# *Novel* Likely Pathogenic Variant in the A3 Domain of von Willebrand Factor Leading to a Collagen-Binding Defect

Salome Fels[1] Doris Boeckelmann[1] Hannah Glonnegger[1] Martin Büchsel[2] Barbara Zieger[1]

[1] Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, Medical Center – University of Freiburg, Germany
[2] Institute of Clinical Chemistry and Laboratory Medicine, Faculty of Medicine, Medical Center – University of Freiburg, Germany

Address for correspondence Barbara Zieger, MD, Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Faculty of Medicine, Medical Center – University of Freiburg, Mathildenstrasse 1, 79106 Freiburg, Germany (e-mail: barbara.zieger@uniklinik-freiburg.de).

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**Abstract**

Von Willebrand disease (VWD) is the most prevalent congenital bleeding disorder. Diagnosis and classification of VWD is complex due to its heterogeneity regarding clinical manifestations and molecular genetic analysis. Genetic investigations became an inherent part of diagnosis and help distinguish different types/subtypes of VWD. Although many variants have been listed being causative for VWD, the genetic etiology remains undefined in a lot of patients. We report about two siblings with severely reduced values for von Willebrand factor collagen-binding activity (VWF:CB). Genetic analysis using panel sequencing identified a heterozygous non-synonymous single nucleotide variant in exon 30. At the protein level, the alteration (p.Ser1731Leu) is located in the A3 collagen-binding domain. The amino acid position is already known to be important for collagen binding because p.Ser1731Thr has been reported to affect the VWF:CB.

**Keywords**

- von Willebrand factor
- von Willebrand disease
- type 2M
- collagen-binding domain

## Introduction

Von Willebrand disease (VWD) is caused by genetic variants in the von Willebrand factor (*VWF*) gene resulting in quantitative (types 1 and 3) and qualitative (type 2) deficiencies of VWF. Main characteristics are prolonged bleeding time, tendency for hematomas, and mucocutaneous bleedings such as epistaxis and menorrhagia. Type 2M VWD is due to autosomal dominant variants either in A1 or A3 domain of VWF. Variants in A1 domain lead to defective binding of VWF to platelet GpIb without loss of high-molecular-weight multimers. Defects in A3 domain are characterized by reduced binding activity of VWF to subendothelial collagen type I and type III.[1] Patients with a collagen-binding defect show low values for VWF:CB (VWF:collagen-binding activity) and VWF:CB/VWF:antigen (Ag) ratio, whereas other VWF parameters and multimeric patterns are normal.[2] Genetic analysis using next-generation sequencing (NGS) for the large *VWF* has replaced former sequential Sanger sequencing.

## Case Report

We investigated two siblings (one girl and one boy) with a bleeding diathesis. The boy (13 years old) suffered from several bleedings after tonsillectomy and recurrent epistaxis. The older sister (16 years old) presented with menorrhagia, intermittent gum bleeding, and easy bruising. Both siblings showed severely decreased values for VWF:CB (≤0.11 U/mL) and VWF:CB/VWF:Ag ratio (0.06 and 0.16, respectively). VWF:CB measured in the patients corresponds to collagen type I (Takeda, Austria GmbH). VWF activity (VWF:GPIbM) was analyzed using VWF:Ac INNOVANCE VWF Ac (Siemens Healthcare Diagnostics). Values for VWF:Ag, VWF:Ac, factor VIII activity (►Table 1), and multimer analysis (►Fig. 1) were

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Georg Thieme Verlag KG,
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**Table 1** VWF diagnostic for the two siblings

| Patient | VWF:Ag [U/mL] normal: 0.6–1.5 | VWF:CB [U/mL] normal: 0.6–1.5 | VWF:CB/VWF:Ag ratio normal: 0.8–1.5 | VWF:Ac [%] normal: 46–179 | Multimer analysis | FVIII activity [%] normal: 60–150 |
|---|---|---|---|---|---|---|
| Brother | 0.70 | 0.09 | 0.13 | 60 | Normal | 100 |
| | 0.83 | 0.05 | 0.06 | 68 | n.d. | n.d. |
| Sister | 0.69 | 0.11 | 0.16 | 57 | Normal | 90 |
| | 0.74 | 0.05 | 0.07 | 67 | n.d. | n.d. |

Abbreviations: n.d., not done; VWF, von Willebrand factor.

