Arteriosclerosis, Thrombosis, and Vascular Biology

BASIC SCIENCES

Acquired von Willebrand Syndrome Hiding Inherited von Willebrand Disease Can Explain Severe Bleeding in Patients With Aortic Stenosis

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OBJECTIVE: Aortic stenosis may be complicated by an acquired von Willebrand syndrome that rarely causes significant bleeding, raising the question of why it does so in a few cases. To seek an explanation, we studied 5 severe bleeder aortic stenosis patients in a cohort of 49 patients, using the flowchart for inherited von Willebrand disease.

APPROACH AND RESULTS: All 5 patients were lacking in large and intermediate VWF (von Willebrand factor) multimers, 3 had reduced plasma and platelet VWF levels, and none showed PFA100 closure. Two patients (those with most multimers missing) also had a short VWF half-life. Genetic analyses on the 3 patients with reduced platelet VWF levels revealed that one carried both the c.1164C>G and the c.7880G>A mutations, and another carried the c.3390C>T mutation, while the third had one of the 2 VWF alleles relatively less expressed than the other (25% versus 75%). No genetic alterations emerged in the other 2 patients. Successful replacement of the stenotic aortic valve, performed in the 2 patients with VWF mutations, did not correct their abnormal VWF multimer picture—unlike what happened in the aortic stenosis patients without bleeding symptoms.

CONCLUSIONS: Our findings suggest that acquired von Willebrand syndrome can develop in patients with hitherto-undiagnosed inherited von Willebrand disease. Since von Willebrand disease is the most common bleeding disorder, this possibility should be considered in aortic stenosis patients—especially those with a more severe bleeding history and more disrupted VWF laboratory patterns—because they risk hemorrhage during aortic valve replacement.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: acquired von Willebrand syndrome ■ aortic valve ■ aortic valve stenosis ■ mutation ■ von Willebrand diseases ■ von Willebrand factor

Aortic valve stenosis (AS) is the most common acquired heart valve disease, affecting 3% to 5% of the population over 65 years old.[1] It can be complicated by hemostatic abnormalities, including a higher risk of thrombotic events (stroke and myocardial infarction) and hemorrhages, the most common being nose and gastrointestinal bleeding (GIB).[2,3] An association between AS and GIB was first reported in 1958 by Edward J. Heyde, who described 10 cases of calcific AS and GIB of unclear origin.[2] Submucosal angiodysplasia was identified as the source of GIB in these patients.[4] A key advance in our understanding of the relationship between angiodysplasia and AS was made thanks to King et al, who found that GIB ceased after aortic valve replacement in 14 patients with AS.[5] Loss of large VWF (von Willebrand factor) multimers was then demonstrated in patients with congenital and acquired aortic stenosis.[6] Warkentin et al[7] first hypothesized that Heyde syndrome could be an acquired von Willebrand syndrome (aVWS) resembling inherited type 2A VWD.[8] Many articles confirmed this hypothesis, demonstrating that these patients' bleeding tendency is not restricted to the gastrointestinal tract.[9–11]

VWF is a large multimeric glycoprotein that mediates the adhesion of blood platelets at the site of vascular injury, also serving as a carrier of coagulation FVIII (factor VIII).[12,13] The main feature of VWF is its polymeric structure,

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The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.120.314656.

For Sources of Funding and Disclosures, see page 2193.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

AS	aortic stenosis
aVWS	acquired von Willebrand syndrome
VWF	von Willebrand factor
VWD	von Willebrand disease
GIB	gastrointestinal bleeding
ADAMTS13	a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
FVIII	factor VIII
VWF:Ag	VWF antigen
VWF:RCo	VWF ristocetin cofactor
VWF:CB	VWF collagen binding
VWFpp	VWF propeptide
ddPCR	digital droplet polymerase chain reaction

