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Unique case of autoantibody mediated inactivation of ADAMTS13 in an Indian TTP patient

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ABSTRACT

A young Indian female visited hospital as a suspected case of thrombotic thrombocytopenic purpura (TTP) with relapsed thrombotic complications with low platelet counts, infarct in middle cerebral artery and thrombi in microvessels. We first confirmed the deficiency of ADAMTS13 metalloprotease in this patient showing improper cleavage of vWF multimers by her plasma unlike her parents and brother. Although patient had very less ADAMTS13 antigen in plasma, but it did not appear to be the cause of deficiency of the enzyme, because her father had similarly low antigen level and he never had prothrombotic complications. While investigating the genetic change in *ADAMTS13*, we observed four homozygous-SNPs (g.420T > C, g.1342C > G, g.1716G > A and g.2280T > C) in exon 5, 12, 15 and 19 respectively in patient and her father unlike the heterozygous form of same SNPs in mother and brother. Further to investigate the cause of ADAMTS13 deficiency, we observed an elevated level of antibody against ADAMTS13 in patient unlike her father and other family members. Our study therefore provides the molecular approach of diagnosis of TTP in this patient and also highlights the use of such techniques in India. More importantly, study provides the clue of alternate treatment such as immunosuppressant therapy to this patient.

1. Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening occlusive disorder characterized by the widespread microvascular platelet-rich thrombi. Although a rare disease, TTP has been a subject of interest among clinicians and researchers owing to its complex pathophysiology and fatal nature in the absence of rapid and aggressive treatment. Lack in the capacity to cleave ultra-large (UL) von Willebrand factor (vWF), which is produced mainly by endothelial cells and partly by platelets, into smaller circulating forms has been attributed to be the underlying cause of TTP development [1]. vWF is the largest (260 kDa monomer) adhesion protein, exists in blood as dimer or multimers, and is normally responsible for bleeding arrest at the site of vessel injury by initiating platelet adhesion to the subendothelium [2,3]. The 13th member of ADAMTS (A Disintegrin And Metalloprotease with ThromboSpondin type I repeats) family, ADAMTS13, cleaves the highly adhesive UL vWF multimers into a series of smaller less adhesive multimers. The deficiency in ADAMTS13 metalloprotease in plasma results in the persistence of UL vWF multimers on endothelial cells [4,5] and in circulating blood [1] predisposing towards unusual platelet aggregation and disseminated vWF/platelet rich thrombus formation [6], the characteristic feature of TTP.

The deficiency in ADAMTS13 enzyme activity can be either due to the presence of autoantibodies against ADAMTS13, manifested as acquired or immune-mediated TTP (iTTP) or because of the mutations in ADAMTS13 gene, known as hereditary or congenital TTP (cTTP). Autoantibodies present in patients with acquired TTP can be either IgG or IgM and ranges from 30 to 83% neutralizing either enzyme activity or accelerating its clearance [7]. Almost all patients with iTTP have anti-ADAMTS13 autoantibodies with an epitope in the spacer domain of the enzyme [8]. In cTTP, heterozygous or homozygous mutations are associated with severe ADAMTS13 deficiency. More than 150 different mutations including missense (~62%), nonsense (~12.5%), splice site (~8%) and frameshift deletions or insertions (~17.5%) have been identified in ADAMTS13 gene showing association with TTP pathophysiology [8,9].

TTP is a rare disorder, an estimated 11 cases among million are reported annually in USA. Mostly (about 75%) the iTTP patients are women and 44% of them are of African origin [10]. However, above such epidemiology reports on TTP is not available in India. Although

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very few reports suggested the deficiency of ADAMTS13 in Indian patients, but a detail molecular insight into disease pathogenesis is missing [11,12]. In an effort to understand the molecular mechanism of iTTP, we report in this study the detail of the pathogenesis of the disease in a 21-year-old female patient suffering from suspected TTP with relapsed thrombotic complications. Although the patient carried four known homozygous-SNPs (g.420T > C, g.1342C > G, g.1716G > A and g.2280T > C) in *ADAMTS13* gene like her healthy father but the presence of elevated autoantibody against ADAMTS13 in her blood appeared to be the cause of inactivation of this protease in developing TTP pathophysiology.

