# Thrombotic thrombocytopenic purpura in children

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**Purpose of review**
Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease in children, due to a severe deficiency of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 repeats, member 13), inherited in congenital TTP or secondary to anti-ADAMTS13 antibodies in acquired TTP. Rapid techniques for ADAMTS 13 assays, long-term follow-up of patients, phenotype–genotype analysis, improved therapeutic schedules, and new therapies have emerged.

**Recent findings**
Rapid techniques for ADAMTS13 assays now permit rapid confirmation of diagnosis. In congenital TTP, mutations affecting the N-terminal domains of ADAMTS13 are associated with lower residual ADAMTS13 activity and more severe phenotype. Early initiation of plasma infusion treatment and lifelong prophylactic plasma infusion have decreased mortality and sequels and prevent relapses. In acquired TTP, a disease of adolescents but also of children less than 2, adding rituximab to plasma exchange is beneficial. Recombinant ADAMTS13 ought to be soon available for congenital TTP, while acquired TTP children might benefit from its administration, alone or in association with rituximab, to avoid or limit plasma exchange duration.

**Summary**
Progress in the understanding of TTP has boosted physicians' awareness that diagnosis and treatment are medical emergencies. New therapies hopefully will decrease treatment burden and improve prognosis.

**Keywords**
ADAMTS13 deficiency, plasma therapy, recombinant ADAMTS13, thrombotic thrombocytopenic purpura, Upshaw–Schulman syndrome

## INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) in children is a rare but life-threatening disease, characterized by microangiopathic hemolytic anemia, thrombocytopenia, and organ involvement. TTP was demonstrated to be secondary to a severe deficiency in ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats, member 13) [1,2]. Although hereditary ADAMTS13 deficiency was considered as the cause of pediatric TTP and antibody-mediated ADAMTS-13 deficiency that of adult TTP, both forms have now been reported in both age groups. This article reviews recent findings, in particular the availability of rapid techniques for ADAMTS13 assays; the understanding of phenotype variability in congenital TTP; the demonstration that acquired TTP is a disease of adolescents but also of children less than 2 and that rituximab allows reduced duration of plasma exchange treatment, but may not prevent late relapses; and the development of new treatments, including recombinant ADAMTS13.

## PATHOGENESIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA

von Willebrand factor (VWF) is a multimeric glycoprotein that plays a key role in high shear stress-associated platelet adhesion and aggregation [3]. The largest VWF multimers have the highest adhesive capacity toward platelets. In 1996, Furlan *et al.* [4] and Tsai [5] independently characterized a

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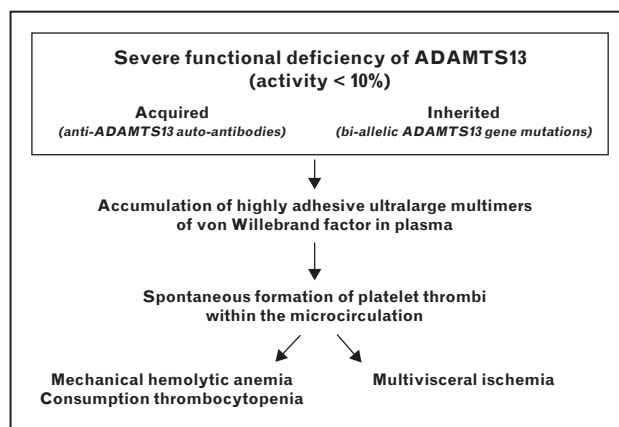
## KEY POINTS

- Congenital TTP (hereditary severe ADAMTS13 deficiency) should be suspected in any newborn child with severe jaundice and thrombocytopenia.

- Acquired TTP (acquired severe ADAMTS13 deficiency due to anti-ADAMTS13 antibodies) is mostly a disease of adolescents, but can occur in children less than 2 years.

- Rapid techniques for ADAMTS13 assays (ADAMTS13 activity and anti-ADAMTS13 immunoglobulin G) are available.

- Congenital TTP requires emergency plasma infusion. Neonatal forms are associated with a high risk of relapses and long-term prophylactic plasma infusions are recommended.

- Acquired TTP requires emergency plasma exchange+corticosteroids±rituximab. Further therapy should be guided by ADAMTS13 activity monitoring.



**FIGURE 1.** Pathogenesis of thrombotic thrombocytopenic purpura. A severe functional deficiency of ADAMTS13 (activity lower than 10%) either acquired (via autoantibodies to ADAMTS13) or inherited (via bi-allelic mutations of *ADAMTS13* gene) induces the accumulation in plasma of highly adhesive ultralarge multimers of von Willebrand factor (VWF). These ultralarge VWF multimers may spontaneously bind to platelets, causing the formation of platelet thrombi within the microvessels. The microthrombi occluding the microcirculation are responsible for the mechanical hemolytic anemia process, the consumption thrombocytopenia, and the multivisceral ischemia observed in thrombotic thrombocytopenic purpura (TTP).

new metalloprotease from human plasma that downregulates VWF activity by cleaving the largest VWF multimers, further identified as the 13th member of the ADAMTS family [6]. In 1998, a severe functional deficiency of ADAMTS13 (<10% of the activity of normal plasma) was shown to be responsible for TTP [1,2]. ADAMTS13 severe deficiency causes the accumulation of highly adhesive ultralarge VWF (ULVWF) multimers in plasma, which bind to platelets and induce platelet thrombi formation in the microcirculation. These microthrombi are responsible for the mechanical hemolytic anemia with schistocytes, the consumption thrombocytopenia, and the multivisceral ischemia defining TTP (Fig. 1). ADAMTS13 severe deficiency is related to two distinct mechanisms. One consists in compound heterozygous or homozygous mutations of the ADAMTS13 gene [7] inducing the inherited form of TTP named Upshaw–Schulman syndrome (USS) [8]. The other is secondary to anti-ADAMTS13 autoantibodies inducing the acquired autoimmune form of TTP [9].

