ADAMTS-13 and bleeding phenotype in von Willebrand disease

Boender, J.; Nederlof, A.; Meijer, K.; Mauser-Bunschoten, E.P.; Cnossen, M.H.; Fijnvandraat, K.; ... ; WiN Study Grp

Received: 5 March 2020 | Revised: 10 May 2020 | Accepted: 20 June 2020

DOI: 10.1002/rth2.12442

ORIGINAL ARTICLE

rpth research & practice in thrombosis & haemostasis

ADAMTS-13 and bleeding phenotype in von Willebrand disease

Johan Boender MD[1] | Angelique Nederlof MSc[1,2] | Karina Meijer MD, PhD[3] | Evelien P. Mauser-Bunschoten MD, PhD[4] | Marjon H. Cnossen MD, PhD[2] | Karin Fijnvandraat MD, PhD[5,6] | Johanna G. van der Bom MD, PhD[7,8] | Joke de Meris[9] | Britta A. P. Laros-van Gorkom MD, PhD[10] | Karin P. M. van Galen MD, PhD[4] | Jeroen Eikenboom MD, PhD[11,12] | Moniek P. M de Maat MSc, PhD[1] | Frank W. G. Leebeek MD, PhD[1] | for the WiN Study Group

[1]Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[2]Department of Pediatric Hematology, Erasmus MC, University Medical Center Rotterdam, Sophia Children's Hospital, Rotterdam, The Netherlands

[3]Department Hematology, University Medical Center Groningen, Groningen, The Netherlands

[4]van Creveld kliniek/Department Benign Hematology, University Medical Center Utrecht, Utrecht, The Netherlands

[5]Department of Pediatric Hematology, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

[6]Department of Plasma Proteins, Sanquin Research, Amsterdam, Netherlands

[7]Department Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

[8]Jon J van Rood Center for Clinical Transfusion Medicine, Sanquin Research, Leiden, The Netherlands

[9]Netherlands Hemophilia Society, Nijkerk, The Netherlands

[10]Department Hematology, Radboud University Medical Center, Nijmegen, The Netherlands

[11]Department Internal Medicine division Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands

Abstract

Background: The bleeding phenotype of von Willebrand disease (VWD) varies highly between patients and can only partly be explained by von Willebrand factor (VWF) parameters. By cleaving large VWF multimers into smaller, less active multimers, ADAMTS-13 is an important regulator of VWF activity. However, it is unknown what the role of ADAMTS-13 is in individuals with VWD.

Objectives: We therefore studied how ADAMTS-13 activity is associated with the laboratory and bleeding phenotype in individuals with VWD.

Methods: We measured ADAMTS-13 activity using the fluorescence resonance energy transfer substrate VWF 73 assay in 638 individuals with VWD in the nationwide cross-sectional Willebrand in the Netherlands study and in 36 healthy controls. The bleeding phenotype was assessed using the Tosetto bleeding score.

Results: ADAMTS-13 activity was similar in individuals with VWD (109% ± 20.6%) and controls (110% ± 19.7%). ADAMTS-13 activity was higher in individuals with VWD with type 3 than those with type 1 (mean difference, 11.8%; 95% confidence interval [CI], 2.9%-20.8%) or type 2 (mean difference, 16.1%; 95% CI, 7.1%-25.1%). ADAMTS-13 activity was not associated with the Tosetto bleeding score (0.1 Tosetto bleeding score increase per 10% ADAMTS-13 increase, 95% CI, −0.2 to 0.3). Furthermore, ADAMTS-13 activity did not differ between individuals with and without a bleeding event during the year preceding blood sampling (mean difference, 1.4%; 95% CI, −2.1% to 4.9%).

Conclusion: ADAMTS-13 activity was highest in individuals with type 3 VWD, but it had only minor associations with VWF parameters. ADAMTS-13 activity does not influence the bleeding phenotype in individuals with VWD.

[12]Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands

Correspondence
Prof. Frank W. G. Leebeek, MD, PhD.
Department of Hematology, Erasmus MC, University Medical Center Rotterdam, PO Box 2040, 3000 CA Rotterdam, The Netherlands.
Email: F.leebeek@erasmusmc.nl

Funding information
Stichting Haemophilia; CSL Behring; European Association for Haemophilia and Allied Disorders, Grant/Award Number: 2017 EAHAD

Handling Editor: Dr Neil Zakai.

KEYWORDS
ADAMTS-13 protein, human, ADAMTS-13 protein, blood coagulation disorders, von Willebrand diseases, von Willebrand factor

Essentials

- Mild reduction of ADAMTS-13 levels are associated with thrombosis, and may be associated with lower risk of bleeding.
- We studied how ADAMTS-13 is associated with the laboratory and bleeding phenotype in individuals with von Willebrand disease (VWD).
- ADAMTS-13 activity was associated with VWD type, but not with von Willebrand factor antigen or activity.
- ADAMTS-13 activity was not associated with the bleeding phenotype of individuals with VWD.

1 | INTRODUCTION

Von Willebrand factor (VWF) plays an important role in both primary and secondary hemostasis. By mediating platelet adhesion and aggregation at sites of vessel damage, VWF is essential in the formation of the platelet plug. Upon stimulation, endothelial cells secrete VWF as ultra-large multimers. These ultra-large multimers are highly procoagulant and, to prevent spontaneous thrombosis, must be cleaved into smaller, less procoagulant multimers. This cleavage is mediated by ADAMTS-13.