2. Materials and methods

2.1. Reagents

Fluorescence labeled monoclonal anti-human antibodies such as CD62P FITC (R&D systems, Minneapolis), PAC1 FITC, annexin V FITC, CD41a PE, CD66b-Percp-cy5.5, CD41 FITC, CD14 FITC, CD16-APC-cy7 (BD Biosciences, San Jose, USA) were used. ADP and ristocetin for platelet aggregation were purchased from BIO/DATA Corporation (Horsham, USA). ELISA kits for determination of ADAMTS13 antigen (R &D Systems, Minneapolis, USA) and autoantibody (Biomedica Diagnostics, Stamford, USA) were purchased.

2.2. Human subjects

The study was approved from the Institutional Ethics Committee for Human Research of All India Institute of Medical Sciences (AIIMS), New Delhi (Reference No. IEC-643/03.11.2017, RP-30/2017) and Regional Centre for Biotechnology (RCB, Reference No. RCB-IEC-H-13). Blood samples (6–8 ml) were collected in vacutainers containing anti-coagulant acid citrate-dextrose (ACD) from a patient with suspected TTP and from her both parents and brother at Department of Hematology, AIIMS. Healthy controls ($n = 3$) for the study were recruited at RCB. Informed consent was provided according to the recommendations of the declaration of Helsinki.

2.3. Preparation of platelet rich plasma (PRP) and platelet aggregation

Whole blood was centrifuged at 500 rpm for 15 min to isolate PRP. Platelet aggregation was measured using agonists such as ADP and ristocetin *ex vivo* using a PAP-8 Platelet Aggregation System from BIO/DATA Corporation. ADP (20 μM) and ristocetin (1.25 mg/ml) were used for aggregation assay.

2.4. Flow cytometry analysis of platelet activation

Platelets from PRP were labeled with FITC conjugated anti-P-selectin and PAC-1 antibody, and annexin V and platelet activation markers were assessed using flow cytometry using BD FACS-Verse instrument as mentioned [13]. For measurement of plasma microparticles (MPs), platelet free plasma was obtained by centrifuging plasma at 5000 rpm for 15 min. Platelet free plasma was incubated with anti-CD41 PE antibody in filtered PBS for 40 min at 37 °C and platelet-derived MPs were measured using flow cytometry. FlowJo software (BD) was used for data analysis as mentioned [13].

2.5. Cleavage of vWF-platelet string under flow condition

The activity of plasma ADAMTS13 was measured using an assay system where the enzyme cleaved vWF-platelet strings on endothelium monolayer under flow shear condition. The human umbilical vein endothelial cells (HUVECs, HiMedia, India) were grown on petri plates coated with human collagen (STEMCELL Technologies, Canada) and were activated with 25 μM histamine (Sigma, USA) in presence of 50 μl plasma from either patient or controls for 3 min at 37 °C before fitting into parallel flow chamber. A syringe pump (Harvard Apparatus Inc., USA) was connected to the outlet port that drew washed-platelets suspension in Tyrode HEPES buffer through the chamber at the shear stress of 25 dyne/cm^2. The flow chamber was mounted onto a Nikon Eclipse Ti-E inverted stage microscope (Nikon, Japan) equipped with a high-speed digital camera. Movie was recorded at magnification 40× and analysed using NIS-Elements version 4.2 software. The length and numbers of platelet-vWF strings on HUVECs were quantified as mentioned [14,15]. The washed platelet preparation and parallel flow chamber assay detail are mentioned in our recent works [15].

2.6. ADAMTS13 gene analysis

Genotyping of all the 29 exons including the intron-exon boundaries and promoter sequence of *ADAMTS13* gene was carried out using polymerase chain reaction (PCR) followed by DNA based sequencing. Genomic DNA was isolated from PBMCs of patient or controls using FlexiGene DNA kit (Qiagen, Hilden, Germany). Primers used for exon amplification were as described somewhere else [16] and promoter region was sequenced using the primers ADAMTS13-PF.1 (GAGACAG GAGAGTCATTCATGC), ADAMTS13-PR.1 (CCTTGTCTTTCCACCCC TCA), ADAMTS13-PF.2 (GCGGCTCTGTGGGTGTAAAA) and ADAMTS13-PR.2 (GGGCCTTGCTCTAAGATGGT). The PCR products were processed using QIAquick gel extraction kit (Qiagen) and sent for DNA sequencing using 96 capillary high throughput sequencer; ABI 3730 XL. The sequence data were analysed using online tool MEGA 7.0 [17].