## ADAMTS13 LABORATORY INVESTIGATION

ADAMTS13 assays are performed mainly in expert laboratories and not available locally in emergency. The clinical presentation is often sufficient for pediatricians to reach a high presumption diagnosis of TTP justifying emergency treatment. In such cases, ADAMTS13 assays should be performed with a few days' delay (from samples collected before treatment initiation) to confirm the diagnosis. However, a clinical diagnosis between TTP and atypical hemolytic uremic syndrome (aHUS) may be difficult due to overlapping symptoms [10,11] (Table 1) [12,13■■,14■■,15–30]. As aHUS in children is currently an indication of eculizumab treatment [30] and TTP an indication of plasma therapy, rapid results of ADAMTS13 assays are required in such cases. A flow-chart for biologic ADAMTS13 investigation is presented in Fig. 2.

Measurement of ADAMTS13 activity is the first assay to perform. All methods rely on the hydrolysis of either a full-length VWF substrate or a shorter VWF peptide including the cleaving site of VWF by ADAMTS13 (VWF73 substrate) with serial dilutions of tested serum/plasma used as ADAMTS13 provider; the amount of residual VWF after cleavage by ADAMTS13 is estimated using electrophoretic, functional, immunologic, or fluorimetric methods [31,32]. Methods using the VWF73 substrate are the most commonly used because they are easier and faster (result in a few hours) [33]. Normal ADAMTS13 activity is more than 50%. In neonates, it may be lower (about 30%) but never less than 10%. In the acute phase of TTP, ADAMTS13 activity is undetectable, that is, less than 10% (if not, the diagnosis of TTP should be reconsidered; Fig. 2).

**Table 1. Clinical characteristics at onset and risk of relapse in children with congenital or acquired thrombotic thrombocytopenic purpura and Shiga toxin _Escherichia coli_-associated or atypical hemolytic uremic syndrome**

| | Congenital TTP [12,13■■,14■■] | Acquired idiopathic TTP [13■■,15–25] | STEC-HUS [26,27] | aHUS [28] |
|---|---|---|---|---|
| Age at onset | Birth | <2 years: 22% | <6 months: 5% | Birth to 6 months: 28% |
| | (neonatal jaundice) | 2–9 years: 16% | 6 months to 3 years: 65% | 6 months to 2 years: 28% |
| | ≥75% | 9–16 years: 62% | >3 years: 30% | 2–15 years: 44% |
| Diarrhea at onset | Possible | Possible | 95% | 40% |
| Progressive onset | Possible (isolated thrombocytopenia) | Possible | No | Possible |
| Hematological characteristics at onset | Platelets | Platelets | Platelets | Platelets |
| | Generally < 30g/l | Generally < 30g/l | Generally > 30g/l | Generally > 30g/l |
| Acute renal failure at onset | Uncommon and mild, no dialysis required[a] | Uncommon and mild, dialysis rarely required[a] | 95%, dialysis required in 50% | 85%, dialysis required in 60% |
| Neurological symptoms at onset[b] | ~35% | 67%[b] | ~20% | 16% |
| Cardiac involvement at onset[c] | Possible | Possible | 2–5% | 2% |
| Familial history | Autosomal recessive inheritance (siblings with the disease) | No | Simultaneous occurrence or a few days–weeks apart (familial contamination) | 14% (years apart) |
| | | | | Autosomal dominant inheritance in the majority (siblings/parent/grand-parent/aunt/uncle with the disease) |
| Relapses | ≥80% without prophylactic plasma therapy | ~30% in treated patients | No | 45% in patients not under eculizumab |
| First-line treatment in 2012 | FFP infusion | PE + corticosteroids | Supportive treatment (eculizumab if CNS/cardiac involvement [29]) | Eculizumab [30] |
| | | ±rituximab | | |

aHUS, atypical hemolytic uremic syndrome; CNS, central nervous system; FFP, fresh frozen plasma; HUS, hemolytic uremic syndrome; PE, plasma exchange; STEC, Shiga toxin-producing _Escherichia coli_; TTP, thrombotic thrombocytopenic purpura. % indicates percentage of patients.
[a]Transient hematuria, hemoglobinuria, proteinuria with serum creatinine normal or slightly elevated are frequent during acute episodes, but acute renal failure rare.
[b]Headaches, altered mental status, visual problems, paresis, seizures, coma. The higher frequency of neurological manifestations in acquired TTP may be partly explained by the high frequency of headaches (36% of patients in [13■■]), a symptom not analyzed in young children with congenital TTP.
[c]Scarcely documented in all subgroups, particularly in TTP.
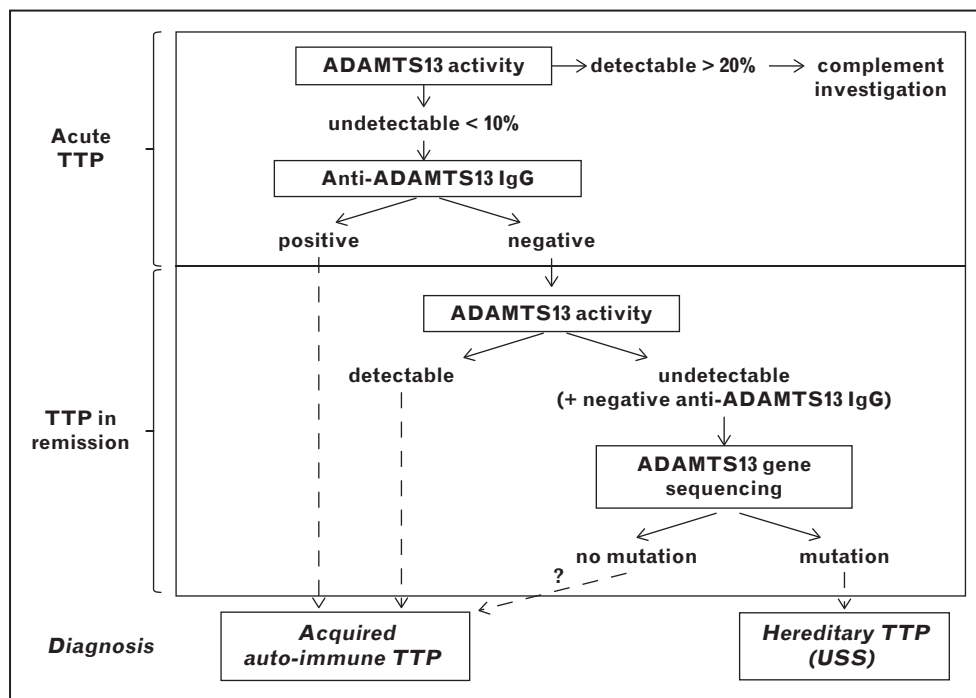
**FIGURE 2.** Flow chart for ADAMTS13 laboratory investigation in thrombotic thrombocytopenic purpura. In the case of thrombotic thrombocytopenic purpura (TTP) suspicion, measurement of serum/plasma ADAMTS13 activity, titration of serum/plasma anti-ADAMTS13 IgG and, if needed, ADAMTS13 gene sequencing should be rationally performed in this sequence in order to both support the diagnosis of TTP and distinguish between the hereditary and the acquired form of TTP. During the acute phase of TTP, the measurement of ADAMTS13 activity should be performed in first intention. If ADAMTS13 activity is detectable (usually higher than 20%), the diagnosis of TTP should be reconsidered, and further biologic investigation devoted to the complement system and the diagnosis of atypical hemolytic uremic syndrome are required. If ADAMTS13 activity is found lower than 10%, the diagnosis of TTP is confirmed, and thus the titration of anti-ADAMTS13 IgG is indicated as second intention test to search for an acquired severe ADAMTS13 deficiency. Values between 10 and 20% are very rarely found; if so, however, they should be considered similarly to values lower than 10%. During remission (first biologic control recommended 1 month after the acute TTP event), both of these assays are repeated. If ADAMTS13 activity has become detectable (usually higher than 20%), the diagnosis of acquired TTP is likely and no genetic investigation is required. In contrast, if ADAMTS13 activity remains undetectable in the absence of anti-ADAMTS13 IgG, *ADAMTS13* gene sequencing should be performed to detect bi-allelic mutations of *ADAMTS13* confirming the suspicion of inherited ADAMTS13 deficiency (Upshaw–Schulman syndrome, USS). Very rarely, this biologic phenotype (still undetectable ADAMTS13 with no anti-ADAMTS13 IgG in remission) is associated with an acquired TTP: in this case, anti-ADAMTS IgG usually becomes positive or ADAMTS13 activity detectable during a longer follow-up and no *ADAMTS13* mutation can be evidenced.

