Haemophilia

WILEY

Haemophilia WFH

ORIGINAL ARTICLE OPEN ACCESS

Von Willebrand disease

Modulation of Haemostatic Balance in Combined von Willebrand Disease and Antithrombin Deficiency: A Comprehensive Family Study

Behnaz Pezeshkpoor[1] | Ronald Fischer[2] | Barbara Preisler[1] | Katrin Hartlieb[1] | Heiko Rühl[1] | Jens Müller[1] | Silvia Horneff[1] | Natascha Marquardt[1] | Anna Pavlova[1] | Johannes Oldenburg[1]

[1]Institute of Experimental Hematology and Transfusion Medicine, Medical Faculty, University Hospital Bonn, University of Bonn, Bonn, Germany | [2]Hemophilia Care Center, SRH Kurpfalzkrankenhaus Heidelberg, Heidelberg, Germany

Correspondence: Johannes Oldenburg (johannes.oldenburg@ukbonn.de)

Received: 5 September 2024 | **Revised:** 22 November 2024 | **Accepted:** 7 December 2024

Funding: This study was supported (partly) by research funding from CSL Behring.

Keywords: antithrombin | haemorrhage | molecular genetics | thrombosis | von Willebrand disease

ABSTRACT

Introduction: Maintaining the balance between procoagulant and anticoagulant factors is essential for effective haemostasis. Emerging evidence suggests a modulation of bleeding tendency by factors in the anticoagulant and fibrinolytic systems.

Aim: This study investigates the clinical and laboratory characteristics of a family with combined von Willebrand disease (VWD) and antithrombin (AT) deficiency.

Methods: The study focused on a 38-year-old female index patient (IP) with severe type 3 VWD and a history of bleeding disorders. Coagulation assays included VWF antigen, platelet-dependent VWF activity, factor VIII activity, thrombin generation assay (TGA) and AT activity. Molecular genetic analyses were conducted by a targeted DNA custom next generation sequencing (NGS) panel.

Results: The IP and one of her sisters suffered type 3 VWD. While the IP presents with a classical severe bleeding phenotype, the sister (II-2) exhibited less severe bleeding symptoms. Extended family members showed type 1 VWD with mild presentations. NGS revealed a homozygous deletion of exon 6 in the *VWF* gene in the IP and her sister (II-2). All other family members carry this genetic variant in a heterozygous state. Additionally, II-2 has a heterozygous variant in the *SERPINC1* gene (c.133C>T, p.Arg45Trp). Both IP and II-2 carry a homozygous prothrombin G20210A variant. TGA results indicated reduced thrombin generation in severe VWD patients, with a pronounced thrombin burst in those with the AT and prothrombin G20210A variant.

Conclusions: AT deficiency appears to modulate bleeding symptoms in severe VWD. This study emphasizes the importance of comprehensive genetic and phenotypic evaluation in managing complex coagulation disorders.

1 | Introduction

Under physiological conditions, a delicate equilibrium exists between procoagulant and anticoagulant factors, essential for effective haemostasis at sites of vascular injury [1, 2]. Despite variations in the detailed mechanisms of coagulation, all models converge on the role of thrombin as the central enzyme necessary for fibrin network formation and clot stabilization

Anna Pavlova and Johannes Oldenburg contributed equally to this study as senior authors.

[3]. The intricate regulation of thrombin generation hinges on a balance between procoagulant and anticoagulant proteins. Genetic defects in any of the haemostatic factors can disrupt this delicate balance, resulting in manifestations of bleeding or thrombosis [4].

Various factors, including gene-gene interactions and interactions with acquired factors, contribute to the phenotypic variability observed in coagulation disorders, either exacerbating or ameliorating their clinical presentations. Emerging evidence suggests that the coinheritance of prothrombotic traits, such as antithrombin (AT) deficiency, in individuals with haemophilia can mitigate the severity of the haemorrhagic disorder and improve event-free bleeding survival rates [5–7].

Von Willebrand factor (VWF) plays a crucial role in primary haemostasis by mediating platelet adhesion and aggregation at sites of vascular injury [8]. Deficient or defective VWF in Von Willebrand factor disease (VWD) leads to impaired platelet plug formation and prolonged bleeding. Conversely, AT acts as a natural inhibitor of thrombin and factor Xa, as well as, to a lesser extent, factors IXa, XIa and XIIa, and other procoagulants, thus regulating the coagulation cascade and preventing excessive blood clot formation. AT deficiency disrupts the haemostatic balance, predisposing individuals to thrombotic events [9–11]. Given its capacity to neutralize multiple targets, AT not only interferes with the generation of thrombin but also efficiently inhibits thrombin once generated [11].

Emerging evidence suggests a modulation of bleeding tendency by factors in the anticoagulant and fibrinolytic systems [5]. Individuals with combined VWD and AT deficiency face an unsteady equilibrium between bleeding and thrombotic risks, influenced by factors such as the type and severity of VWD, as well as the degree of AT deficiency [12]. The interplay between these opposing haemostatic abnormalities can result in complex clinical presentations and management dilemmas.

This variability underscores the importance of individualized assessment and management strategies tailored to each patient's specific haemostatic profile. The diagnosis of combined VWD and AT deficiency may be challenging due to overlapping clinical features and laboratory findings with those of other bleeding or thrombotic disorders. Here we report a large family with VWD and concomitant inheritance of pathogenic variants in anticoagulant genes.

