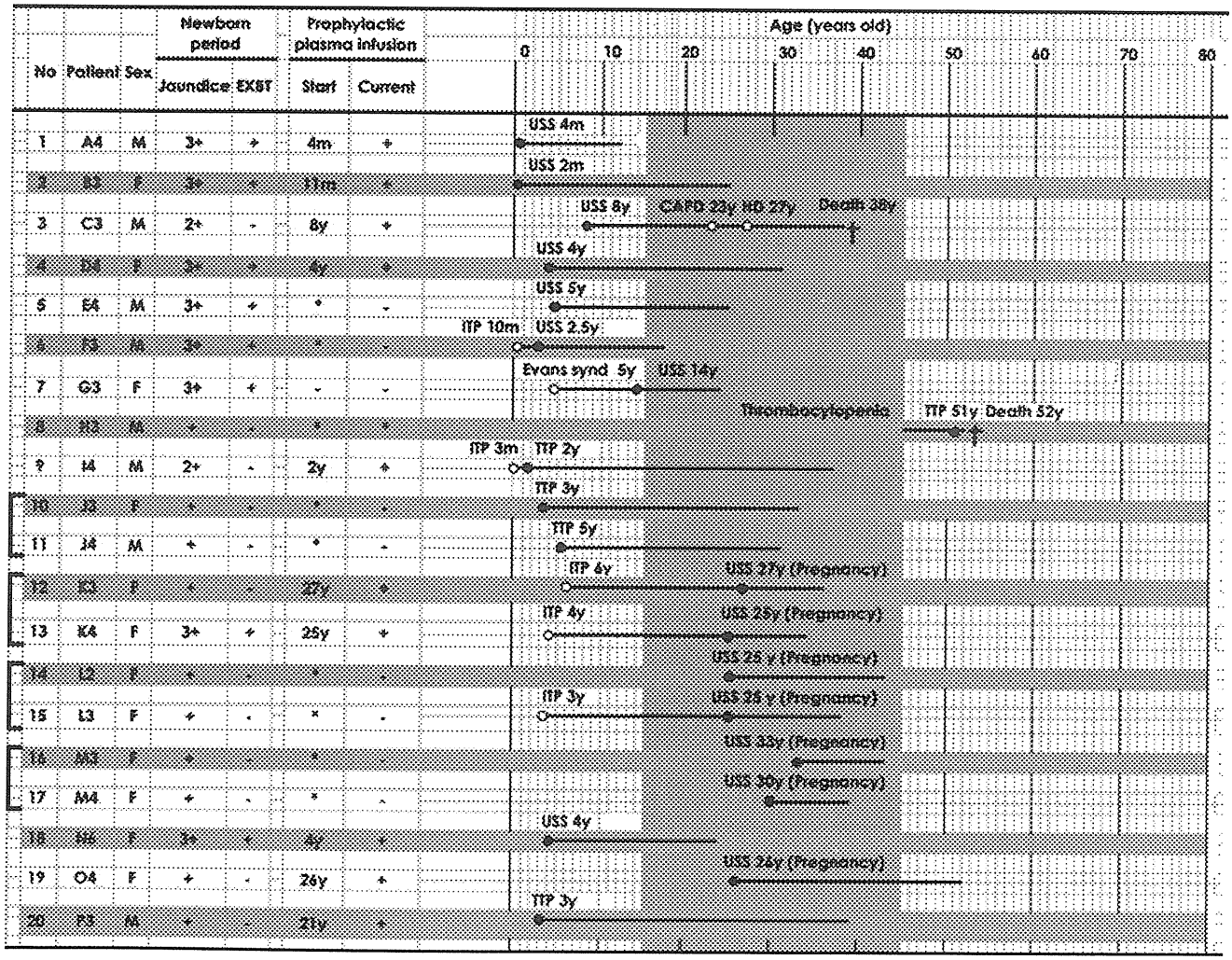
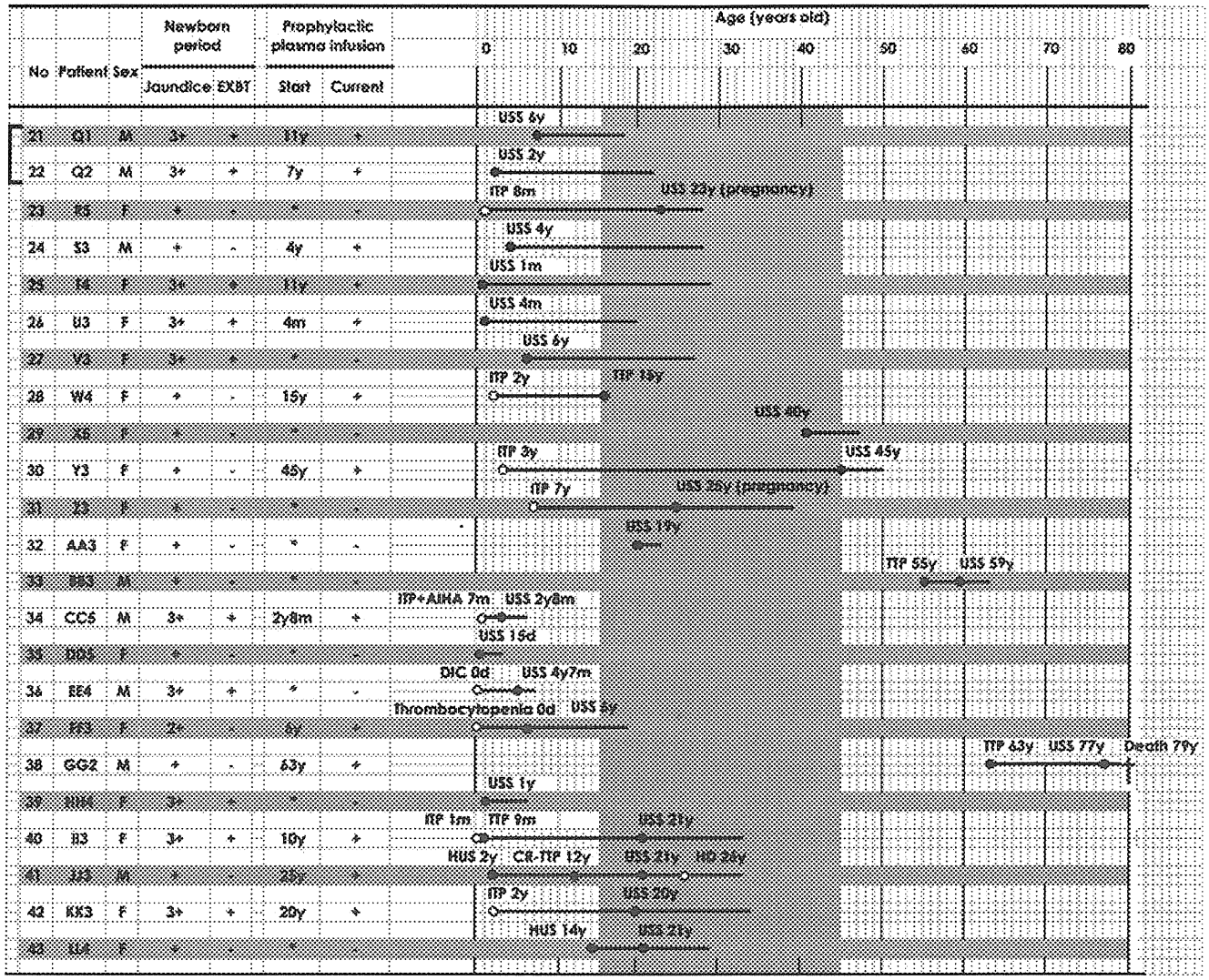


