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

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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.

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
The 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 repolyssine-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


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

Several assays have been developed for the determina-
tion 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 identifi-
cation 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 quan-
titative immunoblotting assay, applied in our laboratory
[21,41].

Fig. 1. Structure of the von Willebrand factor-cleaving protease,
ADAMTS-13. The ADAMTS13 gene contains 29 exons in ~37 kb on chro-
mosome 9q34. Dashed lines show the relationship of the exons with the
ADAMTS-13 protein. Proposed protein domain structure consists of a sig-
nal 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]).

The discovery of two brothers with chronic relapsing
TTP and the linkage of their disease to the complete defi-
cency of ADAMTS-13 activity at the Hemostasis Research
Laboratory, Inselspital, University of Bern [19] and the sub-
sequent identification of the underlying ADAMTS13 muta-
tions [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 dur-
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 polymor-
phisms (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 trig-
gers 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 deficien-
cy 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 affect-
ed 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] sug-
gest, that the prevalence of Upshaw-Schulman syndrome
may have been greatly underestimated: Several siblings of
patients diagnosed in our laboratory had died without estab-
lished diagnosis, a substantial proportion had been diag-
nosed as Evans’ syndrome or ITP resulting in inefficacious
immunosuppressive treatment, and in others diagnosis was
greatly delayed and made only after irreversible organ dam-
age, such as ischemic neurologic deficits or permanent renal
insufficiency had occurred, or even postmortem [44].
Patients with Upshaw-Schulman syndrome respond dramat-
cally 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 with or 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.

References

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