2 | Patients, Materials and Methods

2.1 | Patients

A 38-year-old female index patient (IP) with severe type 3 VWD was investigated in the Haemophilia Centre (HC) in Bonn, Germany. She belongs to a consanguineous family, with a rich history of a bleeding disorder across several generations, encompassing 12 individuals with varying presentations of VWD (Figure 1). All patients were genetically tested after being given informed consent according to the Declaration of Helsinki.

2.2 | Coagulation Assays

Plasma from each available subject was assayed using standard methods by the coagulation laboratory of the University Hospital Bonn. Plasma VWF-antigen (VWF:Ag) and VWF-activity (VWF:GPIbM) were measured by latex particle assays/immunoturbidimetry using an Atellica COAG 360 coagulation analyser (Siemens Healthineers [Siemens], Eschborn, Germany) [13, 14]. Additionally, the ratio between VWF activity and VWF:Ag was calculated, with a ratio > 0.7 indicating VWF dysfunction. Laboratory reference ranges were as follows: non-0 blood type: 65%–165% for VWF:Ag and 64%–150% for VWF activity. Factor VIII activity assay (FVIII:C) measurements were performed on the same analyser using a chromogenic substrate method (Siemens) [15]. VWF propeptide assay (VWF:PP) was performed using an ELISA-based assay (Haemochrom, Essen, Germany) according to the manufacturer's instructions. Laboratory reference ranges were 52.4–296 IU/dL, and for VWF:PP/VWF:Ag, were 0.3–1.8.

Measurements of AT levels were conducted as previously described [16] using the Atellica COAG 360 coagulation analyser (Siemens Healthcare Diagnostics, Eschborn, Germany). AT activity levels were measured using a chromogenic substrate assay based on inhibition of thrombin (Berichrome AT III, Siemens) and another assay based on inhibition of FXa (Innovance Antithrombin, Siemens). Laboratory reference ranges were 80% to 120% for both assays.

2.3 | Thrombin Generation Assay (TGA)

TGA was performed using reagents from Diagnostica Stago (Düsseldorf, Germany). The protocol was followed as described with few modifications [15–17]. Citrated plasma (80 µL) was triggered either with PPP reagent low (1 pM tissue factor [TF]) or PPP reagent (5 pM TF). After the addition of the FLuCa reagent containing calcium chloride and the fluorogenic peptide substrate Z-Gly-Gly-Arg-AMC, the reaction started. A thrombin calibrator was run for each sample in parallel. Fluorescence generation was continuously measured using a Fluoroskan Ascent Microplate Fluorometer (Thermo Fisher Scientific). TGA parameters, including the lag phase (time to thrombin to burst), peak thrombin and endogenous thrombin potential (ETP), were calculated by the Thrombinoscope software.

2.4 | Molecular Genetics Analyses

Genetic analyses were performed at the Department of Molecular Hemostaseology, University Hospital Bonn. Genomic DNA was isolated from peripheral whole blood using the Blood Core Kit (Qiagen, Hilden, Germany). Molecular genetic analyses included the sequencing of all coding regions and intron/exon boundaries of the following genes: *F2* (NM_000506), *F5* (NM_(NM_000130), *SERPINC1* (NM_000488) and *VWF (NM_000552)*. The next-generation sequencing (NGS) analyses were carried out on a Mini-Seq genome sequencer (Illumina, Santa Clara, CA, USA). Data were evaluated using SeqPilot software (JSI medical systems, Ettenheim, Germany). Sequence variants were described following Human Genome Variation Society (HGVS) guidelines.

