

CASE REPORT

Protein S gene mutation c.946C > T (p.R316C) contributed to ischemic stroke in a man with von Willebrand disease type 3 caused by two novel VWF gene mutations, c.2328delT (p.A778Lfs* 23) and c.6521G > T (p.C2174F)

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Abstract

The risk factors for a family with VWD presenting with an ischemic stroke (IS) were explored. FVIII activity (FVIII:C), VWF antigen (VWF:Ag), and protein S activity were measured. Next generation sequencing (NGS) was performed targeting *F8*, *F9*, *VWF*, *PROC*, and *PROS1*. Sanger sequencing validation was performed on family members. The proband and his sister both had low FVIII:C (1 IU/dL) and VWF:Ag (3 IU/dL) levels, confirming the diagnosis of type 3 VWD. His father had nearly normal levels of FVIII:C (58 IU/dL) and VWF:Ag (57 IU/dL). His daughter had type 1 VWD with decreased FVIII:C (46 IU/dL) and VWF:Ag (19 IU/dL). NGS identified a heterozygous VWF c.2328delT (p.A778Lfs*23) frame shift mutation only in the proband and his sister. Another VWF missense mutation, c.6521G > T (p.C2174F), was found heterozygous in all members studied. A *PROS1* mutation, c.946C > T (p.R316C), previously reported to relate to ischemic stroke, was found heterozygous in the patient, his father, and his daughter. Only the proband and daughter have a slightly decreased plasma protein S level. This may be the first case with type 3 VWD with severe VWF/FVIII deficiency presented with ischemic stroke contributed to by a protein S defect.

KEYWORDS

gene mutation, ischemic stroke (IS), protein S (PS), von willebrand disease (VWD)

Baolai Hua, Xiaobo Yan, Bin He contributed equally to this study.

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1 | INTRODUCTION

von Willebrand factor (VWF) is a multimeric plasma glycoprotein that plays a central role in hemostasis. In circulation, VWF can bind to collagen at sites of vascular injury, mediates platelet adhesion and aggregation, and serves as a carrier protein for coagulation factor (F) VIII. von Willebrand Disease (VWD), the most common inherited bleeding disorder, is caused by quantitative (type 1 and 3) or qualitative (type 2) deficiency of VWF activity and is characterized mainly by mucosa-associated bleeding and bleeding after surgery and trauma.^{1,2} Several studies have shown that individuals with high VWF levels have an increased risk of coronary heart disease (CHD) and ischemic stroke (IS),^{3,4} by contrast, patients with VWD may have a reduced risk of arterial thrombosis.⁵ Here, we report an interesting case in which the proband with type 3 VWD experienced an IS, the clinical risk factors for artery thrombosis, as well as gene mutations using next generation sequencing (NGS) method were explored. One protein S mutation reported to cause IS was also found.⁶

2 | PATIENTS AND METHODS

2.1 | Case report

A 44-year-old male patient was admitted to the hospital with history of melena for 12 days. Bleeding history included recurrent epistaxis and hematuria since age 3 years. A diagnosis of type 3 VWD was made at age 15 years based on low factor VIII activity (FVIII:C), VWF antigen (VWF:Ag), and VWF multimers assays. On admission, the patient was alert and oriented, with normal strength, sensation, gait, and coordination. Complete blood count showed normocytic anemia (hemoglobin 74 g/L), normal white cell (and differential), and platelet counts. He had prolonged activated partial thromboplastin time (APTT) (89.4 s (s) [reference range (RR), 28.0–44.0 s]) with greatly decreased FVIII:C (1 IU/dL) and VWF:Ag (3 IU/dL) (Table 1). Abdominal ultrasound scanning revealed fatty liver, normal gallbladder, spleen, pancreas, and kidneys. Four hundred ml of virus inactivated fresh frozen plasma (FFP) was transfused, and his VWF:Ag increased to 6 IU/dL. On Day 2 after admission, the patient abruptly experienced numbness and weakness in his right limb. On physical examination, he was alert and oriented, but had mild weakness (grade 4) in the left limb. The Babinski sign was negative. Cerebral MRI scanning confirmed acute infarction in multiple sites of the left brain, as well as encephalomalacia focus in the left frontal cortex (Figure 1).

