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ADAMTS13 gene; a novel splicing site mutation in a case with thrombotic thrombocytopenic purpura

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ABSTRACT

A plasma protease, *ADAMTS13*, cleaves the von Willebrand factor (VWF) and its deficiency is associated with the pathogenesis of thrombotic thrombocytopenic purpura (TTP). According to the Human Gene Mutation Database (HGMD), about 150 mutations have been identified in the *ADAMTS13* gene. A 23-year-old man, with hematuria and gingival bleeding was admitted to our University Hospital. Four years ago he was diagnosed with a TTP history. During these years, he was under intermittent plasma exchange. A blood sample was taken for genetic study. He effectively responded to one session of fresh frozen plasma replacement and plasma exchange. Genetic study indicated that this case carries two heterozygous mutations in *ADAMTS13* gene; a novel splicing variant (c.2610+5G>A) and a nonsense p.Arg910X mutation that previously is reported to relate to TTP. The novel variant predicted to result in an aberrant *ADAMTS13* transcript processing.

Case Report

Implication for health policy/practice/research/medical education:
The c.2728C>T (p.Arg910X) mutation in *ADAMTS13* gene was found in a patient with congenital TTP. Moreover, we found c.2610+5G>A mutation, a novel splicing variant, that affects exon 20 and encodes for the ADAMTS13's thrombospondin type-1 fifth domain in this case. This novel variant expected to cause aberrant ADAMTS13 transcript processing.

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Introduction

Thrombotic thrombocytopenic purpura (TTP) is identified by small-vessel platelet-rich thrombi and presents with microangiopathic hemolytic anemia, thrombocytopenia, and consequent multiorgan dysfunction (1). Deficiency of a plasma protease ADAMTS13 cleaving the von Willebrand factor (VWF), can result in VWF perseverance that plays an important role in the pathogenesis of TTP (2). ADAMTS13 deficiency is determined by either genetic causes (congenital TTP) or by autoantibodies against the protease (acquired TTP). Congenital TTP, responsible for less than 5% of cases (3, 4), is an autosomal recessive disorder (2) due to compound heterozygous or homozygous mutations in the *ADAMTS13* gene, which is located on chromosome 9.

According to the Human Gene Mutation Database (HGMD), about 150 mutations of the *ADAMTS13* gene have been recognized so far. They included: 113 missense/nonsense mutations, 10 splicing mutations (Table 1), 21 small deletions, 7 small insertions, and 5 gross deletions.

In this report, we describe a case of a young male from Iran suffers from congenital TTP and carries two heterozygous mutations in *ADAMTS13* gene: a novel splicing variant (c.2610+5G>A) and a nonsense p.Arg910X mutation that previously is reported to associate with TTP. The novel variant predicted to result in an aberrant *ADAMTS13* transcript processing.

Case Report

A 23-year-old man, with hematuria and gingival bleeding was admitted to our University Hospital. Four years he was diagnosed with a clinical history of TTP. After TTP was diagnosed, he received intermittent sessions of plasmapheresis (once a week) for one year. Urinalysis

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revealed hematuria and peripheral blood examination disclosed schistocytes. The laboratory findings at the time of admission presented in Table 2. ADAMTS13 enzyme activity was less than 10% during active disease. We did not measure the autoantibody titer of ADAMTS13. The patient received 2 liter per day plasmapheresis with fresh frozen plasma (FFP) replacement and after clinical improvement (platelet count >100 000/mm^3, lactate dehydrogenase (LDH) <600 (units/L)), he was discharged. Thereafter, he was under our frequent follow up and still now he is receiving once weekly plasma exchange (2 liters per session).

DNA was extracted from the patient's peripheral blood cells and genetic analysis was performed at Mario Negri Institute for Pharmacological Research in Bergamo (Italy). DNA from healthy parents and siblings was not available for genotyping.