2.7. Measuring antigen and autoantibody for ADAMTS13 from plasma using ELISA

The antigen and autoantibody levels for ADAMTS13 in plasma were measured using commercial ELISA kits. Plasma **ADAMTS13 antigen** level was measured using a sandwich ELISA Quantikine kit (R&D Systems, USA). Plasma was added into an ELISA plate precoated with anti-ADAMTS13 monoclonal antibody and ADAMTS13 antigen level was detected using HRP-conjugated anti-ADAMTS13 polyclonal antibody as mentioned in other literatures [18–20]. Presence of **ADAMTS13 autoantibodies** in plasma samples was measured using ELISA kit (IMUBIND ADAMTS13 Autoantibody ELISA, Biomedica Diagnostics, Stamford USA) [21]. The ELISA plate was coated with full-length recombinant human ADAMTS13 and IgG type autoantibodies against ADAMTS13 were detected using HRP-labeled goat anti-human IgG antibody. Detail manufacturer's instruction was followed.

2.8. Statistical analysis

The experimental values from at least three independent experiments were presented as mean ± standard error (SEM). Statistical analysis between control and patient sample was performed using either unpaired *t*-test or one-way ANOVA with multiple comparisons. Graph Pad Prism 7.0 software was used for data analysis and P values < 0.05 were considered to be statistically significant.

3. Results

3.1. Clinical history and TTP diagnosis

The 21 year old Indian female patient experienced three episodes of acute focal neurological deficits from May 2015 till March 2017. First episode in May 2015 was an acute onset with inability to speak and difficulty in comprehension. After a moderate recovery patient developed another episode in September 2015, which was an acute onset with right faciobrachial paresis, having low platelet count (60×10^3/μl) along with other prothrombotic complications. Third episode in

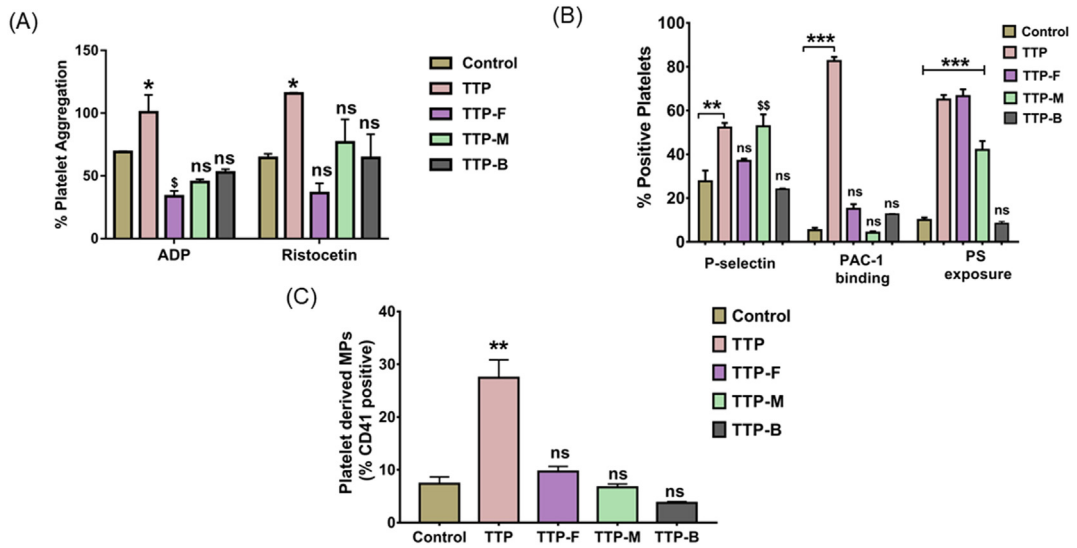


Fig. 1. Platelet function: (A) Platelet aggregation: Platelet-rich plasma (PRP) from patient and others were used for platelet aggregation assay in response to agonists such as ADP and ristocetin. Data present mean ± SEM from 3 independent experiments. One-way ANOVA with multiple comparison tests was used for analysis, for ADP *p = 0.0234, $p = 0.0125 and ns = nonsignificant; for ristocetin *p = 0.0461, compared to control. (B) Platelet activation: Platelets were stained for measuring P-selectin, GPIIbIIIa (PAC-1 binding) and PS (annexin V binding) using flow cytometry. Data calculated as mentioned above, for P-selectin, **p = 0.0017, $$p = 0.0015; for PAC-1 binding, ***p = 0.0003; for PS exposure, ***p < 0.0001. (C) MP generation by platelets: $CD41^+$ MPs were measured in plasma using flow cytometry. The gating strategy is mentioned in Suppl. Fig. 1. Data analysed using unpaired *t*-test, **p = 0.0053.