with oligomers ranging in size from 500 to 20 000 kDa, the largest forms having the greatest hemostatic capacity.[14,15] This explains why inherited VWF defects characterized by the lack of large multimers (VWD types 2A and 2B) are associated with the most severe bleeding diathesis. VWF is synthesized and stored in endothelial cells and megakaryocytes and, after its secretion, it undergoes proteolysis by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), which results in the VWF multimeric structure usually seen in plasma.[8,16] A defective proteolytic process may make patients susceptible to thrombotic events, as in thrombotic thrombocytopenic purpura,[17] with the appearance of ultralarge VWF multimers.[18] An excessive proteolysis, however, prompts the disappearance of large multimers and a tendency to bleed, as seen in the type 2A VWD form characterized by an increased susceptibility to ADAMTS13 (namely type 2A-II).[8,19] It has been suggested that the pathogenesis of aVWS in AS is associated with a greater susceptibility of VWF to ADAMTS13 when it passes through the stenotic valve,[20] because the locally elevated shear stress converts the globular VWF into an elongated, highly asymmetrical protein.[21,22] The change in the conformational state of VWF exposes cryptic exosites to ADAMTS13 binding, thus promoting VWF cleavage, with a consequent reduction in VWF multimer size and the disappearance of ultralarge multimers.[19,20] This hypothesis is confirmed by the observation that VWF defects are more pronounced in patients with the most severe transvalvular pressure.[9] Aortic valve replacement surgery usually completely solves any aVWS.[5,23–25]

Here, we report on the analyses conducted on 5 AS patients with severe bleeding complications in an effort to clarify the pathogenesis of their severe aVWS phenotype.

Highlights

- Some aortic stenosis patients with severe bleeding symptoms are actually undiagnosed cases of inherited von Willebrand disease on which acquired von Willebrand syndrome subsequently develops, exacerbating an already abnormal VWF pattern and bleeding tendency.
- A significant reduction in VWF half-life can also contribute to severely impaired VWF multimer patterns, probably via the removal of VWF at the site of angiodysplasia.
- Several VWF defects may thus be involved in the acquired von Willebrand syndrome of patients with aortic stenosis, and their tendency to bleed.

MATERIALS AND METHODS

Patients and normal subjects were studied in accordance with the Helsinki Declaration after obtaining their written informed consent.

Hemostatic Investigations

Basic hemostatic tests, and platelet function analysis (by PFA100), plasma VWF antigen (VWF:Ag), VWF ristocetin cofactor (VWF:RCo), and VWF collagen binding (VWF:CB) were performed as explained elsewhere.[26] VWF propeptide (VWFpp) was measured with a home-made ELISA assay that uses CLB-Pro 35 as the first antibody and CLB-Pro 14.3-HRP as the second (Sanquin, Netherlands). The values obtained were expressed in U/dL, taking as 100 the first dilution of the reference curve consisting of a pool of normal plasma samples.[27] VWF multimers were analyzed by electrophoresis on 1.6% and 2.2% high-gelling-temperature agarose containing 0.1% sodium dodecyl sulfate, under low- and high-resolution conditions, respectively.[28] Multimers were detected by autoradiography after reaction with purified anti-VWF antibody (DAKO, Denmark) labeled with ^{125}I and analyzed with an Epson DS-50000 scanner.

Genetic Analysis

Patients' genomic DNA was obtained from whole blood using the Maxwell 16 automated DNA extractor and the Maxwell 16 Blood DNA Purification kit (Promega, WI). Total RNA was extracted from patients' platelets with Trizol, and cDNA was generated using the Superscript III kit (Thermo Fisher Scientific, Carlsbad, CA) and random primers, according to the manufacturer's protocol. Polymerase chain reaction (PCR) reactions were performed using the Qiagen HotStartTaq Master Mix and AB2720 thermal cycler (Applied Biosystems, AB, Foster City, CA). The Big Dye Terminator Sequencing Kit v.1.1 (Perkin Elmer, Wellesley, MA) and an ABI 3130 XL genetic analyzer (AB) were used for Sanger sequencing.

Digital Droplet PCR

Digital droplet PCR (DdPCR) was performed in the QX200 ddPCR instrument (Bio-Rad, Hercules, CA) using the ddPCR Supermix

for Probes (Bio-Rad), primers 5′CCCTCCTGAAAGGTGAC3′ and 5′GTAGCTGAGGCGCAC3′ (that amplify the rs1800378 SNP containing region), and 2 different labeled probes designed to distinguish a patient's rs1800378 A and G allele (HEX-labeled 5′CGCATCCAGC***a***TACAGT3′ and FAM-labeled 5′CATCCAGC***g***TACAGTGA3′, respectively). DdPCR reactions were obtained in multiplex according to the standard protocol, starting from 10 ng cDNA, at T=58°C and adding 2% dimethyl sulfoxide to the PCR mix.

Statistical Analysis

The unpaired Student *t* test was used to compare VWF values in AS patients with and without severe bleeding symptoms.