The importance of ADAMTS-13 in the regulation of VWF activity becomes evident in patients with thrombotic thrombocytopenic purpura, a potentially fatal disease in which a severe deficiency of ADAMTS-13 leads to uncontrolled and diffuse formation of microthrombi and microangiopathy.[1] Recently, large prospective population-based cohort studies have linked mild reductions in ADAMTS-13 to arterial thrombosis. In these studies, elderly individuals in the lowest quartile of ADAMTS-13 activity (≤ 81%) had a 49% higher risk of ischemic stroke and a 42% higher risk of coronary heart disease than individuals in the highest quartile of ADAMTS-13 activity during a 10-year follow-up period.[2,3] This increased risk was independent of VWF levels.

A deficiency or dysfunction of VWF causes the bleeding disorder von Willebrand disease (VWD).[4] In VWD type 2A, specific mutations in the *VWF* gene can lead to an increased susceptibility of VWF to cleavage by ADAMTS-13, causing absence of high-molecular-weight multimers and consequently impaired VWF function.

The bleeding phenotype of VWD is highly variable and can only partly be explained by differences in VWF level.[5] One promising candidate that may explain part of this variation is ADAMTS-13, but no studies have been performed yet on the association between ADAMTS-13 and bleeding. Only a few studies have been performed on ADAMTS-13 activity in VWD. In these studies, individuals with type 3 VWD had higher ADAMTS-13 activity than healthy controls, and ADAMTS-13 activity decreased following administration of desmopressin or VWF concentrate.[6,7]

Because ADAMTS-13 is an important regulator of VWF activity, we hypothesized that variations in ADAMTS-13 activity influence VWF parameters and the bleeding phenotype of VWD. We therefore measured ADAMTS-13 activity in a large group of well-defined individuals with VWD.

2 | METHODS

2.1 | Study population

We included individuals from the nationwide cross-sectional Willebrand in the Netherlands (WiN) study. Individuals were included in the WiN study if they had (1) a hemorrhagic diathesis or a positive family history for VWD, and (2) historically lowest VWF antigen (VWF:Ag) and/or VWF activity ≤ 30 U/dL or factor VIII coagulant activity (FVIII:C) ≤40 U/dL (for individuals with type 2N). Details of the study design have been reported elsewhere.[5] Individuals were excluded if they had also been diagnosed with another inherited bleeding disorder. For this project, we included only individuals from whom plasma was obtained at inclusion in the WiN study and excluded individuals who were pregnant (n = 4) or who had used desmopressin or clotting factor concentrates within 72 hours before blood sampling (n = 15). By excluding all individuals who had

received clotting factor concentrate within the past 72 hours before blood sampling, we eliminated potential effects of the small amounts of ADAMTS-13 present in plasma-derived clotting factor concentrates.[8]

Healthy controls were included in a study on the biological variation of hemostasis variables.[9] From 40 healthy individuals who were of similar age and sex distribution as the WiN study population, blood was collected during 13 visits over a 1-year period. One random sample per individual was used for this study.

Both studies were approved by the Medical Ethics Committees at all participating centers and written informed consent was obtained from all participants.

2.2 | ADAMTS-13 activity

Blood was collected in vacuum tubes containing 0.105 M sodium citrate and centrifuged, and the platelet-poor plasma was stored in aliquots at −80°C. ADAMTS-13 activity was measured using a kinetic assay based on the fluorescence resonance energy transfer substrate VWF 73.[10] In this assay, a fluorogenic peptide containing 73 amino acids of VWF that encompasses the cleavage site of VWF for ADAMTS-13 (Tyr1605-Met1606) was added to patient plasma. Fluorescence emitted by the cleaved substrate was then measured against a reference curve of serial dilutions of normal human plasma defined to have an ADAMTS-13 activity of 1 IU/mL and expressed as a percentage of this. The coefficient of variation was 10.6%.

2.3 | Other measurements

2.3.1 | Individuals with VWD

Other laboratory measurements, including VWF:Ag, VWF antibody activity (VWF:Ab), VWF to collagen binding (VWF:CB), FVIII:C, and VWF propeptide (VWFpp) were measured as previously described.[5,11,12]

VWF multimers were quantified by densitometry using ImageQuant TL, version 8.1 (GE Healthcare Life Sciences, Amersham Place, United Kingdom). For each individual, we calculated the VWF large-multimer index as described by de Jong et al,[13] which is a modification of the method introduced by Tamura et al.[14] Briefly, densitometry images were divided into the five smallest bands, intermediate (bands 6-10), and large (all other bands). The VWF large-multimer index was then calculated by dividing the area of the large and intermediate multimers over the total area, relative to the control sample from the same multimer blot. A VWF large-multimer index of 1 reflects a normal multimer pattern, whereas lower index values reflect an increasing relative deficiency of the intermediate and large VWF multimers.

