

Current Status in Diagnosis and Treatment of Hereditary Thrombotic Thrombocytopenic Purpura

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Rec date: April 09, 2014, Acc date: April 09, 2014, Pub date: April 11, 2014

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Editorial

Thrombotic Thrombocytopenic Purpura (TTP) is an acute and potentially fatal hematologic disorder. It is characterized by systemic platelet clumping in the microvasculature and small arterioles, resulting in thrombocytopenia and microangiopathic hemolytic anemia [1]. Some patients may present signs and symptoms that are consistent with end organ dysfunction [2]. The pathology of this disorder derives from the deficiency of plasma metalloenzyme ADAMTS13 (A Disintegrin and Metalloprotease with Thrombo Spondin type-1 repeats, 13) [3,4]. Under physiological conditions, ADAMTS13 cleaves ultra-large VWF (UL-VWF) that is released from endothelial cells upon activation or vascular injury [5-7]. This proteolytic cleavage of UL-VWF by ADAMTS13 in plasma is essential to remove UL-VWF from endothelial surface and to subsequently reduce the size of VWF multimers in circulation (Figure 1).

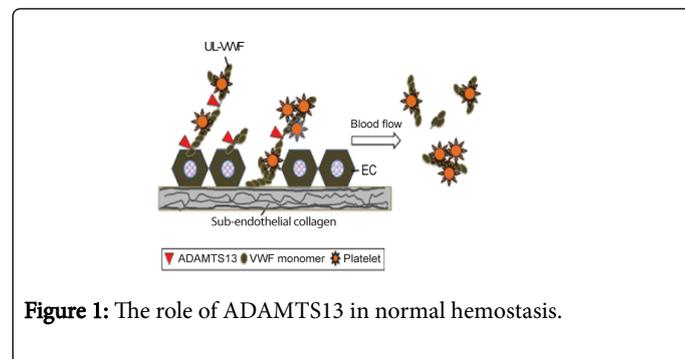


Figure 1: The role of ADAMTS13 in normal hemostasis.

TTP can be divided into at least two major forms: hereditary TTP, also known as Upshaw-Schulman syndrome (USS), is rare, accounting for 2-4% of all TTP cases [4,8,9]; acquired TTP, primarily caused by autoantibody-mediated inhibition of plasma ADAMTS13 activity [10-13], occurs more in adults, particularly in African American women. Interestingly, the manifestation of hereditary TTP is usually seen after an acute illness such as infection or during pregnancy [14,15]. Intermittent plasma infusion is often sufficient for treatment of hereditary TTP [16]. However, plasma exchange is often required for the treatment of acquired autoantibody-associated TTP [2,12,17], which replenishes the deficient plasma ADAMTS13 protease and removes immunoglobulin G-type autoantibodies against ADAMTS13.

Hereditary TTP is primarily seen in neonates and children [8,14,18], but it can occur in adults [8]. A presumptive diagnosis of hereditary TTP can be made if a patient presents with thrombocytopenia and microangiopathic hemolytic anemia (i.e. low hematocrit, low or absence of serum hepatoglobin, elevated serum

lactate dehydrogenase, and fragmentation of red blood cells) with or without organ dysfunctions including central nerve system, cardiac, and renal. If available, plasma ADAMTS13 activity and inhibitor tests are important for the differentiation of TTP from a similar syndrome, atypical hemolytic uremic syndrome (aHUS) [19]. Unlike classic HUS that is commonly caused by toxin-producing *E. coli*, aHUS is found to be associated with abnormalities in one or several complement components such as complement C3 [20] and factor B [21,22] or complement regulatory proteins including factor H, factor I, and membrane cofactor protein [23]. When plasma ADAMTS13 activity is less than 5% with no detectable anti-ADAMTS13 antibodies, a diagnosis of hereditary TTP should be considered. Normal to moderately reduced plasma ADAMTS13 activity does not completely rule out TTP. In this case, plasma infusion or exchange should still be tried initially and a complete blood count should be performed daily to monitor the response to treatment. If a prompt response is not achieved, a diagnosis of aHUS should be considered, and treatment with a humanized monoclonal anti-C5 antibody (i.e. eculizumab) should be considered. Recent studies have demonstrated the excellent efficacy of eculizumab for aHUS that is refractory to plasma exchange therapy [24-26]. The reason why normal or moderately reduced plasma ADAMTS13 activity does not rule out TTP is because the test results for ADAMTS13 activity are method-dependent. A discrepant result has been observed between the FRETs-VWF73 assay that uses a fluorescein-labeled VWF fragment derived from A2 domain as a substrate and the collagen-binding assay [27,28] or VWF multimer assay [29]. The cleavage of a peptidyl substrate VWF73 requires a less intact ADAMTS13 protease for full activity [30,31], and vwf73 may be cleaved non-specifically by other leukocyte proteases such as cathepsin G, proteinase 3, elastase, and MMP9 [29] released during the acute episode of TTP, which results in a falsely high level of plasma ADAMTS13 activity.