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

RESULTS

Patients

Five patients with AS referring to our Hemostasis Center for severe bleeding symptoms between 2010 and 2017 were studied. They were selected from among 49 AS patients investigated during the same period because their hemorrhagic diathesis was more severe, their VWF levels were lower, their large VWF multimers were significantly reduced, and their VWF had failed to return to normal after aortic valve surgery. The 5 cases included 3 females and 2 males, between 69 and 95 years old (Table 1). Their aortic stenosis ranged from moderately severe to severe. Two patients (cases 1 and 4) underwent aortic valve replacement.

Bleeding Symptoms

All 5 patients suffered from GIB and hematomas; 3 had a history of bleeding after surgery (cases 2, 4, and 5); one of postpartum hemorrhage (case 5); one of gingival bleeding (case 2); and 2 of epistaxis (cases 1 and 4, requiring blood transfusion in the latter). Bleeding scores obtained with the ISTH Bleeding Assessment Tool[29] in 2 patients (cases 2 and 5) were high, at 10 and 8, respectively (vs normal range 0–5).

Hemostatic Pattern

Table 1 shows the patients' main hemostatic findings. They showed a prolonged PFA100 time, and low ratios of VWF:CB to VWF:Ag (VWF:CB ratio) and VWF:RCo to VWF:Ag (VWF:RCo ratio), regardless of their VWF:Ag levels (Table 1), which were lower than normal in cases 2, 3, and 4 (45.4, 18, and 34.1 U/dL, respectively, versus normal range 60–160 U/dL) but normal or even increased in the other 2 (Table 1). Platelet VWF:Ag content was measured as well, given the patients' severe hemostatic picture, and also to check for any inherited VWF defect. Three patients—cases 1, 3, and 4—had low platelet VWF level while they were at the lower end of normal range in case 5, and normal in case 2. Multimer analysis of plasma VWF was performed under low-resolution conditions (1.6% agarose gel) to better explore large VWF oligomer representation, and all patients were found lacking in large and intermediate VWF multimers (Figure 1). The greatest loss of VWF multimers was seen in cases 2 and 3, with only a few low-molecular-weight oligomers remaining (Figure 1). The 44 AS patients with only mild or no bleeding symptoms (Table 1) showed increased VWF:Ag and VWF function levels and slightly decreased VWF:CB and VWF:RCo ratios. These values differed significantly from those seen in the severe bleeder AS patients ($P<0.05$).

No ADAMTS13-Induced Proteolysis of VWF

VWF multimer analysis was also performed under high-resolution conditions (2.2% agarose gel) to see whether patients' VWF was more susceptible to the action of ADAMTS13[20] due to the high shear stress conditions induced by their AS. The aim was to better discriminate the VWF oligomer satellite bands, which are the products of the action of ADAMTS13 on VWF. As shown in Figure 2, there was no significant increase in VWF oligomer satellite banding, or in the relative representation of the low-molecular-weight multimers. This was also true in cases 2 and 3, where the loss of large and intermediate multimers was particularly severe. This finding seems to rule out the possibility of an increased VWF proteolysis being responsible for the large multimers' disappearance, unlike the picture seen in inherited type 2A VWD, which is characterized by a greater susceptibility of VWF to ADAMTS13 (2A-II)[9] (Figure 2).

Patients' VWF Survival

VWFpp and the associated VWFpp ratio (a simple way to explore VWF half-life) were measured.[27] The VWFpp ratio was normal or almost normal in cases 1, 4, and 5 (at 1.39, 1.1, and 1.2, respectively, versus normal 1.01±0.25) and higher than normal in cases 2 and 3 (2.4 and 10.3, respectively). The latter 2 patients had the most pronounced loss of large and intermediate VWF multimers. For comparison, 16 AS patients without bleeding symptoms all had a normal VWFpp ratio (mean 0.92±0.27). Interestingly, when case 2 was tested 2 days after the administration of VWF concentrates (Fandhi, 2000 U twice a day for 2 days, then 2000 U once a day for 3 days, followed by 2000 U twice a day for 2 days) was stopped, there was a significant further increase in the patient's VWFpp ratio (from 2.4 to 13.9). This coincided with a significant drop in VWF:Ag and VWF:CB levels, and VWF:CB ratio (26.3, 0.1, and 0.0038 U/dL, respectively) compared with those measured before starting VWF concentrates (Table 1). In case 4, the VWFpp ratio decreased from 1.1 to 0.37 two