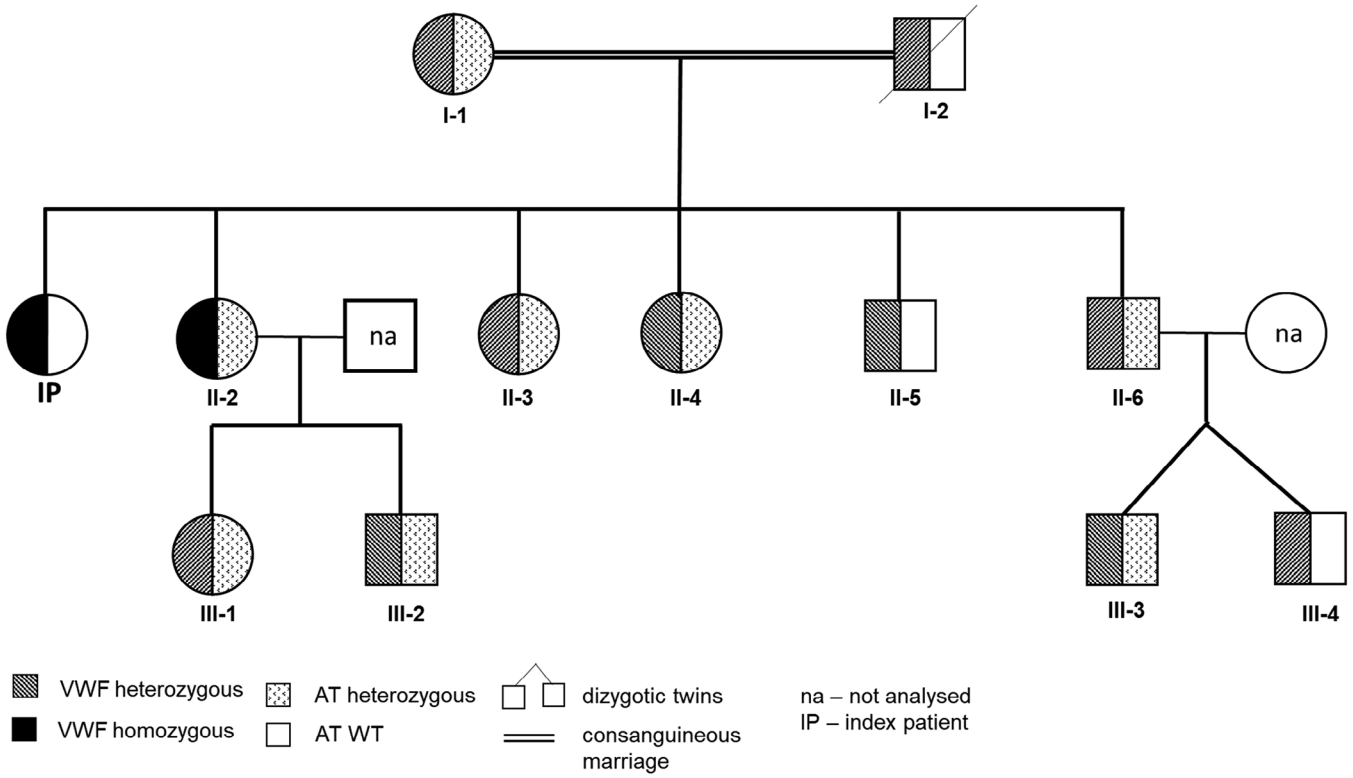


FIGURE 1 | Pedigree of the family with combined von Willebrand factor (VWF) and antithrombin (AT) deficiency.

Variant interpretation followed ACMG (American College of Medical Genetics) and AMP (Association for Molecular Pathology) guidelines [18]. Disease causality was compared using the Human Gene Mutation Database (HGMD) [19, 20] and the ClinGen database [21]. Large deletions and duplications were analysed based on copy number variation (CNV) analysis using SeqPilot.

3 | Results

3.1 | Clinical Characteristic of the Family

The 38-year-old female IP was diagnosed with severe type 3 VWD at age 2, characterized by VWF plasma activity and VWF:Ag levels below 4%. She was referred to the HC at the University Hospital Bonn due to severe bleeding from an adenoidectomy. Throughout her early life, she experienced significant bleeding issues, including epistaxis, easy bruising and bleeding from minor injuries. At menarche (age 12), she suffered severe menstrual bleedings, leading to iron-deficient anaemia. She underwent prophylactic replacement therapy with plasma-derived VWF/FVIII concentrates and oral contraceptives, and her anaemia was treated with iron supplements. At 19, a ruptured ovarian cyst caused severe haemorrhagic complications, necessitating surgery under prophylactic VWF replacement therapy. Since then, continuous prophylactic therapy and oral contraceptives have prevented severe bleeding episodes.

Her younger sister (II-2) also suffers from type 3 VWD with similarly low plasma VWF values but has not experienced severe bleeding episodes. At age 10, she was treated for a traumatic knee injury at the HC, with no bleeding complications following a knee puncture. Apart from this, she had no significant bleeding events. At age 20, she underwent surgery for spinal disc herniation without bleeding issues under low-dose VWF substitution. Her treatment includes on-demand antifibrinolytics (tranexamic acid), oral contraceptives and occasional VWF replacement therapy. She had two pregnancies with uncomplicated deliveries.

Further investigation into coagulation factors revealed consistently reduced FVIII:C levels, while all other coagulation factors were within normal ranges. Notably, the only difference in laboratory analysis was in AT measurements using the FXa-based assay. While for the IP, the AT levels were within the normal range, the sister (II-2) showed reduced FXa-based AT activity (62 IU/dL), whereas AT activity measured by the FIIa-based assay showed normal values for both sisters (Table 1).

Ten extended family members with varying manifestations of VWD were available for analysis. Figure 1 illustrates the detailed relationships among family members, emphasizing the prevalence of VWD throughout the lineage. The extended family members exhibit mild or no bleeding symptoms and were diagnosed with type 1 VWD (Table 1). VWF activity levels range between 34 and 57 IU/dL, with VWF:Ag levels from 54 to 82 IU/dl. AT activity (FXa-based) was found to be reduced in seven family members (range: 62% to 77%). The majority of extended family members showed reduced VWF:PP levels (43 to 51.3 IU/dL). The slightly lower VWF activity levels and decreased VWF PP levels correlate to the location of the large deletion in the propeptide region of the *VWF* gene in Exon 6. Thus, this comprehensive assessment highlights the diverse clinical presentations of type 3 and type 1 VWD within the family, ranging from mild to severe.

TABLE 1 | Characteristic of the patients including the laboratory parameters and genetic defects.