Table 2 Continued



EXBT, exchange blood transfusion; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; ITP, idiopatic thrombocytopenic purpura; DIC, disseminated intravasclar coagulation; AIHA, autoimmune hemolytic anemia; CAPD, continuous ambulatory peritoneal dialysis; HD, hemodialysis; USS, Upshaw–Schulman syndrome. *Undertermined.

Brief clinical data

USS-G3 (*ADAMTS13* genotype: **p.R1123C/c.686+1G>A**) was born as the third of four children to non-consanguineous parents. Her history before she reached 14 years of age was previously described [40]. Of note, her elder sister (second child) had severe jaundice soon after birth and received an exchange blood transfusion. She died of intracranial bleeding after a trivial traffic accident when she was 8 years old. USS-G3 received prophylactic FFP infusions after she was diagnosed with USS, but the infusion intervals gradually increased. Under these circumstances, she became pregnant at 21 and 22 years of age. During the first pregnancy, she had a spontaneous abortion at 5 weeks of gestation. During the second pregnancy, she delivered a premature baby girl (1446 g BW) at 35 weeks of gestation after a caesarean section under extensive prophylactic FFP infusions (normal Japanese female baby at 35 weeks of gestation has a BW of median 2173 g). Interestingly, many of the placental small vessels were occupied with hyaline thrombosis (communication with Dr Michiko Kajiwara, details will be published elsewhere by the physicians in charge).

Family USS-H

Patient

One male (USS-H3) born in 1951.

Brief clinical data

USS-H3 (*ADAMTS13* genotype: **p.A250V/c.330+1G>A**) was born to non-consanguineous parents, but the details were unclear [42]. At 51 years of age, he visited a nearby

hospital because of a convulsive seizure after haemorrhoidectomy where he was diagnosed with TTP. He had an episode of childhood thrombocytopenia, but there is no additional information. After 51 years of age, he had two episodes of overt TTP, and both were efficiently treated with FFP infusions. In July 2002, he experienced a fourth episode of overt TTP that developed after cholecystectomy, followed by gastrointestinal bleeding that was unsuccessfully treated with FFP infusions and further complicated by renal failure, which ultimately resulted in death at 52 years of age.

Family USS-I

Patient

One male (USS-I4) born in 1972.

Brief clinical data

USS-I4 (*ADAMTS13* genotype: **p.H234Q/p.R1206X**) was born as the second of two children to non-consanguineous parents. His elder brother died at 2 years of age with clinical signs that were compatible with TTP, as previously described [43]. At the age of 3 months, USS-I4 developed thrombocytopenia after receiving the diphtheria/pertussis/tetanus vaccine and was diagnosed with idiopathic thrombocytopenic purpura (ITP). Since he was 2 years old, he has experienced repeated overt TTP that has been treated with plasma infusions.

Family USS-J

Patients

One female (USS-J3) born in 1977 and one male (USS-J4) born in 1979.

Brief clinical data

USS-J3 (*ADAMTS13* genotype: **p.R312C/c.3198delCT**) and -J4 (*ADAMTS13* genotype: **p.R312C/c.3198delCT**) are the first and second of three children to non-consanguineous parents, respectively. For these two patients, severe jaundice was not noted during the newborn period. At 3 years of age, USS-J3 developed a cold followed by purpura with thrombocytopenia and was diagnosed with disseminated intravascular coagulation (DIC). Since then, she has experienced repeated episodes of thrombocytopenia and haemolytic anaemia, and was diagnosed with CR-TTP at 6 years of age. USS-J4 had an episode of purpura and thrombocytopenia when he was 5 years old. In 2000, both patients were shown to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. These two patients were not given prophylactic FFP infusions.

Family USS-K

Patients

Two females (USS-K3 born in 1976 and USS-K4 born in 1978).

Brief clinical data

Patients USS-K3 (*ADAMTS13* genotype: **p.Y304C**/p.T339R-p.Q448E-**p.G525D**-p.P618A) and -K4 (*ADAMTS13* genotype: **p.Y304C**/p.T339R- p.Q448E-**p.G525D**-p.P618A) were the first and second of two children of non-consanguineous parents, respectively. The history of these two patients was previously reported [12]. In 2003, USS-K3 became pregnant at 27 years old and developed overt TTP at 25 weeks of gestation. She experienced intrauterine foetal death followed by a caesarean delivery with a hysterectomy. On this occasion, she was diagnosed with USS. Since then, she has received prophylactic FFP infusions (80–120 mL) every 4 weeks in an out-patient clinic with a good clinical course. However, at the end of 2010, she had H1N1 influenza A virus infection that remarkably aggravated thrombocytopenia and was hospitalised for treatment (communication with Dr Junji Tomiyama).