The lifelong bleeding phenotype of the patients was determined using a self-administered condensed Tosetto bleeding score (BS) as previously described.[5] In short, 12 bleeding symptoms were systematically evaluated on occurrence and severity, with scores per symptom ranging from −1 or 0 to 4. Higher scores reflect more severe bleeding. The bleeding phenotype was additionally determined by patient-reported occurrence of bleeding requiring hemostatic treatment in the year before inclusion and blood sampling in the WiN study.[15]

2.3.2 | Healthy controls

VWF:Ag, VWF ristocetin cofactor (VWF:RCo), VWF:CB, and FVIII:C were measured as previously described.[9]

2.4 | Definitions

We classified every individual with VWD according to current guidelines.[4,16] Individuals with a VWF:Ab/VWF:Ag ratio >0.60 were classified as type 1 VWD, while those with a VWF:Ab/VWF:Ag ratio ≤0.60 were classified as type 2A or 2B or 2M VWD. Type 2 subtypes were further defined as abnormal multimer pattern with normal ristocetin-induced platelet agglutination (RIPA) test (2A); abnormal multimer pattern with abnormal RIPA test (2B); normal multimer pattern (2M); FVIII:C/VWF:Ag ratio ≤0.60 and reduced VWF:FVIIIB (2N). Individuals were classified as type 3 VWD if they had both VWF:Ag and VWFpp <5 U/dL. Individuals with *VWF* mutations consistently described in the literature as a certain VWD type were classified as such even if the laboratory phenotype suggested another VWD type.

This classification differs from previous publications from the WiN study. Notably, in this study we used a more stringent cutoff value for the VWF activity/antigen ratio (0.7 in previous publications) to distinguish type 1 and 2 VWD. Moreover, individuals had to have both VWFpp and VWF:Ag <5 U/dL (vs only VWF:Ag in previous publication) to be classified as having type 3 VWD.

Based on the causal *VWF* mutation, individuals with type 2A VWD were further divided into those with impaired multimerization and those with increased susceptibility to ADAMST-13–mediated cleavage.[17]

2.5 | Statistical analysis

ADAMTS-13 activity in healthy controls and patients with VWD was compared by independent *t* test. The association between ADAMTS-13 and continuous variables was examined using linear regression models. Differences in ADAMTS-13 activity between patients with different VWD types were assessed using linear regression models adjusting for age; type 2 subtypes were compared using the Kruskal-Wallis test and post hoc Mann-Whitney U test with Bonferroni correction. All analyses were performed using SPSS, version 21 (IBM Corporation, Armonk, NY, USA).

3 | RESULTS

3.1 | ADAMTS13 activity in individuals with VWD and healthy controls

Demographics of the 638 individuals with VWD and 36 controls are shown in Table 1. ADAMTS13 activity did not differ between individuals with VWD (mean, 109% ± 20.6%) and controls (mean, 110% ± 19.7%), 95% confidence interval (CI) −8.3%-5.5% (Figure 1A).

In the individuals with VWD, ADAMTS-13 activity was negatively associated with age and decreased by 2.1% per 10-year age increase (95% CI, 1.2-2.9). ADAMTS-13 activity was similar in men and women (mean difference, −0.58%; 95% CI, −3.9 to 2.7) and in blood group O versus non-O (mean difference, 0.91%; 95% CI, −2.4 to 4.2). In a subgroup analysis of type 1 VWD, there was no difference in ADAMTS-13 activity between the 270 blood group O individuals and 120 blood group non-O individuals (mean difference, 1.8%; 95% CI, −2.6 to 6.3); adjusting for age and sex had no impact on this analysis.

After adjusting for age, ADAMTS-13 activity was higher in individuals with type 3 VWD than in individuals with type 1 (mean difference, 11.8%; 95% CI, 2.9-20.8) or type 2 VWD (mean difference, 16.1%; 95% CI, 7.1-25.1); individuals with type 1 VWD had higher ADAMTS-13 activity than individuals with type 2 VWD (mean difference, 4.7%; 95% CI, 1.4-8.0) (Figure 1B). ADAMTS-13 activity also differed between the type 2 subtypes (χ^2 [3] = 13.2; P = .004); after Bonferroni correction, ADAMTS-13 activity differed only between types 2B and 2N (P = .02) and between types 2M and 2N (P = .02) with 2N individuals having higher ADAMTS-13 activity (Figure 1C).

3.2 | ADAMTS-13 activity and the laboratory phenotype of VWD

ADAMTS-13 activity was not associated with VWF:Ag, VWF:Ab, VWF:CB, or FVIII:C (Table 2). After adjusting for age, there was no association between ADAMTS-13 activity and these VWF parameters.

ADAMTS-13 activity was associated with VWFpp (age-adjusted 0.8% decrease per 10 U/dL VWFpp increase, 95% CI, 0.5-1.2); ADAMTS-13 activity was also associated with VWFpp in type 1 VWD (1.0% decrease per 10 U/dL VWFpp increase, 95% CI, 0.3-1.4) (Table 2).

After exclusion of individuals with type 3 VWD (ie, individuals without VWF multimers), ADAMTS-13 activity was associated with the VWF large-multimer index (0.8% increase per 0.1 point increase in VWF large-multimer index, 95% CI, 0.2-1.3) (Table 2). ADAMTS-13 activity was not associated with the VWF large-multimer index in individuals with type 1 VWD.

Type 2A VWD can be caused by mutations that lead to intracellular multimerization defects or that increase the susceptibility of VWF for proteolysis by ADAMTS-13. To test if ADAMTS-13 activity differs between these type 2A subgroups, we stratified individuals with type 2A into these two groups, based on the causal *VWF* mutation[17] (list of mutations in Table S1). After adjusting for age, ADAMTS-13 activity did not differ between 46 individuals with a mutation that causes increased proteolysis by ADAMTS-13 and 32 individuals with a mutation that impairs VWF multimerization rather than proteolysis (mean difference, −6.7%; 95% CI, −14.9 to 1.4).

