

Two novel heterozygote missense mutations of the *ADAMTS13* gene in a child with recurrent thrombotic thrombocytopenic purpura

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Background. Thrombotic thrombocytopenic purpura is a rare, life-threatening disease characterised by microangiopathic haemolytic anaemia, thrombocytopenia and symptoms related to organ ischaemia, mainly involving the brain and the kidney. It is associated with a deficiency of ADAMTS13, a plasma metalloprotease that cleaves von Willebrand factor. The congenital form (Upshaw-Schulman syndrome) is rare and is associated with mutations of the *ADAMTS13* gene on chromosome 9q34. The clinical symptoms of congenital thrombotic thrombocytopenic purpura are variable, with some patients developing their first episode during the neonatal period or childhood and others becoming symptomatic in adulthood.

Materials and methods. We describe a case of thrombotic thrombocytopenic purpura, who presented to our attention with a relapsing form of the disease: the first episode occurred at the age of 13 months. Phenotype and genotype tests were performed in the patient and his family.

Results. The undetectable level of ADAMTS13 in the patient was caused by two novel heterozygote missense mutations on the *ADAMTS13* gene: one mutation is c.788C > T (p.Ser263Phe) on exon 7 and the second is c.3251G > A (p.Cys1084Tyr) on exon 25 of the *ADAMTS13* gene. All the relatives who have been investigated were found to carry one of these missense mutations in a heterozygous state.

Discussion. Although Upshaw-Schulman syndrome is a rare disease, it should be considered in all children with thrombocytopenia and jaundice in the neonatal period. In fact, once a child is confirmed to carry mutations of the *ADAMTS13* gene causing early thrombotic thrombocytopenic purpura, prophylactic treatment should be started to avoid recurrence of symptoms. Genotype tests of relatives would also be important for those women in the family who could be carriers of ADAMTS13 mutations, particularly during pregnancy.

Keywords: ADAMTS13, thrombotic thrombocytopenic purpura, mutation, Upshaw-Schulman syndrome, TTP.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare disease characterised by thrombocytopenia, microangiopathic haemolytic anaemia and microvascular thrombosis, which causes ischaemic damage in multiple organs¹⁻³.

The hereditary form, also called Upshaw-Schulman syndrome, is rare (accounting for about 5% of all cases) and is caused by mutations on the gene encoding for ADAMTS13, a zinc metalloprotease of the ADAMTS family (a disintegrin-like and metalloprotease with thrombospondin type 1 motif), responsible for the cleavage of von Willebrand factor (VWF).

More than 70 *ADAMTS13* gene mutations have been described so far in patients with congenital TTP. Most patients are compound heterozygotes but a few homozygotes have also been reported, particularly in

consanguineous families. The majority are missense mutations.

The clinical presentation of this form of TTP is variable; the first episode can happen in childhood or in adulthood and the patient can have a single episode or a chronic-relapsing form. In this report, we describe a case of a familial form of chronic-relapsing TTP caused by two novel heterozygote missense mutations on the *ADAMTS13* gene.

Materials and methods

The patient

In October 2010, a 3-year old boy was referred to our Haematology Clinic with a clinical and laboratory diagnosis of chronic relapsing TTP. In his family history, a paternal aunt was reported to have had an episode of microangiopathic anaemia during pregnancy, which was diagnosed as pre-eclampsia.

The boy's personal history included severe neonatal jaundice treated with phototherapy. At the age of 13 months (November 2008) he had his first episode of thrombocytopenia and microangiopathic haemolytic anaemia, starting with fever and petechiae. His blood tests revealed a platelet count of 5,000/mL and lactate dehydrogenase concentration of 914 U/L (haemoglobin levels were not available); serological analyses were consistent with a recent infection by Epstein-Barr virus. The patient was treated with steroids and platelet transfusions, and discharged on steroid therapy. In January 2009, during steroid tapering, his blood samples revealed a new decrease of platelet count, which was treated with plasma infusions, steroids and intravenous immunoglobulins. In February, after a further episode, maintenance therapy was started with infusions of cryoprecipitate two or three times a week for 2 months. The patient did not experience any other episodes until June 2009, after vaccination against measles, mumps and pertussis. After a new relapse in August of the same year, prophylactic therapy was started with weekly plasma infusions for 8 months, during which the patient achieved clinical remission. The patient experienced numerous other acute episodes of thrombocytopenia and microangiopathic haemolytic anaemia, associated with fever, when plasma prophylactic therapy was suspended.

The patient was then referred to our Haematology Clinic for ADAMTS13 evaluation. We performed assays of ADAMTS13 activity and anti-ADAMTS13 antibody, followed by analysis of the *ADAMTS13* gene of the patient and members of his family.

Assay of ADAMTS13 activity and its inhibitor

Plasma ADAMTS13 activity was measured using the residual VWF collagen binding activity assay (CBA), a method based upon the indirect detection of cleavage products of full-length plasma-derived VWF exposed to the patient's plasma, performed using urea as a denaturing agent to promote the susceptibility of full-length VWF to cleavage. The normal range of ADAMTS13 activity is 46-160%⁴.