March 2017 was an acute onset of hemiparesis and infarct in right middle cerebral artery (MCA). Multiple small thrombi were observed in small blood vessels and patient was suffering from severe thrombocytopenia (45×10^3/μl). Peripheral blood smear showed elevated numbers of schistocytes (3%), increased LDH (440), positive antinuclear antibodies (1:160) and anemia. Patient was diagnosed with suspected TTP and received plasma exchange in April 2017. Suppl. Table 1 shows the clinical parameters in the patient before and after receiving the plasma exchange.

3.2. Elevated platelet activation and aggregation in patient

To understand the molecular basis of relapsed and refractory episodes of thrombotic complications, we collected blood samples from patient in June 2018 and performed following studies. Patient's platelet rich plasma (PRP) showed significant platelet aggregation in response to agonists such as ristocetin and ADP unlike her parents and brother (Fig. 1A). Further, the surface expression of activation markers such as P-selectin, phosphatidylserine (PS) and PAC-1 binding was elevated in platelets of patient compared to her family members and healthy controls (Fig. 1B). Elevated level of platelet-derived microparticles (MP) in patient's plasma further supported the molecular basis for platelet activation and related thrombotic complications in patient, unlike others including her family members (Fig. 1C). The TTP patient also had elevated levels of neutrophil-platelet aggregates and monocyte-platelet aggregates (Suppl. Fig. 2).

3.3. Significant decrease in ADAMTS13 activity in patient

We measured the activity of ADAMTS13 from patient's plasma using *in vitro* assay of cleaving the vWF-platelet strings on endothelium monolayer under flow shear condition. Our data showed significantly less cleavage of vWF-platelet strings by patient's plasma when compared with plasma ADAMTS13 activity of her family members (Fig. 2A). More numbers (Fig. 2B) as well as larger length (Fig. 2C) of vWF-platelet strings observed in patient than others including her family members. Study also determined the ADAMTS13 antigen level in plasma using ELISA. A low plasma antigen level for the enzyme was detected in patient as well as her father unlike her mother and brother (Fig. 2D).

3.4. Patient carries SNPs of ADAMTS13

In order to investigate any genetic basis of the ADAMTS13 deficiency in this patient, we identified the homozygous SNPs with nucleotide substitutions 420T > C, 1342C > G, 1716G > A and 2280T > C in exon 5, 12, 15 and 19 respectively in *ADAMTS13* gene of patient as well as her father unlike the heterozygous form of same SNPs in mother and brother (Fig. 3). Out of these four SNPs, g.1342C > G (Q448E) is a known non-synonymous SNP and g.420T > C (A140A), g.1716G > A (T572T) and g.2280T > C (G760G) are known synonymous SNPs [16,22]. We did not find other known SNPs (NCBI reference sequence: NG_011934.2) or new variants in *ADAMTS13* in this patient. Thus, above observations including antigen level and SNPs in *ADAMTS13* clearly indicated the association of some other mechanism (s) that resulted in low activity of this enzyme in patient but not her father.

3.5. Elevated autoantibody against ADAMTS13 in patient

In order to investigate the association of autoantibody against ADAMTS13 with the deficiency of this enzyme in patient, we observed significantly high level of antibody against ADAMTS13 in her plasma unlike her father or other family members (Fig. 4), which appeared to be the cause for this enzyme deficiency.

