

# The von Willebrand Factor-Cleaving Protease (ADAMTS-13) and the Diagnosis of Thrombotic Thrombocytopenic Purpura (TTP)

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## Key Words

Thrombotic thrombocytopenic purpura · von Willebrand factor · von Willebrand factor-cleaving protease · ADAMTS-13

## Abstract

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by microangiopathic hemolytic anemia and thrombocytopenia as a result of microvascular platelet clumping often accompanied by ischemic organ dysfunctions such as neurological abnormalities or renal insufficiency, and fever. Until the sixties of the 20th century TTP remained an almost universally fatal disorder. The introduction of plasma exchange therapy (PE) with replacement of fresh frozen plasma has dramatically improved the survival of patients with acute TTP from less than 10% to about 80-90% and is now considered the therapy of choice. Severe deficiency of the von Willebrand factor (VWF)-cleaving protease, now denoted as ADAMTS-13, prevents normal processing of unusually large VWF multimers released from endothelial cells and it is assumed that their persistence is responsible for the formation of platelet thrombi in the microvasculature, a pathophysiological hallmark of acute TTP. An ADAMTS-13 activity of <5% of the normal is a specific finding for acute classical TTP. However, the sensitivity

of this finding for the clinical diagnosis of TTP is equivocal with reported prevalences ranging from 33 - 100%. Today, two forms of classical TTP are distinguished. Hereditary TTP, also known as Upshaw-Schulman syndrome, is caused by severe constitutional ADAMTS-13 deficiency due to compound heterozygous or homozygous mutations of the ADAMTS13 gene and patients often present with a chronic relapsing course. The acquired or sporadic form of TTP is caused by circulating autoantibodies inhibiting ADAMTS-13 activity. Relapses are also frequent in acquired TTP occurring in about 35-50% of survivors of a first bout. Despite improved treatment modalities, patients suffering from acute bouts of TTP constitute a challenge for any clinician as mortality and morbidity rates are still considerably high.

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Thrombotic thrombocytopenic purpura (TTP) was first described by Moschcowitz in 1924 when he reported the case of a 16-year-old girl who died within a fortnight after abrupt onset of petechiae, anemia, micro-hematuria, fever and coma [1]. Autopsy revealed widespread "hyaline" microthrombi in the terminal arterioles and capillaries which were interpreted as agglutinated and hyalinized erythrocytes triggered by "a powerful poison which had both

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agglutinative and hemolytic properties". Subsequent reports of similar cases led to the classic description of this syndrome by Singer et al. in 1947 [2]. TTP is characterized by microangiopathic hemolytic anemia with schistocytes on the blood smear, thrombocytopenia due to intravascular platelet clumping resulting in ischemic organ manifestations, typically neurological disturbances, renal insufficiency and fever [3]. Clinically often indistinguishable from TTP is the hemolytic uremic syndrome (HUS), which is often associated with enterohemorrhagic *E. coli* infection and predominantly although not exclusively diagnosed in children and elderly persons. Until the late sixties of the 20th century TTP remained an almost universally fatal disorder [4]. The empirical introduction of plasma exchange therapy with replacement of fresh frozen plasma has dramatically improved survival of patients suffering from acute TTP from <10% [5] to about 80-90% [6,7].

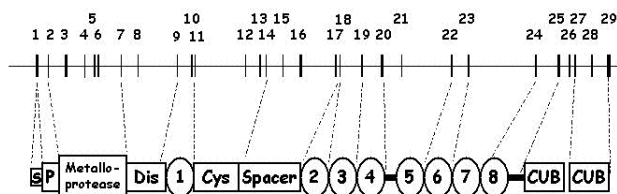
During the last decade the understanding of the pathophysiology of thrombotic microangiopathies, especially TTP, has increased considerably. The first indication that von Willebrand factor (VWF) was involved in the pathogenesis of TTP came from the observation by Moake et al. [8] of unusually large VWF multimers in the plasma of patients with a chronic relapsing form of TTP. VWF is a multimeric glycoprotein composed of identical disulfide-linked 250kD subunits synthesized by endothelial cells and megakaryocytes and plays an important role in primary hemostasis by mediating initial platelet adhesion to the subendothelium of the damaged vessel wall at high shear rates. From the storage organelles (Weibel-Palade bodies) of endothelial cells, VWF is secreted in the form of extremely adhesive ultralarge VWF multimers into the circulation, where they are slowly but constantly attacked by plasma protease(s) and degraded into multimers ranging in size from 500 to ~20'000kD [9]. Proteolytic cleavage occurs physiologically between the tyrosine residue at position 842 and the methionine residue at position 843 within the A2 domain of the mature VWF subunit [10]. In 1996 a specific VWF-cleaving protease was isolated from normal human plasma that cleaved purified VWF in vitro to the same fragments as those observed in vivo [11,12]. The N-terminal protein sequence of the purified VWF-cleaving protease allowed its identification as a member of the ADAMTS family of metalloproteases [13-15], named for the combination of a *disintegrin-like and metalloprotease with thrombospondin type 1 motifs*. As the thirteenth member of this family the VWF-cleaving protease was named ADAMTS-13 [15-18]. Subsequently, it was shown that most patients diagnosed with classical TTP had a severely deficient activity of this VWF-cleaving protease (<5% of normal) [19-22]. Today, two forms of classical TTP are distinguished. Acquired TTP is caused by circulating autoantibodies,

mainly IgG, generally neutralizing ADAMTS-13 activity [21-23] while hereditary TTP (Upshaw-Schulman syndrome) is caused by severe constitutional deficiency of ADAMTS-13 [16,24-30].