Patient	Platelets (×109/μL)	VWF:Ag (IU/dL)	VWF: GPIbM (IU/dL)	VWF: GPIbM/ VWF:Ag ratio	VWF:PP (IU/dL)	VWF:PP/ VWF:Ag ratio	FVIII: C (IU/dL)	AT (IIa) (%)	AT(Xa) (%)	F2 (%)	PTT (s)	Blood group	VWF defect (large deletion exon 6)	*SERPINC1 Defect p.(Arg45Trp)*	Prothrombin G20210A
I-1	218	82	57	0.70	43.8	0.53	103	103	111	138	25.8	n.d.	Heterozygous	wt.	Heterozygous
I-2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Heterozygous	Heterozygous	n.d.
II-1 (IP)	**214**	**< 4**	**< 4**	n.d.	0.7	n.a.	**2**	**104**	**114**	**168**	**42.5**	**A**	**Homozygous**	**wt**.	**Homozygous**
II-2	331	< 4	< 4	n.d.	0.7	n.a.	3	95	62	144	42	A	Homozygous	Heterozygous	Homozygous
II-3	289	55	39	0.71	47.0	0.85	145	129	77	160	23.7	A	Heterozygous	Heterozygous	Heterozygous
II-4	302	62	48	0.77	n.d.	n.d.	154	115	73	159	22	A	Heterozygous	Heterozygous	Heterozygous
II-5	236	44	39	0.89	44.9	1.02	86	105	110	144	22.6	n.d.	Heterozygous	wt.	Heterozygous
II-6	285	57	41	0.72	41.3	0.72	138	120	70	141	27.9	A	Heterozygous	Heterozygous	Heterozygous
III-1	372	55	49	0.89	51.3	0.93	125	114	69	149	26.9	n.d.	Heterozygous	Heterozygous	Heterozygous
III-2	396	48	34	0.71	43.6	0.91	116	106	68	n.d.	25.9	n.d.	Heterozygous	Heterozygous	Heterozygous
III-3	402	63	77	1.22	66.8	1.06	117	92	61	129	24.5	A	Heterozygous	Heterozygous	n.d.
III-4	259	76	73	0.96	84.3	1.11	104	94	101	150	27	A	Heterozygous	wt.	n.d.

Abbreviations: AT, antithrombin; F2: Factor 2; FVIII:C: FVIII activity; IU: international units; s, seconds; n.a., not applicable; n.d., no data; PTT, partial thromboplastin time; VWF:Ag, VWF antigen; VWF:GPIbM, VWF activity; VWF:PP, VWF propeptide levels; wt., wildtype.

3.2 | Concomitant Inheritance of Pro and Anticoagulant Genetic Defects

Genetic analyses were carried out using NGS, offering a comprehensive view of the genetic landscape underlying their respective coagulation disorders within the family. NGS screening of the IP did not reveal any genetic variant within the coding regions of the *VWF* gene. However, a more detailed examination employing CNV analysis unveiled a significant genetic alteration—the complete deletion of exon 6 within the *VWF* gene. This deletion was present in a homozygous state in both the IP and her sister (II-2), both diagnosed with type 3 VWD. All other family members possessed this deletion in a heterozygous state (Table 1).

Due to the detected pathological values for AT, extended analyses of the NGS data for the IP and her sister (II-2) were performed. The results showed additional genetic defects in the *SERPINC1* gene (encoding the AT protein) as well as the *F2* gene (encoding the prothrombin protein). The genetic variant (c.133C>T, p.Arg45Trp.) in the *SERPINC1* gene was identified in a heterozygous state in exon 2, situated near the heparin binding site (HBS) of AT. This genetic defect was not found in the IP but was present in her sister (II-2) and seven additional family members (Figure 1 and Table 1). Additionally, the FV Leiden variant -G1691A, p.Arg534Gln- in Exon 10 of the *F5* gene was excluded in all family members. These findings align with the milder clinical presentation in these patients, suggesting a potential modulatory effect of this variant on bleeding tendencies. Furthermore, a second genetic variant known as *prothrombin G20210A w*as detected in all tested family members. This variant was in a homozygous state in the IP and II-2, while the other family members carry it in a heterozygous state.

3.3 | TGA

Next, we conducted TGA to investigate the impact of combined VWF and AT deficiencies on the thrombin generation potential. The tests were performed with 1 and 5 pM TF to evaluate the respective defects in *VWF* and *SERPINC1*, respectively. The analyses were performed on plasma samples from four patients: the two sisters with type 3 VWD and homozygous p*rothrombin G20210A* defect, one with and one without AT deficiency. Furthermore, two family members with type 1 VWD, each heterozygous for the p*rothrombin G20210A* defect, again one with and one without AT deficiency, were tested. The results, including the lag time, ETP and peak height, are summarized in Figure 2.

Figure 2A illustrates the TGA results for the IP and her sister (II-2) using 1 pM TF. Both patients showed a significant decrease in thrombin generation compared to a control plasma pool, as indicated by a reduced thrombin peak. There was no substantial difference between the patient with AT deficiency (II-2) and the one without (IP).

However, when TGA was performed with 5 pM TF, there was a marked reduction in lag time compared to the results obtained with 1 pM TF, approaching normal plasma levels (Figure 2B). The peak height and ETP values were higher in both patients carrying the homozygous *prothrombin G20210A* variant compared to the control pool plasma, with a more pronounced effect in the IP's sister (II-2), who also carries the genetic variant in *SERPINC1*. Specifically, the peak thrombin concentrations were 419 nM in the IP and 495 nM in II-2 versus 314 nM in the control pool plasma. The ETP values were 6316 nM/min in the IP and 5574 nM/min in II-2 versus 2582 nM/min in the control pool plasma. The overall increased thrombin generation potential correlates well with high prothrombin levels due to the homozygous *prothrombin G20210A* variant (Table 1).

