Received: 28 June 2023 | Revised: 7 August 2023 | Accepted: 24 August 2023

https://doi.org/10.1016/j.rpth.2023.102200

rpth research & practice in thrombosis & haemostasis

RESEARCH LETTER

No von Willebrand factor domains other than A1 are involved in type 2B von Willebrand disease: what the p.R924Q and p.A2178S variants teach us

1 | INTRODUCTION

von Willebrand factor (VWF) is a high-molecular weight multimeric glycoprotein with a role in primary hemostasis. It mediates platelet adhesion at the site of vascular injury [1] and serves as a carrier of coagulation factor VIII (FVIII). The molecule has a multimeric structure, with oligomers that range from 500,000 to 20 million Dalton, and large VWF multimers characterized by a high-hemostatic capacity. Each VWF subunit comprises of 4 repetitive domains named from A to D, with different structural and functional roles. The A1 domain is involved in the interaction with platelet glycoprotein Ib (GPIb) [2].

von Willebrand disease (VWD)—the most common of all the inherited bleeding disorders [3]—is associated with VWF deficiencies or abnormalities classified as type 1, 2, or 3, according to the type of VWF defect involved. In type 2B VWD, there is a greater affinity between VWF and platelet GPIb [4] associated with gain-of-function mutations on exon 28 of the *VWF* gene [5] that encodes for the A1 domain of VWF. *In vivo*, this defect prompts type 2B VWF to bind spontaneously to platelet; this causes a loss of large VWF multimers, and leads to moderate or severe, persistent or transient thrombocytopenia [4]. Type 2B is revealed, *in vitro*, by a more marked ristocetin-induced platelet aggregation, which is an indication of how well a patient's platelet-rich plasma can aggregate at very low-ristocetin concentrations [6]. The VWF of patients with type 2B VWD may have a greater affinity for platelets even if their multimer pattern is normal and they have mutations in the A1 domain, but never thrombocytopenia [7]. It was recently posited that domains other than A1, and especially the D'D3 and D4 domains, may be involved in VWF–platelet interaction in individuals carrying both the p.R924Q and the p.A2178S variants [8].

Here, we describe our patients with VWD carrying the combined p.R924Q and p.A2178S variants that present as a typical type 1 VWD and not a type 2B phenotype.

2 | RESULTS

Five subjects from 3 unrelated families having life-long mild bleeding symptoms were studied. The patients showed a normal ristocetin-induced platelet aggregation at 1.2 mg/mL, and the lowest dose eliciting a platelet response to ristocetin was 1.0 mg/mL, so not dissimilar to what happens in normal subjects (Table).

The patients had slightly reduced plasma VWF:antigen (VWF:Ag), VWF:collagen binding, and VWF:ristocetin cofactor levels, with normal VWF:collagen binding to VWF:Ag ratio, and VWF: ristocetin cofactor to VWF:Ag ratio (Table). These findings would suggest a normal VWF multimeric structure. Analyses of the circulating VWF multimers in patients demonstrated a homogeneous, mild reduction of all oligomers, with a preserved representation of high-molecular weight multimers, and no accumulation of lower molecular weight VWF forms (Figure). The patients' platelet counts were normal, ranging from 228×10^3μL to 275×10^3/μL (Table). Their platelet VWF content was within normal range, suggestive of a normal VWF synthesis. VWF propeptide (VWFpp), measured to explore VWF half-life under steady-state conditions, was found slightly shorter than normal, but the VWFpp to VWF:Ag ratio appeared to be normal in all 5 patients, consistent with a normal VWF survival. The patients studied were thus classified as a mild form of type 1 VWD.

Sanger sequencing of the whole *VWF* gene revealed the presence of 2 variants: the c.2771G>A, p.R924Q, in exon 21; and the c.6532G>T, p.A2178S in exon 37, at heterozygous level. No other variants came to light anywhere in the *VWF* gene. The c.2771G>A variant induces the substitution p.R924Q in the D'D3 domain. The c.6532G>T prompts the substitution p.A2178S in the D4 domain. These substitutions were transmitted as a dominant defect, since 3 members of the same family from 2 generations were found to carry both variants, and to express a mild VWF defect.