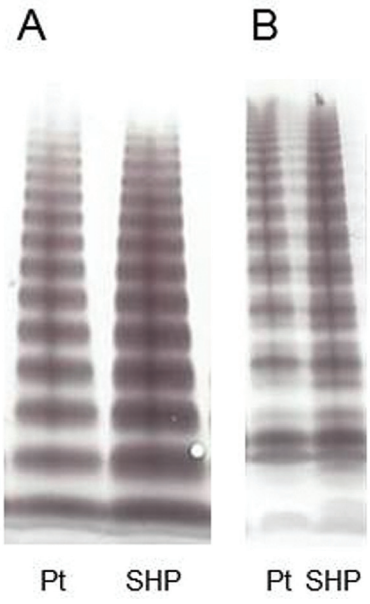


**Fig. 1** SDS agarose gel electrophoresis of von Willebrand factor multimers, visualized by enzyme immunostaining after capillary transfer onto PVDF (polyvinylidene difluoride) membranes. Multimeric analysis was performed by SDS-agarose gel electrophoresis in 1.0% (**A**) and in 2.2% (**B**) SDS-agarose gels (demonstrated for the boy).

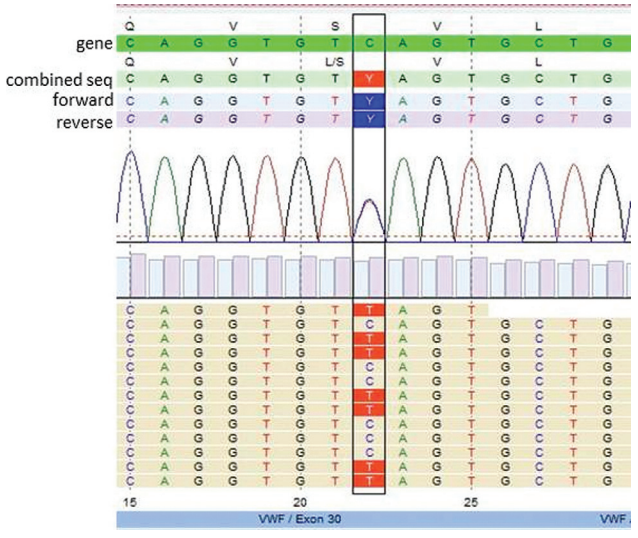


**Fig. 2** Molecular genetic analysis; NM_000552.3(VWF):c.5192[C > T];[=] (p.S1731L).

normal. For genetic analysis, we performed NGS using enriched gene panel diagnostics (Nextera Rapid Custom Enrichment Kit followed by sequencing on a MiSeq; both Illumina, San Diego, California, United States). The coverage for the enriched sequence of the *VWF* reached 99% for 20× and 97% for 100×. In exon 30 (NM_000552.3), we identified a heterozygous nsSNV (c.5192C > T) (►**Fig. 2**). The nsSNV is listed in dbSNP (rs764077750) and gnomAD (genome aggregation database) with rare minor allele frequency (ALL: 0.0012%). The variant is leading to an amino acid substitution from serine to leucine at position 1731 (p.Ser1731Leu) within the A3 domain of VWF. The A3 domain is considered to be the major binding site for collagen types I and III. In silico pathogenicity prediction is concordant pathogenic (SIFT, MutationTaster, PolyPhen2, CADD; ►**Table 2**). The variant is absent in the locus-specific database EAHAD and the public version of HGMD (Human Gene Mutation Database) (accessed July 10, 2021). However, the amino acid position p.1731 has been described with threonine instead of wild-type serine (p.Ser1731Thr) to be present in two patients with reduced

**Table 2** In silico pathogenicity prediction for VWF variant p.Ser1731Leu

| Program | Output |
|---|---|
| SIFT | Deleterious (score: 0.01, median: 3.34) |
| MutationTaster | Disease causing (prob: 1) |
| PolyPhen2 HumDiv | Probably damaging (score: 0.983) |
| CADD score | 26.5 |

Abbreviation: VWF, von Willebrand factor.

binding of VWF to collagen. Expression of the VWF mutant p.Ser1731Thr in COS7 cells confirmed the functional defect.[3] Based on these findings, we classified p.Ser1731Leu as *novel* likely pathogenic variant within the A3 domain of VWF causing VWD type 2M CBD.

## Conflict of Interest

B.Z. received research funding from Biotest AG, Takeda, and CSL Behring and honoraria for lectures from Bayer and CSL Behring. M.B. received travel support from Takeda, CSL Behring, SOBI, and Bayer.

 

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