Table 1. Main Hemostatic Findings of the AS Patients With Severe Bleeding Symptoms Compared With Patients Without Bleedings

Patients	Sex/ Age	Age	ABO Blood Group	Platelet Count (×10³/μL)	PFA100, s	aPTT, s	FVIII, U/dL	VWF:Ag, U/dL	VWF:RCo, U/dL	VWF:RCo ratio	VWF:CB, U/dL	VWF:CB, ratio	VWFpp ratio	Platelet VWF, U/dL	Mutation
1	M	68	A	235	>300	40.7	80.0	120.4	NP	NP	70.5	0.6	1.40	43.5	c.3390C>T (p.P1127_ G1180delinsR)
2	F	69	A	102	>198	36.0	45.7	45.4	15.7	0.3	7.1	0.2	2.40	79.5	NF
3	F	95	O	382	NP	37.1	20.5	18.0	4.6	0.3	1.3	0.1	10.30	56.5	Unbalanced allele expression
4	M	67	O	163	>300	39.7	101.9	34.1	26.2	0.8	21.0	0.6	1.10	12.0	c.1164C>G (p.C388W); c.7880G>A (p.C2627Y)
5	F	84	O	359	>300	36.1	181.8	265.0	179.3	0.7	132.5	0.5	1.20	67.7	NF
AS w/t bleeding	23/M; 21/F	75±15		NP	NP	31.3±7.9	192±75	191±89	161±81	0.8±0.1	145±66	0.8±0.2	0.92±0.27	NP	
Normal range				150–350	94–193	24–36	60–160	60–160	60–130	≥0.75	65–150	≥0.75	O 1.07–1.49; non-O 0.79–1.17	70.0–140.0	

Ag indicates antigen; AS, aortic stenosis; CB, collagen binding; F, female; FVIII, factor VIII; M, male; NF, not found; NP, not performed; Rco, ristocetin cofactor; VWF, von Willebrand factor; VWFpp, VWF propeptide; and w/t, without.

days after heart surgery, while the patient's VWF levels rose from 34.1 to 176 U/dL.

Hemostatic Patterns Failing to Return to Normal After Surgery

Aortic valve replacement surgery was performed in cases 1 and 4, with no significant perioperative bleeding. These patients were studied before the procedure and again 24 hours and 3 months afterwards. Table 2 shows the time courses of their VWF:Ag, VWF:CB levels, and VWF:CB ratios compared with 30 AS patients with no bleeding symptoms. The former 2 patients' VWF:CB ratios were significantly reduced on the day before surgery and did not return to normal on the day after surgery, despite a significant increase in VWF:Ag and VWF:CB levels. This picture differs from that of AS patients without bleeding symptoms, whose VWF:CB ratio increased after surgery (from a mean 0.79±0.1 before to 1.22±0.24 afterwards; Table 2), along with a return of the large multimers and the appearance of ultralarge forms. Such ultralarge multimers were never seen in the cases of AS with severe bleeding symptoms (Figure 3). Three months later, there was still no change in the abnormal VWF:CB ratios and no significant improvement in the multimer patterns for cases 1 and 4 (Figure 3).

Search for VWF Mutations

Since their severe VWD phenotype and clinical pattern, reduced synthesis of VWF, and failure to return to a normal multimer pattern after successful aortic valve surgery suggested an inherited bleeding disorder, the patients' VWF gene was explored. VWF mutations were found in 2 of the 5 patients investigated (cases 1 and 4). One (case 1) had the c.3390C>T mutation at heterozygous level, a synonymous substitution that prompts the skipping of exon 26 at mRNA level by altering the strength of an exon splicing silencer motif. The exclusion of exon