TABLE 1 Coagulation and anticoagulation parameters and gene mutation

Unit	Gender	Age	BAT score	Arterial thrombosis	Coagulant		Anticoagulant		APC-R	VWF gene	PROS1 gene
					FVIII:C	VWF:Ag	PS	PC			
RR					IU/dL	IU/dL	%	%			
					60–150	50–160	76–135	70–140	>2.1	p.A778Lfs*23	p.R316C
Proband	M	44	16	IS	1	3	58	100	2.9	Het	Het
Older sister	F	47	13	N	1	3	128	111	2.9	Het	No
Father	M	79	0	N	58	57	88	104	3.0	No	Het
Daughter	F	16	0	N	46	19	72	100	2.9	No	Het

Abbreviations: APC-R, activated protein C resistance; AT, antithrombin III; BAT, International Society for Thrombosis and Hemostasis (ISTH) bleeding assessment tool; FVIII, C Factor VIII activity; Het, Heterozygous; Homo, homozygous; IS, ischemic stroke; PC, protein C; PS, protein S; RR, reference range; VWF, Ag von Willebrand factor antigen.

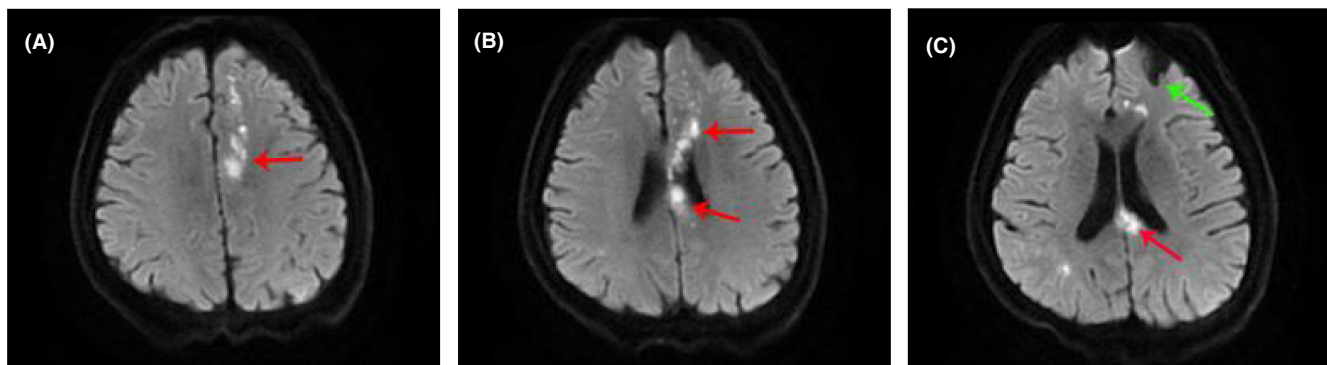


FIGURE 1 MRI images showing acute cerebral infarction in multiple sites of the left brain: in the semioval center (A) the Corpus Callosum (B and C) as well as old encephalomalacia in the left frontal cortex (C). Red arrows point to the acute cerebral infarction areas; green arrow points to the old encephalomalacia area

Transcranial doppler (TCD) showed severe stenosis of the right middle cerebral artery. Ultrasound doppler for vertebral and carotid arteries was normal. Computed tomography angiography (CTA) was not performed.

The patient had a more than 10 years history of smoking, alcohol consumption, hyperlipidemia, and hypertension. His hypertension was not well controlled despite treatment (for over 10 years) with metoprolol, nifedipine, and valsartan.

The patient was treated with infusion of FFP, cryoprecipitate, and packed erythrocytes. The patient's muscle strength recovered, but the left limb numbness and weakness remained at discharge 7 days later.

His father denied excessive bleeding and thrombosis but was on clopidogrel for carotid artery stenosis (up to 90%) and had undergone 2 uneventful major surgical procedures (gastric perforation repair and abdominal aortic aneurysm stenting) without excessive bleeding. His mother underwent tumor resection and splenectomy without excessive bleeding. Case record, however, did show that both his father and mother had a prolonged bleeding time, and a positive aspirin tolerance test.