Sequencing analysis of ADAMTS13 gene may be performed if hereditary TTP is considered to confirm the presence of causative mutations. To date, more than 150 different mutations (~60% missense, ~20% small deletions and insertions, and a small percentage of nonsense and splice site mutations) have been reported in patients with congenital TTP [32-34]. While genotype-phenotype correlation has not been well established, the age of onset of the disease phenotype appears to correlate with the residual plasma ADAMTS13 activity. One study has shown that ADAMTS13 lower levels of activity (less than 3%) were associated with earlier age at first TTP episode requiring plasma infusion, more frequent recurrences, and prescription of fresh-frozen plasma prophylaxis [35]. Despite of the compound heterozygous mutations in ADAMTS13 gene found in most patients with hereditary TTP, homozygous mutations in these patients were

reported [36,37]. A majority of ADAMTS13 mutants appear to have folding problems that result in an intracellular retention of the misfolded proteins [9,36].

Upon stimulation or vascular injury, UL-VWF is released from endothelial cells or adhered to sub-endothelial collagen matrix. Platelets are recruited to the sites of activation or injury via interaction with UL-VWF. While cleavage of cell bound UL-VWF does not require shear, fluidic shear stress accelerates the removal or cleavage of UL-VWF on endothelium and at the sites of thrombus formation. Binding of platelets and/or coagulation factor VIII to UL-VWF or soluble VWF dramatically enhances its proteolytic cleavage by ADAMTS13 under flow. This regulated process of UL-VWF or large soluble VWF by ADAMTS13 is essential for maintaining normal blood flow. Deficiency of plasma ADAMTS13 activity either resulting from mutations in ADAMTS13 gene or acquired autoantibodies against ADAMTS13 results in accumulation of UL-VWF on endothelium and at the sites of injury, leading to exaggerated platelet aggregation and thrombus formation in small arterioles and capillaries, the characteristic pathological feature of TTP.

Hereditary TTP can be prevented or treated by plasma infusions. Because of a long half-life (2-3 days) for infused plasma ADAMTS13 in patients with hereditary TTP [38], an intermittent infusion of fresh frozen plasma every two to three weeks appears to be sufficient to maintain a plasma levels of ADAMTS13 activity above 5% that prevents relapses of the disease [39]. The relapses of hereditary TTP are often triggered by infection and/or pregnancy, because there may be inflammatory cytokines or bacterial toxin in circulation that increases the synthesis and release of UL-VWF multimers [40,41]. Further, bacterial toxin has been shown to block the cleavage of UL-VWF by ADAMTS13 in vitro [42].

While plasma infusion is the mainstay of treatment for hereditary TTP, recombinant ADAMTS13 is under development for therapy [43] that may minimize the exposure of human plasma and reduce the allergic reactions. AAV8-mediated gene therapy may be curative for hereditary TTP. Animal study has demonstrated a long-term expression of a C-terminal truncated ADAMTS13 variant at therapeutic levels [44]. Other agents such as anti-VWF aptamer [45] or anti-glycoprotein 1b (GP1b) nanobody [46], anti-GP1b snake venom (i.e. anfibatide) [47], and a disulfide bond reducing agent such as N-acetylcysteine [48] may be developed as novel therapeutics for hereditary TTP.

In conclusion, the identification and cloning of ADAMTS13 enzyme has provided an invaluable tool for further understanding of the molecular mechanism and developing a novel diagnostic and therapeutic strategy for hereditary TTP.

Acknowledgment

The work is partially supported by grants from T32HL007971 (to H.H.) and R01HL115187-01A1 (to X.Z.)

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