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Two novel *ADAMTS13* gene mutations in thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome (TTP/HUS)

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Two novel *ADAMTS13* gene mutations in thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome (TTP/HUS).

Background. Thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS) are now considered to be variants of one single syndrome called thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome (TTP/HUS). Key features are thrombocytopenia, hemolytic anemia, and subsequently impaired function of different organs, especially the kidneys and the central nervous system (CNS). One possible reason is the deficiency of von Willebrand factor-cleaving protease (vWF-CP) resulting in persistence of uncleaved, ultralarge von Willebrand factor multimers (ULvWFM).

Methods. We report a patient who was initially diagnosed with Evans syndrome (hemolytic anemia and autoimmune thrombocytopenia) as infant. At 10 years of age he developed HUS-like disease with gastrointestinal tract infection, hemolytic anemia, thrombocytopenia,and acute renal failure. However, enteropathogenic *Escherichia coli*–like or Shiga-like toxins were not detected.

Results. Further investigations revealed severe deficiency (<3%; normal >40%) of vWF-CP activity caused by compound heterozygosity of two novel ADAMTS13 gene mutations (1170 G>C [W390C] and 3735 G>A [W1245X]. vWF-CP autoantibodies were not detected. Periodic (every 2 weeks) treatment with fresh frozen plasma (FFP) maintained both platelet level and kidney function within normal range and prevented new episodes of TTP/HUS.

Conclusion. Enteropathogenic *E. coli*– and Shiga-like toxin-negative patients who present with hemolytic or thrombocytopenic episodes and HUS like symptoms should be tested for vWF-CP deficiency and other noninfectious reasons for TTP/HUS since plasma substitution possibly provides an efficient therapeutic option for this subgroup of patients.

Key words: thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), von Willebrand factor-cleaving protease (vWF-CP), *ADAMTS13* gene mutation.

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Today, thrombotic thrombocytopenic purpura (TTP), first described by Moschcowitz in 1924 [1], and hemolytic-uremic syndrome (HUS), first described by Gasser et al in 1955 [2], are considered to be variants of one single syndrome called thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome (TTP/HUS) [3]. Common symptoms are formation of hyaline thrombi containing large amounts of von Willebrand factor (vWF), associated with thrombocytopenia, hemolytic anemia, and subsequently impaired function of different organ systems. TTP often is complicated by neurologic symptoms, whereas renal abnormalities prevail in HUS [3, 4].

Responsible are ultralarge vWF multimers (ULvWFM) [5], which were first identified in a patient with TTP [6], but later also detected in patients with HUS [7]. Persistence of ULvWFM is caused by a processing defect of vWF through an acquired (autoantibodies) or congenital (*ADAMTS13* gene mutations) deficiency of vWF-cleaving protease (vWF-CP). vWF-CP is a vWF-specific metalloprotease [8, 9] of the ADAMTS (a disintegrin and metalloprotease, with thrombospondin-1–like domains) family [10–13], which physiologically cleaves vWF at a defined position (Tyr1605 – Met1606) after synthesis and secretion by endothelial cells [14]. If vWF-CP is deficient, ULvWFM persist and mediate adhesion and aggregation of circulating platelets at sites of high intravascular shear stress [15]. As a result, large, potentially occlusive platelet thrombi appear [5].

We present the case of a boy with severe vWF-CP deficiency caused by compound heterozygosity of two novel *ADAMTS13* gene mutations (1170 G>C [W390C] and 3735 G>A [W1245X] with both parents being heterozygous for one mutation, each.

METHODS

Patient

We report a boy of a German father and a Portuguese mother. The parents are healthy and not consanguineous. At the age of 3 years the child developed recurrent episodes of hemolytic anemia and thrombocytopenia. Screening for autoantibodies against vWF-CP remained negative. Malignant disease of the hematopoetic system was excluded by bone marrow biopsy. Therapy, before the diagnosis of vWF-CP deficiency was made at 10 years of age, included initially steroids and repeated transfusions of platelets; splenectomy was performed at 5 years of age; afterwards, the clinical course was uneventful for years.

At 10 years of age he developed HUS-like disease with diarrhea and typical laboratory findings, including hemolytic anemia (hemoglobin 7.1 g/dL), fragmented red cells, thrombocytopenia (<15,000/μL), elevated lactate dehydrogenase (LDH) (5510 U/L), low fibrinogen levels (<50 mg/dL), and acute renal failure [serum creatinine 756.7 μmol/L and blood urea nitrogen (BUN) 274 mg/dL]. To prevent bleeding, platelets and fresh frozen plasma (FFP) were given. Hemodialysis was performed for 7 days. Furthermore, liver [glutamate-oxalacetate-transaminase (GOT) 1290 U/L and glutamate-pyruvate-transaminase (GPT) 428 U/L] and pancreatic (amylase 980 U/L and lipase 2800 U/L) enzymes were elevated. Increased creatine kinase (5317 U/L) and troponin T (26.4 μg/L) indicated affection of skeletal and cardiac muscle. Echocardiographic examination showed pericardiac effusion and markedly impaired shortening fraction.