In a second step, if ADAMTS13 activity is less than 10%, anti-ADAMTS13 immunoglobulin G (IgG) should be searched for and titrated using an ELISA method [31]. Several commercial ELISA kits are available (positivity threshold for anti-ADAMTS13 IgG titer about 15 U/ml). In hereditary TTP, anti-ADAMTS13 IgG are negative. In acquired TTP, anti-ADAMTS13 IgG are positive during the acute phase and decrease according to ADAMTS13 activity increase in remission (Fig. 2).

In a third step, if ADAMTS13 activity remains less than 10% in remission together with undetectable anti-ADAMTS13 IgG, ADAMTS13 gene sequencing has to be performed to confirm inherited TTP (USS; Fig. 2). As no hot spot of ADAMTS13 mutations has been demonstrated, sequencing includes the totality of the gene (29 exons and all exon–intron boundaries). USS patients are homozygous or compound heterozygous for recessive bi-allelic mutations of ADAMTS13 gene.

## CLINICAL COURSE OF CONGENITAL THROMBOTIC THROMBOCYTOPENIC PURPURA (UPSHAW–SCHULMAN SYNDROME)

The Japanese TTP registry included 41 cases of USS patients, of whom 78% had thrombocytopenia since childhood, 61% were diagnosed as TTP before age 15, and 39% beyond childhood (age 19–63)

[13■■]. Four European TTP registries (Milan, London, Bergamo, and Paris) included 29 USS patients, of whom 55% had the first TTP episode requiring plasma therapy before age 15 and 45% between age 18 and 32 [14■■].

Neonatal onset with severe jaundice and thrombocytopenia, hemolytic anemia with schistocytosis, and a negative Coombs test are typical hallmarks of USS. Repeated episodes of thrombocytopenia occur in 80% of patients [13■■,14■■,34■■,35■] (Table 1). Neonatal onset has been demonstrated to be associated with the highest frequency of relapses and more intensive treatment requirements [14■■,36■■]. Patients were often misdiagnosed as having idiopathic thrombocytopenic purpura or Evans syndrome. Improved awareness of physicians and biological/molecular investigations now allow diagnosis.

One-third of patients from historical series had brain ischemic events – with neurologic sequels in half of them – and 20% progressed to chronic or end-stage renal disease (ESRD) in adolescence or adult age [12,37,38]. Importantly, neurological events as well as fatal outcomes have not been reported in patients under well-observed prophylactic plasma infusions [13■■,14■■]. Only two patients (6%) from the Japan cohort progressed to ESRD at age 24 and 26 (after late initiation of prophylactic plasma infusion in both and poor observance in one) ([13■■] and communication of H. Yagi to CL, with permission). Also, only 10% of patients from the European cohort had persistent renal/neurologic damage [14■■]. However, a significant number of undiagnosed patients may die unknown to specialized centers.

Whether renal, cardiac, cognitive, or vascular complications might develop in the long term despite prophylactic plasma therapy (or in untreated patients) is an important concern that deserves forthcoming studies [39].