4. Discussion

This study reports the molecular insight into the pathogenesis of idiopathic TTP (iTTP) with relapsed thrombotic episodes in a young Indian woman. Also study is the very first to describe a detail molecular basis of diagnosis of iTTP in an Indian patient, although earlier studies have reported the similar molecular diagnosis of this rare disorder among other ethnic populations including African and Caucasian [23]. This patient had several acute episodes of thrombotic complications with very low platelet counts and significant platelet thrombi in microcirculations as well as infarct in right middle cerebral artery (MCA) territory as observed through MR angio and MRI respectively. Although

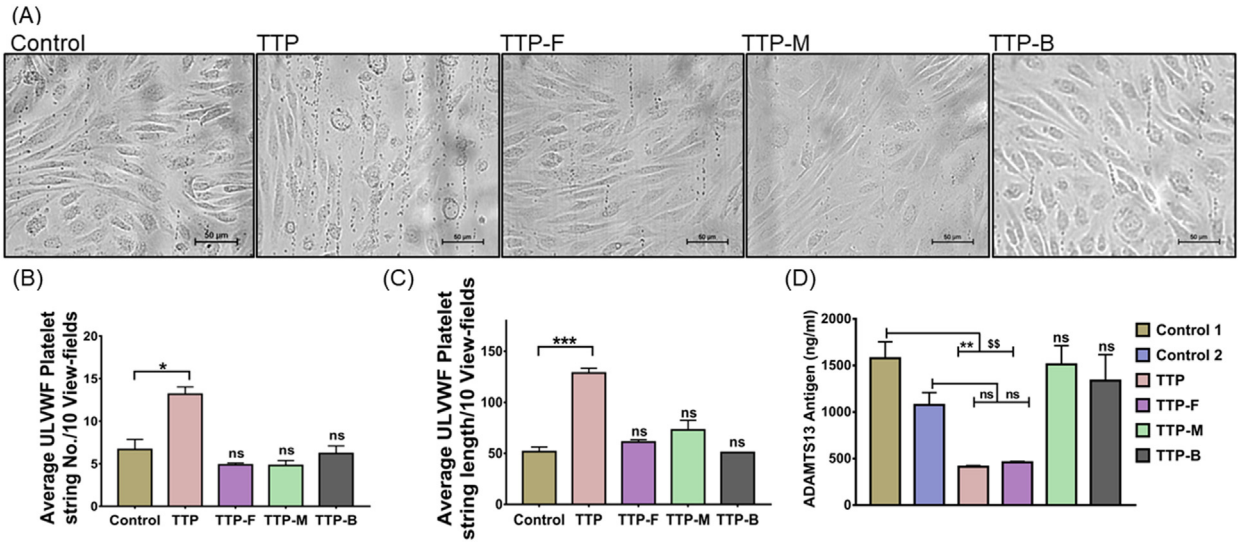


Fig. 2. ADAMTS13 activity and antigen levels: (A) Enzyme activity was measured using assay under flow condition where plasma ADAMTS13 cleaves ULVWF-platelets strings on histamine-activated HUVECs monolayer under shear stress of 25 dyne/cm^2. Representative pictures at 40× resolution. (B) String number (*p = 0.0118) and (C) string length (***p = 0.0003) were calculated from 10 images from each of the three different experimental sets. Data presented as mean ± SEM and unpaired t-test was used for analysis. (D) ADAMTS13 antigen levels were determined using ELISA. Data analysed using one-way ANOVA with multiple comparisons, **p = 0.0015, $^{\$\$}$p = 0.0021.

the patient was diagnosed as suspected case of TTP but due to the lack of any molecular evidence no specific treatment other than plasma transfusion was recommended. In mechanism, we observed the improper cleavage of vWF multimers by patient's plasma suggesting a clear deficiency in ADAMTS13 protease unlike her parents, brother or healthy individuals. Although our data showed a very low antigen level of ADAMTS13 in plasma of the patient, but it did not appear to be the cause of deficiency of the enzyme, in other word, cause of impaired cleavage of vWF multimer by this plasma protease, because the patient's father also displayed similarly low antigen level of the enzyme in plasma, and her father never had any such prothrombotic complication. Further our investigation on the genetic basis of deficiency of the enzyme showed four homozygous-SNPs such as g.420T > C (A140A), g.1342C > G (Q448E), g.1716G > A (T572T) and g.2280T > C (G760G) in exon 5, 12, 15 and 19 respectively in *ADAMTS13* gene of the patient. The patient's father also carried the homozygous form of same SNPs. Unfortunately, the frequency of these SNPs as well as their association with prevalence of TTP has never been studied among Indian. Although several other studies have described insignificant association of SNPs including g.1342C > G with secretion as well as proteolytic activity of ADAMTS13 in TTP patients in other ethnic