For the two family members with a heterozygous *VWF* defect (type 1 VWD) and a heterozygous *prothrombin G20210A* variant, TGA with 1 pM TF showed no difference in all parameters between the patients with and without the *SERPINC1* variant and the control pool plasma (Figure 2C). When TGA was conducted with 5 pM TF, the type 1 VWD patient with AT deficiency (II-6) exhibited a higher peak height (332 nM) compared to the family member without the *SERPINC1* defect (II-5) (232 nM) (Figure 2D).

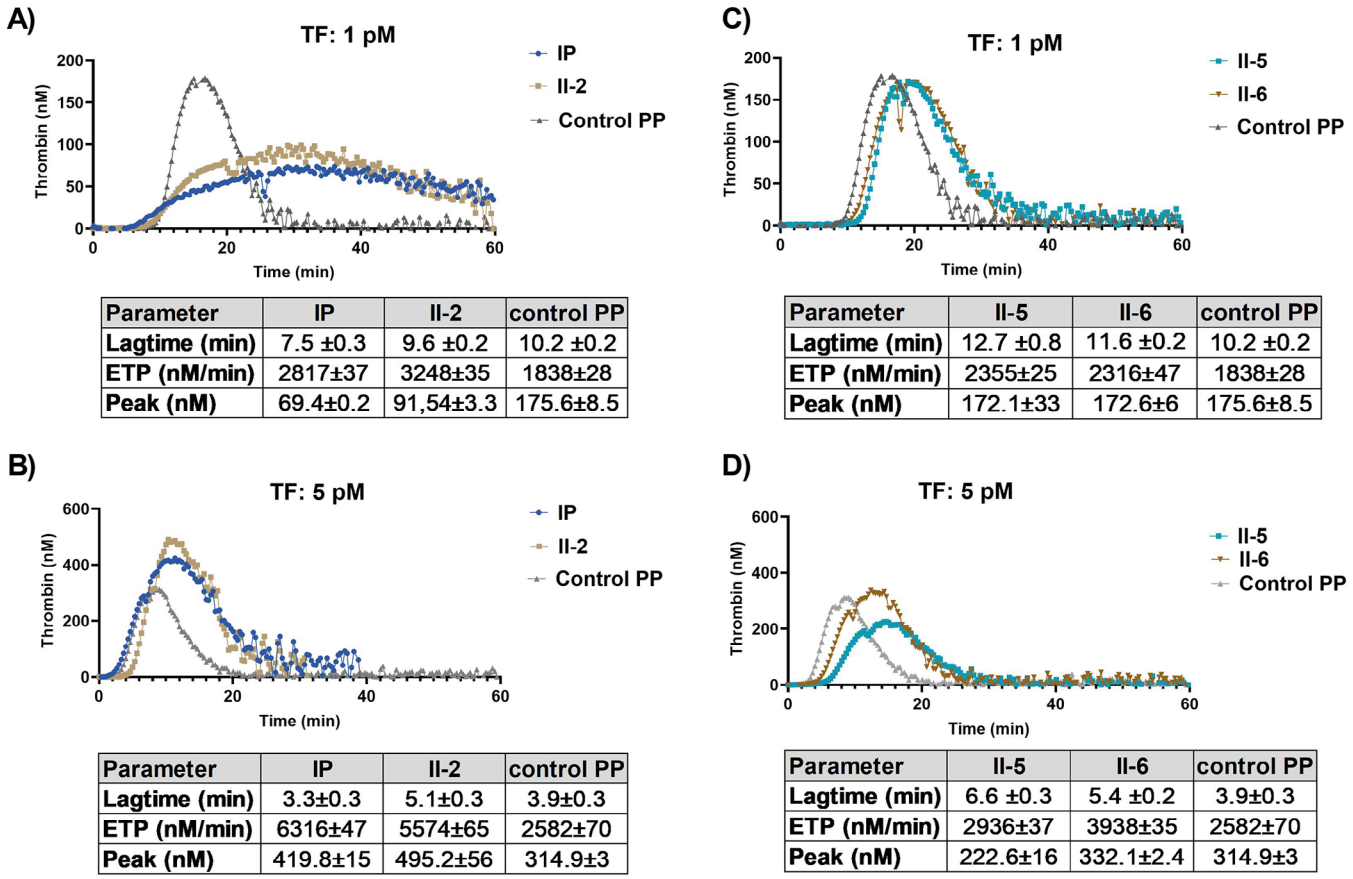
4 | Discussion

The interaction between AT and VWF in patients with concurrent VWD and AT deficiency significantly impacts haemostatic balance, influencing both clinical presentation and management of bleeding phenotypes. This dual deficiency can range from mild mucocutaneous bleeding to severe haemorrhages. Interestingly, some studies have reported thrombosis in VWD patients with inherited prothrombotic risk factors. *Girolami* et al. suggested that the concomitant presence of VWD in a patient with AT deficiency might have a protective effect against thrombotic manifestations [12].

In the analysed family, VWD and AT defects segregated independently, presenting four distinct phenotypes: AT deficiency combined with type 3 or type 1 VWD, and isolated type 3 or type 1 VWD. The AT defect appeared to mitigate bleeding symptoms, with the type 3 VWD patient (II-2) exhibiting only mild bleeding similar to type 1 VWD, and those with type 1 VWD experiencing no or only minimal bleedings. No thrombotic incidents were reported, suggesting that the AT deficiency may not always lead to clinically significant thrombosis in the context of VWD. Indeed, there is increasing evidence in the literature that in some cases the co-existence of acquired or inherited prothrombotic risk factors may modulate this balance, overcome the bleeding tendency, and lead to a milder clinical phenotype and, in rare cases, to an increased probability of developing thrombotic complications [22].