His sister had a history of hypertension and excessive bleeding with menorrhagia. His daughter denied history of excessive bleeding.

The laboratory results and bleeding score of the patient and his family members are listed in [Table 1](#).

2.2 | Methods

The study was approved by the Ethics Review Committee of the Clinical Medical College, Yangzhou University, and was conducted in accordance with the Declaration of Helsinki. Written informed consents were obtained from the proband and his family members for the study.

2.2.1 | Routine coagulation tests

FVIII activity (FVIII:C) was measured by the one-stage APTT-based method, VWF:Ag was detected by immunoturbidimetric assay, antithrombin III (AT III), and protein C (PC) were assayed by colorimetric method, and protein S (PS) by clotting assay. All tests were carried out using the Stago STA-R automated coagulation analyzer using commercial reagents from Stago.

2.2.2 | NGS and sanger sequencing

NGS was performed targeting all exons and intronic flanking regions of the 128 genes, including *F8* (OMIM: *300841, NM_000132.3), *F9* (OMIM: *300746, NM_000133.3), *VWF* (OMIM: *613160, NM_000552.4), *PROC* (NM_000312.3), *PROS1* (NM_000313.3) genes.

DNA libraries preparation, targeted genes enrichment, and sequencing, bioinformatics analysis, variants selection were all carried out by MyGenostics.

Sanger sequencing validation was also performed on his family members.

2.2.3 | Bleeding score

Bleeding score was assessed using ISTH/SSC bleeding assessment tool.^{7,8}

3 | RESULTS

The results of coagulant and anticoagulant proteins are summarized in [Table 1](#). The proband had a low FVIII:C (1 IU/dL) and VWF:Ag (3 IU/dL) levels, consistent with his previous test results, and confirming the diagnosis of

type 3 VWD. His sister had a similarly low FVIII:C (1 IU/dL) and VWF:Ag (3 IU/dL) results, but their father has a nearly normal FVIII:C (58 IU/dL) and VWF:Ag (57 IU/dL) levels. His daughter has type 1 VWD with decreased FVIII:C (46 IU/dL) and VWF:Ag (19 IU/dL). The ISTH bleeding score was 16 for the patient, 13 for his sister, and was 0 for both his father and his daughter.

NGS analysis identified a heterozygous *VWF* c.2328delT (p.A778Lfs* 23) (exon18, NM_000552) frame shift mutation in the proband and his older sister, but not present in his father and daughter. Another *VWF* missense gene mutation, c.6521G > T (p.C2174F), were found heterozygous in each of the family members studied. No mutation was found in the *F8* or *F9* gene in this family. We were unable to obtain blood from the patient's mother for our phenotypic and genotypic studies.

The acute ischemic stroke was confirmed in the proband by the symptoms, signs, and cerebral MRI imaging study (Figure 1).

The patient was negative for ANA, ds-DNA, and anti-phospholipid antibody. Thrombophilia study showed all studied family members to have normal levels of AT-III activity and PC activity as well as negative activated protein C resistance (APC-R) (Table 1). PS level was slightly decreased in the proband (58%, RR 76%–135%) and his daughter (72%) (Table 1), but normal in the other 2 family members. The results on these anticoagulant proteins were also confirmed by another reference laboratory (Peking Union Medical College Hospital).

To further explore the risk factors for thrombosis, NGS was carried out and found that the patient, his father, and his daughter were all heterozygous for a PS gene (*PROS1*) mutation, c.946C > T (p.R316C) (Table 1).

4 | DISCUSSION

NGS has been widely used in the detection of bleeding disorders including VWD.^{9–11} In our case, we used NGS to screen the genetic defects for VWD and arterial thrombosis in the available family members, and found two novel *VWF* gene mutations, c.2328delT (p.A778Lfs* 23) and c.6521G > T (p.C2174F), and a *PROS1* mutation, c.946C > T (p.R316C) previously shown to relate to IS.⁶

The type 3 VWD phenotype of the patient and his sister is related to their double heterozygosity for 2 different novel *VWF* mutations, with *VWF* c.2328delT (p.A778Lfs* 23) from their father and *VWF* c.2328delT (p.A778Lfs* 23) mutation presumably from their mother. *VWF* c.2328delT (p.A778Lfs* 23) mutation was assumed likely pathogenic according to the ACMG guideline although their mother denied having a history of bleeding diathesis. This mutation was not transmitted to the

patient's daughter. The *VWF* c.6521G > T (p.C2174F) mutation present in the patient's father was transmitted to the patient as well as the patient's sister and his daughter.