In 2003, USS-K4, the younger sibling, became pregnant 2 months after her elder sister. She developed mild thrombocytopenia without significant clinical signs at 22 weeks of gestation. She underwent ADAMTS13 analysis, which confirmed a diagnosis of USS. While being treated with FFP infusions, she delivered a premature baby by a caesarean section [12]. Since then, she has received FFP infusions of 80 mL every 3 weeks. In 2008, 5 years after her first pregnancy, USS-K4 became pregnant for the second time and received more frequent FFP infusions (160 mL biweekly). At 29 weeks of gestation, her platelet count suddenly and severely dropped. Thus, at 30 weeks of gestation, a caesarean section was performed, and she delivered a baby (1522g BW) with congenital heart failure due to a ventricular septum defect (details will be published elsewhere by the physicians in charge).

Family USS-L

Patients

Two females (USS-L2 born in 1967 and USS-L3 born in 1972).

Brief clinical data

Both patients USS-L2 (*ADAMTS13* genotype: p.618A-**p.Q1302X/p.R125fsX6**-p.T339R-p.Q448E) and -L3 (*ADAMTS13* genotype: p.618A-**p.Q1302X/p.R125fsX6**-p.T339R-p.Q448E) were born as the second and fifth of five children to non-consanguineous parents. The history of these two patients was previously described [12]. At 27 years of age, USS-L2 became pregnant. At 27 weeks of gestation, she had intrauterine foetal death due to a suspected diagnosis of HELLP (haemolysis, elevated liver-enzymes, low platelets) syndrome. However, she

subsequently had four children who were all premature and uniformly born at approximately 30 weeks of gestation by a caesarean section with oral aspirin. Patient USS-L3, the younger sister of USS-L2, was diagnosed with ITP at 3 years of age. She had two pregnancies at 25 and 27 years of age. However, she lost both babies at 23 and 24 weeks of gestation, respectively, under a suspected diagnosis of 'habitual abortion'.

Family USS-M

Patients

Two females (USS-M3 born in 1969 and USS-M4 born in 1971).

Brief clinical data

Patients USS-M3 (*ADAMTS13* genotype: **p.R193W/p.R349C**) and USS-M4 (*ADAMTS13* genotype: **p.R193W/p.R349C**) were born as the second and third of four children to non-consanguineous parents. The history of USS-M3 was previously described [12]. USS-M3 was primigravida at 33 years of age, and at 20 weeks of gestation she miscarried with overt TTP. The history of her younger sister, USS-M4, was also previously reported [12]. However, recently Kato *et al.* [44] reported a more detailed account of the pregnancy of USS-M4, to which we have to make some corrections. According to that report, USS-M4 became primigravida at 28 years of age. Until 28 weeks of gestation, the pregnancy was uneventful when she suddenly stopped feeling foetal movement, resulting in intrauterine foetal death and a subsequent diagnosis of HELLP syndrome. One year later, at the age of 29, she became pregnant for the second time. She was diagnosed with ITP and treated with prednisolone therapy until 37 weeks of gestation, but with incremental low platelet counts (approximately 23×10^9 L^{-1}). Soon after this, she underwent a caesarean section after receiving concentrated platelet infusions that transiently increased her platelet counts to 96×10^9 L^{-1}. As a result, she delivered a healthy baby. At 32 years of age, she became pregnant for the third time. At 20 weeks of gestation, she developed DIC followed by multi-organ failure, despite extensive treatments, including platelet transfusions. By this time, she had been diagnosed with USS and had undergone ADAMTS13 analysis, along with her elder sister, USS-M3. At the age of 36, USS-M4 became pregnant for a fourth time. With extensive FFP infusions, she continued her pregnancy until 36 weeks of gestation and delivered a healthy baby (2506 g BW) by natural birth with a skin incision [44].

Family USS-N

Patient

One female (USS-N6) born in 1986.

Brief clinical data

Patient USS-N6 (*ADAMTS13* genotype: p.**H234R**-p.P475S/**c.3220delTACC**) was born as the last of four children to non-consanguineous parents. She had a history of severe neonatal jaundice and childhood thrombocytopenia. Her clinical data were previously reported [11,37]. Of note, she developed a thrombotic occlusion of the left carotid artery at 11 years of age that resulted in right hemiparesis. Subsequently, she developed hypertension and proteinuria, but these clinical signs have significantly improved during a long clinical course with prophylactic FFP infusions, although some neurological sequelae have persisted (communication with Dr Seiji Kinoshita).

Family USS-O

Patient

One female (USS-O4) born in 1958.

Brief clinical data

Patient USS-O4 (*ADAMTS13* genotype: **p.I178T/p.Q929X**) was the second of two children to non-consanguineous parents. The history of USS-O4 was previously described [12]. At the age of 26, USS-O4 became pregnant. At 23 weeks of gestation, she developed thrombocytopenia and delivered a premature infant at 25 weeks of gestation who died soon after birth. After delivery, she developed overt TTP that was rescued with plasma exchange. At 31 years of age, she became pregnant for the second time while receiving prophylactic FFP infusions every 1–2 weeks. At 8 weeks of gestation, she developed proteinuria and thrombocytopenia, and therefore received more frequent FFP infusions. At 36 weeks of gestation, she delivered a healthy baby girl.

Family USS-P

Patient

One male (USS-P3) born in 1971.

Brief clinical data

The clinical data for patient USS-P3 (*ADAMTS13* genotype: **p.C908Y/p.C322G-p.T323R-p.F324L**, *de novo* mutation) were previously described [45]. Briefly, USS-P3 was the second of four children to non-consanguineous parents. The first and fourth siblings died of an abortion at 6 and 22 weeks of gestation, respectively, due to unknown causes. At 3 years of age, USS-P3 had clinical signs of overt TTP, which was efficiently treated with FFP infusions. He was repeatedly treated with FFP infusions when overt TTP developed. Thus, after 21 years of age, the prophylactic FFP infusions were continued.

Family USS-Q

Patients

Two males, (USS-Q1) born in 1983 and (USS-Q2) born in 1988.

Brief clinical data

Patients USS-Q1 (*ADAMTS13* genotype: **p.G227R**-p.G1181R/**p.C908Y**) and -Q2 (*ADAMTS13* genotype: **p.G227R**-p.G1181R/**p.C908Y**) were the first and third of three children to non-consanguineous parents. Their detailed clinical data during childhood were reported in 1990 [46].

Family USS-R

Patient

One female (USS-R5) born in 1982.