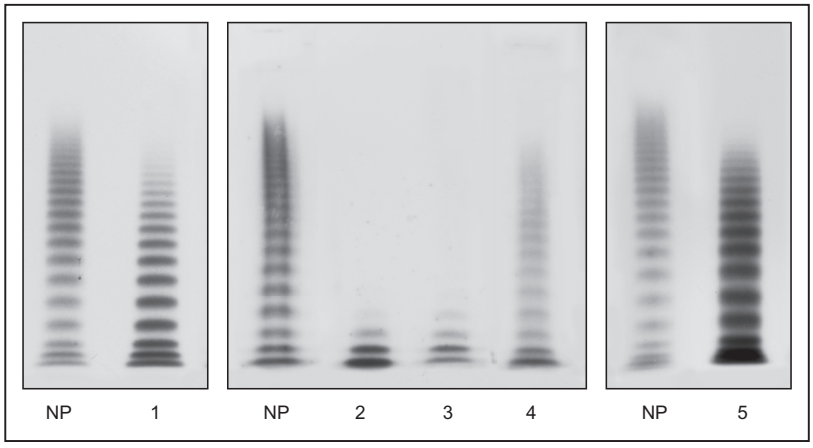

Figure 1. VWF (von Willebrand factor) multimer patterns observed in the 5 aortic stenosis (AS) patients studied compared with normal plasma (NP).
Large VWF multimers are at the **top**, small ones at the **bottom**. The analysis was performed under low-resolution conditions, using 1.6% agarose gel with 0.1% SDS, to better explore the presence of large VWF multimers. Oligomers were detected with ^{125}I-conjugated anti-VWF polyclonal antibody. Note the significant loss of large and intermediate VWF multimers in all patients, and the absence of almost all VWF in cases 2 and 3.

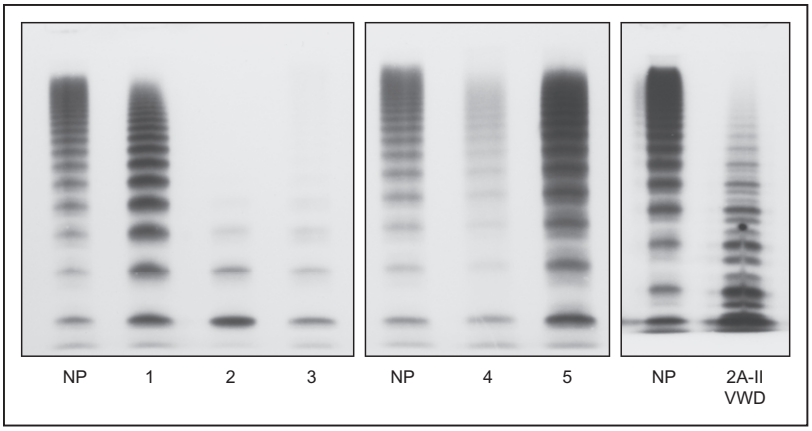

Figure 2. VWF (von Willebrand factor) multimer patterns obtained under high-resolution electrophoretic conditions (2.2% low-gelling-temperature agarose gel) to better explore the composition of each oligomer, and especially the presence of satellite bands, which are the products of the proteolytic action of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). The loss of large and intermediate multimers did not coincide with a greater representation of VWF satellite bands or accumulation of low-molecular-weight multimers (unlike what happens in inherited type 2A-II von Willebrand disease [VWD] variant, that is, characterized by a VWF more susceptible to ADAMTS13).

26 leads to an in-frame deletion of 159 bp, with the loss of 54 amino acids, and the insertion of an arginine (p.P1127_G1180delinsR),[30] (Figure 4). The other (case 4) carried both the c.1164C>G and the c.7880G>A mutations, which are responsible for the amino acid substitutions p.C388W and p.C2627Y, respectively (Figure 4). In silico prediction (with Polyphen-2 software) of the pathogenic role of the p.C388W and p.C2627Y mutations strongly identified them as damaging substitutions, with scores of 0.99 and 1.0, respectively, on a scale of 0 to 1. One patient (case 3) had no VWF gene mutations, but the electropherograms of her heterozygous common polymorphisms (rs1063857, rs1053523, and rs1800378) differed when genomic DNA or cDNA were analyzed. The double peak identifying each heterozygous SNP in the patient's genomic DNA shifted strongly towards one of the 2 nucleotides in her cDNA (Figure 4), a condition that may indicate a defective expression of one of the 2 VWF alleles. To test this hypothesis, the patient's VWF alleles were quantitatively analyzed by ddPCR, using labeled probes designed to discriminate between her rs1800378 A/G alleles. ddPCR showed that the rs1800378 G allele was significantly less represented than the A-allele (25% versus 75%, respectively), whereas no such difference in the relative representation of the 2 alleles was seen in a normal subject carrying the same SNP who was analyzed as a control, and showed 48% versus 52% for the A and G alleles, respectively. This would account for the biased double peak observed in the patient's cDNA. No mutations were found in the other 2 patients studied (cases 2 and 5).