## PHENOTYPE/GENOTYPE CORRELATIONS

Phenotypic variability in USS, appreciated by indicators like age at first TTP episode requiring plasma therapy, rate of episodes, or use of prophylactic plasma therapy, is likely to be related to multifactorial causes, either genetic or environmental.
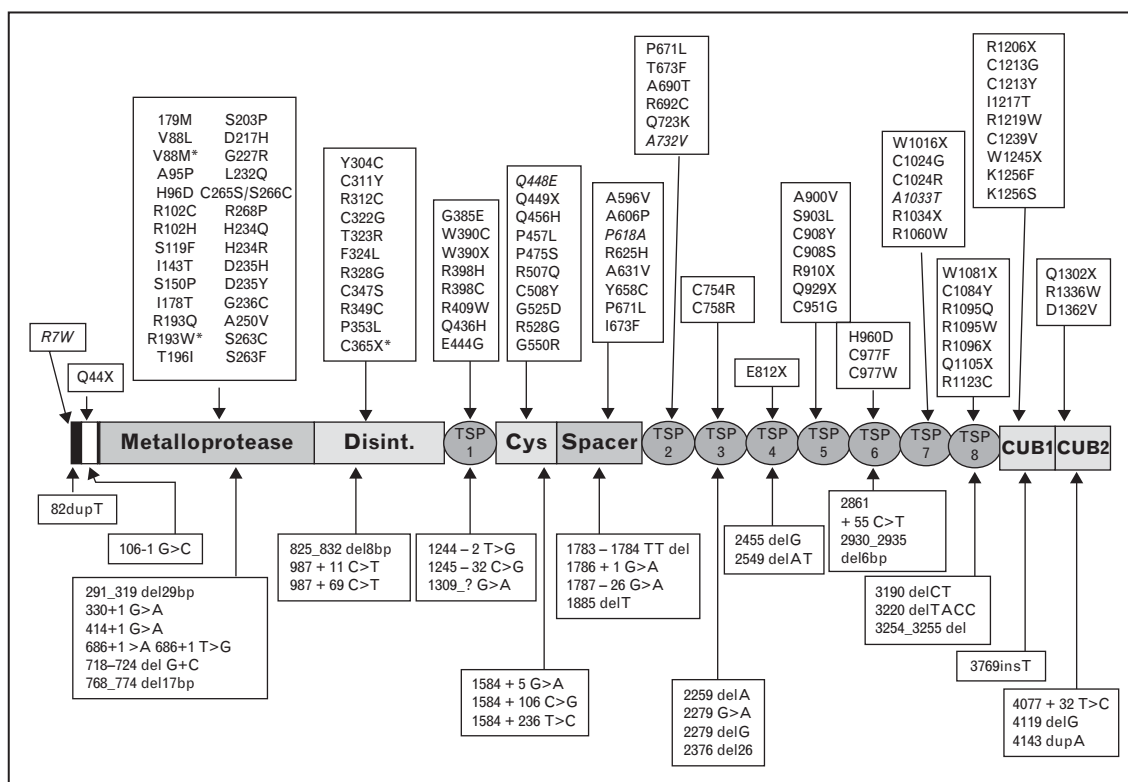
About 150 candidate mutations spreading all over the *ADAMTS13* gene have been identified in USS [14■■] (Fig. 3). Two recent studies [14■■,36■■] have demonstrated that mutations affecting the highly conserved N-terminal domains of ADAMTS13 are associated with lower residual ADAMTS13 activity and a more severe clinical phenotype in an allelic dose-dependent manner. In addition,

an approximately 2% ADAMTS13 activity threshold [measured by surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry] appears to be a disease-severity marker of potential clinical relevance [14■■]. Conversely, mutations located in the C-terminal part of ADAMTS13 may be associated with a less severe clinical expression. In that regard, one mutation located in the TSP1–7 domain, p.Arg1060Trp, is specifically associated with an adult onset of USS and found with a very high prevalence in pregnancy-associated TTP [40]. The role of *ADAMTS13* polymorphisms in the modulation of the deleterious effect of associated *ADAMTS13* mutations has also been suggested [41,42]. Other genetic modifiers, that is, genetic systems regulating baseline VWF levels (ABO blood group, for example), may also influence the susceptibility to TTP flares in USS patients, as suggested by the study of animal models [43]. Accordingly, in patients with USS, clinical conditions associated with increased VWF plasma levels secondary to VWF release from endothelial cells (i.e., pregnancy via hormonal modifications, infections via cytokines secretion) are well-established triggering factors of TTP flares and may be considered as environmental modifiers of disease severity [9].

These genetic and environmental factors are likely to act in concert to explain the phenotypic heterogeneity of USS between siblings or between families [42]. Furthermore, the association of hereditary ADAMTS13 severe deficiency with a rare complement factor H variant has been reported in one family [44].

## TREATMENT OF CONGENITAL THROMBOTIC THROMBOCYTOPENIC PURPURA

Treatment should be initiated as early as possible on the clinical presumption of USS [34■■,35■]. Except for newborns who require exchange blood transfusions to treat severe jaundice, USS patients only require plasma infusion (10–15 ml/kg) to replace deficient ADAMTS13, allowing the increase of platelet count within 24 h and cessation of hemolysis within a few days. Although ADAMTS13 has a half-life of only 2–3 days [45], plasma infusions every 2–3 weeks maintain normal platelets and hemoglobin. In recent years, approximately 80% of USS neonates have been put on prophylactic plasma infusion after the first episode [13■■,14■■]. The question remains whether one should wait for a second episode of thrombocytopenia to initiate prophylactic plasma therapy only if the free interval is less than a few months [39]. Considering the risk

Metalloprotease | Disint. | TSP 1 | Cys | Spacer | TSP 2 | TSP 3 | TSP 4 | TSP 5 | TSP 6 | TSP 7 | TSP 8 | CUB1 | CUB2

Missense/nonsense mutations (top):

R7W, Q44X

| 179M S203P | Y304C | | | Q448E | | P671L | R1206X |
| V88L D217H | C311Y | G385E | | Q449X | A596V | T673F | C1213G |
| V88M* G227R | R312C | W390C | | Q456H | A606P | A690T | C1213Y |
| A95P L232Q | C322G | W390X | | P457L | P618A | R692C | I1217T |
| H96D C265S/S266C | T323R | R398H | | P475S | R625H | Q723K | R1219W |
| R102C R268P | F324L | R398C | | R507Q | A631V | A732V | C1239V |
| R102H H234Q | R328G | R409W | | C508Y | Y658C | | W1245X |
| S119F H234R | C347S | Q436H | | G525D | P671L | | K1256F |
| I143T D235H | R349C | E444G | | R528G | I673F | | K1256S |
| S150P D235Y | P353L | | | G550R | | | |
| I178T G236C | C365X* | | | | | | |
| R193Q A250V | | | | | | | |
| R193W* S263C | | | | | | | |
| T196I S263F | | | | | | | |

C754R, C758R · E812X · A900V, S903L, C908Y, C908S, R910X, Q929X, C951G · H960D, C977F, C977W · W1016X, C1024G, C1024R, A1033T, R1034X, R1060W · W1081X, C1084Y, R1095Q, R1095W, R1096X, Q1105X, R1123C · Q1302X, R1336W, D1362V

Splice/frameshift mutations (bottom):

82dupT

106-1 G>C

291_319 del29bp
330+1 G>A
414+1 G>A
686+1 >A 686+1 T>G
718–724 del G+C
768_774 del17bp

