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ORIGINAL ARTICLE

# A first case of congenital TTP on the African continent due to a new homozygous mutation in the catalytic domain of ADAMTS13

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**Abstract** Hereditary thrombotic thrombocytopenic purpura (TTP) is a rare disorder characterized by occlusive microvascular thrombosis, consumptive thrombocytopenia, and microangiopathic hemolytic anemia. Homozygous or compound heterozygous mutations in the *ADAMTS13* gene result in a congenital severe ADAMTS13 deficiency and subsequent accumulation of ultra-large von Willebrand factor multimers, which tend to form platelet thrombi in the microcirculation. We report a first case of congenital TTP on the African continent with a new, homozygous mutation in the metalloprotease domain of ADAMTS13. An initially oligo- symptomatic presentation was followed by acute exacerbation with ischemic stroke and acute renal failure highlighting the severity of this syndrome.

**Keywords** ADAMTS13 · Thrombotic thrombocytopenic purpura · TTP · Hereditary · Upshaw-Schulman syndrome

## Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare, but devastating disorder characterized by thrombosis of the microcirculation along with consumptive thrombocytopenia and microangiopathic hemolytic anemia [1]. Severe deficiency of ADAMTS13 proteolytic activity leads to persistence of unusually large von Willebrand factor (VWF) multimers promoting platelet aggregation under fluid shear stress.

In the more prevalent acquired form of TTP, severe ADAMTS13 deficiency results from anti-ADAMTS13 autoantibodies inhibiting the enzyme's function. In hereditary TTP, homozygous or compound heterozygous *ADAMTS13* mutations result in severe congenital ADAMTS13 deficiency [2]. More than 80 different mutations spread over the whole *ADAMTS13* gene have been identified in families with hereditary TTP. The majority of these mutations are restricted to single families, homozygous mutations being found predominantly in offspring of consanguineous marriages.

So far, 13 different *ADAMTS13* mutations have been observed in homozygous form. They are of particular interest as they facilitate the search for a correlation between the underlying *ADAMTS13* genotype and the clinical phenotype.

Here, we report a first case of congenital TTP on the African continent with a new homozygous mutation located in the ADAMTS13 catalytic domain. The relapsing course with serious cerebrovascular and renal complications demonstrates the threat of this disease.

## Case report

The patient was studied in the context of our hereditary TTP/Upshaw-Schulman registry (more information available at www.insel.ch/hzl), which had been approved by the Kantonale Ethikkommission, Bern, Switzerland. The pa-

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tient and his family members gave written informed consent for the study and publication of results.

The patient, a previously healthy Tunisian male of Arabic origin, presented for the first time at the age of 17 years with petechial bleeding and epistaxis. Laboratory investigations revealed a pronounced thrombocytopenia (platelet count $21\times10^9$/l) and anemia (hemoglobin 85 g/l) without signs of hemolysis or schistocytes on the peripheral blood smear and a negative direct Coombs' test. The bone marrow smear was normal except for an increased number of megakaryocytes. A diagnosis of idiopathic thrombocytopenic purpura (ITP) was made, and the patient was treated with corticosteroids. Eventually, the platelet count improved ($50–100\times10^9$/l) and hemoglobin levels normalized.

Three years later, the patient relapsed with severe thrombocytopenia (platelet count $13\times10^9$/l) together with pronounced hemolytic anemia (Hb 77 g/l; total bilirubin 107 μmol/l; and LDH 1,095 IU, normal range 91–180 IU). Again, schistocytes were absent, and the direct Coombs' test was negative. Corticosteroid pulse therapy had no effect, and 2 months later, the patient developed an ischemic stroke with generalized seizures. A computed tomography scan revealed lacunar lesions in the lenticular and capsular region with mild cerebral edema. A diagnosis of acute acquired TTP with a differential diagnosis of Evans' syndrome was considered. A treatment with polyvalent intravenous immunoglobulins for 2 days and two sessions of plasmapheresis resulted in the normalization of clinical symptoms and laboratory parameters (i.e., platelet count $245\times10^9$/l).