In addition, just as the presence of prothrombotic factors may modulate the clinical phenotype of severe haemophilia [23], some authors have found that the severity of bleeding symptoms in type 1 VWD depends on functional defects in platelet aggregation [24] or DNA polymorphisms in the platelet membrane proteins integrin a2, αIIbβ3 and GPVI [25].

The mechanism by which AT deficiency reduces bleeding in severe VWD is not fully understood but may involve compensatory haemostatic mechanisms, enhanced platelet activation, and altered thrombin dynamics. In individuals with severe VWD, the absence or dysfunction of VWF impairs primary haemostasis, leading to prolonged bleeding times and a predisposition to



Parameter	IP	II-2	control PP
Lagtime (min)	7.5 ±0.3	9.6 ±0.2	10.2 ±0.2
ETP (nM/min)	2817±37	3248±35	1838±28
Peak (nM)	69.4±0.2	91,54±3.3	175.6±8.5

Parameter	II-5	II-6	control PP
Lagtime (min)	12.7 ±0.8	11.6 ±0.2	10.2 ±0.2
ETP (nM/min)	2355±25	2316±47	1838±28
Peak (nM)	172.1±33	172.6±6	175.6±8.5

Parameter	IP	II-2	control PP
Lagtime (min)	3.3±0.3	5.1±0.3	3.9±0.3
ETP (nM/min)	6316±47	5574±65	2582±70
Peak (nM)	419.8±15	495.2±56	314.9±3

Parameter	II-5	II-6	control PP
Lagtime (min)	6.6 ±0.3	5.4 ±0.2	3.9±0.3
ETP (nM/min)	2936±37	3938±35	2582±70
Peak (nM)	222.6±16	332.1±2.4	314.9±3

FIGURE 2 | Thrombin generation assay (TGA) using PPP low (1 pM TF) and PPP high (5 pM TF). (A)–(B) Both patients with severe type 3 VWD (IP and her sister) were analysed (C)–(D) with her bothers with type 1 VWD with and without the *SERPINC1* defect. control PP, control pool plasma; IP, index patient; min, minutes; nM, nanomolar; pM, picomolar.

bleeding episodes. AT deficiency, by promoting a procoagulant state through impaired inhibition of thrombin and other coagulation factors, may partially compensate for the deficient primary haemostatic function of VWF. This compensatory mechanism could enhance secondary haemostasis and clot stability, potentially reducing the severity or duration of bleeding episodes [26].

Additionally, enhanced platelet activation could contribute to improvement of bleeding tendency in these patients. AT deficiency may lead to increased thrombin generation and subsequent platelet activation. Thrombin, in addition to its role in fibrin formation, is a potent platelet agonist that induces platelet activation and aggregation. In the absence of VWF-mediated platelet adhesion, enhanced platelet activation driven by thrombin may promote the formation of unstable platelet plugs at sites of vascular injury, mitigating bleeding tendencies in individuals with severe VWD [27].

Diagnosing combined VWD and AT deficiency is challenging due to overlapping clinical features and variable diagnostic sensitivity of AT activity assays. Regardless of the specific genetic variant in the *SERPINC1* gene, the preferred method for detecting AT deficiency in plasma typically involves chromogenic assays. These assays measure the inhibition of FXa or thrombin (FIIa)—hence referred to as FXa-based and FIIa-based assays, respectively—in the presence of heparin. However, numerous studies have highlighted the variable diagnostic sensitivity of these AT activity assays across different AT deficiency subtypes, which complicates the laboratory diagnosis of this condition [28, 29]. Particularly difficult are type II AT deficiencies, like HBS variants, which may not significantly alter thrombin inhibition in FIIa-based assays, leading to underdiagnosis. This study highlights the need for a range of diagnostic tests, especially when clinical suspicion remains despite inconclusive results. Employing both FIIa- and FXa-based AT activity in first-line setting assays can enhance diagnostic accuracy, ensuring that subtle variations in AT function, particularly at the HBS, are detected [30]. This approach is also particularly valuable in cases where genetic testing is not available, as performing both tests can help infer the location of the variant based on discrepancies between the results. Moreover, since HBS variants are reported to be less thrombogenic, laboratory tests can directly assist in evaluating the patient's risk of thrombosis.

The dual nature of these two disorders necessitates careful interpretation of both bleeding and thrombotic risks. TGA can illustrate the interaction between altered coagulation initiation in VWD and enhanced propagation due to reduced thrombin inhibition in AT deficiency [31]. Our results underline that TGA is useful [32] for investigating the impact of combined VWF and AT deficiencies. Notably, there was no significant difference between type 3 VWD patients with and without AT deficiency under 1 pM TF conditions, which predominantly reflects the intrinsic coagulation system influenced by reduced FVIII activity. In 5 pM TF settings, the thrombin burst was more pronounced in patients with

the *SERPINC1* genetic defect, regardless of the *VWF* variant state. The increased peak height in our IP without an AT defect may be explained by the homozygous defect in the prothrombin gene.

Additionally, the implication of genetic testing in the diagnostic panel could play a crucial role in the diagnostic process, identifying pathogenic variants and correlating genotype with phenotype. Although genetic testing is neither mandated nor required for a diagnosis of VWD, integrating NGS into clinical practice enhances our understanding of such complex disorders and facilitates a more precise diagnosis and management of affected individuals [33, 34].