DISCUSSION

In the present study, we demonstrate that AS patients with severe bleeding symptoms may be cases of hitherto-undiagnosed VWD, whose AS-related aVWS enhances the expression of an inherited VWF defect. We also found that a significant reduction in VWF half-life could contribute to the hemorrhagic diathesis but apparently not an increased proteolysis of VWF.

Most AS patients have an abnormal VWF multimer pattern, mainly involving a loss of large VWF multimers, but a few experience severe hemorrhagic symptoms. In an effort to understand why this happens, we studied the 5 most severe bleeders in our cohort of AS patients using the same flowchart that we use to characterize patients with VWD.

All 5 patients analyzed lacked large and intermediate VWF multimers, and sometimes even most of the VWF multimers—a picture resembling severe inherited forms of VWD. Although most AS patients usually have fewer large VWF multimers than normal, it is rare to find their VWF levels reduced as well (as seen in some of our patients). This combined quantitative and qualitative VWF impairment explains the pronounced hemorrhagic

Table 2. Main Hemostatic Findings Before and 24 Hours and 3 Months After Aortic Valve Surgery in AS Patients Carrying Inherited VWD, as Compared With AS Patients With No Significant Bleeding History and Normal Subjects

	Patient 1			Patient 4			Other AS (30 Subjects)			Normal Range
	0	24 h	3 mo	0	24 h	3 mo	0	24 h	3 mo	
FVIII, U/dL	80.0	160.0	118.0	101.9	281.3	109.1	141.6±62.4	176.0±0.6	200.0±54	60–160
VWF:Ag, U/dL	120.4	195.1	72.5	34.1	173.0	38.8	162.7±73	247.0±87.0	217.0±108.0	60–160
VWF:CB, U/dL	70.5	117.9	50.4	21.0	89.8	25.3	128.7±71.6	306.0±15.2	206.0±98.0	65–150
VWF:CB ratio	0.6	0.6	0.7	0.6	0.5	0.6	0.8±0.1	1.2±0.2	0.9±0.1	≥0.75
Large multimers	–	–	–	–	–	–	–	+	+	+
Ultralarge multimers	–	–	–	–	–	–	–	+	–	–

– indicates absent; +, present; Ag, antigen; AS, aortic stenosis; CB, collagen binding; FVIII, factor VIII; VWD, von Willebrand disease; and VWF, von Willebrand factor.

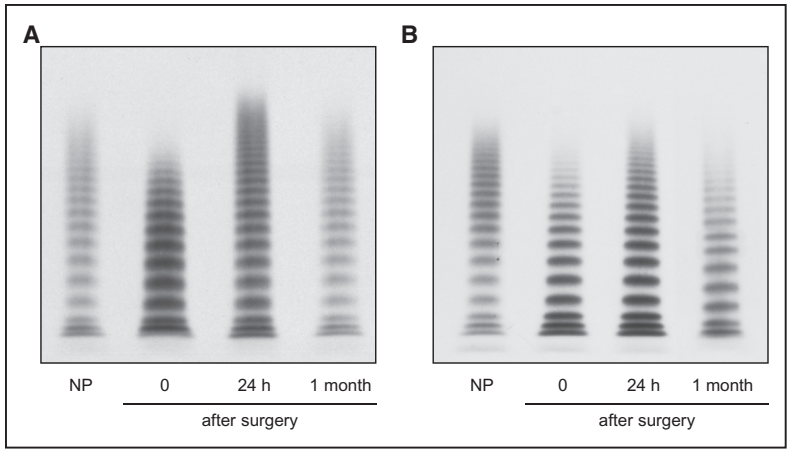

Figure 3. Time courses of plasma VWF (von Willebrand factor) multimer patterns before (0'), and 24 h and 3 mo after aortic valve replacement surgery.
An aortic stenosis (AS) patient with no significant bleeding symptoms and higher than normal plasma VWF levels (**left**, **A**), and case 1 carrying the c.3390C>T VWF mutation (**right**, **B**). Before surgery, both patients showed loss of large multimers compared with normal plasma (NP). Large multimers were restored and ultralarge components were visible 24 h after surgery in the patient in **A**, but not in case 1 in **B**. Three months later, the multimer pattern in case 1 was still almost identical to the one seen before surgery, while it was normal in the patient in **A**.