3.3 | ADAMTS-13 activity and bleeding

When investigating all participants in the WiN study, ADAMTS-13 activity was not associated with the Tosetto BS, adjusting for age and sex (0.1 BS increase per 10% ADAMTS-13 activity increase; 95% CI, −0.2 to 0.3) (Figure 2A and Table 3). Similarly, ADAMTS-13 was not associated with the Tosetto BS in individuals with type 1 VWD (0.0 BS increase per 10% ADAMTS-13 activity increase, 95% CI, −0.3 to 0.3 after adjusting for age and sex). Adjusting additionally for VWF:Ab had no effect on the observed associations between ADAMTS-13 activity and the Tosetto BS (Table 3).

ADAMTS-13 activity was similar in individuals with VWD who had (n = 188) and individuals who had not had (n = 420) a bleeding episode requiring treatment during the year before inclusion in the WiN study

TABLE 1 Participant demographics

	VWD patients		Healthy controls		*P* value
Number	638		36		
Age, y	44	(29-57)	38	(25-53)	.23
Females	398	(62.4)	24	(66.7)	.61
VWF:Ag	29	(18-45)	103	(80-118)	<.001
VWF activity [a]	22	(8-52)	121	(104-146)	<.001
VWF:CB	22	(7-51)	163	(131-195)	<.001
FVIII:C	51	(32-73)	98	(83-118)	<.001
VWD type					
1	393	61.6	NA		
2	223	35.0	NA		
2A	130		NA		
2B	57		NA		
2M	22		NA		
2N	14		NA		
3	22	3.4%	NA		

Note: Categorical values are shown as n (%); continuous variables are shown as median (interquartile range).

P values were calculated with χ^2 test (categorical variables) or Mann-Whitney U test (continuous variables).

Abbreviations: FVIII:C, factor VIII coagulant activity; NA, not applicable; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ab, von Willebrand factor antibody; VWF:Ag, von Willebrand factor antigen; VWF;CB, von Willebrand factor to collagen binding; VWF:RCo, von Willebrand factor ristocetin cofactor.

[a]VWF activity was measured with VWF:Ab (individuals with VWD) or VWF:RCo (controls).

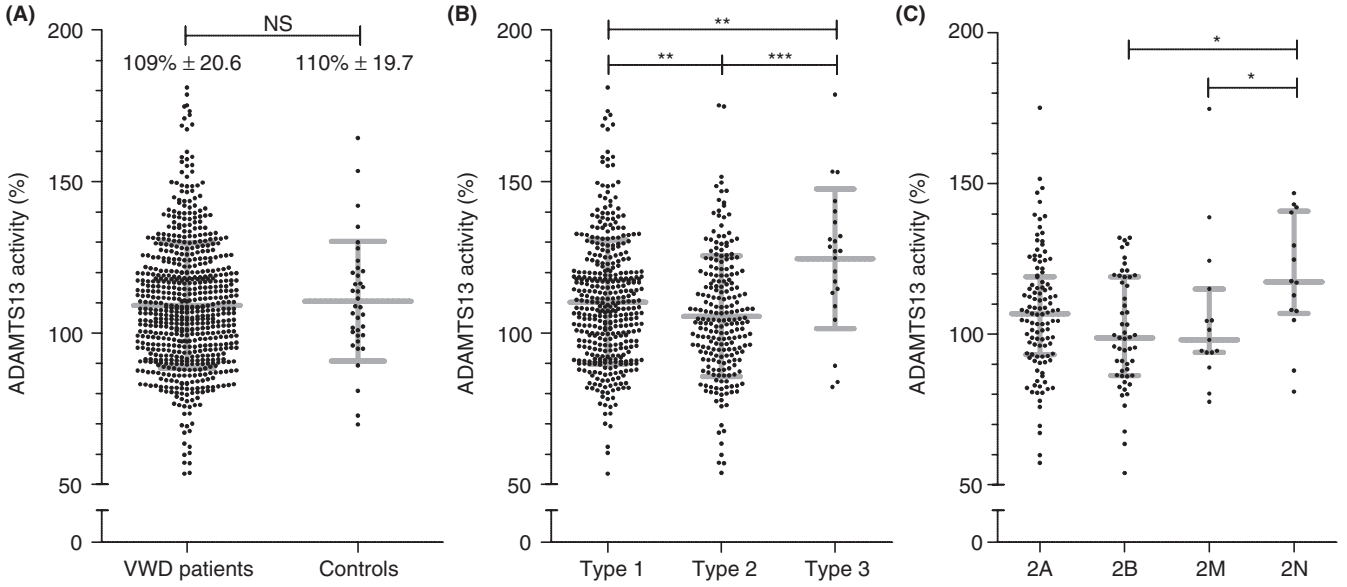

FIGURE 1 ADAMTS-13 activity in individuals with VWD and healthy controls A, ADAMTS-13 activity was similar in healthy controls and individuals with VWD (compared with independent *t* test). B, ADAMTS-13 activity was higher in individuals with type 3 than in type 1 or 2 VWD (compared using linear regression adjusting for age). C, ADAMTS-13 activity was higher in individuals with type 2N than in type 2B or 2M VWD (compared using Kruskal-Wallis and Mann-Whitney U test with Bonferroni correction). NS, not significant; $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. Gray lines depict mean ± standard deviation (A, B) or median and interquartile range (C)

(age- and sex-adjusted mean difference, 1.6%; 95% CI, −1.9 to 5.0) (Figure 2B and Table 4). Similarly, among patients with type 1 VWD, ADAMTS-13 did not differ between individuals with and without such a bleed (age- and sex-adjusted mean difference, 0.5%; 95% CI, −4.5 to 5.4). Adjusting additionally for VWF:Ab had no impact on these results.