Brief clinical data

USS-R5 (*ADAMTS13* genotype: **p.R193W**/p.T339R-p.Q448E-**p.A606P**-p.P618A) was the last of three children to non-consanguineous parents. The history of USS-R5 was previously reported [12]. Briefly, at 23 years of age, she became pregnant. At 23 weeks of gestation, she developed mild thrombocytopenia, and at 31 weeks of gestation, she had sudden intrauterine foetal death. After a caesarean section, she developed overt TTP, which was treated with plasma exchange and steroids. On this occasion, she was diagnosed with USS after her ADAMTS13 activity and ADAMTS13 inhibitor status were analysed. This patient did not receive prophylactic FFP infusions.

Family USS-S

Patient

One male (USS-S3) born in 1982.

Brief clinical data

USS-S3 (*ADAMTS13* genotype: undetermined) was born to non-consanguineous parents. Neither his childhood nor family history have been obtained. The patient was clinically diagnosed with USS at a nearby hospital when he was 4 years old. Since then, he has received prophylactic FFP infusions every 1 weeks at the same hospital. In 2002, USS-S3 was confirmed to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. Furthermore, the ADAMTS13 activities for his father and mother were 34.2% and 47.6%, respectively. This family has not been examined for *ADAMTS13* gene mutations.

Family USS-T

Patient

One female (USS-T4) born in 1981.

Brief clinical data

USS-T4 (*ADAMTS13* genotype: **c.3220delTACC/c.3220del-TACC**) was born as the second of two children to non-consanguineous parents. Soon after birth, she developed severe neonatal jaundice and received exchange blood transfusion for three times [47]. One month after birth, she developed haematuria with thrombocytopenia, which led to a clinical diagnosis of USS. She received DDAVP infusion once at the age of 4, by which her platelet count promptly dropped and her clinical signs were aggravated, in accord with a transient disappearance of larger VWFMs from plasma [47]. Thus, she has received prophylactic FFP infusions every 2 weeks since 1992.In 1998, USS-T4 was confirmed to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. She had a homozygous *ADAMTS13* gene mutation of **c.3220del TACC/c.3220delTACC** (exon 24).

Family USS-U

Patient

One female (USS-U3) born in 1990.

Brief clinical data

USS-U3 (*ADAMTS13* genotype: **c.2259delA/c.2259delA**) was born as the second of two children to consanguineous parents (second cousins). Soon after birth, she developed severe neonatal jaundice that required an exchange transfusion. She was clinically diagnosed with USS at 4 months of age. In 1998, USS-U3 was confirmed to have a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. She was homozygous for an *ADAMTS13* gene mutation of **c.2259delA/c.2259delA** (exon 19). This patient has continued prophylactic FFP infusions.

Family USS-V

Patient

One female (USS-V3) born in 1983.

Brief clinical data

USS-V3 (*ADAMTS13* genotype: **p.W1081X/p.R193W**) was born as the second of two children to non-consanguineous parents. Soon after birth, she developed severe neonatal jaundice that required an exchange blood transfusion. She was clinically diagnosed with USS at 4 years of age. In 1998,

USS-V3 was confirmed to have a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. She had a compound heterozygous *ADAMTS13* gene mutation with **p.W1081X** (exon 24) from her father and **p.R193W** (exon 6) from her mother. The patient has been administered FFP infusions on demand.

Family USS-W

Patient

One female (USS-W4) born in 1990.

Brief clinical data

USS-W4 (*ADAMTS13* genotype: p.Q448E-**p.G550R**/p.P475S) was born as the second of two children to non-consanguineous parents. She did not have episodes of severe jaundice as a newborn. At 2 years of age, she developed pneumonia followed by thrombocytopenia. Since then, she has had repeated episodes of thrombocytopenia and haemolytic anaemia that have coincided with various infections, resulting in a diagnosis of Evans syndrome. In 2005, USS-W4 was confirmed to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. *ADAMTS13* gene analysis in USS-W4 suggested that she was a compound heterozygote with a **p.G550R** (exon 14) mutation from her father and an unidentified DCM from her mother. This patient has received prophylactic FFP infusions every 2 weeks.

Family USS-X

Patient

One female (USS-X5) born in 1963.

Brief clinical data

USS-X5 (*ADAMTS13* genotype: p.G1181R/p.P475S) was the last of four children to non-consanguineous parents. She did not have severe neonatal jaundice or childhood thrombocytopenia. She had two pregnancies at the ages of 24 and 26 years that yielded two children. During her first pregnancy, she had pregnancy-induced hypertension, but the details are unknown. At 32 years of age, she developed nephrotic syndrome, followed by repeated haemolytic anaemia and thrombocytopenia of an unknown cause. None of the laboratory markers were indicative of connective tissue disease. She underwent a splenectomy at the age of 36. In 2004, she had a relapse of nephrotic syndrome with haemolytic anaemia and thrombocytopenia that was treated with high-dose steroid therapy with limited success. At this time, her plasma ADAMTS13 activity levels and ADAMTS13 inhibitor status were examined, and she was determined to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. The same results were obtained 6 months later with a different plasma specimen. An *ADAMTS13* gene analysis in USS-X5 identified no DCMs, but revealed two SNPs of p.P475S from her mother and p.G1181R from her father. In 2007, she developed systemic lupus erythematosus (SLE) and was moved to a different hospital, after which we were unable to follow her clinical and laboratory data. From these results, USS-X5 could be considered to be a possible USS.

Family USS-Y

Patient

One female (USS-Y3) born in 1960.

Brief clinical data

USS-Y3 (*ADAMTS13* genotype: **p.G385E/p.R1206X**) was the last of three children to non-consanguineous parents. It is unclear whether this patient had a history of severe neonatal jaundice. However, during childhood she had an episode of thrombocytopenia and was diagnosed with ITP. She has a history of fresh whole blood transfusions, although the details are unclear. Since then, she had no remarkable changes. However, at 45 years of age, she suddenly developed thrombocytopenia and haemolytic anaemia, leading to a diagnosis of Evans syndrome. On this occasion, her physician noted many schistocytes on her blood film, and USS-Y3 was confirmed to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. An *ADAMTS13* gene analysis determined that she was a compound heterozygote with **p.G385E** (exon 10) from her father and **p.R1206X** (exon 26) from her mother.

Family USS-Z

Patient

One female (USS-Z3) born in 1971.