5 | Conclusion

In conclusion, we report rare cases of combined VWD and AT deficiency within a consanguine family. The mechanism by which AT deficiency reduces bleeding episodes in severe VWD likely involves compensatory haemostatic mechanisms, enhanced platelet activation, and altered thrombin dynamics. The interplay between these haemostatic abnormalities requires careful evaluation and personalized treatment. Comprehensive laboratory evaluation, including assays for VWF and AT levels, specialized tests like TGA, and NGS approaches, is essential to clarify the haemostatic profile and to guide therapeutic decisions.

Author Contributions

A.P., B.P., B.Pr., and J.O. designed the study. cK.H. performed the experiments. A.P., B.P. analysed and interpreted data. A.P. and B.P. wrote the first draft of the manuscript. All authors contributed to data analysis, laboratory/correlative analyses, and manuscript editing and evaluation and read and approved the final manuscript.

Acknowledgements

This study was supported (partly) by research funding from CSL Behring. We would like to thank Judith Bergmoser for technical assistance.

Ethics Statement

This article complies with all ethical requirements for data review and analysis.

Conflicts of Interest

Behnaz Pezeshkpoor reports having received grants for research from Biotest, Octapharma and NovoNordisk as well as personal fees for lectures and advisory board meetings from NovoNordisk and Octapharma. Johannes Oldenburg reports having received grants for studies and research from Bayer, Biotest, CSL-Behring, Octapharma, Pfizer, SOBI and Takeda, and travel support as well as personal fees for lectures and advisory board meetings from Bayer, Biogen Idec, Biomarin, Biotest, CSL-Behring, Chugai, Freeline, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, Swedish Orphan Biovitrum and Takeda. All other authors declare no conflicts of interest.

Data Availability Statement

All data referred to in this article are available for review as required.

References

1. J. P. Riddel, B. E. Aouizerat, C. Miaskowski, and D. P. Lillicrap, "Theories of Blood Coagulation," *Journal of Pediatric Oncology Nursing: Official Journal of the Association of Pediatric Oncology Nurses* 24, no. 3 (2007): 123–131.

2. B. Pezeshkpoor, J. Oldenburg, and A. Pavlova, "Experiences in Routine Genetic Analysis of Hereditary Hemorrhagic, Thrombotic, and Platelet Disorders," *Hamostaseologie* 42, supplement, no. S1 (2022): S5–S12.

3. M. Hoffman, "A Cell-Based Model of Coagulation and the Role of Factor VIIa," *Blood Reviews* 17, supplement, no. S1 (2003): S1–5.

4. J. S. O'Donnell, J. M. O'Sullivan, and R. J. S. Preston, "Advances in Understanding the Molecular Mechanisms That Maintain Normal Haemostasis," *British Journal of Haematology* 186, no. 1 (2019): 24–36.

5. S. Shetty, S. Vora, B. Kulkarni, et al., "Contribution of Natural Anticoagulant and Fibrinolytic Factors in Modulating the Clinical Severity of Haemophilia Patients," *British Journal of Haematology* 138, no. 4 (2007): 541–544.

6. E. Castoldi, J. W. P. Govers-Riemslag, M. Pinotti, et al., "Coinheritance of Factor V (FV) Leiden Enhances Thrombin Formation and Is Associated With a Mild Bleeding Phenotype in Patients Homozygous for the FVII 9726+5GA (FVII Lazio) Mutation," *Blood* 102, no. 12 (2003): 4014–4020.

7. K. Ghosh, S. Shetty, and D. Mohanty, "Milder Clinical Presentation of Haemophilia A With Severe Deficiency of Factor VIII as Measured by One-Stage Assay," *Haemophilia the Official Journal of the World Federation of Hemophilia* 7, no. 1 (2001): 9–12.

8. L. L. Swystun and D. Lillicrap, "How Much Do We Really Know About von Willebrand Disease?," *Current Opinion in Hematology* 23, no. 5 (2016): 471–478.

9. S. Butenas, C. van't Veer, and K. G. Mann, "'Normal' Thrombin Generation," *Blood* 94, no. 7 (1999): 2169–2178.

10. V. Regnault, H. C. Hemker, D. Wahl, and T. Lecompte, "Phenotyping the Haemostatic System by Thrombography–Potential for the Estimation of Thrombotic Risk," *Thrombosis Research* 114, no. 5–6 (2004): 539–545.

11. C. Negrier, M. Shima, and M. Hoffman, "The central Role of Thrombin in Bleeding Disorders," *Blood Reviews* 38 (2019): 100582.

12. A. Girolami, M. G. Cappellato, M. A. Vicarioto, S. Casonato, and F. Marafioti, "Associated von Willebrand Disease as a Possible Cause of Lack of Thrombosis in an AT III Abnormality (AT III Trento)," *Blut* 52, no. 1 (1986): 29–33.

13. A. Sedaghat, H. Kulka, J.-M. Sinning, et al., "Transcatheter Aortic Valve Implantation Leads to a Restoration of von Willebrand Factor (VWF) Abnormalities in Patients With Severe Aortic Stenosis—Incidence and Relevance of Clinical and Subclinical VWF Dysfunction in Patients Undergoing Transfemoral TAVI," *Thrombosis Research* 151 (2017): 23–28.