825_832 del8bp
987 + 11 C>T
987 + 69 C>T

1244 – 2 T>G
1245 – 32 C>G
1309_? G>A

1584 + 5 G>A
1584 + 106 C>G
1584 + 236 T>C

1783 – 1784 TT del
1786 + 1 G>A
1787 – 26 G>A
1885 delT

2259 del A
2279 G>A
2279 delG
2376 del26

2455 delG
2549 delAT

2861 + 55 C>T
2930_2935 del6bp

3190 delCT
3220 delTACC
3254_3255 del

3769insT

4077 + 32 T>C
4119 delG
4143 dupA

**FIGURE 3.** ADAMTS13 mutations in inherited thrombotic thrombocytopenic purpura (Upshaw–Schulman syndrome). ADAMTS13 is a multiple domain protein that consists of a metalloprotease domain followed by a disintegrin-like motif, a first thrombospondin-1 (TSP-1) repeat, Cys-rich and spacer domains, seven additional TSP-1 repeats, and two Complement components C1r/C1s, Urinary epidermal growth factor, and Bone morphogenic protein-1 (CUB) domains. About 150 candidate mutations of ADAMTS13 spread all over the gene have been reported in the inherited form of thrombotic thrombocytopenic purpura (Upshaw–Schulman syndrome). Most mutations are located within the N-terminal region of the protease comprising the metalloprotease domain to the Cys-rich-spacer domain of the protease. The N-terminal part of ADAMTS13 has been shown to be the in-vitro active part of the protein; the C-terminal part of ADAMTS13, not crucial for ADAMTS13 activity *in vitro*, is, however, essential for in-vivo normal function of ADAMTS13. Mutations leading to amino acid substitutions (missense mutations) are present in about two-thirds of cases and truncating mutations (nonsense mutations inducing stop codon or slice/frameshift mutations) are also described. Missense and nonsense mutations are represented at the top of the figure, whereas slice/frameshift mutations are represented at the bottom of the figure.

of visceral complications in case of relapse and the demonstration that neonatal onset is associated with the highest risk of relapse and more intensive plasma requirements [14■■,36■■], we would rather recommend not to postpone initiation of prophylactic plasma infusion in neonatal forms [35■]. The interval between plasma infusions should be adapted to maintain permanent normal platelet count. However, an interval of more than 1 month is not recommended. In the few children having the first episode later in childhood or mild thrombocytopenia as the only manifestation, plasma infusion in response to early signs of relapse (such as a decrease in platelet count) may be proposed rather than prophylactic plasma infusion. This implies medical evaluation in any stress situation (infection, surgery, trauma, vaccination).

## CLINICAL COURSE OF ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

Experience of acquired TTP in children is limited. In the Japanese registry including 195 idiopathic acquired TTP cases, 7.2% of patients were children less than 15, whereas three of 46 (6.5%) with connective tissue disease-TTP were children aged 8–12 [13■■]. A total of 37 idiopathic cases have been published in children [13■■,15–25] and a few associated to systemic lupus erythematosus or mixed connective tissue disease [13■■,46]. Analysis of data from these 37 cases indicates that idiopathic acquired TTP is mostly a disease of preadolescent and adolescent children (62% between ages 9 and 16). However, pediatricians should be aware of its possible occurrence in children less than 2 years

(36% of cases in the Japanese series with a minimal age of 9 months [13▪▪,16,22]; 13% in non-Japanese patients). Presenting features are similar to those of USS (Table 1), including triggering infections (one-third of patients), fever (up to 93% of patients), and neurological symptoms (67.5% of patients, including cerebral bleeding or infarction in five children) [13▪▪,16,17,23,25]. Two patients had raised troponin levels and one had abnormal electrocardiogram [13▪▪]. However, cardiac involvement, a severe complication in adults [34▪▪,35▪], is scarcely documented in children. Importantly, many children younger than 2 years were initially misdiagnosed as having idiopathic thrombocytopenic purpura, HUS, hemophagocytic syndrome, or paroxysmal nocturnal hemoglobinuria. Mortality during acute episodes (8%) is similar to that in adults (8–11%) [47–49]. While 30–40% of acquired TTP adults have a chronic relapsing course [35▪], the proportion was lower in Japanese children (14%) [13▪▪], suggesting the immune process might be a self-limited disease in children. However, 43% of non-Japanese children had relapses, showing that children are at risk of relapses just like adults in western countries.

## TREATMENT OF ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

Acquired TTP is a medical emergency [34▪▪,35▪]. First-line treatment is daily plasma exchange with plasma (plasma volume × 1.5, 60 ml/kg). Plasma exchange in young children is associated with a high risk of hemodynamic and catheter-related complications [50]. When plasma exchange cannot be realized in emergency, plasma infusion (10–15 ml/kg) may suffice to obtain a rapid increase in platelet count. It is generally accepted that corticosteroids (prednisone, 1 mg/kg per day) remain important. Pulse methylprednisolone ($1000 \, mg/1.73 \, m^2$ for a few days) may be used in severe cases [13▪▪,35▪,51▪].

Plasma exchange schedule has been deeply modified with the advent of rituximab since 2005 [35▪,51▪]. Adults treated with rituximab for plasma exchange refractory TTP or relapsing TTP [51▪,52–55,56▪] or as first-line treatment have shortened time to remission and plasma exchange treatment duration and reduced rate of 1-year relapses. Rituximab has been used in 13 children [13▪▪,19–21,23–25] (outcome documented in 12): 83% were free of relapses during a mean follow-up of 19.7 months (3–60) after rituximab, and two had a relapse after 12 and 19 months. Relapses beyond 1 year (up to 30–34 months after rituximab) have also been reported in 10–15% of adult patients [51▪,57▪▪].

With this experience, rituximab (generally $375 \, mg/m^2$ weekly × 4) should be considered in children in association with plasma exchange as first line in life-threatening forms, and as second line if no improvement is obtained after five to seven plasma exchanges (as recommended in adults [35▪]) or in case of exacerbation. Moreover, the association of rituximab as soon as at first episode could be particularly beneficial in children, to limit duration of plasma exchange. Last, administration of rituximab at greater than weekly intervals over a longer period of time may be more appropriate to reduce long-term relapses [51▪].