The ADAMTS-13 gene is located on chromosome 9q34, spans ~37kb and contains 29 exons. Congenital ADAMTS-13 deficiency is the result of compound heterozygous or homozygous mutations in the ADAMTS-13 gene. The primary translation product consists of 1427 amino acid residues and consists of a signal peptide and a propeptide, followed by the motifs defining the ADAMTS family: a repolysine-like metalloprotease domain, a disintegrin-like domain, a thrombospondin type 1 (TSP1) repeat, a characteristic cysteine-rich domain, an ADAMTS spacer domain followed by an unique combination of seven TSP1 repeats and two CUB domains (Figure 1) [15]. These various domains are conserved in other vertebrates and presumably required for ADAMTS-13 function.

Investigation into the structure-function aspects of ADAMTS-13 and into the VWF-ADAMTS-13 interaction have been initiated only recently. Using recombinant ADAMTS-13 fragments, Zheng and co-workers [31] demonstrated that constructs truncated after the metalloprotease domain, the disintegrin domain, the first TSP1 repeat or the cysteine-rich domain were devoid of any proteolytic activity towards VWF. Addition of the spacer domain restored enzymatic activity to 50%, and further extension of the protein beyond the remaining seven TSP1 motifs restored activity to 80% of full-length wild type ADAMTS-13. The importance of the cysteine-rich/spacer domain is further stressed by the finding that this region is consistently involved in antibody reactivity in patients with acute acquired TTP [32,33].

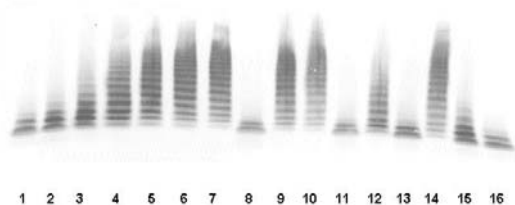
Several other ADAMTS-13 domains have been implicated in binding to other macromolecules, especially extracellular matrix and endothelial cells. In the circulation ADAMTS-13 docks to the surface of endothelial cells, where endothelium-anchored unusually large VWF multimers are cleaved [34,35]. Proteolytic degradation of VWF on endothelial cells is ~1000-fold enhanced compared to cleavage in the resting fluid phase [34]. Although the protein domain(s) involved in ADAMTS-13 binding to endothelial cells have not yet been identified it seems likely that the TSP1 repeats participate in this process as they may interact with a number of possible binding sites on endothelial cells, including CD36 (glycoprotein IV) or different glycosaminoglycans. In this context, it is noteworthy that anti-glycoprotein IV antibodies have been found in patients with acute TTP [36,37]. Binding of VWF to the endothelial cell surface is assisted by P-selectin, an adhesive protein stored in and secreted together with VWF from the Weibel-Pallade bodies upon activation of endothelial cells [38]. Protein - protein



**Fig. 1.** Structure of the von Willebrand factor-cleaving protease, ADAMTS-13. The ADAMTS13 gene contains 29 exons in ~37 kb on chromosome 9q34. Dashed lines show the relationship of the exons with the ADAMTS-13 protein. Proposed protein domain structure consists of a signal peptide (S), a propeptide (P), a metalloprotease, a disintegrin domain (Dis), thrombospondin type 1 repeats (numbered 1-8), a cysteine-rich domain (Cys), a spacer domain and two CUB domains (adapted from [54]).

interaction between ADAMTS-13 and VWF is mediated by the two CUB domains - unique in the ADAMTS superfamily - and adjacent thrombospondin type 1 repeats and the VWF-A3 domain [35].

Several assays have been developed for the determination of ADAMTS-13 activity in plasma. All assays consist of two principal steps: first proteolysis of VWF substrate by patient's plasma ADAMTS-13 followed by quantification of digestion products or residual VWF activity (reviewed in [39]). A multicenter study comparing several of these assays found a generally good agreement concerning the identification of severely deficient ADAMTS-13 activity, although some false-positive and one false-negative result(s) were reported by laboratories using the very delicate collagen-binding assay [40]. Figure 2 gives an example of the quantitative immunoblotting assay, applied in our laboratory [21,41].