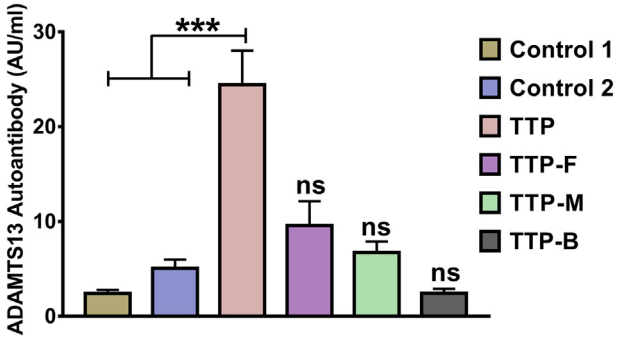
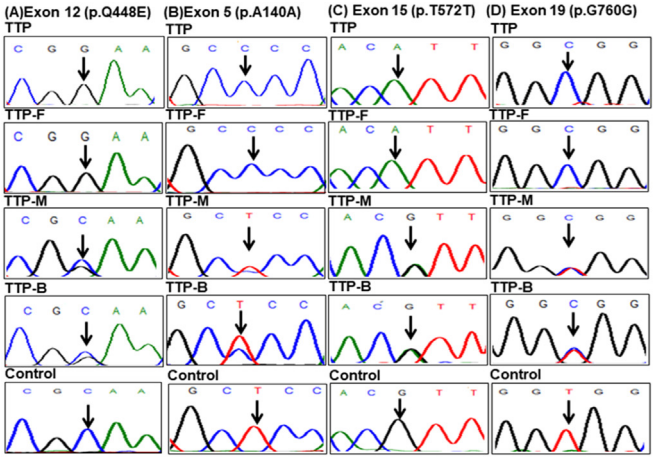


Fig. 4. ADAMTS13 autoantibody level was determined using ELISA kit. Data is presented as mean ± SEM and analysed using one-way ANOVA with multiple comparisons, ***p < 0.0001.



	Exon 5 420T>C Silent	Exon 12 1342C>G Q448E	Exon 15 1716G>A Silent	Exon 19 2280T>C Silent
TTP	CC	GG	AA	CC
TTP-F	CC	GG	AA	CC
TTP-M	CT	CG	GA	TC
TTP-B	CT	CG	GA	TC
Control	TT	CC	GG	TT

Fig. 3. ADAMTS13 polymorphisms: (A-D) Sequence chromatograms of PCR-amplified exons showing polymorphisms in exons 12, 5, 15 and 19 in the patient, her family members and healthy control. Arrow indicates the homozygosity/heterozygosity at indicative position. Region containing the polymorphism was repeated twice with fresh template each time.

populations [24,25]; another study showed that both non-synonymous and synonymous mutations in ADAMTS13 gene can affect its expression and functional levels [22].

In an attempt to further investigate the mechanism of ADAMTS13 deficiency in this patient, we observed an elevated level of antibody against ADAMTS13 in patient unlike her father as well as other family members. Therefore, our observation highlights the possibility of change in conformation of cryptic epitope of spacer domain of the enzyme that carries the autoantigenic core [26,27]. The autoantibody against ADAMTS13 might have inhibited the enzyme activity predisposing towards the accumulation of UL vWF and platelet-rich microthrombi in microcirculations as observed in this patient. Also, it could be that the germ-line mutations in *ADAMTS13* gene have higher likelihood of developing autoantibodies as found in this propositus leading to iTTP; as is well described in coagulation factor VIII deficiency where mutations in the *F8* gene have been shown to form elevated factor VIII inhibitors [28–30]. Thus, our observations together describe the molecular way of diagnosis of TTP in this patient and also highlight the use of such unique techniques to detect this rare disorder in India. Besides, study also strongly suggests the clue of alternate treatment such as immunosuppressant drug therapy to this patient other than regular plasmapheresis.

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Authorship contributions

TB performed most of the experiments. RS and GKA developed critical tools and analysed data. AS, MM and RS conceptualised the clinical components of the work, supervised the data collection, and analysed the data. TB and PG conceptualised the approach, designed the experiments, analysed the data, and wrote the manuscript. All authors read, edited and approved the final manuscript.