The prognostic value of anti-ADAMTS13 antibodies still remains controversial. Severe ADAMTS13 deficiency together with anti-ADAMTS13 IgG high titers is associated with a higher risk of relapses [58–63]. In remission, persistent severe ADAMTS13 deficiency increased the risk of relapse three-fold in one series [60], and 38.5% of patients with ADAMTS13 less than 15% relapsed versus 5% of those with ADAMTS13 more than 15%, in another series [58]. Monitoring of ADAMTS13 during remission should guide therapeutic decisions. Indeed, persistent severe ADAMTS13 deficiency leads increasingly to the administration of rituximab or other immunosuppressive drugs to prevent relapses.

## TREATMENTS FOR THE FUTURE

Recombinant ADAMTS13 (rADAMTS13) is in development by Baxter, Inc. In a new animal model of TTP-like disease in ADAMTS13-knockout mice challenged by the administration of recombinant human VWF containing ULVWF multimers, prophylactic administration of human rADAMTS13 protected mice from developing TTP [64▪▪]. rADAMTS13 normalizes VWF-cleaving activity in plasma of acquired TTP patients by overriding the inhibitory activity of anti-ADAMTS13 IgG, opening the way to ADAMTS13 as an adjunctive therapy in acquired TTP [65]. A phase I clinical trial of rADAMTS13 in USS patients is scheduled to begin in 2013. Considering the risks of plasma exchange in children, avoidance of plasma exchange is the aim for the near future.

Drugs targeting the VWF–platelet interaction such as N-acetylcysteine [66], aptamers [67], anti-VWF nanobodies [68], or monoclonal antibody [69] are being developed or studied in trials.

Gain-of-function ADAMTS13 variants that resist inhibition by anti-ADAMTS13 IgG from patients with acquired TTP may be further developed [70].

Recent studies suggest complement activation through the alternative pathway at the acute phase of acquired TTP [71,72]. Preliminary data suggest

that eculizumab may be efficient in acquired TTP [73]. This view requires further prospective studies.

## CONCLUSION

Progress has been made in the diagnosis and treatment of TTP in children, but improvements are still required. The first one is to develop access to rapid ADAMTS13 assays, so that the diagnosis between USS, acquired TTP, and aHUS and treatment decisions should not rely only on an often confounding clinic. The second one is recombinant ADAMTS13, which hopefully will benefit USS patients, as well as patients with acquired TTP.

### Conflicts of interest

*C.L. has received fees from Alexion Pharmaceuticals for participation in teaching courses and expert meetings and for serving on the French Scientific Advisory Board of Alexion Pharmaceuticals; has been coordinator for France of the Alexion trials of Eculizumab for atypical hemolytic uremic syndrome C08-002, C08-003, C10–003 and C10–004 (fees to her institution).*
*P.C. has received fees for being on Scientific Advisory Boards of Baxter and Alexion Pharmaceuticals and grants to his institution from Baxter and Alexion Pharmaceuticals.*
*A.V. has no conflicts of interest to declare.*

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 281–282).

1. Furlan M, Robles R, Galbusera M, *et al.* von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med 1998; 339:1578–1584.
2. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med 1998; 339:1585–1594.
3. Sadler JE. von Willebrand factor: two sides of a coin. J Thromb Haemost 2005; 3:1702–1709.
4. Furlan M, Robles R, Lamie B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood 1996; 87:4223–4234.
5. Tsai HM. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. Blood 1996; 87:4235–4244.
6. Zheng X, Chung D, Takayama TK, *et al.* Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem 2001; 276:41059–44163.
7. Levy GG, Nichols WC, Lian EC, *et al.* Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature 2001; 413:488–494.
8. Kinoshita S, Yoshioka A, Park YD, *et al.* Upshaw-Schulman syndrome revisited: a concept of congenital thrombotic thrombocytopenic purpura. Int J Hematol 2001; 74:101–108.
9. Tsai HM. Pathophysiology of thrombotic thrombocytopenic purpura. Int J Hematol 2010; 91:1–19.
10. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis 2011; 6:60.
11. Loirat C, Saland J, Bitzan M. Management of hemolytic uremic syndrome. Presse Med 2012; 41 (3 Pt 2):e115–e135.
12. Loirat C, Girma JP, Desconclois C, *et al.* Thrombotic thrombocytopenic purpura related to severe ADAMTS13 deficiency in children. Pediatr Nephrol 2009; 24:19–29.
13. Yagi H, Matsumoto M, Fujimura Y. Paradigm shift of childhood thrombotic
■■ thrombocytopenic purpura with severe ADAMTS13 deficiency. Presse Med 2012; 41:e137–e155.
The report from the Japanese TTP registry, focused on TTP in a total of 42 children, including 25 with congenital TTP (USS) and 17 with acquired TTP: a unique source of information.
14. Lotta LA, Wu HM, Mackie IJ, *et al.* Residual plasmatic activity of ADAMTS13 is
■■ correlated with phenotype severity in congenital thrombotic thrombocytopenic purpura. Blood 2012; 120:440–448.
A study issued from the collaboration of four European TTP registries (Milan, London, Bergamo, and Paris), allowing phenotype–genotype correlations in 29 cases of congenital TTP (USS). Certain mutations in the N-terminal domains of ADAMTS13 were shown to be associated with a lower residual ADAMTS13 activity and a more severe phenotype (earlier age at first TTP episode requiring plasma infusion, more frequent relapses, and prescription of prophylactic plasma).
15. Robson WL, Tsai HM. Thrombotic thrombocytopenic purpura attributable to von Willebrand factor-cleaving protease inhibitor in an 8-year-old boy. Pediatrics 2002; 109:322–325.
16. Ashida A, Nakamura H, Yoden A, *et al.* Successful treatment of a young infant who developed high-titer inhibitors against VWF-cleaving protease (ADAMTS-13): important discrimination from Upshaw-Schulman syndrome. Am J Hematol 2002; 71:318–322.
17. Horton TM, Stone JD, Yee D, *et al.* Case series of thrombotic thrombocytopenic purpura in children and adolescents. J Pediatr Hematol Oncol 2003; 25:336–339.
18. Schneppenheim R, Budde U, Hassenpflug W, Obser T. Severe ADAMTS-13 deficiency in childhood. Semin Hematol 2004; 41:83–89.
19. Curtillet C, Poullin P, Doré E, *et al.* Paediatric case report of an acquired autoimmune thrombotic thrombocytopenic purpura. Arch Pediatr 2006; 13:1521–1524.
20. Albaramki JH, Teo J, Alexander SI. Rituximab therapy in two children with autoimmune thrombotic thrombocytopenic purpura. Pediatr Nephrol 2009; 24:1749–1752.
21. McDonald V, Liesner R, Grainger J, *et al.* Acquired, noncongenital thrombotic thrombocytopenic purpura in children and adolescents: clinical management and the use of ADAMTS 13 assays. Blood Coagul Fibrinolysis 2010; 21:245–250.
22. Sato A, Hoshi Y, Onuma M, *et al.* A 9-month-old infant with acquired idiopathic thrombotic thrombocytopenic purpura caused by inhibitory IgG-autoantibody to ADAMTS13. Pediatr Hematol Oncol 2010; 27:53–58.
23. Harambat J, Lamireau D, Delmas Y, *et al.* Successful treatment with rituximab for acute refractory thrombotic thrombocytopenic purpura related to acquired ADAMTS13 deficiency: a pediatric report and literature review. Pediatr Crit Care Med 2011; 12:e90–e93.
24. Jayabose S, Dunbar J, Nowicki TS, *et al.* Rituximab therapy to prevent relapse in chronic relapsing thrombotic thrombocytopenic purpura (TTP) in a child. Pediatr Hematol Oncol 2011; 28:167–172.
25. Narayanan P, Jayaraman A, Rustagi RS, *et al.* Rituximab in a child with autoimmune thrombotic thrombocytopenic purpura refractory to plasma exchange. Int J Hematol 2012; 96:122–124.
26. Espié E, Grimont F, Mariani-Kurkdjian P, *et al.* Surveillance of hemolytic uremic syndrome in children less than 15 years of age, a system to monitor O157 and non-O157 Shiga toxin-producing Escherichia coli infections in France, 1996–2006. Pediatr Infect Dis J 2008; 27:595–601.
27. Pennington H. Escherichia coli O157. Lancet 2010; 376:1428–1435.
28. Fremeaux-Bacchi V, Fakhouri F, Garnier A, *et al.* Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. Clin J Am Soc Nephrol 2013. doi: 10.2215/CJN. 04760512. [Epub ahead of print]

29. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Complement blockade in severe Shiga-toxin-associated HUS. N Engl J Med 2011; 364:2561–2563.

30. Zuber J, Fakhouri F, Roumenina LT, et al. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. Nat Rev Nephrol 2012; 8:643–657.

31. Peyvandi F, Palla R, Lotta LA, et al. ADAMTS13 assays in thrombotic thrombocytopenic purpura. J Thromb Haemost 2010; 8:631–640.

32. Palla R, Valsecchi C, Bajetta M, et al. Evaluation of assay methods to measure plasma ADAMTS13 activity in thrombotic microangiopathies. Thromb Haemost 2011; 105:381–385.

33. Kokame K, Nobe Y, Kokubo Y, et al. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. Br J Haematol 2005; 129:93–100.

34. ■■ Scully M, Hunt BJ, Benjamin S, et al. Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. Br J Haematol 2012; 158:323–335.
An outstanding publication of the guidelines established by the British Committee for Standards in Haematology for the diagnosis and management of TTP, including all the practical aspects for diagnosis and treatment, presented in a very didactic way. Absolutely recommended to all physicians concerned by TTP.

35. ■ Coppo P, Veyradier A. Current management and therapeutical perspectives in thrombotic thrombocytopenic purpura. Presse Med 2012; 41:e163–e176.
A didactic and useful review relying on the large clinical experience from the French Reference Center for TTP.

36. ■■ Camilleri RS, Scully M, Thomas M, et al. A phenotype-genotype correlation of ADAMTS13 mutations in congenital thrombotic thrombocytopenic purpura patients treated in the United Kingdom. J Thromb Haemost 2012; 10:1792–1801.
A study of phenotype–genotype correlations in 17 cases of congenital TTP, including five with neonatal onset, six with onset later during childhood, and six pregnancy-associated TTP, suggesting that certain mutations cause more severe clinical phenotype than other abnormalities in ADAMTS13.

37. Deschênes G, Veyradier A, Cloarec S, et al. Plasma therapy in von Willebrand factor protease deficiency. Pediatr Nephrol 2002; 17:867–870.

38. Loirat C, Veyradier A, Girma JP, et al. Thrombotic thrombocytopenic purpura associated with von Willebrand factor-cleaving protease (ADAMTS13) deficiency in children. Semin Thromb Hemost 2006; 32:90–97.

39. George JN. Forecasting the future for patients with hereditary TTP. Blood 2012; 120:243–244.

40. Moatti-Cohen M, Garrec C, Wolf M, et al. Unexpected frequency of Upshaw-Schulman syndrome in pregnancy-onset thrombotic thrombocytopenic purpura. Blood 2012; 119:588–597.

41. Plaimauer B, Fuhrmann J, Mohr G, et al. Modulation of ADAMTS13 secretion and specific activity by a combination of common amino acid polymorphisms and a missense mutation. Blood 2006; 107:118–125.

42. Lotta LA, Garagiola I, Palla R, et al. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. Hum Mutat 2010; 31:11–19.

43. Motto DG, Chauhan AK, Zhu G, et al. Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. J Clin Invest 2005; 115:2752–2761.

44. Noris M, Bucchioni S, Galbusera M, et al. Complement factor H mutation in familial thrombotic thrombocytopenic purpura with ADAMTS 13 deficiency and renal involvement. J Am Soc Nephrol 2005; 16:1177–1183.

45. Furlan M, Robles R, Morselli B, et al. Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in patients with thrombotic thrombocytopenic purpura. Thromb Haemost 1999; 81:8–13.

46. Thampi S, Salmi D, Imashuku S, et al. Thrombotic thrombocytopenic purpura in a child with systemic lupus erythematosus. J Pediatr Hematol Oncol 2011; 33:221–223.

47. Scully M, Yarranton H, Liesner R, et al. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. Br J Haematol 2008; 142:819–826.

48. Lotta LA, Mariani M, Consonni D, et al. Different clinical severity of first episodes and recurrences of thrombotic thrombocytopenic purpura. Br J Haematol 2010; 151:488–494.