A broad spectrum investigation for viral [including cytomegalovirus (CMV), Epstein-Barr virus (EBV), Parvo B19 virus, BK virus, adenovirus, and Coxsackie virus] and bacterial infection (including leptospirosis) was performed with negative result. Both, enteropathogenic *Escherichia coli*–like and Shiga-like toxins were negative.

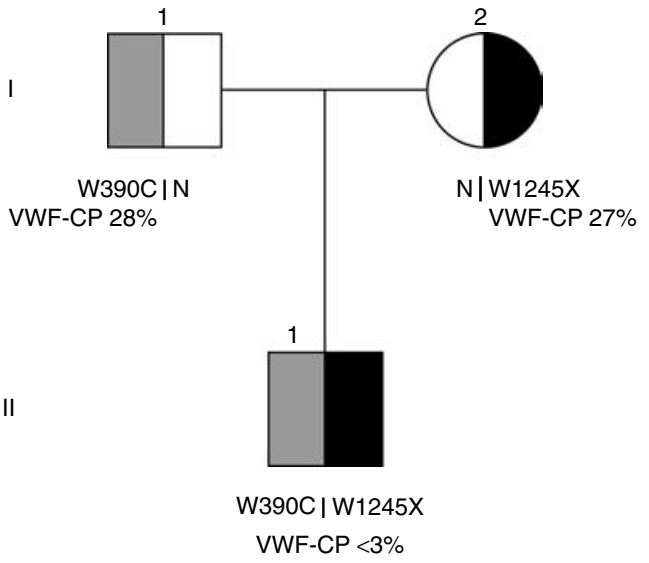

Fig. 1. Pedigree of the index patient (II).

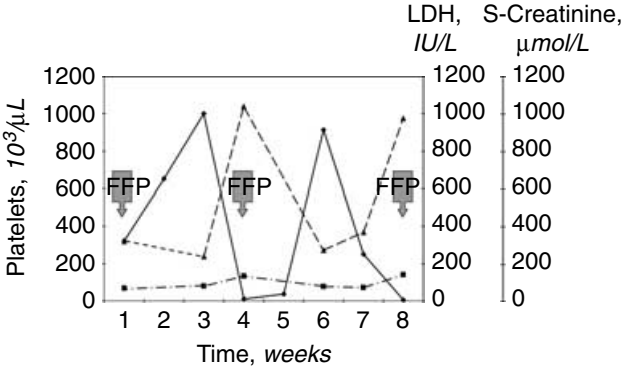

Fig. 2. Time course of platelet (◆), lactate dehydrogenase (LDH) (▲), and serum creatinine (■) over two completed treatment cycles. With about 10 mL fresh frozen plasma (FFP) per kilogram body weight given only following 3 to 4 weeks, platelets dropped to <10,000/μL prior to the next infusion. Therefore, 2-week treatment cycles were chosen for the future.

RESULTS

vWF-CP deficiency

Serologic examinations revealed severe deficiency of vWF-CP activity (<3%; normal >40%), while acitivity of vWF itself was increased (>200%; normal 50% to 150%). vWF-CP autoantibodies were not present. However, molecular genetic analysis revealed heterozygosity for two novel *ADAMTS13* gene mutations (1170 G>C [W390C] and 3735 G>A [W1245X]. The patient's father and mother showed vWF-CP levels of 28% and 27% of normal, respectively, which is characteristic for unaffected heterozygous carriers of *ADMTS13* mutations (Fig. 1) (R. Schneppenheim, personal communication).

Since so far no curative treatment is available for VWF-CP deficiency, the missing plasma factor has to be replaced by plasma substitution. Therefore we induced periodical (every 2 weeks) infusion of about 10 mL fresh frozen plasma (FFP) per kilogram body weight. Under this treatment, kidney function recovered completely, and hemodialysis could be discontinued (Fig. 2). Increased enzymes, pathologic changes in plasma coagulation, and impaired heart function were also transient and returned to normal without specific treatment.

Further investigations revealed minimal abnormalities in the electroencephalography (EEC) without seizures, which can be interpreted as the result of a generalized vasculopathy with symptoms not only in the kidney, but also in the central nervous system (CNS). Magnetic resonance imaging (MRI) examination of the brain revealed three small white matter lesions (centra semiovalia) but no intracerebral bleeding.

Regular FFP infusions so far maintained both platelet levels and kidney function within normal range and prevented new episodes of TTP/HUS. Up to now it remains open whether substitution of vWF-CP will also improve the CNS findings.