4 | DISCUSSION

We hypothesized that variations in ADAMTS-13 activity could affect VWF parameters and subsequently influence the bleeding phenotype in individuals with VWD. In this first-ever study on ADAMTS-13 and bleeding, we found no such association.

To our knowledge, only three studies have thus far investigated ADAMTS-13 in individuals with VWD. In 2001, Mannucci et al[18] measured ADAMTS-13 activity in six individuals with type 1 Vicenza, five with type 3, and four with type 2A VWD. They found that the ADAMTS-13 activity measured in these individuals was in the normal range but did not provide further data. They also found a negative association between ADAMTS-13 activity and VWF:Ag (Pearson *r*, −.40) and VWF:CB (*r*, −.42). Their analysis included not only healthy individuals but also pregnant women and individuals with cirrhosis, chronic renal insufficiency, acute inflammatory states, and surgery, with extremely high VWF levels that sometimes exceeded 1000 U/dL.[18]

In a subsequent study, Mannucci and colleagues[6] compared ADAMTS-13 activity in 33 individuals with type 3 VWD and 33 healthy controls matched for sex, age, and blood group. In their study, the individuals with type 3 had a mean ADAMTS-13 activity of 136%, which was 38% higher than in healthy controls. They also observed unchanged ADAMTS-13 activity after desmopressin infusion in three individuals with severe VWD, which was in contrast to the 10%-20% ADAMTS-13 decrease after desmopressin infusion in 10 healthy volunteers in their study. Based on these results, they suggested that ADAMTS-13 levels are regulated by circulating VWF.[6]

In another study, Reiter et al[7] measured ADAMTS-13 activity after desmopressin infusion in three individuals with type 1 VWD and 10 healthy volunteers. They observed an ~60% decrease in ADAMTS-13 activity in both groups, which returned to baseline values after a few hours.

Our observation that ADAMTS-13 activity was not associated with VWF levels contradicts the association reported by Mannucci et al; this may be explained by an effect of severe disease on ADAMTS13 and VWF levels, rather than a direct interaction between the two proteins. In large population-based studies, ADAMTS-13 also was not associated with VWF.[2,19] The only "physiological" circumstances in which VWF and ADAMTS-13 activity are associated seem to be when VWF is absent (type 3 VWD, as observed by us and Mannucci et al[6]), or secretion of VWF following desmopressin (as observed by Reiter et al[7]). This is supported by the association between ADAMTS-13 and VWF propeptide as a marker of VWF secretion in the absence of an association between ADAMTS-13 activity and VWF antigen or activity.

Individuals with type 2N VWD had higher ADAMTS-13 activity than individuals with other type 2 subtypes. In individuals with hemophilia A, ADAMTS-13 decreases after FVIII infusion.[20] This suggests that differences in FVIII:C levels could explain the higher ADAMTS-13 activity in individuals with type 2N, but is contradicted by the absence of an association between ADAMTS-13 activity and

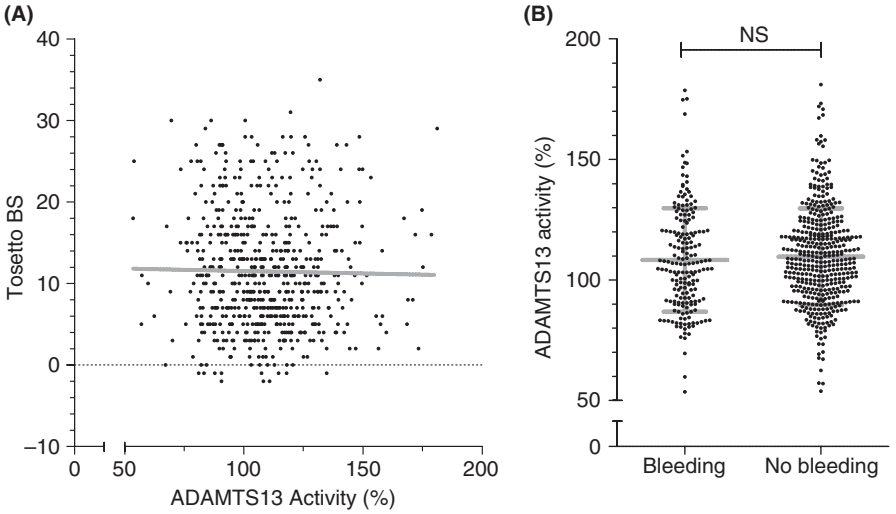

FIGURE 2 ADAMTS-13 activity and bleeding in individuals with VWD. A, Association between ADAMTS-13 activity and the Tosetto BS. The linear regression line is shown after adjusting for age and sex (−0.1 BS increase per 10% ADAMTS-13 activity increase, 95% CI −0.4 to 0.2). B, ADAMTS-13 activity in individuals with and without a bleeding episode requiring hemostatic treatment during a 1-year period. BS, bleeding score; NS, not significant. Lines depict linear regression line or mean ± standard deviation

FVIII:C in our study. Studies with more individuals with type 2N VWD are needed to confirm our finding and to explain the higher ADAMTS-13 activity in type 2N.