Brief clinical data

USS-Z3 (*ADAMTS13* genotype: **p.R193W/p.R193W**) was the last of three children to consanguineous parents (second cousins). Her clinical data were previously described [12]. Briefly, she became pregnant for the first time at 25 years of age, and at 12 weeks of gestation, she developed thrombocytopenia and was diagnosed with pregnancy-associated ITP. At 32 weeks of gestation, she had a live birth by caesarean section, and then developed overt TTP, which was treated with daily plasma exchange. This patient was referred to our laboratory in 1998, and USS-Z3 was confirmed to have a severe deficiency in ADAMTS13 activity in the absence of ADAMTS13 inhibitors. This patient did not receive prophylactic FFP infusions, and she had more than five TTP episodes between 1998 and 2005. Each episode was treated with 320 mL plasma infusions. She has been receiving prophylactic FFP infusions every 2 weeks.

Family USS-AA

Patient

One female (USS-AA3) born in 1987.

Brief clinical data

USS-AA3 (*ADAMTS13* genotype: not performed) was the first of two children born to non-consanguineous parents. She had neither an apparent history of severe neonatal jaundice nor thrombocytopenia during childhood. At 19 years of age, she suddenly developed petechiae, and her laboratory data indicated severe thrombocytopenia and haemolytic anaemia. Thus, her ADAMTS13 activity was examined and revealed a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. Plasma exchange therapy was performed, and her platelet counts normalised. One month later, her ADAMTS13 activity and ADAMTS13 inhibitor status were re-tested and yielded the same results. In addition, her family members had the following ADAMTS13 activities: father (32%), mother (53%), and younger sister (46%). An *ADAMTS13* gene analysis was not performed in this family because permission was not obtained. In 2009, we determined that USS-AA3 had a normal platelet count (201×10^9 L^{-1}), but her ADAMTS13 activity was still very low (< 0.5% of normal) with no ADAMTS13 inhibitors. Since this point, we have been unable to obtain more up-dated information on this patient.

Family USS-BB

Patient

One male (USS-BB3) born in 1947.

Brief clinical data

USS-BB3 (*ADAMTS13* genotype: **p.R193W/p.R193W**) was the first of three children to consanguineous parents (first cousins). His younger sister died of 'purpura of unknown cause' at 23 years of age. It is unclear whether USS-BB3 experienced episodes of severe jaundice as a newborn or childhood thrombocytopenia. He was married and had three children. At 55 years of age, he developed overt TTP, which was successfully treated with plasma exchange. When he was 59 years old, he developed haematuria and was admitted to a nearby hospital, where an ADAMTS13 analysis showed that he had a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. An *ADAMTS13* gene analysis indicated that he was a homozygote with **p.R193W** (exon 6) (communication with Dr Toshi Imai, details will be reported by the physicians in charge).

Family USS-CC

Patient

One male (USS-CC5) born in 2004.

Brief clinical data

USS-CC5 (*ADAMTS13* genotype: **p.Q723K/p.R398C**) was the last of three children to non-consanguineous parents. Soon after birth, he developed Coombs-negative haemolytic anaemia and was treated with an exchange blood transfusion. At 7 months of age, he became infected with influenza A virus that aggravated his thrombocytopenia and haemolytic anaemia. At 32 months of age, he suddenly developed a transient disturbance in his ability to walk and converse. On this occasion, an ADAMTS13 analysis revealed that USS-CC5 had a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. An *ADAMTS13* gene analysis indicated that he was a compound heterozygote with **p.Q723K** (exon 18) from his father and **p.R398C** (exon 10) from his mother. Since he was diagnosed with USS, he has received prophylactic FFP infusions every 2 weeks.

Family USS-DD

Patient

One female (USS-DD5) born in 2007.

Brief clinical data

USS-DD5 (*ADAMTS13* genotype: **p.R268P/p.Y304C**) was born as the last of three children to non-consanguineous parents. One day after birth, the patient developed haematuria, petechiae, moderate jaundice, and thrombocytopenia, suggesting immune thrombocytopenia. A platelet transfusion was performed that subsequently aggravated her jaundice, which was ameliorated with albumin infusions and phototherapy from three directions. Therefore, an exchange blood transfusion was not performed. Her platelet counts were maintained around $60–100 \times 10^9$ L^{-1}, and at 15 days of age the physician infused FFP at a dose of 10 mL kg^{-1} due to suspected USS. This treatment markedly increased her platelet counts (written information from Dr Hitoshi Miyabayashi). One month after birth, ADAMTS13 analysis showed that the patient had a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. An *ADAMTS13* gene analysis determined that USS-DD5 was a compound heterozygote with **p.R268P** (exon 7) from her father and **p.Y304C** (exon 8) from her mother. The patient did not receive prophylactic FFP infusions.

Family USS-EE

Patient

One male (USS-EE4) born in 2003.

Brief clinical data

USS-EE4 (*ADAMTS13* genotype: **c.2259delA/c.2259delA**) was born as the second child of bi-ovular twins by a caesarean delivery at 37 weeks of gestation to consanguineous parents (second cousins). Soon after birth, USS-EE4 received an exchange blood transfusion under a diagnosis of DIC. However, the other twin did not have these complications. Since then, USS-EE4 has continued to experience mild thrombocytopenia. At 18 months of age, his platelet count dropped to 11×10^9 L^{-1}, and schistocytes appeared on a blood film when the patient had a rotavirus infection. The patient subsequently experienced repeated episodes of thrombocytopenia and haemolytic anaemia associated with a variety of infectious diseases. At the age of 4 years and 7 months, the patient was admitted to a nearby hospital because of exacerbated asthmatoid bronchitis together with severe thrombocytopenia (4×10^9 L^{-1}). After being diagnosed with ITP, the patient was administered high-dose immunoglobulin therapy with steroid therapy, but there was no clinical improvement (written information from Dr Masahiro Migita). ADAMTS13 analysis showed severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. *ADAMTS13* gene analysis in USS-EE4 identified a homozygous mutation of **c.2259delA** (exon 19). This patient did not receive prophylactic FFP infusions.

Family USS-FF

Patient

One female (USS-FF3) born in 1991.

