Familial acquired thrombotic thrombocytopenic purpura: ADAMTS-13 inhibitory autoantibodies in identical twins

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- Revised manuscript -

Short title: Familial acquired TTP

Key words: Von Willebrand factor-cleaving protease; ADAMTS-13; inhibitor; acquired TTP; familial clustering

Scientific heading: Hemostasis, thrombosis, and vascular biology

Manuscript category: Brief report

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Grant support: This work was supported by a grant from the Swiss National Foundation for Scientific Research (32-66756.01 to B.L.)

Word count: Abstract 95 words; text 1200 words

Number of figures: 2
Abstract

Thrombotic thrombocytopenic purpura (TTP) occurs either in a congenital form caused by ADAMTS13 gene mutations, or is acquired and most often due to ADAMTS-13 inhibitory autoantibodies. In congenital TTP siblings are often affected while acquired TTP occurs sporadically and familial clustering has not been described so far. We report identical twin sisters suffering from acquired TTP due to IgG autoantibodies inactivating ADAMTS-13, suggesting an important role of hitherto unidentified genetic determinants of ADAMTS-13 inhibitor formation. These cases also demonstrate that familial clustering is not sufficient for unambiguously diagnosing hereditary ADAMTS-13 deficiency and congenital TTP.
Introduction

The metalloprotease ADAMTS-13 (A disintegrin and metalloprotease with thrombospondin type 1 domains-13) \(^1\) specifically cleaves the von Willebrand factor (VWF) subunit at the peptide bond Tyr842-Met843 \(^6,7\). A severely deficient ADAMTS-13 activity (<5% of that in normal plasma) was found to be a strong risk factor for thrombotic thrombocytopenic purpura (TTP) \(^8,9\).

Two fundamental mechanisms are known to cause severe ADAMTS-13 deficiency. Homozygous or compound heterozygous mutations of the ADAMTS13 gene lead to hereditary ADAMTS-13 deficiency in congenital TTP \(^3,10-14\), often affecting siblings. In contrast, most cases of acquired TTP are caused by autoantibodies inactivating ADAMTS-13 \(^8,9,15\). It occurs sporadically, and familial clustering has not been described so far. Distinction between both forms is important as their management may differ. While regular infusion of limited amounts of fresh frozen plasma (FFP) usually reverts or prevents disease manifestations in congenital TTP, plasma exchange with FFP replacement, often combined with corticosteroids or other immunosuppressants, is the current therapy of choice in acute acquired TTP \(^16\).

We report identical twin sisters suffering from acquired TTP due to severe ADAMTS-13 deficiency caused by circulating inhibitory IgG autoantibodies.
Study design

Patients

Previously healthy identical twin sisters suffered from a first episode of acute TTP at 23 (sister 1) and 24 (sister 2) years of age. Sister 1 presented with fever, neurological symptoms, thrombocytopenia, and microangiopathic hemolytic anemia. Since therapy was initiated promptly, symptoms were less pronounced in sister 2. These episodes resolved under plasma exchange with FFP replacement and corticosteroids; sister 1 received vincristine, in addition. Follow-up for now 37 (sister 1) and 25 (sister 2) months was uneventful except for a short relapse in sister 1 which occurred 13 months after the initial episode and was treated by plasma exchange. Since then, no plasma has been administered to either of the sisters.

Both sisters are otherwise healthy without indication of another autoimmune disease, especially systemic lupus erythematosus (SLE), and have the same living and working conditions. No pregnancy has so far occurred in either of them. No drugs, including oral contraceptives, were taken in association with these episodes, and no other potential precipitating factors could be identified.

The study was conducted according to the responsible ethical committee’s guidelines on research on human subjects (Kantonale Ethische Kommission, Bern, Switzerland). Written consent was obtained from both sisters.

ADAMTS-13 activity and inhibitors

ADAMTS-13 activity and inhibitory autoantibodies were determined by immunoblotting of purified VWF substrate degraded by BaCl₂-activated ADAMTS-13 in citrated plasma taken during acute TTP and in remission as described, with slight modifications.

ADAMTS-13 activity was estimated by visual comparison of the extent of VWF degradation by 1:20-diluted patient plasma and by dilutions of a normal human plasma pool (NHP) between 1:20 (100% activity) to 1:640 (3%), and buffer control (0%).
For inhibitor detection, patient plasma (heat-inactivated for 30 min at 56°C, centrifuged for 15 min at 15,000 g) or purified IgG were mixed 1:1 (v:v) with NHP and incubated for 2 h at 37°C. This mixture was diluted 1:10, resulting in a final NHP dilution of 1:20. Inhibitor titers were estimated by visual comparison of the residual VWF degradation with the degradation obtained with NHP mixed 1:1 (v:v) with heat-inactivated NHP, incubated for 2 h at 37°C and diluted to final NHP concentrations of 1:20 (100% activity), 1:40 (50%), 1:80 (25%), and buffer control (0%). One Bethesda unit (BU) of inhibitor reduced the ADAMTS-13 activity of an equal volume of NHP by 50%.

**Purification of ADAMTS-13 inhibitory IgG**

Total IgG was purified from plasma taken during acute TTP by adsorption to protein A Sepharose CL-4B (Pharmacia, Uppsala, Sweden), and concentrated to its original plasma level by centrifugation through filter membranes (Millipore, Bedford, MA; molecular weight cut-off 30 kDa). The ADAMTS-13 inhibitory effect of purified IgG and of IgG-depleted plasma was tested by immunoblotting assay.\(^{15,17}\)

**Autoimmune serology and HLA typing**

Screening for circulating autoantibodies included antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (c-/p-ANCA), and rheumatoid factor (RF) in samples taken during acute TTP and in remission, using routine assays.