Conflict of interest

Authors have no financial or other interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcmd.2019.03.003.

References

[1] J.L. Moake, C.K. Rudy, J.H. Troll, M.J. Weinstein, N.M. Colannino, J. Azocar, R.H. Seder, S.L. Hong, D. Deykin, Unusually large plasma factor VIII:von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura, N. Engl. J. Med. 307 (1982) 1432–1435.
[2] J.E. Sadler, von Willebrand factor, J. Biol. Chem. 266 (1991) 22777–22780.
[3] D.D. Wagner, V.J. Marder, Biosynthesis of von Willebrand protein by human endothelial cells: processing steps and their intracellular localization, J. Cell Biol. 99 (1984) 2123–2130.
[4] A.K. Chauhan, D.G. Motto, C.B. Lamb, W. Bergmeier, M. Dockal, B. Plaimauer, F. Scheiflinger, D. Ginsburg, D.D. Wagner, Systemic antithrombotic effects of ADAMTS13, J. Exp. Med. 203 (2006) 767–776.
[5] J.F. Dong, Structural and functional correlation of ADAMTS13, Curr. Opin. Hematol. 14 (2007) 270–276.
[6] G.A. Hosler, A.M. Cusumano, G.M. Hutchins, Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome are distinct pathologic entities. A review of 56 autopsy cases, Arch. Pathol. Lab. Med. 127 (2003) 834–839.
[7] H.M. Tsai, E.C. Lian, Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura, N. Engl. J. Med. 339 (1998) 1585–1594.
[8] J.A. Kremer Hovinga, P. Coppo, B. Lammle, J.L. Moake, T. Miyata, K. Vanhoorelbeke, Thrombotic thrombocytopenic purpura, Nat. Rev. Dis. Primers. 3 (2017) 17020.
[9] E. De Cock, C. Hermans, J. De Raeymaecker, K. De Ceunynck, B. De Maeyer, N. Vandeputte, A. Vandenbulcke, H. Deckmyn, H. Rottensteiner, M. De Maeyer, S.F. De Meyer, K. Vanhoorelbeke, The novel ADAMTS13-p.D187H mutation impairs ADAMTS13 activity and secretion and contributes to thrombotic thrombocytopenic purpura in mice, J. Thromb. Haemost. 13 (2015) 283–292.
[10] D.R. Terrell, S.K. Vesely, J.A. Kremer Hovinga, B. Lammle, J.N. George, Different disparities of gender and race among the thrombotic thrombocytopenic purpura and hemolytic-uremic syndromes, Am. J. Hematol. 85 (2010) 844–847.
[11] A. Goel, V. Raghupathy, G.J. Amirtharaj, A. Chapla, A. Venkatraman, B. Ramakrishna, A. Ramachandran, N. Thomas, K.A. Balasubramanian, I. Mackie, E. Elias, C.E. Eapen, ADAMTS13 missense variants associated with defective activity and secretion of ADAMTS13 in a patient with non-cirrhotic portal hypertension, Indian J. Gastroenterol. 36 (2017) 380–389.
[12] S. Yadav, S. Shetty, B. Kulkarni, A novel homozygous frameshift mutation in exon 7 of the ADAMTS13 gene in a patient with congenital thrombotic thrombocytopenic purpura from India: a case report, Transfusion 57 (2017) 2712–2714.
[13] R. Singhal, G.K. Annarapu, A. Pandey, S. Chawla, A. Ojha, A. Gupta, M.A. Cruz, T. Seth, P. Guchhait, Hemoglobin interaction with GP1balpha induces platelet activation and apoptosis: a novel mechanism associated with intravascular hemolysis, Haematologica 100 (2015) 1526–1533.
[14] G.K. Annarapu, R. Singhal, A. Gupta, S. Chawla, H. Batra, T. Seth, P. Guchhait, HbS binding to GP1balpha activates platelets in sickle cell disease, PLoS One 11 (2016) e0167899.
[15] A. Ojha, D. Nandi, H. Batra, R. Singhal, G.K. Annarapu, S. Bhattacharyya, T. Seth, L. Dar, G.R. Medigeshi, S. Vrati, N.K. Vikram, P. Guchhait, Platelet activation determines the severity of thrombocytopenia in dengue infection, Sci. Rep. 7 (2017) 41697.