Brief clinical data

USS-FF3 (*ADAMTS13* genotype: **p.Q449X/p.Q449X**) was born as the first of two children to non-consanguineous parents [48]. As a newborn, the patient had moderate jaundice that required phototherapy, but no exchange blood transfusion was required. She also had a history of chronic thrombocytopenia as a newborn, but did not receive specific treatment. At 6 years of age, she developed severe thrombocytopenia and haemolytic anaemia, and ADAMTS13 analysis revealed a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. *ADAMTS13* gene analysis was performed at the laboratory of Dr David Ginsburg, where a homozygous mutation of **p.Q449X** (exon 6) was identified ([40] and written communication with Dr Yoji Sasahara). Since the USS diagnosis was confirmed, the patient has received FFP infusions (5 mL kg^{-1}) every 2 weeks.

Family USS-GG

Patient

One male (USS-GG2) born in 1931.

Brief clinical data

USS-GG2 (*ADAMTS13* genotype: **p.C1024R/p.C1024R**) was born as the fifth of seven children to consanguineous parents (first cousins). The ancestors of this family can be traced back to Kochi on Shikoku Island. The first two siblings died of an unknown aetiology during childhood. Interestingly, USS-GG2 suddenly developed overt TTP with neurological signs at 63 years of age and was admitted to a nearby hospital. Before this, he had never had an episode of anaemia or thrombocytopenia. He was treated with plasma infusions because plasma exchange was not readily available at that hospital. The next day, his neurological signs dramatically improved. He subsequently has experienced repeated episodes of overt TTP, resulting in a clinical diagnosis of CR-TTP, which was treated with biweekly prophylactic FFP infusions (320–480 mL per each). However, at 77 years of age, he had cerebellar bleeding. Thus, he received an ADAMTS13 analysis that showed a significant reduction in ADAMTS13 activity (2.4–3.4% of normal on three different occasions) but no ADAMTS13 inhibitors. An *ADAMTS13* gene analysis revealed that he was a **p.C1024R/p.C1024R** (exon 24) homozygote, confirming the USS diagnosis. Under prophylactic FFP infusions, he was alive until 79 years old, but he suddenly died of stroke in 2011 at the age of 79 (communication with Dr Fumihiro Taguchi, details will be published elsewhere by the physician in charge).

Family USS-HH

Patient

One female (USS-HH4) born in 2003.

Brief clinical data

USS-HH4 (*ADAMTS13* genotype: **p.Q449X/c.4119delG**) was born as the second of two children to non-consanguineous parents. Soon after birth, she developed Coombs-negative haemolytic anaemia that was treated with an exchange blood transfusion. In 2005, she had three episodes of thrombocytopenia and haemolytic anaemia that occurred concomitantly with fever or the chicken pox. Therefore, her ADAMTS13 activity was assayed, and she was determined to have a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. *ADAMTS13* gene analysis revealed that she was a compound heterozygote with **p.Q449X** (exon 12) from her father and **c.4119delG** (exon 29) from her mother. Although she had a history of severe neonatal jaundice followed by an exchange blood transfusion, she subsequently has only had mild clinical signs and has not received prophylactic FFP infusions. She receives FFP infusions only when her platelet count severely drops.

Family USS-II

Patient

One female (USS-II3) born in 1977.

Brief clinical data

USS-II3 (*ADAMTS13* genotype: not performed) was born by a caesarean section as the fourth and final pregnancy of her mother at 40 weeks of gestation. Her parents were non-consanguineous. Her mother had previously had two abortions (5 and 3 months of gestation) and a stillbirth (9 months of gestation) before USS-II3 was born. On the second day after birth, USS-II3 was treated with an exchange blood transfusion because of severe jaundice and thrombocytopenia. One month later, the patient was discharged but the thrombocytopenia continued, suggesting ITP. Since then, she has received whole blood transfusions when her platelet counts have dropped to 10×10^3 L^{-1}. At 9 months of age, the patient was clinically diagnosed with TTP. She was administered FFP infusion when severe thrombocytopenia developed. At 10 years of age, she underwent a splenectomy but there was no clinical improvement. The prophylactic FFP infusions have continued. At 21 years of age, she was diagnosed with USS after it was determined that she had a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. She currently receives prophylactic FFP infusions (120 mL) every week.

Family USS-JJ

Patient

One male (USS-JJ3) born in 1980.

Brief clinical data

USS-JJ3 (*ADAMTS13* genotype: **c.1885delT/p.C908Y**) was born as the last of four children to non-consanguineous parents. He had no history of exchange blood transfusions as a newborn. At 2 years of age, he suddenly complained of abdominal pain and developed haemolytic anaemia, haematuria, and thrombocytopenia. On this occasion, he was diagnosed with acute renal insufficiency due to diarrhoea-negative atypical HUS at a nearby hospital. Under this diagnosis, he received conservative therapy, including heparin, anti-platelet drugs, and red blood cell transfusion, but no platelet or FFP infusions. Over the next 14 years, he occasionally experienced overt HUS. At 12 years of age, his physician noticed that the FFP infusions were highly effective and improved his clinical manifestations, suggesting a clinical diagnosis of CR-TTP. Since 1996, he has received FFP infusions (160–240 mL per each) when his platelet counts have dropped below 100×10^9 L^{-1}, and has been administered FFP infusions of greater volumes (320–480 mL) during instances of overt TTP. In 1998, he was diagnosed with USS after an ADAMTS13 analysis revealed a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. Furthermore, he was a heavy drinker, which increased the frequency of overt TTP. Under these unhealthy conditions, the prophylactic FFP infusions were sometimes interrupted. Thus, when he was 25 years old, he experienced a cerebral infarction and the prophylactic FFP infusions were re-started. Nevertheless, 1 year later, he had severe renal insufficiency that required haemodialysis. Thus, he currently receives maintenance dialysis therapy and prophylactic FFP infusions of 240 mL per week. In 2009, an ADAMTS13 activity analysis revealed a severe deficiency in ADAMTS13 activity but no ADAMTS13 inhibitors. *ADAMTS13* gene analysis revealed that he was a compound heterozygote with **c.1885delT** (exon 16) and **p.C908Y** (exon 21), but that was not performed to his parents.

Family USS-KK

Patient

One female (KK3) born in 1976.