Interestingly, age was the most important parameter that was associated with ADAMTS-13 activity in the individuals with VWD. Age as a determinant of ADAMTS-13 in the general population is well established, having first been described by Kokame et al[10,21] and reproduced in large population-based studies.[2]

In our study, ADAMTS-13 activity was not associated with lifelong bleeding phenotype determined with the Tosetto BS and bleeding incidence during the year before blood draw. The absence of an association between ADAMTS-13 and bleeding may be explained by the properties of the VWF protein. The A2 domain of VWF, in which the Tyr1605-Met1606 cleavage site for ADAMTS-13 is located, needs to unfold before cleavage by ADAMTS-13 can occur.[22,23] This unfolding is dependent on stress exerted by the blood flow on VWF,[24] to which ultra-large VWF multimers are much more sensitive than smaller multimers.[25] It is likely that the decreased susceptibility of smaller VWF multimers for cleavage by ADAMTS-13 rather than ADAMTS-13 quantity is the rate-limiting step for further cleavage.

TABLE 2 ADAMTS-13 and VWF parameters

		Total VWD cohort (n = 638)		Type 1 VWD (n = 393)	
		B (95% CI)	Mean of VWF parameter	B (95% CI)	Mean of VWF parameter
VWF:Ag	Crude	−0.7 (−1.5 to −0.0)	33 IU/dL	−0.7 (−1.5 to 0.2)	38.3 IU/dL
	Adjusted	−0.3 (−1.0 to 0.5)		−0.2 (−1.2 to 0.7)	
VWF:Ab	Crude	−0.1 (−0.6 to 0.5)	34 IU/dL	−0.5 (−1.1 to 0.2)	47.6 IU/dL
	Adjusted	0.2 (−0.3 to 0.8)		−0.2 (−0.8 to 0.5)	
VWF:CB	Crude	−0.8 (−0.6 to 0.5)	32 IU/dL	−0.5 (−1.2 to 0.2)	44.9 IU/dL
	Adjusted	0.2 (−0.4 to 0.7)		−0.2 (−0.9 to 0.5)	
FVIII:C	Crude	−0.0 (−0.5 to 0.5)	56 IU/dL	0.1 (−0.5 to 0.8)	67 IU/dL
	Adjusted	0.3 (−0.2 to 0.8)		0.5 (−0.2 to 1.1)	
VWFpp	Crude	−1.0 (−1.3 to −0.6)	97 U/dL	−1.0 (−1.5 to −0.4)	94 U/dL
	Adjusted	−0.8 (−1.2 to −0.5)		−0.8 (−1.4 to −0.3)	
VWF large-multimer index	Crude	0.8[a] (0.2 to 1.3)	0.86	0.5[b] (−1.2 to 2.1)	1.0
	Adjusted	0.8[a] (0.2 to 1.3)		0.6 [b] (−1.1 to 2.2)	

Note: Table shows the association of ADAMTS-13 with VWF parameters with and without adjusting for age. B: change in ADAMTS-13 activity (%) per 10 U/dL increase of each VWF parameter or 0.1 VWF large multimer index increase. Significant associations are shown in *italics*.

Abbreviations: CI, confidence interval; FVIII:C, factor VIII coagulant activity; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ab, von Willebrand factor antibody; VWF:Ag, von Willebrand factor antigen; VWF;CB, von Willebrand factor to collagen binding; VWFpp, von Willebrand factor propeptide.

[a]After exclusion of type 3 VWD patients and failed densitometric analysis (n = 523).

[b]n = 319.

TABLE 3 ADAMTS-13 and Tosetto BS

		Total VWD cohort (n = 604)		Type 1 VWD (n = 377)	
		B (95% CI)	Mean Tosetto BS	B (95% CI)	Mean Tosetto BS
Tosetto BS	Crude	−0.1 (−0.4 to 0.2)	11.5	−0.1 (−0.5 to 0.2)	10.1
	Model 1	0.0 (−0.2 to 0.3)		0.0 (−0.3 to 0.4)	
	Model 2	0.1 (−0.2 to 0.3)		0.0 (−0.3 to 0.4)	

Note: Model 1, adjusted for age and sex; Model 2, adjusted for age, sex and VWF:Ab. B reflects change in Tosetto BS per 10% increase in ADAMTS-13 activity.

Abbreviations: BS, bleeding score; CI, confidence interval; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor antibody.

TABLE 4 ADAMTS-13 and bleeding incidence

		Total VWD cohort (n = 604)		Type 1 VWD (n = 377)	
		Mean difference	95% CI	Mean difference	95% CI
ADAMTS-13 activity	Crude	1.9	−1.7 to 5.4	1.1	−3.8 to 6.1
	Model 1	1.6	−1.9 to 5.0	0.5	−4.5 to 5.4
	Model 2	1.4	−2.1 to 4.9	0.5	−4.7 to 5.4

Note: Table shows the mean difference in ADAMTS-13 activity between individuals with VWD with and without a bleeding episode during the year before inclusion in the WiN study. A positive difference reflects a higher ADAMTS-13 activity in patients without a recent bleeding. Model 1, adjusted for age and sex; Model 2, adjusted for age, sex, and VWF:Ab.

Abbreviations: CI, confidence interval; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor antibody; WiN, Willebrand in the Netherlands.

Our observation that ADAMTS-13 is not associated with bleeding is in line with data from a recently published phase 1 trial for recombinant ADAMTS-13 in individuals with congenital thrombotic thrombocytopenic purpura. In this study, none of the 15 included individuals reported bleeding after regular infusions of recombinant ADAMTS-13.[26]

A number of limitations need to be mentioned. In this substudy, we used different classification criteria than in previous projects in the WiN study. While using the most contemporary criteria makes for a better extrapolation of our data, caution should be used when comparing our results with previous reports from the WiN study, especially those on the association between fibrinolysis and bleeding.[27,28] We have previously shown that the requirement of VWF propeptide levels <5 U/dL in addition to low VWF antigen and activity levels improves the classification of type 3 VWD.[11]

Moreover, it can be argued that the bleeding phenotype is insufficiently measured with the Tosetto BS, which has inherent limitations. It is a cumulative score with a so-called ceiling effect, and it is influenced by recall bias. However, we have addressed these issues by also determining the incidence of bleeding requiring hemostatic treatment during the year before inclusion in the WiN study. This bleeding incidence, which we consider a better reflection of the current bleeding phenotype, also was not associated with ADAMTS-13 activity.