Molecular genetic analysis

After diagnosis of severe vWF-CP deficiency in the absence of *ADAMTS13* autoantibodies, molecular genetic analysis was carried out by polymerase chain reaction (PCR) and subsequent direct sequencing of all 29 exons of the *ADAMTS13* gene as previously described [16]. Two previously undescribed candidate mutations were detected by this approach. The first one was 1170G>C in exon 10, predicting the exchange of tryptophane for cysteine [W390C] and the second one was 3735G>A in exon 27, resulting in the nonsense mutation W1245X. W390C was identified in the patient's father and W1245X in the patient's mother, respectively (Fig. 1). Whereas the nonsense mutation can be considered causative, W390C can only be regarded as candidate mutation since the causative nature has not been proven by expression studies, yet. Interpretation as common polymorphisms, however, is rather unlikely since screening of 100 normal alleles did not detect the here described new mutations [16].

DISCUSSION

TTP/HUS was only recently understood as a single syndrome. The cascade of events of several different pathomechanisms merge to the formation of potentially occlusive platelet aggregations associated with thrombocytopenia and hemolytic anemia [3, 5]. Different organ systems are affected: ischemia of the brain or the gastrointestinal tract is common and renal dysfunction may occur [5].

Responsible is decreased vWF-CP activity (acquired, IgG autoantibodies; congenital, *ADAMTS13* gene mutations) or deficiency of factors regulating the activation of the alternative pathway of the complement system (e.g., complement factor H (CFH) and membrane cofactor protein (MCP) [5, 17–19]. It seems likely that in the future even more factors will be identified and it has to be assumed that TTP/HUS complex was, and probably currently still is, underdiagnosed [16].

In support of this, Schneppenheim et al [16] recently identified eight different *ADAMTS13* gene mutations suggesting the hereditary form of TTP in two patients who were misdiagnosed before with idiopathic thrombocytopenic purpura (ITP) or Evans syndrome, and in five out of eight patients with TTP, respectively. vWF-CP plasma levels are not discriminating between specific diagnoses: in patients with HUS they vary from normal to decreased values [3, 20, 21]. However, severe vWF-CP deficiency (<5% of normal) seems to be indicative for TTP rather than HUS [16]. Furthermore, vWF-CP levels in patients carrying compound heterozygous *ADAMTS13* gene mutations were usually not found to be higher than 5% [16].

Treatment of TTP/HUS depends on the exact diagnosis. If the syndrome is caused by a defect in the vWF-CP system, both, deficiency of vWF-CP caused by *ADAMTS13* gene mutations [16], or decreased activity of vWF-CP caused by vWF-CP autoantibodies are possible [22]. Patients with vWF-CP autoantibodies need more aggressive therapy, including plasma exchange and immunosuppression, while patients with the congenital form can solely be treated by replacement of vWF-CP by means of FFP [23–25].

In our patient, severe vWF-CP deficiency with vWF plasma level of <3% was caused by compound heterozygosity for two novel *ADAMTS13* gene mutations (1170 G>C [W390C] and 3735 G>A [W1245X]. Periodic (every 2 weeks) infusion of about 10 mL FFP per kilogram body weight raised vWF-CP activity from 5% (prior to infusion) to 10% 2 hours after infusion. vWF-CP activity, however, decreased to 4% 8 hours after infusion. Whereas the only small increase of vWF-CP is expected and in accordance with the infused FFP volume (10 mL/kg), the fast decline in vWF-CP activity occurs unexpectedly. Binding of vWF-CP to endothelial surfaces, thereby reducing vWF-CP plasma levels without reducing available intact vWF-CP, might explain this observation. To prevent clinical manifestation of new TTP/HUS episodes, vWF-CP activity of >3% seems to be sufficient. The 2-week treatment cycle in our patient is in accordance with the literature [26, 27] and fits with the half-life of vWF-CP of 2 to 3 days [28]. Recently, our patient developed another TTP/HUS crisis when the treatment interval was mistakenly increased to more than 2 weeks. Severe thrombopenia (platelets 14,000/μL with petechial hemorrhages) and increase of LDH (maximum 2900 U/L) were accompanied by impaired kidney function (maximum serum creatinine 460 μmol/L). Infusion of about 25 mL FFP per kilogram body weight as single therapy immediately stopped further decrease of platelets and normalized kidney function within 4 to 5 days (serum creatinine 71 μmol/L).

CONCLUSION

We conclude that in patients with unclear episodes of hemolytic or thrombocytopenic episodes or HUS-like symptoms, screening for severe vWF-CP deficiency or for other factors causing atypical TTP/HUS should be performed. Patients identified with a defect in vWF-CP can be treated, possibly with restitutio ad integrum, by regular FFP infusions. Furthermore, patients who presented

with hemolytic or thrombocytopenic episodes or HUS-like symptoms in the past should be re-evaluated, at least if diagnosis was not clear, or if restitutio ad integrum was not achieved.

For the future, it has to be expected that the identification of even more factors causing hemolytic or thrombocytopenic episodes or HUS-like symptoms will lead to a complete new understanding and nomenclature of this family of diseases [25].

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