3 | DISCUSSION

The A1 domain plays a key part in binding VWF to platelet GPIb, promoting the adhesion and aggregation of platelets on an injured vessel wall. However, it was recently posited that domains other than A1, and especially the D'D3 and D4 domains, may be involved in VWF–platelet interaction, in patients with type 2B VWD carrying the p.R924Q and the p.A2178S variants [8]. This observation seemed to

TABLE Main hemostatic findings in the patients with VWD carrying the p.R924Q and the p.A2178S mutations combined

Family/ subject	Gender/ ABO/ age	Platelet count $\times 10^3$/uL	RIPA 1.2 mg/mL %	MADR mg/mL	FVIII U/dL	VWF:Ag U/dL	VWF:RCo U/dL	VWF:CB U/dL	VWF: FVIIIB U/dL	Platelet VWF:Ag	VWF propeptide U/dL
1/1	M/A/38	210	81.5	1.0	40.7	43.2	63.5 (1.47)	45.3 (1.05)	52 (1.20)	78.2	47.6 (1.10)
1/2	F/A/10	265	NP	NP	40.7	42.6	69.7 (1.64)	43.5 (1.02)	42.3 (0.99)	73.5	50.2 (1.18)
1/3	F/O/3	228	79.8	1.0	42.9	30.3	25.2 (0.83)	31 (1.02)	25.5 (0.84)	NP	NP
2/1	F/O/32	NP	93.7	1.0	40.1	46.4	43.2 (0.93)	53 (1.14)	46 (0.99)	93.1	NP
3/1	M/NP/5	275	NP	NP	53.5	47.3	NP	46.5	46.1 (0.97)	85	45.9 (0.96)
Normal range		150-350	60-84	1.0	60-160	60-160	60-130 (>0.70)	65-150 (>0.70)	60-160 (≥0.74)	70-140	60-160 (0.8-1.3)

The values inside the brackets represent the corresponding ratios.

CB, collagen binding; MADR, minimal aggregating dose of ristocetin; NP, not performed; RCo, ristocetin cofactor; RIPA, ristocetin-induced platelet aggregation; VWF, von Willebrand factor.

cast doubts on the dogma that gain-of-function type 2B variants are always and only located in the A1 domain of VWF.

The p.R924Q and the p.A2178S variants reportedly induce a VWF polarity change, promoting the transition toward the open conformational state needed for VWF to interact with platelets, even in the absence of shear forces [8]. Such a suggestion was strongly supported by the *in vitro* functional behavior of recombinant mutated VWF, and by the demonstration of a partially stretched and open conformation of mutated VWF on molecular modeling, transmission electron microscopy, and atomic force microscopy. These *in vitro* findings were fully consistent with the patient' phenotype, involving hyper-responsiveness to ristocetin, the absence of large VWF multimers [4,7], thrombocytopenia and increased VWFpp ratio [9]—the typical features of type 2B VWD. This observation could have opened up new scenarios for treating patients with VWD by taking action through novel VWF domains.

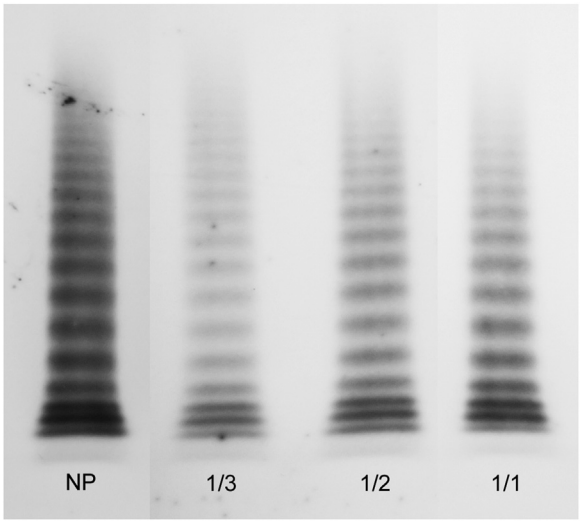

FIGURE Plasma VWF multimeric pattern obtained on 1.8% Sodium Dodecyl Sulfate (SDS) agarose gel electrophoresis under nonreducing conditions. Patients 1, 2, and 3 from family 1 are identified by the numbers entered in the Table, as compared with normal plasma (NP). The multimers were detected using ^{125}I-conjugated anti-VWF antibody, followed by autoradiography. High-molecular weight multimers are at the top, low-molecular weight VWF forms at the bottom. VWF, von Willebrand factor.