In conclusion, ADAMTS-13 activity was higher in individuals with type 3 VWD, but it had only minor associations with VWF parameters. ADAMTS-13 activity does not influence the bleeding phenotype in individuals with VWD.

5 | WIN STUDY GROUP MEMBERS

Amsterdam UMC, Amsterdam: K. Fijnvandraat, M. Coppens, A. Kors, S. Zweegman; Netherlands Hemophilia Society, Nijkerk: J. de Meris; Amphia Hospital, Breda: G.J. Goverde, M.H. Jonkers; Catharina Hospital, Eindhoven: N. Dors; Maxima Medical Center, Eindhoven: L. Nieuwenhuizen; University Medical Center Groningen, Groningen: K. Meijer, R.Y.J. Tamminga; Kennemer Gasthuis, Haarlem: P.W. van der Linden; HagaZiekenhuis, The Hague: P.F. Ypma; Leiden University Medical Center, Leiden: J.G. van der Bom, J. Eikenboom, F.J.W. Smiers; Maastricht University Medical Center: B. Granzen, E. Beckers; Radboud University Medical Center, Nijmegen: P. Brons, B.A.P. Laros-van Gorkom; Erasmus University Medical Center, Rotterdam: M.H. Cnossen, F.W.G. Leebeek (principal investigator), J. Boender, F. Atiq; Van Creveld Kliniek, University Medical Center, Utrecht: E.P. Mauser-Bunschoten (chairman steering committee), K.P.M. van Galen.

ACKNOWLEDGMENTS

This project was supported by a 2017 EAHAD research grant. The WiN study is supported by research funding from the Dutch Hemophilia Foundation (Stichting Haemophilia) and CSL Behring

(unrestricted grant). A complete list of the members of the WiN study appears in the appendix.

RELATIONSHIP DISCLOSURE

JB started working at Sobi after finishing this research project. FWGL received research support from CSL Behring and Shire/Takeda for performing the WiN study, and from uniQure and Sobi for other studies. He is consultant for uniQure, Novo Nordisk, and Shire/Takeda, of which the fees go to the institution, and has received a travel grant from Sobi. He is also a DSMB member for a study by Roche. HCJE received research support from CSL Behring, and he has been a teacher on educational activities of Roche. KPMG received unrestricted research support from CSL Behring and Bayer and speakers fee from Takeda. JGB has been a teacher on educational activities of Bayer. MHC has received grants from governmental research institutes, such as the Dutch Research Institute (NOW), ZonMW, Innovation fund, and NWO-NWA and unrestricted investigator-initiated research grants as well as educational and travel funding from various companies over the years (Pfizer, Baxter/Baxalta/Shire, Bayer Schering Pharma, CSL Behring, Sobi Biogen, Novo Nordisk, Novartis, and Nordic Pharma) and has served as a member on steering boards of Roche and Bayer. All grants, awards, and fees go to the Erasmus MC as an institution. MM has received travel support and speaker fees from Roche Diagnostics, Sysmex, Siemens, and Werfen. The institution of KF has received unrestricted research grants from CSL Behring, Sobi, and Novo Nordisk, and her institution received consultancy fees from Grifols, Takeda, Novo Nordisk, and Roche. KM received research support from Bayer, Sanquin, and Pfizer; speaker fees from Bayer, Sanquin, Boehringer Ingelheim, BMS, and Aspen; and consulting fees from Uniqure, of which all fees go to the institution. BAPLG has received unrestricted educational grants from Baxter and CSL Behring. All other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

FWGL designed the study, interpreted data, and wrote the article. JB designed the study, analyzed and interpreted data, and wrote the article. MPMdM interpreted data and wrote the article. A. Nederlof performed experiments, analyzed and interpreted data, and wrote the article. KPMG interpreted data and critically revised the article. KM, EPM-B, MHC, KF, JGB, JM, BAPLG, and JE designed the study and critically revised the article.

ORCID

Johan Boender https://orcid.org/0000-0003-3415-6903
Jeroen Eikenboom https://orcid.org/0000-0002-3268-5759
Moniek P. M de Maat https://orcid.org/0000-0001-7749-334X
Frank W. G. Leebeek https://orcid.org/0000-0001-5677-1371