At the age of 21 years, a third hemolytic crisis (Hb 87 g/l) occurred, which was complicated by acute renal failure (creatinine 290 μmol/l, normal range 53–115 μmol/l). For the first time, schistocytes (16%) were found on the peripheral blood smear. A diagnosis of acute TTP was considered, which was confirmed by the finding of a severe ADAMTS13 deficiency of <5% of the normal in the absence of an inhibitor. The patient was treated with repeated infusions of fresh frozen plasma (FFP), which promptly resulted in partial recovery of renal function, thrombocytopenia ($110\times10^9$/l), and anemia (hemoglobin 100 g/l). As a diagnosis of hereditary TTP due to congenital ADAMTS13 deficiency seemed likely, the patient was put on prophylactic plasma infusions of 10 ml/kg FFP every 2 weeks. Today, after a follow-up of 2.5 years, the patient is in sustained remission (platelet count $>100\times10^9$/l) with persistent mild renal insufficiency (serum creatinine 150 μmol/l), however, without neurological sequelae.

The parents are first-degree relatives, and the patient's sister had been diagnosed with another rare recessively inherited disorder, Rosai–Dorfman syndrome, also known as sinus histiocytosis with massive lymphadenopathy. None of the family members had a history of a TTP-like disorder, thrombosis, or bleeding episodes.

## Methods and results

For genetic analysis, all 29 exons with flanking intron–exon boundaries of the *ADAMTS13* gene were amplified and sequenced using published primers [2, 3]. The propositus was found to be homozygous for a new missense mutation, cDNA 356 C>T in exon 4, resulting in the amino acid exchange of Ser 119 Phe in the metalloprotease domain of ADAMTS13. Besides the mutation, the patient was found to be homozygous for all known coding and silent *ADAMTS13* SNPs, as well as for the ABO blood group B-allele (Ready Gene AB0, Inno-train Diagnostik GmBH, Kronberg, Germany). The *ABO* locus is in close proximity to *ADAMTS13* on chromosome 9q34, and we found that the Ser 119 Phe mutation segregated with the ABO blood group B-allele in this family. The mother and the patient's sister were found to be heterozygous carriers of Ser 119 Phe, which explains their ADAMTS13 activity in the lower normal range (ADAMTS13 activity of 66% and 68%,

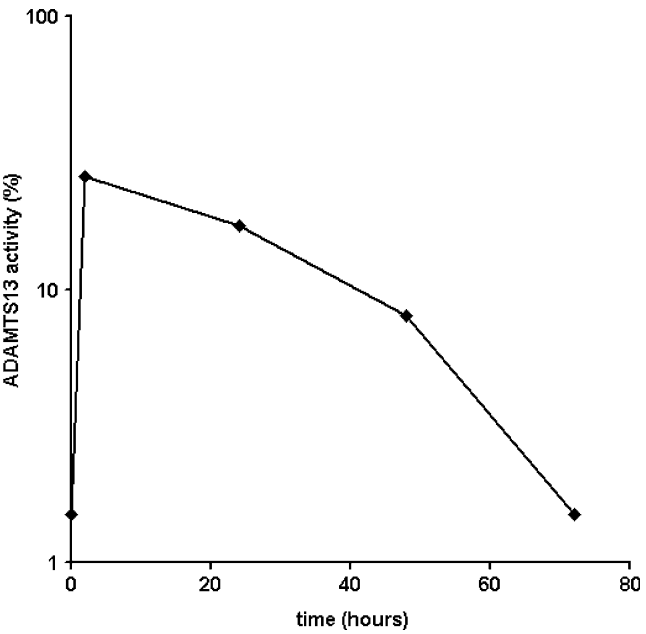


**Fig. 1** ADAMTS13 recovery and half-life upon infusion of FFP. The patient is on a prophylactic regimen with infusions of FFP (10 ml per kg body weight) every 2 weeks. Plasma samples for ADAMTS13 activity determination were withdrawn immediately before, 2, 24, 48, and 72 h, as well as 5 and 7 days (the latter times are not shown) after starting the FFP infusion. Initial ADAMTS13 activity was 1.5%, and there were no indications of an ADAMTS13 inhibitor in the patient's plasma; FFP infused had an ADAMTS13 activity of 50%. ADAMTS13 half-life was calculated from the slope of the decay curve to be about 37 h

respectively), while the patient's father was not available for study. The mutation was not found in 108 control alleles sequenced.