49. Benhamou Y, Assié C, Boelle PY, et al. Development and validation of a predictive model for death in acquired severe ADAMTS13 deficiency-associated idiopathic thrombotic thrombocytopenic purpura: the French TMA Reference Center experience. Haematologica 2012; 97:1181–1186.

50. Michon B, Moghrabi A, Winikoff R, et al. Complications of apheresis in children. Transfusion 2007; 47:1837–1842.

51. ■ George JN. Corticosteroids and rituximab as adjunctive treatments for thrombotic thrombocytopenic purpura. Am J Hematol 2012; 87 (Suppl 1): S88–91.
A didactic review of the benefit from rituximab associated with corticosteroids in acquired TTP.

52. Fakhouri F, Vernant JP, Veyradier A, et al. Efficiency of curative and prophylactic treatment with rituximab in ADAMTS13-deficient thrombotic thrombocytopenic purpura: a study of 11 cases. Blood 2005; 106:1932–1937.

53. Scully M, Cohen H, Cavenagh J, et al. Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. Br J Haematol 2007; 136:451–461.

54. Elliott MA, Heit JA, Pruthi RK, et al. Rituximab for refractory and or relapsing thrombotic thrombocytopenic purpura related to immune-mediated severe ADAMTS13-deficiency: a report of four cases and a systematic review of the literature. Eur J Haematol 2009; 83:365–372.

55. Caramazza D, Quintini G, Abbene I, et al. Rituximab for managing relapsing or refractory patients with idiopathic thrombotic thrombocytopenic purpura: haemolytic uraemic syndrome. Blood Transfus 2010; 8:203–210.

56. ■ Froissart A, Buffet M, Veyradier A, et al. Efficacy and safety of first-line rituximab in severe, acquired thrombotic thrombocytopenic purpura with a suboptimal response to plasma exchange. Experience of the French Thrombotic Microangiopathies Reference Center. Crit Care Med 2012; 40:104–111.
A demonstration of the benefit from rituximab in acquired TTP with a suboptimal response to plasma exchanges.

57. ■■ Scully M, McDonald V, Cavenagh J, et al. A phase 2 study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. Blood 2011; 118:1746–1753.
The results of a prospective trial demonstrating the benefit from the association of rituximab to plasma exchanges and steroids within the first 3 days of TTP, to obtain more rapid remission, reduced duration of plasma exchange treatment, and reduced number of relapses during the first year, compared with historical controls.

58. Ferrari S, Scheiflinger F, Rieger M, et al. Prognostic value of anti-ADAMTS 13 antibody features (Ig isotype, titer, and inhibitory effect) in a cohort of 35 adult French patients undergoing a first episode of thrombotic microangiopathy with undetectable ADAMTS 13 activity. Blood 2007; 109:2815–2822.

59. Jin M, Casper TC, Cataland SR, et al. Relationship between ADAMTS13 activity in clinical remission and the risk of TTP relapse. Br J Haematol 2008; 141:651–658.

60. Peyvandi F, Lavoretano S, Palla R, et al. ADAMTS13 and anti-ADAMTS13 antibodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission. Haematologica 2008; 93:232–239.

61. Kremer Hovinga JA, Vesely SK, Terrell DR, et al. Survival and relapse in patients with thrombotic thrombocytopenic purpura. Blood 2010; 115:1500–1511.

62. Bettoni G, Palla R, Valsecchi C, et al. ADAMTS-13 activity and autoantibodies classes and subclasses as prognostic predictors in acquired thrombotic thrombocytopenic purpura. J Thromb Haemost 2012; 10:1556–1565.

63. Mannucci PM, Franchini M. Advantages and limits of ADAMTS13 testing in the prognostic assessment of thrombotic thrombocytopenic purpura. Presse Med 2012; 41:e157–e162.

64. ■■ Schiviz A, Wuersch K, Piskernik C, et al. A new mouse model mimicking thrombotic thrombocytopenic purpura: correction of symptoms by recombinant human ADAMTS13. Blood 2012; 119:6128–6135.
The demonstration that recombinant human ADAMTS13 is efficient to prevent the TTP-like disease obtained in ADAMTS13-knockout mice by the administration of VWF containing ULVWF. It opens the way to treatment of USS patients by recombinant ADAMTS13.

65. Plaimauer B, Kremer Hovinga JA, Juno C, et al. Recombinant ADAMTS13 normalizes von Willebrand factor-cleaving activity in plasma of acquired TTP patients by overriding inhibitory antibodies. J Thromb Haemost 2011; 9:936–944.

66. Chen J, Reheman A, Gushiken FC, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. J Clin Invest 2011; 12:593–603.

67. Cataland SR, Peyvandi F, Mannucci PM, et al. Initial experience from a double-blind, placebo-controlled, clinical outcome study of ARC1779 in patients with thrombotic thrombocytopenic purpura. Am J Hematol 2012; 87:430–432.

68. Callewaert F, Roodt J, Ulrichts H, et al. Evaluation of efficacy and safety of the anti-VWF nanobody ALX-0681 in a preclinical baboon model of acquired thrombotic thrombocytopenic purpura. Blood 2012; 120:3603–3610.

69. Feys HB, Roodt J, Vandeputte N, et al. Inhibition of von Willebrand factor-platelet glycoprotein Ib interaction prevents and reverses symptoms of acute acquired thrombotic thrombocytopenic purpura in baboons. Blood 2012; 120:3611–3614.

70. Jian C, Xiao J, Gong L, et al. Gain-of-function ADAMTS13 variants that are resistant to autoantibodies against ADAMTS13 in patients with acquired thrombotic thrombocytopenic purpura. Blood 2012; 119:3836–3843.

71. Ruiz-Torres MP, Casiraghi F, Galbusera M, et al. Complement activation: the missing link between ADAMTS-13 deficiency and microvascular thrombosis of thrombotic microangiopathies. Thromb Haemost 2005; 93:443–452.

72. Réti M, Farkas P, Csuka D, et al. Complement activation in thrombotic thrombocytopenic purpura. J Thromb Haemost 2012; 10:791–798.

73. Chapin J, Weksler B, Magro C, et al. Eculizumab in the treatment of refractory idiopathic thrombotic thrombocytopenic purpura. Br J Haematol 2012; 157:772–774.