Brief clinical data

USS-KK3 (*ADAMTS13* genotype: not performed) was born as the second of three children to non-consanguineous parents. She had no history of exchange blood transfusion during the newborn period. At the age of 2, she developed thrombocytopenia and was diagnosed of ITP. She received a steroid therapy for thrombocytopenia at the age of 17 but without improvement, and then received splenectomy. As a university student at the age of 20, she developed thrombocytopenia and haemolytic anaemia after heavily drinking alcohol, and on this occasion she was clinically diagnosed of TTP at Shinshu university hospital in Nagano. A diagnosis of CR-TTP was made by Dr Miha Furlan at University of Bern in 1998, after ADAMTS13 analysis, which showed a severe deficiency of the activity but without its inhibitors (these results were re-confirmed in March 2011 using chromogenic act-ELISA). Her mother and two siblings had a slightly decreased ADAMTS13 activity (25–50%) (communication with Drs Fumihiro Ishida and Hikaru Kobayashi). Now, the patient receives the prophylactic FFP infusions (240 mL per each) every 3 weeks. *ADAMTS13* gene analysis has not been performed.

Family USS-LL

Patient

One female (LL4) born in 1981.

Brief clinical data

USS-LL4 (*ADAMTS13* genotype: **p.C438S**/p.T339R-p.Q448E-p.P618A-**p.G909R**) was born as the last of two children to non-consanguineous parents. She had no history of exchange blood transfusion during the newborn period. At the age of 14, she was diagnosed of HUS of unknown aetiology,

and received haemodialysis. During 1996–2001, she repeated overt TTP when she had various infectious diseases, and in each occasion she was treated with FFP infusions. In 2002, she was diagnosed of USS after analysing ADAMTS13, showing a severe deficiency of the activity but without its inhibitors in our laboratory. Since then, however, a low-titer ADAMTS13 inhibitor (< 1.4 BU mL^{-1}) was detected on a few occasions, but its clinical significance was not well evaluated. Her parents and elder sister are asymptomatic and have a slightly decreased ADAMTS13 activity (27–57%). The *ADAMTS13* gene analysis in this patient revealed a compound heterozygote of **p.C438S** (exon 12) from her father and **p.G909R** (exon 21) from her mother. She had been treated with FFP infusions on demand. Most recently, she has become pregnant, and her inhibitor titers have remained below 0.5 BU mL^{-1}. Thus, the prophylactic FFP infusions (10 mL kg BW^{-1}) have been started biweekly, and so far no increase of ADAMTS13 inhibitor titer has been observed (communication with Dr Yoshiyuki Ogawa).

Characterisation and allelic numbers of ADAMTS13 gene mutations in Japanese patients with USS

Of our 43 USS-patients, 39 received an *ADAMTS13* gene analysis while it was not performed in four patients (USS-S3, AA3, II3 and KK3). Nine of these 39 USS-patients were homozygous for *ADAMTS13* gene mutations, and 29 were the compound heterozygotes, including one patient (USS-W4) with **p.G550R** mutation on one allele while DCM on the other allele was unidentified. In the remaining patient (USS-X5), two SNPs (p.P475S/p.G1181R) but no DCMs were identified on each allele. Of these 39 USS-patients, five were siblings that each belonged to different families. Thus, the 65 [$2 \times (39 - 5) - 3$] allelic numbers of DCMs in these patients are summarised in Table 3. Interestingly, these mutations are quite different from those reported in the US and Western countries [3,49–66], except for **p.R268P**. However, the **p.R349C** mutation was previously reported in a Chinese USS patient in Hong Kong [67], and **c.330 + 1G > A** was identified in a Korean patient [68]. Thus, it is likely that specific *ADAMTS13* gene mutations are more common among certain ethnicities. In this regard, the mutation of **p.R268P** is quite unique, as the same mutation was reported by Veyradier *et al.* [55] in France, but in a Haitian patient.

The *ADAMTS13* gene mutation with the highest frequency in Japan was **p.R193W** ($n = 8$), followed by the remaining alleles in order of descending frequency: **p.Q449X** ($n = 5$), **p.C908Y** ($n = 4$), **c.2259delA** ($n = 4$), etc. The **p.Q449X** mutation was localised to the northern part (Tohoku) of Honshu, **c.2259delA** to Kyushu, **p.C908Y** to western Japan, and **p.R193W** to a relatively wide area across Japan but more frequently in western Japan, suggesting some geographical specificity in these mutations (Fig. 1).

Plasma levels of ADAMTS13 activity, ADAMTS13 inhibitor, and IgG-type anti-ADAMTS13 binding antibody in USS-patients

Most of our USS-patients had the plasma levels of ADAMTS13 activity with a $< 0.5\%$ of the normal (Table 1), but USS-GG2 alone had the ADAMTS13 activity of 2.4–3.4% of the normal, measured in three different occasions, as described above. Further, seven USS-patients (USS-F3, J3, K3, H3, Q1, S3, and LL4) had a trace amount of ADAMTS13 activity (0.6–1.8% of the normal) on some occasions, of whom four patients (USS-J3, K3, Q1, and LL4) had the ADAMTS13 activity below 0.5% of the normal in different occasions. The reason for this slight variation of plasma ADAMTS13 activity in our patients is presently unknown.

As for the ADAMTS13 inhibitors, all of our USS-patients had plasma levels of < 0.5 BU mL^{-1}, with one exception (USS-LL4), who showed the inhibitor titers ranging from < 0.5–1.4 BU mL^{-1}.

In regard to the IgG-type anti-ADAMTS13 binding antibody, 36 of 43 USS-patients did not have it (shown as the titer of 25 or $< 25 \times$ in Table 1). However, seven patients (USS-K3, K4, X5, AA3, EE4, II3, and LL4) had the antibody titers ranged from 50 to 400 $\times$ on some occasions. Clinical significance of the IgG-type anti-ADAMTS13 binding antibody is also unclear at moment, but notably six of these seven patients are female.