[16] K. Kokame, T. Miyata, Genetic defects leading to hereditary thrombotic thrombocytopenic purpura, Semin. Hematol. 41 (2004) 34–40.
[17] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874.
[18] M. Miura, K. Kaikita, M. Matsukawa, K. Soejima, S. Fuchigami, Y. Miyazaki, T. Ono, T. Uemura, K. Tsujita, S. Hokimoto, H. Sumida, S. Sugiyama, K. Matsui, H. Yamabe, H. Ogawa, Prognostic value of plasma von Willebrand factor-cleaving protease (ADAMTS13) antigen levels in patients with coronary artery disease, Thromb. Haemost. 103 (2010) 623–629.
[19] M.K. Freynhofer, V. Bruno, R. Jarai, S. Gruber, T. Hochtl, I. Brozovic, S. Farhan, J. Wojta, K. Huber, Levels of von Willebrand factor and ADAMTS13 determine clinical outcome after cardioversion for atrial fibrillation, Thromb. Haemost. 105 (2011) 435–443.
[20] S.B.M. El-Hady, M.H. Farahat, E. Almasry, ADAMTS-13 contents in therapeutic plasma products, Egypt. J. Haematol. 38 (2013) 68–73.
[21] H.M. Tsai, L. Rice, R. Sarode, T.W. Chow, J.L. Moake, Antibody inhibitors to von Willebrand factor metalloproteinase and increased binding of von Willebrand factor to platelets in ticlopidine-associated thrombotic thrombocytopenic purpura, Ann. Intern. Med. 132 (2000) 794–799.
[22] N.C. Edwards, Z.A. Hing, A. Perry, A. Blaisdell, D.B. Kopelman, R. Fathke, W. Plum, J. Newell, C.E. Allen, G. S, A. Shapiro, C. Okunji, I. Kosti, N. Shomron, V. Grigoryan, T.M. Przytycka, Z.E. Sauna, R. Salari, Y. Mandel-Gutfreund, A.A. Komar, C. Kimchi-Sarfaty, Characterization of coding synonymous and non-synonymous variants in ADAMTS13 using ex vivo and in silico approaches, PLoS One 7 (2012) e38864.
[23] S. Martino, M. Jamme, C. Deligny, M. Busson, P. Loiseau, E. Azoulay, L. Galicier, F. Pene, F. Provot, A. Dossier, S. Saheb, A. Veyradier, P. Coppo, Thrombotic thrombocytopenic purpura in black people: impact of ethnicity on survival and genetic risk factors, PLoS One 11 (2016) e0156679.
[24] K. Kokame, M. Matsumoto, K. Soejima, H. Yagi, H. Ishizashi, M. Funato, H. Tamai, M. Konno, K. Kamide, Y. Kawano, T. Miyata, Y. Fujimura, Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11902–11907.
[25] B. Plaimauer, J. Fuhrmann, G. Mohr, W. Wernhart, K. Bruno, S. Ferrari, C. Konetschny, G. Antoine, M. Rieger, F. Scheiflinger, Modulation of ADAMTS13 secretion and specific activity by a combination of common amino acid polymorphisms and a missense mutation, Blood 107 (2006) 118–125.
[26] K. South, B.M. Luken, J.T. Crawley, R. Phillips, M. Thomas, R.F. Collins, L. Deforche, K. Vanhoorelbeke, D.A. Lane, Conformational activation of ADAMTS13, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 18578–18583.
[27] M.R. Thomas, R. de Groot, M.A. Scully, J.T. Crawley, Pathogenicity of anti-ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura, EBioMedicine 2 (2015) 942–952.
[28] C.R. Hay, Factor VIII inhibitors in mild and moderate-severity haemophilia A, Haemophilia 4 (1998) 558–563.
[29] K.E. Knobe, B.O. Villoutreix, L.I. Tengborn, P. Petrini, R.C. Ljung, Factor VIII inhibitors in two families with mild haemophilia A: structural analysis of the mutations, Haemostasis 30 (2000) 268–279.
[30] J. Astermark, FVIII inhibitors: pathogenesis and avoidance, Blood 125 (2015) 2045–2051.