HLA types were determined using polymerase chain reaction-based routine assays.
Results and discussion

ADAMTS-13 activity was severely deficient (<3% of the normal) in plasma of both sisters taken during the initial episode of their thrombotic microangiopathy, confirming classical TTP (Figure 1).

Inhibition of the ADAMTS-13 activity in normal plasma by plasma of both sisters indicated the presence of inactivating autoantibodies. The titer was estimated to be ~1BU/mL in sister 1 and >2BU/mL in sister 2 (Figure 2). The purified total IgG had an ADAMTS-13 inhibitory effect comparable to that of the original plasma. No inhibition was found for the IgG-depleted plasma (Figure 2), confirming that the observed inhibition was due to IgG autoantibodies.

Investigation of follow-up samples taken during clinical remission 17 (sister 1) and 5 (sister 2) months after the initial TTP episodes revealed ADAMTS-13 activities of <5% and the persistence of inhibitors, though of a lower titer. Concordant with other observations of consistent remission or absence of events despite prolonged severe ADAMTS-13 deficiency, this indicates that additional, as yet unknown triggers may be necessary for the onset of acute TTP episodes, at least in some patients.

In samples taken 35 and 37 months (sister 1) and 23 and 25 months (sister 2) after the initial episodes, ADAMTS-13 activity had completely normalized in both sisters (Figure 1), and no inhibitor was detectable although plasma therapy had not been administered for more than a year in either of them.

The TTP episodes described here occurred in identical twin sisters at a similar age and were in both cases associated with severe ADAMTS-13 deficiency, a constellation typical for a hereditary cause. However, ADAMTS-13 inhibitory IgG autoantibodies were detected in both sisters, and normalization of the severely deficient ADAMTS-13 activity, without preceding plasma therapy, was observed during follow-up. These findings ruled out hereditary ADAMTS-13 deficiency and confirmed an acquired cause. Consequently, familial clustering alone may not allow classifying patients unambiguously as congenital TTP. Distinction between congenital and acquired TTP should be based on additional investigations such as screening for ADAMTS-13 autoantibodies, inhibitory as well as non-inhibitory in a fluid-phase assay, determination of ADAMTS-13 kinetics after plasma infusion, and investigation of the ADAMTS13 gene in suitable cases.
ADAMTS-13 inhibitors have been reported in SLE, although this association seems to be rare. SLE shows a strong familial aggregation and a concordance in identical twins, and could therefore explain a familial clustering of ADAMTS-13 inhibitors. Apart from the described TTP episodes both sisters are healthy without indication of SLE or other systemic autoimmune disease. Screening for autoantibodies including ANA, ANCA, and RF in samples taken during acute TTP and in remission did not give pathological results.

Investigation of the sisters’ HLA types revealed A*0201/0301, B*3901/5101, Cw*12/1502/07, DRB1*1201/1401, DQB1*0503/0301/04/09, DPB1*0401/0402. A possible association of ADAMTS-13 autoantibodies with HLA types has not been investigated so far. A previous study reported a lower frequency of HLA-DR53 in TTP/HUS patients as compared with controls, suggesting a protective role of this type. However, ADAMTS-13 was still unidentified at the time of that study.

Our observations suggest a genetic predisposition and hitherto unidentified genetic determinants for ADAMTS-13 autoantibody formation. These may include an association with certain HLA types. This could be an important area of future research, providing a deeper insight into the molecular and immunologic basis of ADAMTS-13 autoimmunity.
Acknowledgment

We thank Dr. Zsuzsanna Beleznay, Institute of Immunology, Inselspital, Bern, for her valuable help with autoimmune serology.
References


7. 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


Legends to figures

Figure 1  Determination of ADAMTS-13 activity by immunoblotting of VWF substrate degraded by BaCl₂-activated ADAMTS-13 in patient plasma (diluted 1:20).

Lanes 1-7: assay calibration by normal plasma dilutions 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%).

Sister 1: acute initial TTP episode (lane 8); clinical remission 17, 35 and 37 months after the initial episode (lanes 9-11).
Sister 2: acute initial TTP episode (lane 12); clinical remission 5, 23 and 25 months after the initial episode (lanes 13-15).

Figure 2  Detection of ADAMTS-13 inhibitory autoantibodies by immunoblotting of VWF substrate degraded by BaCl₂-activated ADAMTS-13 in mixtures of normal plasma (NHP) with patient plasma obtained at acute initial TTP, or IgG purified from patient plasma (final NHP dilution, 1:20).

Lanes 1-4: assay calibration by NHP dilutions of 1:20 (100% activity), 1:40 (50%, equivalent to an inhibitor titer of 1BU/mL), 1:80 (25%), and buffer control (0%).

Sister 1: mixtures of NHP 1:1 (v:v) with patient plasma (lane 5), with IgG purified from patient plasma (lane 6), and with IgG-depleted patient plasma (lane 7).
Sister 2: mixtures of NHP 1:1 (v:v) with patient plasma (lane 8), with IgG purified from patient plasma (lane 9), and with IgG-depleted patient plasma (lane 10).
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