Anti-ADAMTS13 antibodies were searched for by western blotting as previously reported⁵. The presence of anti-ADAMTS13 antibodies with neutralising activity was expressed as inhibitor activity against ADAMTS13 and was measured as previously described by Furlan *et al.*, based on the Bethesda method⁶. One unit of inhibitor was defined as the amount that reduced the ADAMTS13 activity to 50% of the control activity. The normal value of inhibitor activity against ADAMTS13 is <1 U/mL.

ADAMTS13 gene analysis

After obtaining informed consent from relatives, molecular analysis was performed starting with the extraction of genomic DNA from peripheral blood

leucocytes according to standard protocols. The coding regions, intron/exon boundaries and 5' and 3' untranslated regions were amplified by polymerase chain reaction (PCR). Following the amplification the PCR products were sequenced by a Big Dye Terminator cycle sequencing kit (Applied Biosystems, Warrington, UK) on an ABI 3130 genetic analyser (PE Applied Biosystems). Mutations identified by direct sequencing were confirmed by repeat sequencing in the relatives too. Mutations were named in accordance with the standard international nomenclature guidelines recommended by the Human Genome Variation Society (HGVS at: <http://www.hgvs.org/mutnomen/recs.html>), with nucleotide +1 as the A of the ATG translation initiation codon.

Results

ADAMTS13 activity (CBA) of the patient's plasma was <6% of that of pooled normal plasma. Western blotting analysis resulted negative with undetectable inhibitor activity against ADAMTS13 (<1 U/mL). The genetic analysis identified two mutations that have not been reported previously. One is a point mutation in exon 7 (c.788C > T) that results in an amino acid substitution of p.Ser263Phe in the metalloprotease domain. The other is a point mutation in exon 25 (c.3251G > A) that causes the amino acid substitution of p.Cys1084Tyr in the eighth thrombospondin type 1 (TSP-1) domain. The exon 7 mutation (c.788 C > T) was inherited from his mother and the other mutation on exon 25 (c.3251G > A) was inherited with a paternal allele. All the other relatives still alive have been investigated: the c.788 C > T (p.Ser263Phe) mutation on exon 7 was also found in the boy's maternal grandmother, in a maternal sibling (one sister) and in the patient's brother; the c.3251G > A (p.Cys1084Tyr) mutation on exon 25 was also found in all paternal siblings (one brother and two sisters). All the relatives were tested for ADAMTS13 activity levels and only the paternal uncle and maternal aunt were found to have low ADAMTS13 activity levels (24% and 43%, respectively), in the absence of a medical history of haematological disorders (Figure 1).

Discussion

TTP is a rare, life-threatening disease characterised by acute episodes of thrombocytopenia and microangiopathic haemolytic anaemia due to disseminated microvascular thrombosis with variable symptoms and signs related to organ ischaemia¹⁻³. The main pathogenic mechanism of this disorder is the excess of ultralarge multimers of VWF in the microcirculation, a condition that leads to uncontrolled platelet aggregation and formation of intravascular thrombi. The excess of ultralarge multimers of VWF is due to the inherited or acquired deficiency of the activity of ADAMTS13, a circulating metalloprotease of the family of "a disintegrin and metalloprotease with

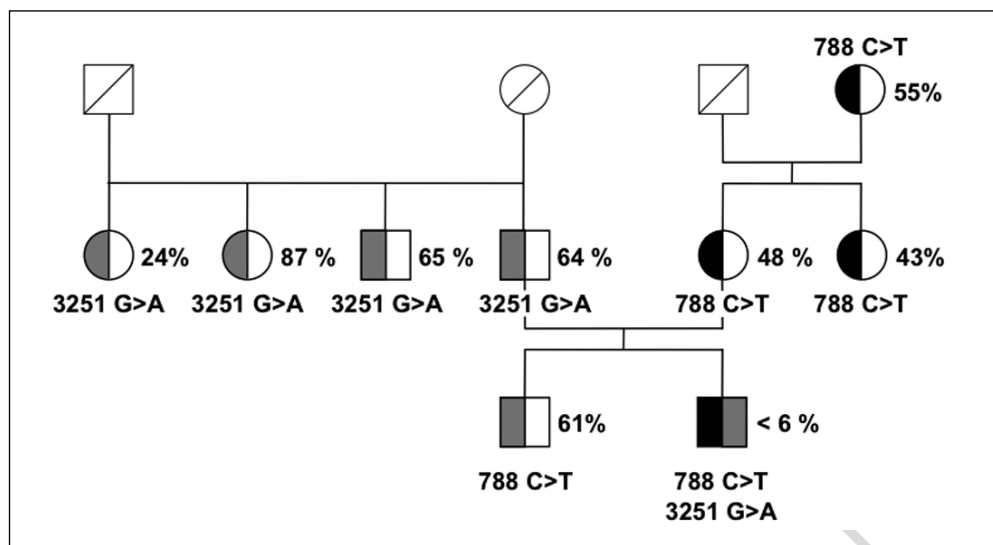


Figure 1 - Pedigree of the family with indication of gene mutation and plasma ADAMTS13 activity (% CBA).