diathesis seen in the patients examined. As we do when exploring cases of inherited VWD, we measured platelet VWF content in the 5 patients with severe bleeding symptoms (a test never done in AS patients) because its value is not influenced by environmental or congenital factors (mainly ABO blood group and age) known to regulate plasma VWF levels. Three of our 5 cases revealed low platelet VWF levels, suggesting a defective VWF synthesis. Genetic analysis performed in the light of this finding revealed that they were carrying VWF genetic alterations. One had a synonymous substitution responsible for an abnormal VWF gene splicing that caused the skipping of exon 26.[30] Another patient had 2 amino acid substitutions (p.C388W and p.C2627Y) causing the loss of 2 cysteine residues in the propeptide (D2 domain) and C-terminal (C2 domain) regions, respectively[31]; the VWF cysteines are known to have a key role in VWF dimerization and multimerization processes. The third patient showed an unbalanced VWF allele expression that might explain her reduced plasma and platelet VWF

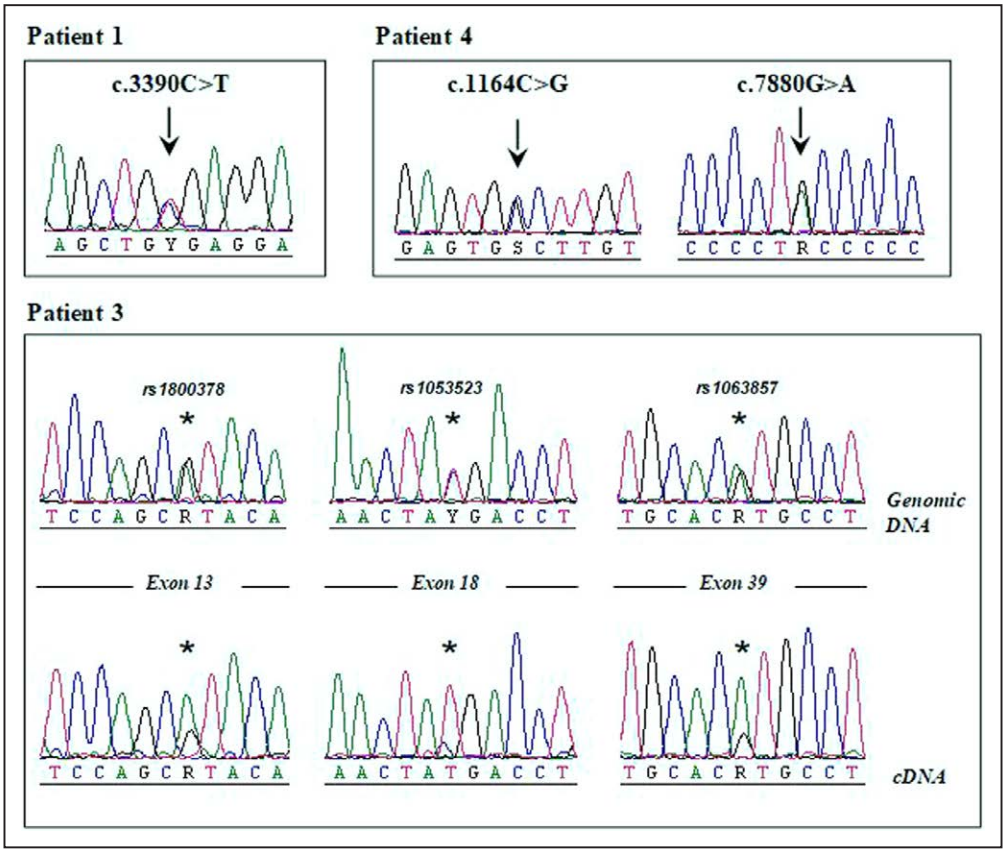

Figure 4. VWF (von Willebrand factor) genetic alterations in cases 1, 4, and 3.
The c.3390C>T VWF gene mutation (p.P1127_G1180delinsR) was found in case 1, and the c.1164C>G and c.7880G>A mutations (p.C388W and p.C2627Y, respectively) was found in case 4. No VWF mutations were identified for case 3, but the double peak identifying the patient's heterozygous SNPs rs1800378, rs1053523, and rs1063857 (*), in exons 13, 18, and 39, respectively, were strongly biased towards the A, T, and A SNPs in her cDNA, suggesting a differential expression of the 2 VWF gene alleles.

levels, although we were unable to identify any genetic alterations responsible for her mild form of VWD (which might originate from an alteration in the far promoter, an as yet uncharacterized regulatory element, or some form of miRNA interference).[32] An inherited VWD was thus revealed in these 3 patients by the onset of an acquired AS-related VWF defect that resulted in more severe bleeding symptoms. These patients had already experienced bleeding episodes in the past, but they had presumably not been severe enough to cause concern. These findings suggest that, among patients with AS, those with particularly severe bleeding and the most abnormal hemostatic patterns might actually have an inherited VWD or other hitherto-undiagnosed hemostatic disorder. AS is not uncommon, especially in the elderly, and VWD is the most common inherited bleeding disorder, so it is hardly surprising to find them in combination.