Type 3 VWD is autosomal recessive inherited bleeding disorder with interaction of two recessive genes to confer the severe quantitative phenotype. Amino acid 778 is located at the D'-D3 region of the VWF protein and serves as the FVIII binding site.¹¹ Small deletion in the *VWF* gene, c.2328delT (p.A778Lfs* 23), causes a frame shift, leading to a VWF defect. The amino acid 2174 is located at the C1 domain which includes the "RGDS" (Arg - Gly - Asp - Ser) site that binds to platelet GPIIb/IIIa.¹² The missense genetic defect, c.6521G > T (p.C2174F), identified was heterozygous in the proband's father who had normal levels of VWF:Ag and FVIII:C with no history of bleeding diathesis. This mutation, however, did contribute to type 3 VWD phenotype in his two (double) heterozygous children (interacting with c.2328delT mutation) and type 1 VWD (with low VWF:Ag) in his heterozygous granddaughter. The observations suggest this mutation may have incomplete penetrance. The alternative explanation is that the current level of VWF/FVIII results of this grandfather represent age-related increased levels from mild type 1 VWD levels which he might have at his younger age. Both mutations were not reported in VWF mutation database and are considered novel mutations.^{13,14}

PS is a vitamin K-dependent plasma glycoprotein that acts as a cofactor for activated protein C (APC) in the inactivation of the procoagulant factor (F) Va and FVIIIa.^{15,16} Protein S deficiency (PSD) is considered a risk factor for venous thrombosis.^{6,17} Its role in arterial thrombosis, such as arterial ischemic stroke, remains uncertain. Recently, a systematic review and meta-analysis suggested that PSD, was associated with a significant but small increase in the risk of arterial ischemic stroke in adults, particularly in young patients.¹⁷ In our case, the heterozygous protein S gene mutation (*PROS1*), c.946C > T (p.R316C), found in the proband as well as his father and daughter by NGS, and confirmed by Sanger sequencing, has been reported to be associated with ischemic stroke.⁶ Arg316 is located in the SHBG-liked domain of PS and is important in the interactions of PS with C4b-binding protein (C4BP) and with FV during the APC-mediated inactivation of FVIIIa.¹⁸ Arg275 is highly conserved among species. Its change to Cys would likely disrupt protein folding of PS and its function as a cofactor for APC.

Arterial ischemic stroke is a multicausal disease that involves complex interactions of genetic and environmental risk factors. The proband also had several other risk factors for artery thrombosis, such as hypertension, hyperlipidemia, and smoking in the setting of right cerebral artery stenosis, all of which may contribute to his ischemic stroke.

The cost of management of this complex patient in China is considerable for our patient, and included ~2100

RMB (~300USD) for routine blood work (including coagulation screening, CBC, chemistry) and coagulant/anticoagulant proteins assays and monitoring (including VWF workup, protein C, S, AT, etc), ~1500 RMB (~224USD) for all imaging studies (including ultrasound and MRI), ~2800 RMB (~418USD) for NGS and Sanger sequencing validation, ~3300 RMB (~490USD) for treatment blood products (include plasma, cryoprecipitate, and red cells), and ~2800 (~418USD) for hospital admission cost, to a total of about 12,500 RMB (~1865USD).

In conclusion, to the best of our knowledge, this may be the first case with type 3 VWD with severe VWF/FVIII deficiency presented with ischemic stroke contributed to by a protein S defect.

AUTHOR CONTRIBUTIONS

BLH and MCP designed the study. XBY and BH collected and interpreted the clinical data. LJS performed the coagulation and anticoagulation tests. XBY and BLH prepared the manuscript. MCP gave critical reviewed and revised the manuscript. All authors approved the manuscript.

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CONFLICTS OF INTEREST

The authors stated that they had no competing interests which might be perceived as posing a conflict or bias.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSENT

Written informed consent was obtained from the patient to publish this report.

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