**Fig. 2.** Determination of ADAMTS-13 activity by immunoblotting assay [21].

Lanes 1-7: Assay calibration with dilutions of normal plasma of 1:20 (100% activity), 1:40 (50%), 1:80 (25%), 1:160 (12.5%), 1:320 (6.25%), 1:640 (3%), and buffer control (0%).

Lanes 8-16: ADAMTS-13 activity determination in 1:20 dilutions of different patient plasmas displaying ADAMTS-13 activities of ~100%; 3%; <3%; ~100%; 15%; 100%; <3%; ~35% and 100%, respectively.

The discovery of two brothers with chronic relapsing TTP and the linkage of their disease to the complete deficiency of ADAMTS-13 activity at the Hemostasis Research Laboratory, Inselspital, University of Bern [19] and the subsequent identification of the underlying ADAMTS13 mutations [26] prompted many clinicians to refer plasma and whole blood samples of similar cases. As of May 2004 we have identified 38 patients with a severe constitutional ADAMTS-13 deficiency from 29 families in 13 countries (worldwide about 60 families). Analysis of patient histories revealed a striking age-dependent clustering of the first TTP attack. Half of the patients suffered from their first acute bout of TTP between the first day of life and the age of about five years (early onset), while the other half remained asymptomatic into early adulthood and suffered from a first acute TTP episode at the age of 20-40 years (late onset) [42]. In most of the families with two or more affected siblings the age at initial disease manifestation was comparable. Six female patients from four families had their first attack during a first pregnancy.

Today, over 70 different ADAMTS-13 mutations have been reported of which 2 thirds are missense mutations [16,24-30]. In addition, several single nucleotide polymorphisms (SNPs) have been identified. These mutations and SNPs are not restricted to a specific domain but distributed throughout the whole protein. So far, about one third of the reported missense and nonsense mutations have been expressed and were not or merely marginally secreted [24,29,43]. Despite the apparent familial clustering of age at disease onset there is no link between the clinical phenotype and the underlying genotype. Besides severe ADAMTS-13 deficiency apparently additional, hitherto unidentified triggers are necessary for the onset of an acute TTP episode, at least in some patients. This is supported by the observation of two unrelated males with severe ADAMTS-13 deficiency who remained asymptomatic into their fourth and fifth decades of life although both had affected sisters [42]. However, regardless of the age at disease onset, once affected individuals developed a first bout of TTP they usually had a chronic relapsing course [42].

Hereditary TTP is considered an extremely rare disorder, however, our own observations and those of others [25] suggest, that the prevalence of Upshaw-Schulman syndrome may have been greatly underestimated: Several siblings of patients diagnosed in our laboratory had died without established diagnosis, a substantial proportion had been diagnosed as Evans' syndrome or ITP resulting in inefficacious immunosuppressive treatment, and in others diagnosis was greatly delayed and made only after irreversible organ damage, such as ischemic neurologic deficits or permanent renal insufficiency had occurred, or even postmortem [44]. Patients with Upshaw-Schulman syndrome respond dramati-

ically to simple FFP infusion [42,44-48] and can be maintained for many years in good health by regular FFP infusion every 2-3 weeks [42,44,48].

Although severe deficiency of ADAMTS-13 activity (<5% of normal) is a specific finding for acute idiopathic TTP, the sensitivity of this finding for the clinical diagnosis of idiopathic TTP remains equivocal. In several retrospective studies clinically diagnosed acute TTP was associated with severe ADAMTS-13 deficiency in 52-100% of patients [21,22,49-51]. A similar prevalence of severe ADAMTS-13 deficiency of 71% was found in a prospective study [52], while a considerably lower prevalence of only 33% (16/48 patients) was reported recently in an inception cohort study of 142 consecutive adult patients [53]. In this latter study patients were diagnosed as having acute idiopathic TTP-HUS on the basis of thrombocytopenia and microangiopathic hemolytic anemia without another apparent etiology without distinction between TTP or HUS. Apparently other, hitherto unidentified pathogenetic factors may lead to a condition clinically indistinguishable from that seen in severe

ADAMTS-13 deficiency [3,42]. Therefore, TTP with severe ADAMTS-13 deficiency and TTP without severe ADAMTS-13 deficiency may well represent two distinct disease entities. This seems to be supported by the notion of a considerably higher mortality in patients suffering from acute TTP without (67%) compared to that in those with severe ADAMTS-13 deficiency (17%) despite appropriate treatment regimens [49], suggesting that plasma exchange might not be the optimal treatment for the former patients. This is refuted, however, by the Oklahoma TTP-HUS registry [53], where TTP-HUS related mortality, defined as mortality within the first 30 days of completion of plasma therapy, was similar in patients suffering from acute idiopathic TTP-HUS irrespective of the presence or absence of severe ADAMTS-13 deficiency. Therefore, plasma exchange therapy with replacement of fresh frozen plasma remains mandatory for all patients presenting with an acute bout of TTP regardless of their ADAMTS-13 activity.

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