The failure of 2 patients' VWF patterns to return to normal after successful aortic valve replacement also points to the presence of an inherited VWF defect. The aVWS associated with AS almost always disappears quickly after surgery, ensuring a prompt restoration of the large VWF multimers.[10] This did not happen in the patients who had an inherited VWD behind their aVWS. Their reduced VWF:CB ratios and abnormal multimer patterns persisted despite successful valve surgery, exposing them to the risk of bleeding complications during and after the surgical procedure.[33,34]

The significant loss of large and intermediate VWF multimers in our patients (most of the VWF oligomers were missing in some cases) also prompted us to explore the contribution of VWF proteolysis to the development of aVWS. We found that proteolysis did not significantly influence the loss of VWF multimers, as we had already suggested.[10] Indeed, we found no accumulation of low-molecular-weight VWF multimers or greater representation of the satellite bands of each oligomer, as we might have expected if proteolysis by ADAMTS13 had been involved. We, therefore, speculate that a selective removal of large VWF multimers on the deranged aortic valve (lacking in endothelial cell lining and recognizable as a foreign surface) and/or VWF-platelet binding in the setting of shear-induced platelet aggregation contribute to the abnormal multimer pattern in AS.

The question of whether VWF survival is involved in aVWS is more complex, as 3 of our patients had a normal VWFpp ratio,[27] suggesting a normal VWF survival,[10] and the same picture was seen in AS patients with no bleeding symptoms. The other 2 patients with severe bleeding symptoms, however, had high VWFpp ratios, indicating a short VWF half-life. These 2 latter patients were those with the most severely abnormal VWF multimer patterns. Interestingly, one of them showed an even more dramatic increase in her VWFpp ratio after the infusion of VWF concentrates, associated with a further decrease in VWF levels. In other words, prophylaxis with VWF concentrates seemed to worsen the patient's VWF pattern, significantly reducing the survival of both exogenous and endogenous VWF. These findings suggest that an increased VWF clearance may contribute to the aVWS in some AS patients, especially when associated with a more severely abnormal VWF multimer pattern. The site where VWF is removed might well be the site of angiodysplasia, which—together with the deranged aortic valve—could be involved in the disappearance of VWF multimers and the development of the aVWS of AS.

Finally, there is the question of when we should suspect an inherited VWD lurking behind an acquired VWF defect in AS patients. When patients report significant bleeding symptoms, or reveal low VWF:Ag levels, or a significant decrease in VWF:CB or VWF activity ratios, there is reason to wonder if this might be the case. It is important to emphasize that lifelong bleeding episodes are not always reported, or may be considered unimportant—as in the case of our patients. But the picture changed when a second, acquired VWF defect further affected their hemostatic capacity.

In conclusion, our findings demonstrate that an undiagnosed inherited VWD may explain the severe bleeding symptoms experienced by some patients with AS. The number of patients studied was not enough to estimate the prevalence of combined inherited and acquired VWF defects in the most severe AS bleeders. A large-scale cohort of well-characterized patients will enable to estimate the true dimension of this phenomenon. Whatever the frequency, however, it is important to identify such patients to adopt the most appropriate strategies to avoid unexpected perioperative bleeding complications, since the strategies usually used may be useless, or even harmful in such cases. These patients can be identified by obtaining a detailed bleeding history, preferably administering the Bleeding Assessment Tool, and accurately studying patients' VWF laboratory patterns (especially platelet VWF and VWFpp, whenever possible).

ARTICLE INFORMATION

Received February 6, 2020; accepted June 23, 2020.

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Acknowledgments

A. Casonato designed the study, discussed the results, and wrote the article; E. Galletta performed the hemostatic tests; G. Cella discussed the results; G. Barbon performed genetic analysis; V. Daidone performed the genetic analysis, analyzed the data, and discussed the results.

Sources of Funding

This work was supported by a grant from MURST (Ministero dell'Università e della Ricerca Scientifico-Tecnologica; 60%, 2015).

Disclosures

None.

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