All coding exons and flanking intronic regions of the *ADAMTS*13 gene were analyzed by Sanger sequencing. We recognized a heterozygous mutation, located at exon 21, leading to the insertion of a premature stop codon (c.2728C>T, NM_139025.4, p.Arg910X). This mutation is absent in public databases (dbSNP151, 1000 Genomes Project, Exome Aggregation Consortium, The Genome Aggregation Database), but it has been previously reported in a case of TTP with childhood onset (2). We identified a second heterozygous mutation (c.2610+5G>A) laying in intron 20, predicted to probably affect the splicing of exon 20. This variant has been reported neither in public databases nor in TTP patients. *In silico* analyses predicted that the possibility of the exon 20 correct splicing declines from 0.983 to 0 *versus* the wild-type sequence (GenScan software) (5) in the mutant sequence. Furthermore, an aberrant exon 20 characterized by the retention of 21-base pairs was predicted with a probability of 0.572, leading to an in-frame insertion of 7 amino acids (VNALGMR) in the protein sequence.

Additional predictions with the Human Splicing Finder software (6) confirmed that this mutation causes the break of the wild-type donor splicing site.

Table 1. Reported mutations in splicing sites of *ADAMTS*13 gene TTP patients

Accession Number	HGMD splicing mutation	References
CS126162	IVS1 as -1 G-C (c.106-1G>C)	van Dorland et al (8)
CS043116	IVS3 ds +1 G-A (c.330+1G>A)	Uchida et al (9)
CS040521	IVS4 ds +1 G-A (c.414+1G>A)	Matsumoto et al (10)
CS040522	IVS6 ds +1 G-A (c.686+1G>A)	Matsumoto et al (10)
CS058243	IVS6 as -2 A-G (c.687-2A>G)	van Dorland et al (8)
CS040523	IVS10 ds +2 T-G (c.1244+2T>G)	Matsumoto et al (10)
CS126173	IVS11 ds -1 G-C	van Dorland et al (8)
CS044745	IVS11 as -1 G-A	Veyradier et al (11)
CS013075	IVS13 ds +5 G-A (c.1584+5G>A)	Levy et al (12)
CS123086	IVS15 ds +1 G-A (c.1786+1G>A)	Prestidge et al (13)

Discussion

Here, we describe the clinical course of a patient with congenital TTP in whom we identified the underlying genetic abnormality. The c.2728C>T (p.Arg910X) mutation found in our patient was previously reported and its functional effect was shown. However, the c.2610+5G>A mutation, which affects exon 20 and encodes for the ADAMTS13's thrombospondin type-1 fifth domain, is novel. As shown, it is predicted to interfere with normal *ADAMTS13* mRNA splicing but future studies are needed to confirm the functional effect of this substitution. An accurate splicing of pre-mRNA is a serious stage in protein translation and post-transcriptional regulation. Consensus "cis-acting" splicing sequences existence in exon-intron boundaries is essential for recognition by splicing machinery. By creating new splicing sites, splicing regulatory sequences, disruption of existing splice sites, or activating the cryptic splicing sites,

Table 2. The laboratory findings of the case at the time of admission

Hematological tests	(Normal range)	Serological tests	(Normal range)
PLC/mm^3	22 000	Creatinine (mg/dL)	1.1
WBC/mm^3	8800	URA (mg/dL)	44(19-44)
M.C.V (fl)	79 (77-97)	Sodium (meq/L)	144
M.C.H (pgm)	26 (26-32)	Potassium (meq/L)	4.4
M.C.H.C (%)	34 (32-36)	ALP (IU/L)	107
SGOT (IU/L)	29 (0-31)	Calcium (mmol/L)	1.07 (1.13-1.30)
SGPT (IU/L)	19 (0-41)	Phosphors (mg/dL)	2.7 (2.6-4.5)
Bilirubin, Total (mg/dL)	3.7 (0.1-1.2)	LDH level (U/L)	2772
Bilirubin, Direct (mg/dL)	0.8 (0-0.4)	PTT (seconds)	33
Bilirubin, Indirect (mg/dL)	2.9 (0.1-0.8)	PT (seconds)	13
Hemoglobin	12.3 mg/mL		

PLC: platelet count, WBC: white blood cell count, LDH: lactate dehydrogenase, ALP: Alkalin phosphatase, PTT: partial thromboplastin time, MCV: mean corpuscular volume, PT: prothrombin time.

mutations on these sequences can result in an aberrant transcript of the mutated gene or inappropriate removal of intron, and modifications of the open reading frame. The impact of the specific mutation needs to be confirmed with functional studies (7).