A time-course of ADAMTS13 recovery and half-life following the administration of a plasma infusion of 10 ml/kg body weight was performed (Fig. 1). At the end of the biweekly interval between prophylactic plasma infusions, a severely deficient ADAMTS13 activity (1.5%; determined by the slightly modified FRETS-VWF73 assay [4, 5]) was found. Within 2 h of FFP (having an ADAMTS13 activity of 50%) infusion, ADAMTS13 activity increased to 26% and declined to baseline levels over a period of 3 days. The calculated half-life time of the infused ADAMTS13 of ~37 h was somewhat shorter than had been expected (2–3 days [6]), however, still excluded the presence of an ADAMTS13 inhibitor.

## Discussion

So far, congenital TTP was diagnosed mainly in Caucasian and Asian families. We have presented here the case of a young Tunisian who suffered from TTP with serious cerebrovascular and renal complications as a young adult. The severe ADAMTS13 deficiency in the absence of an inhibitor, the observed ADAMTS13 recovery and half-life upon plasma infusion, the homozygous *ADAMTS13* gene mutation, Ser119Phe, as well as the analyses of the patient's relatives, confirmed the diagnosis of congenital severe ADAMTS13 deficiency and hereditary TTP (Upshaw-Schulman syndrome) in the propositus, which is the first case with this diagnosis on the African continent to our knowledge.

The patient's clinical course is exemplary. Oligo-symptomatic presentations (even without observation of schistocytes on the peripheral blood smear) are not seldom observed in patients later diagnosed with congenital TTP and may lead to misdiagnoses, such as ITP or Evans' syndrome, and idle treatment [7, 8]. Once, however, a first acute TTP bout has occurred, most patients tend to present with a relapsing course [9]. As has been demonstrated for several hereditary TTP cases and the patient reported here, relapses, which may eventually result in cerebrovascular and renal complications, can be prevented by regular FFP infusions every 2–3 weeks [10, 11].

The patient was found to be homozygous for a new *ADAMTS13* missense mutation, Ser 119 Phe. Replacement of an amino acid with a polar, medium-sized side chain by one with a non-polar phenolic ring structure very likely results in a conformational change within the crucial ADAMTS13 catalytic domain. Due to the proximity to two calcium binding sites, at aa82 and aa173, Ser119Phe may very well also affect ADAMTS13 function. A negative effect of the found mutation on ADAMTS13 function is also predicted by two sequence homology-based bioinformatic tools, *Sort Intolerant from Tolerant* (SIFT version 2.1 http://blocks.fhcrc.org/sift/SIFT.html) and *Polymorphism Phenotype* (PolyPhen, http://coot.embl.de/PolyPhen/). It is noticeable that this potentially damaging mutation in the metalloprotease domain led to a first acute TTP bout when the patient was a young adult and was not associated with clinically overt TTP in early childhood. Apparently additional disease-triggering factors seem to be necessary to induce a first bout of acute TTP, at least in some hereditary TTP cases.

In conclusion, this exemplary case of congenital TTP reveals a new *ADAMTS13* mutation in homozygous state, which probably interferes with ADAMTS13 function. The characteristic course in a first patient from the African continent highlights the difficulties and the importance of a timely diagnosis and treatment of congenital TTP.

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**Conflict of interest statement** J.A.K.H. and B.L. received a consultancy fee from Baxter Healthcare; the other authors have no competing financial interests to disclose.

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