Unfortunately, we cannot corroborate such findings of a noncanonical type 2B VWD because our 5 patients (from 3 unrelated families) carrying both the variants p.R924Q and the p.A2178S, and no others anywhere in the *VWF* gene, showed a clear type 1 VWD phenotype. For a start, they did not have a low-platelet count or lack of large VWF multimers; they showed a homogeneous reduction in the quantity of VWF multimers and no accumulation of low-molecular weight forms. That said, it is well known that some type 2B variants in the A1 domain do not induce the consumption of large VWF multimers, despite an increased affinity of VWF for platelets [7], so the patient carrying the combined p.R924Q and p.A2178S variants described by Sacco et al. [8] may belong to this group. However, our patients showed no platelet hyper-responsiveness to ristocetin, the phenotypic hallmark of type 2B variants in patients with a normal or abnormal VWF multimer pattern [7], excluding the possibility of them being cases of type 2B VWD with a normal multimer pattern. Our patients also had normal platelet VWF levels, suggesting a normal VWF synthesis, and thereby ruling out any likelihood of an altered splicing site in exon 28, reportedly associated with the p.R924Q variant [10], explaining the reduction in circulating VWF levels. Finally, VWF survival seems to be normal in our patients, judging from their normal VWFpp ratio values, again in contrast with the patient findings of the study by Sacco et al. [8], and in patients with type 2B [9]. We have no explanation for the discrepancy between the phenotype seen in our patients and the one described by Sacco et al. [8]. It would be tempting to assume that the patient described by Sacco et al. [8] had some other, unidentified mutation(s) that would explain this picture, but that cannot be the case because the authors found the recombinant VWF carrying the combined p.R924Q and p.A2178S variants hyper-responsive to ristocetin, especially when expressed in the homozygous state.

In conclusion, our findings mean that p.R924Q and p.A2178S in the VWF D'D3 and D4 cannot be responsible for type 2B VWD, not even in a noncanonical form, so for now, no known variants away from

the A1 domain can have gain-of-function effects on VWF–platelet interaction. This highlights how complex and heterogeneous VWD can be, and how difficult it can be to characterize these patients.

FUNDING

This work was supported by a grant from the MURST (ex 60%, 2019).

AUTHOR CONTRIBUTIONS

C.A. designed the study, discussed the results and wrote the paper; G.L. performed analysis and interpretation of the data; R.D. performed genetic analysis; G.E. and D.V. performed acquisition of the data and interpretation and revision of the results. All the authors have reviewed the final manuscript, agree on its contents, and approve its submission to *Research and Practice in Thrombosis and Haemostasis* for possible publication.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

Casonato Alessandra
Gianesello Lisa
Regazzo Daniela
Galletta Eva
Daidone Viviana

Department of Medicine, First chair of Internal Medicine, University of Padua Medical School, Padua, Italy

Handling Editor: Dr Johnny Mahlangu.

Correspondence
Alessandra Casonato, Department of Medicine, First chair of Internal Medicine, University of Padua Medical School, via Ospedale Civile 105, Padova, Italy.
Email: sandra.casonato@unipd.it

TWITTER

Regazzo Daniela @DanielaRegazzo

REFERENCES

[1] Sadler JE. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem*. 1998;67:395–424.
[2] Fujimura Y, Titani K, Holland LZ, Russell SR, Roberts JR, Elder JH, et al. von Willebrand factor. A reduced and alkylated 52/48-kDa fragment beginning at amino acid residue 449 contains the domain interacting with platelet glycoprotein Ib. *J Biol Chem*. 1986;261:381–5.
[3] Leebeek FWG, Eikenboom JCJ. Von Willebrand's disease. *N Engl J Med*. 2016;375:2067–80.
[4] Ruggeri ZM, Pareti FI, Mannucci PM, Ciavarella N, Zimmerman TS. Heightened interaction between platelets and factor VIII/von Willebrand factor in a new subtype of von Willebrand's disease. *N Engl J Med*. 1980;302:1047–51.
[5] Randi AM, Rabinowitz I, Mancuso DJ, Mannucci PM, Sadler JE. Molecular basis of von Willebrand disease type IIB. Candidate mutations cluster in one disulfide loop between proposed platelet glycoprotein Ib binding sequences. *J Clin Invest*. 1991;87:1220–6.
[6] De Marco L, Mazzuccato M, Grazia Del Ben M, Budde U, Federici AB, Girolami A, et al. Type IIB von Willebrand factor with normal sialic acid content induces platelet aggregation in the absence of ristocetin. Role of platelet activation, fibrinogen, and two distinct membrane receptors. *J Clin Invest*. 1987;80:475–82.
[7] Casonato A, Daidone V, Galletta E, Bertomoro A. Type 2B von Willebrand disease with or without large multimers: a distinction of the two sides of the disorder is long overdue. *PLoS One*. 2017;12:e0179566.
[8] Sacco M, Lancellotti S, Ferrarese M, Bernardi F, Pinotti M, Tardugno M, et al. Noncanonical type 2B von Willebrand disease associated with mutations in the VWF D'D3 and D4 domains. *Blood Adv*. 2020;4:3405–15.
[9] Casonato A, Gallinaro L, Cattini MG, Pontara E, Padrini R, Bertomoro A, et al. Reduced survival of type 2B von Willebrand factor, irrespective of large multimer representation or thrombocytopenia. *Haematologica*. 2010;95:1366–72.
[10] Berber E, James PD, Hough C, Lillicrap D. An assessment of the pathogenic significance of the R924Q von Willebrand factor substitution. *J Thromb Haemost*. 2009;7:1672–9.