Congenital TTP is an autosomal recessive disorder. Thus, mutations have to affect both alleles of *ADAMTS13*. Unfortunately, DNA from patient's parents was not available to assess the segregation of the c.2728C>T and the c.2610+5G>A. However, his ADAMTS13 activity was less than 10%, leading us to reasonably assume that the two mutations are carried on different chromosomes, and to speculate that the c.2610+5G>A disrupts the correct splicing of *ADAMTS13* transcript.

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Authors' contribution

MRA was the principal investigator of the study who prepared the concept and design. BZ and SZV prepared the draft of the manuscript. AC and JE revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. The patient gave the consent to publish as a case report.

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References

1. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem. 2001;276:41059-63. doi 10.1074/jbc.C100515200
2. Schneppenheim R, Budde U, Oyen F, Angerhaus D, Aumann V, Drewke E, et al. von Willebrand factor cleaving protease and ADAMTS13 mutations in childhood TTP. Blood. 2003;101:1845-50. doi:10.1182/blood-2002-08-2399
3. Romao de Souza V, Jr., Beatriz Cavalcante de Oliveira A, Maria Vanderlei A, Queiroz da Mota Silveira Aroucha A, Pontes Duarte B, Nunes Machado A, et al. Inherited thrombotic thrombocytopenic purpura mimicking immune thrombocytopenic purpura during pregnancy: a case report. J Med Case Rep. 2018;12:15. doi:10.1186/s13256-017-1545-3
4. Moatti-Cohen M, Garrec C, Wolf M, Boisseau P, Galicier L, Azoulay E, et al. Unexpected frequency of Upshaw-Schulman syndrome in pregnancy-onset thrombotic thrombocytopenic purpura. Blood. 2012;119(24): 5888-97. doi:10.1182/blood-2012-02-408914
5. Burge C, Karlin S. Prediction of complete gene structures in human genomic DNA. J Mol Biol. 1997;268:78-94. doi:10.1006/jmbi.1997.0951
6. Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009;37:e67. doi:10.1093/nar/gkp215
7. Anna A, Monika G. Splicing mutations in human genetic disorders: examples, detection, and confirmation. J Appl Genet. 2018;59(3):253-268. doi: 10.1007/s13353-018-0444-7
8. van Dorland HA, Taleghani MM, Sakai K, Friedman KD, George JN, Hrachovinova I. The International Hereditary Thrombotic Thrombocytopenic Purpura Registry: key findings at enrollment until 2017, Haematologica. 2019;104:2107-15. doi: 10.3324/haematol.2019.216796.
9. Uchida T, Wada H, Mizutani M, Iwashita M, Ishihara H, Shibano T, et al. Identification of novel mutations in ADAMTS13 in an adult patient with congenital thrombotic thrombocytopenic purpura. Blood. 2004;104:2081-3. doi: 10.1182/blood-2004-02-0715
10. Matsumoto M, Kokame K, Soejima K, Miura M, Hayashi S, Fujii Y, et al. Molecular characterization of ADAMTS13 gene mutations in Japanese patients with Upshaw-Schulman syndrome. Blood. 2004;103:1305-10. doi: 10.1182/blood-2003-06-1796
11. Veyradier A, Lavergne JM, Ribba AS, Obert B, Loirat C, Meyer D, et al. Ten candidate ADAMTS13 mutations in six French families with congenital thrombotic thrombocytopenic purpura (Upshaw-Schulman syndrome). J Thromb Haemost. 2004;2:424-9.
12. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413:488-94. doi:10.1038/35097008
13. Prestidge TD, Rurali E, Wadsworth L, Wu JK, Moore JC, Bresin E. Congenital thrombotic thrombocytopenic purpura (cTTP) with two novel mutations. Pediatr Blood Cancer. 2012;59:1296-8. doi:10.1002/pbc.24159