Squares and circles indicate male and female, respectively. The half-solid circles and squares indicate asymptomatic heterozygotes.

thrombospondin type 1 motif" enzymes. This protease cleaves VWF at its Y1605-M1606 peptidyl bond in a shear-dependent manner⁷.

The congenital form of TTP results from homozygous or double heterozygous mutations of the *ADAMTS13* gene, which spans 29 exons and ~37 kb and is located at chromosome 9q34. Most of the mutations are missense mutations (~50%), followed by splice site, nonsense, and frameshift mutations². Most mutations have not been investigated by *in vitro* mutagenesis and expression analysis, but those that have been functionally characterised mainly cause secretion defects; nonetheless, some ADAMTS13 mutants are secreted but are less functional or non-functional, supporting the concept that other possible mechanisms of mutations involve biosynthesis or proteolytic activity. The phenotypic result is a severe reduction or absence of this circulating enzyme.

A congenital form of TTP should, therefore, be suspected in patients who present with TTP and severe ADAMTS13 deficiency in the absence of anti-ADAMTS13 autoantibodies⁸. This could be particularly important in children with thrombocytopenia and jaundice who could remain misdiagnosed for many years.

The clinical phenotype of congenital TTP is variable, ranging from patients developing their first episode during the neonatal period or childhood to others who become clinically symptomatic in adulthood, during the second or third decade of life⁸.

After the first episode, patients with the congenital form of TTP often experience a chronic relapsing course. From a therapeutic point of view, congenital forms of

TTP differ from acquired forms since plasmapheresis is not required for the former; the acute episodes are treated and subsequently prevented by the infusion of fresh-frozen plasma or cryoprecipitate-poor plasma. In fact, once the congenital deficiency of ADAMTS13 is diagnosed, it requires monthly prophylactic treatment, particularly if the patient has early-onset disease.

Our patient was diagnosed with familial TTP and was given regular prophylactic infusions of plasma (virus-inactivated) every 3 weeks. Our patient was found to have two heterozygote missense mutations, neither of which, to our knowledge, has been previously reported in the literature. Their potential to cause harm was assessed and confirmed through PolyPhen-2 software (<http://genetics.bwh.harvard.edu/pph2/>).

The point mutation in exon 7 (c.788C > T) results in an amino acid substitution of p.Ser263Phe in the metalloprotease domain, which is crucial for the cleavage of the Y1605-M1606 peptide bond in the VWF-A2 domain. So far 19 mutations have been identified in the metalloprotease domain⁸ and nine of them have been characterised by *in vitro* expression studies⁹. Most of them lead to an alteration of the secretion pathway and/or affect the specific activity of the enzyme, sometimes resulting in the absence of enzymatic activity due to Zn²⁺ binding site alterations^{10,11}.

The point mutation in exon 25 (c.3251G > A) causes the amino acid substitution of p.Cys1084Tyr in the eighth TSP-1 domain. The functional significance of the eighth TSP-1 domain has not been completely understood so far. On one hand, a few studies support the view that the seventh and eighth TSP-1 repeats and the CUB domains are not required for protease activity *in*

*vitro*¹². On the other hand, other authors have postulated that these domains have a functional role under fluid shear stress or under more physiological conditions *in vivo*: Bernardo *et al.* demonstrated that peptides from the CUB domains inhibited VWF-cleaving protease activity under flow and Majerus *et al.* showed that the most C-terminal TSP-1 and CUB domains modulate the binding of ADAMTS13 to immobilised VWF¹³⁻¹⁵. Moreover, a recent study by Palla *et al.* supported a functional role of one of the C-terminal TPS-1 repeats, demonstrating that a homozygous mutation leading to amino acid changes in the sixth TPS-1 repeat domain leads to a secretion pathway defect of the mutant protein, with no intracellular accumulation.

In conclusion, we describe a family case of chronic-relapsing TTP, caused by two not previously reported heterozygous mutations in the *ADAMTS13* gene. Both functional and population studies of these sequence variations should be performed in order to determine their biological role in the pathogenesis of TTP. The results of this study are also important for those women in the family who could be carriers of *ADAMTS13* mutations, particularly during pregnancy. Finally, we could advise paediatric neonatologists that all children with thrombocytopenia and jaundice at birth need to be analysed for *ADAMTS13* level with the hypothesis of TTP. Once a patient is confirmed to carry an *ADAMTS13* gene mutation that could lead to early onset of the disease, early prophylactic treatment should be started.

The Authors declare no conflicts of interest.

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