14. E. J. Favaloro and S. Mohammed, "Towards Improved Diagnosis of von Willebrand Disease: Comparative Evaluations of Several Automated von Willebrand Factor Antigen and Activity Assays," *Thrombosis Research* 134, no. 6 (2014): 1292–1300.

15. B. Pezeshkpoor, M. Gazorpak, A.-C. Berkemeier, et al., "In Silico and in Vitro Evaluation of the Impact of Mutations in Non-Severe Haemophilia A Patients on Assay Discrepancies," *Annals of Hematology* 98, no. 8 (2019): 1855–1865.

16. S. Reda, J. Müller, A. Pavlova, et al., "Functional Characterization of Antithrombin Mutations by Monitoring of Thrombin Inhibition Kinetics," *International Journal of Molecular Sciences* 22, no. 4 (2021).

17. H. C. Hemker, P. Giesen, R. Al Dieri, et al., "Calibrated Automated Thrombin Generation Measurement in Clotting Plasma," *Pathophysiology of Haemostasis and Thrombosis* 33, no. 1 (2003): 4–15.

18. S. Richards, N. Aziz, S. Bale, et al., "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology," *Genetics in Medicine Official Journal of the American College of Medical Genetics* 17, no. 5 (2015): 405–424.

19. P. D. Stenson, M. Mort, E. V. Ball, K. Shaw, A. Phillips, and D. N. Cooper, "The Human Gene Mutation Database: Building a Comprehensive Mutation Repository for Clinical and Molecular Genetics, Diagnostic Testing and Personalized Genomic Medicine," *Human Genetics* 133, no. 1 (2014): 1–9.

20. P. D. Stenson, M. Mort, E. V. Ball, et al., "The Human Gene Mutation Database (HGMD®): Optimizing Its Use in a Clinical Diagnostic or Research Setting," *Human Genetics* 139, no. 10 (2020): 1197–1207.

21. H. L. Rehm, J. S. Berg, L. D. Brooks, et al., "ClinGen–The Clinical Genome Resource," *New England Journal of Medicine* 372, no. 23 (2015): 2235–2242.

22. M. Franchini, "Thrombotic Complications in Patients With Hereditary Bleeding Disorders," *Thrombosis and Haemostasis* 92, no. 2 (2004): 298–304.

23. K. van Dijk, J. G. van der Bom, K. Fischer, D. E. Grobbee, and H. M. van den Berg, "Do Prothrombotic Factors Influence Clinical Phenotype of Severe Haemophilia? A Review of the Literature," *Thrombosis and Haemostasis* 92, no. 2 (2004): 305–310.

24. H. J. Weiss, "The Bleeding Tendency in Patients With Low von Willebrand Factor and Type 1 Phenotype Is Greater in the Presence of Impaired Collagen-Induced Platelet Aggregation," *Journal of Thrombosis and Haemostasis JTH* 2, no. 1 (2004): 198–199.

25. T. J. Kunicki, A. B. Federici, D. R. Salomon, et al., "An Association of Candidate Gene Haplotypes and Bleeding Severity in von Willebrand Disease (VWD) Type 1 Pedigrees," *Blood* 104, no. 8 (2004): 2359–2367.

26. P. M. Mannucci, "Treatment of von Willebrand's Disease," *New England Journal of Medicine* 351, no. 7 (2004): 683–694.

27. T. Szanto, V. Nummi, A. Jouppila, H. J. M. Brinkman, and R. Lassila, "Platelets Compensate for Poor Thrombin Generation in Type 3 von Willebrand Disease," *Platelets* 31, no. 1 (2020): 103–111.

28. K. Javela, S. Engelbarth, L. Hiltunen, P. Mustonen, and M. Puurunen, "Great Discrepancy in Antithrombin Activity Measured Using Five Commercially Available Functional Assays," *Thrombosis Research* 132, no. 1 (2013): 132–137.

29. S. R. Kristensen, B. Rasmussen, S. Pedersen, and L. Bathum, "Detecting Antithrombin Deficiency May be a Difficult Task–More Than One Test Is Necessary," *Journal of Thrombosis and Haemostasis JTH* 5, no. 3 (2007): 617–618.

30. B. Kovács, Z. Bereczky, Z. Oláh, et al., "The Superiority of Anti-FXa Assay Over Anti-FIIa Assay in Detecting Heparin-Binding Site Antithrombin Deficiency," *American Journal of Clinical Pathology* 140, no. 5 (2013): 675–679.

31. R. Kremers, M. Ninivaggi, I. v. Moort, M. P. M. d. Maat, and B. d. Laat, "Tailoring the Effect of Antithrombin-Targeting Therapy in Haemophilia A Using in Silico Thrombin Generation," *Scientific Reports* 11, no. 1 (2021): 15572.

32. L. Rugeri, S. Beguin, C. Hemker, et al., "Thrombin-Generating Capacity in Patients With von Willebrand's Disease," *Haematologica* 92, no. 12 (2007): 1639–1646.

33. J. Batlle, A. Pérez-Rodríguez, I. Corrales, et al., "Update on Molecular Testing in von Willebrand Disease," *Seminars in Thrombosis and Hemostasis* 45, no. 7 (2019): 708–719.

34. B. Preisler, B. Pezeshkpoor, A. Banchev, et al., "Familial Multiple Coagulation Factor Deficiencies (FMCFDs) in a Large Cohort of Patients-A Single-Center Experience in Genetic Diagnosis," *Journal of Clinical Medicine* 10, no. 2 (2021).