REFERENCES

1. Sadler JE. Pathophysiology of thrombotic thrombocytopenic purpura. Blood. 2017;130(10):1181–8.
2. Sonneveld MA, de Maat MP, Portegies ML, Kavousi M, Hofman A, Turecek PL, et al. Low ADAMTS13 activity is associated with an increased risk of ischemic stroke. Blood. 2015;126(25):2739–46.
3. Sonneveld MA, Kavousi M, Ikram MA, Hofman A, Rueda Ochoa OL, Turecek PL, et al. Low ADAMTS-13 activity and the risk of coronary heart disease - a prospective cohort study: the Rotterdam Study. J Thromb Haemost. 2016;14(11):2114–20.
4. Leebeek FW, Eikenboom JC. Von Willebrand's disease. N Engl J Med. 2016;375(21):2067–80.
5. de Wee EM, Sanders YV, Mauser-Bunschoten EP, van der Bom JG, Degenaar-Dujardin ME, Eikenboom J, et al. Determinants of bleeding phenotype in adult patients with moderate or severe von Willebrand disease. Thromb Haemost. 2012;108(4):683–92.
6. Mannucci PM, Capoferri C, Canciani MT. Plasma levels of von Willebrand factor regulate ADAMTS-13, its major cleaving protease. Br J Haematol. 2004;126(2):213–8.
7. Reiter RA, Knobl P, Varadi K, Turecek PL. Changes in von Willebrand factor–cleaving protease (ADAMTS13) activity after infusion of desmopressin. Blood. 2003;101(3):946–8.
8. Allford SL, Harrison P, Lawrie AS, Liesner R, MacKie IJ, Machin SJ. Von Willebrand factor–cleaving protease activity in congenital thrombotic thrombocytopenic purpura. Br J Haematol. 2000;111(4):1215–22.
9. de Maat MP, van Schie M, Kluft C, Leebeek FW, Meijer P. Biological Variation of Hemostasis Variables in Thrombosis and Bleeding: Consequences for Performance Specifications. Clin Chem. 2016;62(12):1639–46.
10. Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. Br J Haematol. 2005;129(1):93–100.
11. Sanders YV, Groeneveld D, Meijer K, Fijnvandraat K, Cnossen MH, van der Bom JG, et al. von Willebrand factor propeptide and the phenotypic classification of von Willebrand disease. Blood. 2015;125(19):3006–13.
12. Boender J, Eikenboom J, van der Bom JG, Meijer K, de Meris J, Fijnvandraat K, et al. Clinically relevant differences between assays for von Willebrand factor activity. J Thromb Haemost. 2018;16(12):2413–24.
13. de Jong A, Dirven RJ, Oud JA, Tio D, van Vlijmen BJM, Eikenboom J. Correction of a dominant-negative von Willebrand factor multimerization defect by small interfering RNA-mediated allele-specific inhibition of mutant von Willebrand factor. J Thromb Haemost. 2018;16(7):1357–68.
14. Tamura T, Horiuchi H, Imai M, Tada T, Shiomi H, Kuroda M, et al. Unexpectedly high prevalence of acquired von Willebrand syndrome in patients with severe aortic stenosis as evaluated with a novel large multimer index. J Atheroscler Thromb. 2015;22(11):1115–23.
15. Sanders YV, Giezenaar MA, Laros-van Gorkom BA, Meijer K, van der Bom JG, Cnossen MH, et al. Von Willebrand disease and aging: an evolving phenotype. J Thromb Haemost. 2014;12(7):1066–75.
16. Nichols WL, Hultin MB, James AH, Manco-Johnson MJ, Montgomery RR, Ortel TL, et al. Von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA). Haemophilia. 2008;14(2):171–232.
17. de Jong A, Eikenboom J. Von Willebrand disease mutation spectrum and associated mutation mechanisms. Thromb Res. 2017;159:65–75.
18. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. Blood. 2001;98(9):2730–5.
19. Crawley JT, Lane DA, Woodward M, Rumley A, Lowe GD. Evidence that high von Willebrand factor and low ADAMTS-13 levels independently increase the risk of a non-fatal heart attack. J Thromb Haemost. 2008;6(4):583–8.

20. van Bladel ER, Tuinenburg A, Roest M, de Groot PG, Schutgens RE. Factor VIII concentrate infusion in patients with haemophilia results in decreased von Willebrand factor and ADAMTS-13 activity. Haemophilia. 2014;20(1):92–8.
21. Kokame K, Sakata T, Kokubo Y, Miyata T. von Willebrand factor-to-ADAMTS13 ratio increases with age in a Japanese population. J Thromb Haemost. 2011;9(7):1426–8.
22. Crawley JT, de Groot R, Xiang Y, Luken BM, Lane DA. Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor. Blood. 2011;118(12):3212–21.
23. Springer TA. Von Willebrand factor, Jedi knight of the bloodstream. Blood. 2014;124(9):1412–25.
24. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood. 2002;100(12):4033–9.
25. Zhang X, Halvorsen K, Zhang CZ, Wong WP, Springer TA. Mechanoenzymatic cleavage of the ultralarge vascular protein von Willebrand factor. Science. 2009;324(5932):1330–4.
26. Scully M, Knobl P, Kentouche K, Rice L, Windyga J, Schneppenheim R, et al. Recombinant ADAMTS-13: first-in-human pharmacokinetics and safety in congenital thrombotic thrombocytopenic purpura. Blood. 2017;130(19):2055–63.
27. De Wee EM, Klaij K, Eikenboom HC, Van Der Bom JG, Fijnvandraat K, Laros-Van Gorkom BA, et al. Effect of fibrinolysis on bleeding phenotype in moderate and severe von Willebrand disease. Haemophilia. 2012;18(3):444–51.
28. Abdul S, Boender J, Malfliet J, Eikenboom J, Fijn van Draat K, Mauser-Bunschoten EP, et al. Plasma levels of plasminogen activator inhibitor-1 and bleeding phenotype in patients with von Willebrand disease. Haemophilia. 2017;23(3):437–43.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Boender J, Nederlof A, Meijer K, et al. ADAMTS-13 and bleeding phenotype in von Willebrand disease. *Res Pract Thromb Haemost*. 2020;4:1331–1339. https://doi.org/10.1002/rth2.12442