Table 3 Summary of 65 allelic numbers of ADAMTS13 disease-causing gene mutation out of 69 mutations in 35 Japanese patients with USS (five siblings)

≥ 2 Allelic numbers ($n = 11$)	Allelic numbers	One allelic number ($n = 28$)	
p.R193W	8	p.I178T	p.A606P
p.Q449X	5	p.G227R	p.Q723K
p.C908Y	4	p.H234R	p.G909R
c.2259del A	4	p.H234Q	p.Q929X
c.414 + 1G > A	3	p.A250V	p.W1081X
c.3220delTACC	3	p.R312C	p.R1123C
p.R268P	2	p.C322G/p.T323R/ p.F324L	p.Q1302X
p.Y304C	2	p.R349C	c.372insGT
p.I673F	2	p.G385E	c.1885delT
p.C1024R	2	p.R398C	c.3198delCT
p.R1206X	2	p.C438S	c.4119delG
		p.C508Y	c.330 + 1G > A
		p.G525D	c.686 + 1G > A
		p.G550R	c.1244 + 2T > G

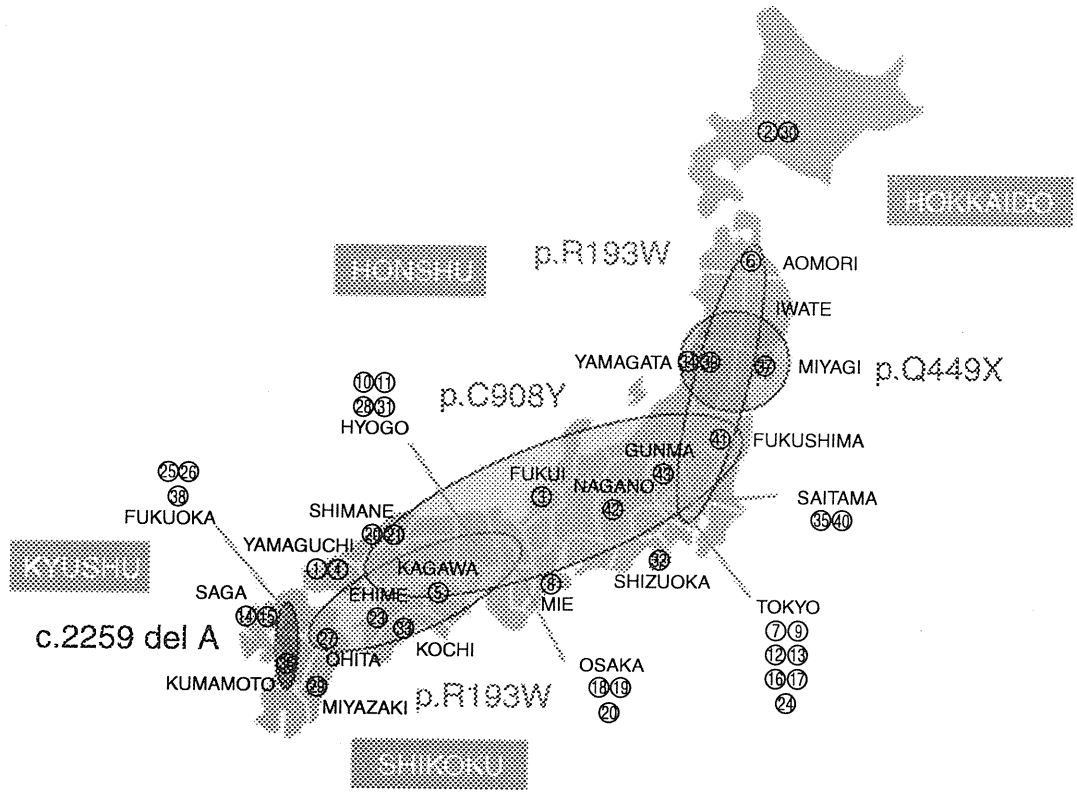


Fig. 1. Geographical distribution of 43 Japanese patients with USS and their *ADAMTS13* gene mutations. Among 43 USS patients, an *ADAMTS13* gene analysis was performed in 39 patients. Nine of the 39 USS patients had homozygous *ADAMTS13* gene mutations, and 29 were the compound heterozygotes, including one patient (USS-W4: patient no 28) with disease-causing mutation (DCM) on one allele while the other was unidentified. In the remaining one patient (USS-X5: patient no 29), two single nucleotide polymorphisms (SNPs), p.P475S and p.G1181R, but not DCMs were identified on each allele. The p.Q449X mutation localised to the northern part (Tohoku) of Honshu, c.2259delA to Kyushu, p.C908Y to western Japan, and p.R193W to a relatively wide area across Japan. Circled numbers indicate the patients shown in Tables 1 and 2.

Discussion

Since ADAMTS13 was originally discovered, one major question has been why USS-patients who consistently lack ADAMTS13 activity do not always experience acute symptoms of overt TTP. Furthermore, symptoms often become evident only when the patients have infections or become pregnant [12,69]. In both instances, vascular endothelial cell injury may be involved, and these cases have been indirectly associated with elevated plasma levels of cytokines or soluble thrombomodulin [70]. Consistent with these observations, studies on two different groups of *ADAMTS13* gene knockout mice revealed that UL-VWFMs were detectable in the blood, although the mice did not exhibit acute symptoms [71,72]. Considering these results, investigators have assumed that a deficiency in ADAMTS13 activity is prothrombotic, but alone is insufficient to provoke acute symptoms. Thus, second hits or triggers must exist. Related to this hypothesis, it has been said that there are two clinical features of USS, termed the 'early-onset' and 'late-onset' phenotypes. To partially address this question, we have extensively analysed the natural histories and *ADAMTS13* genotypes of 43 Japanese patients with USS.

This study has two advantages. One advantage is that Japan basically has four small islands, Hokkaido, Honshu, Shikoku, and Kyushu that make tracing the ancestral roots of a targeted USS family favourable. This is because USS patients tend to live near their parents or healthy relatives to receive medical support when they develop overt signs of TTP. In fact, before ADAMTS13 was discovered in 2001, nine patients were clinically diagnosed with USS or congenital CR-TTP in Japan, and none of these patients have moved to other areas or countries. The other advantage of this study can be attributed to the development of two convenient ADAMTS13 activity assays in our country, FRETS-VWF73 [29] and the chromogenic ADAMTS13-ac-ELISA [28]. Both assays are now used worldwide, and in 1998 Nara Medical University started voluntarily using the VWFM assay to meet the requests of clients across Japan. In 2005, the act-ELISA shortened the time required to diagnose TTP, and more importantly facilitated the identification of new USS-patients in Japan.

Although severe neonatal jaundice that requires exchange blood transfusion has been a hallmark of USS, this clinical sign was only present in 18 of 43 (42%) patients in this study. Because of this just four (/18) physicians correctly diagnosed their patient with USS before the patient reached 6 months of age, whereas 10 (/18) physicians required 6 years to reach a diagnosis of USS. On the other hand, among 25 USS patients without severe newborn jaundice, two (/25) were correctly diagnosed within