

A Novel Homozygous Canonical Splice-Donor VWF Variant Causing Type 3 von Willebrand Disease in an Iranian Patient: Case Report

Narjes Bakhtari

Blood Diseases Research Center (BDRC), Iranian Comprehensive Hemophilia Care Center, Iran
University of Medical Sciences, Tehran, Iran

Morvarid Ataebi

Blood Diseases Research Center (BDRC), Iranian Comprehensive Hemophilia Care Center, Iran
University of Medical Sciences, Tehran, Iran

Azam Bolhassani

Blood Diseases Research Center (BDRC), Iranian Comprehensive Hemophilia Care Center, Iran
University of Medical Sciences, Tehran, Iran

Shadi Tabibian

sh.tabibian@gmail.com

Blood Diseases Research Center (BDRC), Iranian Comprehensive Hemophilia Care Center, Iran
University of Medical Sciences, Tehran, Iran

Case Report

Keywords: Type 3 von Willebrand disease, VWF gene, whole-exome sequencing, canonical splice-donor variant, genetic counselling

Posted Date: October 21st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10487540/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

A Novel Homozygous Canonical Splice-Donor VWF Variant Causing Type 3 von Willebrand Disease in an Iranian Patient: Case Report

Narjes bakhtari¹, Morvarid Ataebi¹ Azam Bolhassani^{1,2}, Shadi Tabibian¹

- 1- Blood Diseases Research Center (BDRC), Iranian Comprehensive Hemophilia Care Center, Iran University of Medical Sciences, Tehran, Iran
- 2- Department of Hepatitis, AIDS and Blood-borne Diseases, Pasteur Institute of Iran, Tehran, Iran.

Corresponding author: Shadi Tabibian

Blood Disease Research Center (BDRC), Iranian Comprehensive Hemophilia Care Center, Iran University of Medical Sciences, Tehran, Iran.

Sh.tabibian@gmail.com

Abstract:

Type 3 von Willebrand disease (VWD) is the most severe form of VWD, characterised by a near-complete deficiency of von Willebrand factor (VWF) and severe bleeding manifestations. We report a 16-year-old Iranian female born to consanguineous parents who presented with recurrent mucocutaneous bleeding, including epistaxis, gingival bleeding, and prolonged bleeding following minor trauma. Laboratory evaluation revealed markedly reduced VWF antigen and activity together with decreased factor VIII activity (all below 5%), consistent with type 3 VWD. Whole-exome sequencing identified a previously unreported homozygous canonical splice-donor variant in VWF (NM_000552.5:c.2186+1G>A), which was confirmed by Sanger sequencing, with both parents shown to be heterozygous carriers on segregation analysis. The variant is predicted to disrupt normal pre-mRNA splicing and thereby impair production of functional VWF, and was classified as pathogenic according to the ACMG/AMP framework. This case expands the mutational spectrum of VWF and highlights the value of molecular diagnosis for carrier detection, family studies, and genetic counselling in severe inherited bleeding disorders.

Keywords: Type 3 von Willebrand disease; VWF gene; whole-exome sequencing; canonical splice-donor variant; genetic counselling.

Introduction

Von Willebrand disease (VWD) is the most common inherited bleeding disorder and results from quantitative or qualitative defects in von Willebrand factor (VWF) (1). VWF plays a central role in primary haemostasis by mediating platelet adhesion to exposed subendothelial collagen at sites of vascular injury. It also serves as the carrier protein for coagulation factor VIII (FVIII), protecting it from premature proteolytic degradation and thereby maintaining its stability in the circulation (2,3).

VWD is clinically and genetically heterogeneous and is classified into three principal types: types 1, 2, and 3 (4). Type 3 VWD is the rarest and most severe form of the disease and is typically inherited in an autosomal recessive pattern. It is characterised by a near-complete deficiency of circulating VWF, accompanied by a marked secondary reduction in FVIII activity (5). Affected individuals commonly present early in life with severe mucocutaneous bleeding and, in some

cases, potentially life-threatening haemorrhage (6). The *VWF* gene is located on chromosome 12p13.31, spans approximately 178 kb, and comprises 52 exons encoding a precursor protein of 2,813 amino acids (7). Type 3 VWD is most commonly caused by biallelic loss-of-function variants, including nonsense, frameshift, canonical splice-site, and large deletions. These variants may impair *VWF* transcription, pre-mRNA processing, protein synthesis, or secretion, ultimately resulting in absent or markedly reduced circulating VWF (8). Molecular characterisation of such variants is particularly important in patients with severe VWD because it can confirm the diagnosis, clarify the inheritance pattern, facilitate carrier detection, and support genetic counselling.

In this report, we describe the clinical and molecular findings in an Iranian patient with type 3 VWD in whom whole-exome sequencing identified a previously unreported homozygous canonical splice-donor variant, NM_000552.5:c.2186+1G>A, affecting the invariant +1 nucleotide of intron 16. We also present the familial segregation analysis, in silico splicing predictions, and classification of the variant according to the ACMG/AMP framework.

Materials and Methods

DNA extraction

Peripheral blood samples were collected from the proband and both parents in EDTA-containing tubes. Genomic DNA was extracted using the RIBO-prep Nucleic Acid Extraction Kit according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Extracted DNA was stored at -20°C until further analysis.

Whole-Exome Sequencing and Bioinformatics Analysis

Whole-exome sequencing (WES) was performed using genomic DNA obtained from the proband. Parental DNA samples were used for targeted Sanger sequencing to assess segregation of the identified variant. Exonic regions and adjacent exon–intron boundaries were enriched using the

Twist Comprehensive Exome Panel (Twist Bioscience, South San Francisco, CA, USA). Sequencing was performed on an Illumina NovaSeq 6000 platform using 2×150 -bp paired-end reads.

The mean sequencing depth across the targeted regions was approximately $150\times$, and approximately 96% of sequenced bases achieved a Phred quality score of ≥ 30 (Q30). Raw sequencing reads were aligned to the human reference genome GRCh38/hg38 using the Burrows–Wheeler Aligner maximal exact match algorithm (BWA-MEM). Sequence alignment files were converted to BAM format, sorted, indexed, and processed using SAMtools and Picard Tools. Variant calling was performed according to the Genome Analysis Toolkit Best Practices workflow using GATK version 4.3.0.0.

Detected variants were annotated using the Ensembl Variant Effect Predictor. Population allele frequencies and previously reported clinical interpretations were assessed using gnomAD, ClinVar, dbSNP, the Human Gene Mutation Database Professional, the EAHAD VWF Variant Database, and the Leiden Open Variation Database (LOVD). Gene–disease associations were reviewed using Online Mendelian Inheritance in Man (OMIM). All databases were accessed in July 2026, and gnomAD version 4.1 was used.

Candidate variants were prioritised based on allele frequency, zygosity, predicted molecular consequence, expected inheritance pattern, and concordance with the patient’s clinical and laboratory phenotype. Variants considered potentially relevant were visually reviewed using Integrative Genomics Viewer to assess read quality and exclude apparent sequencing or alignment artefacts.

The predicted functional effect of the identified variant was assessed using Combined Annotation Dependent Depletion (CADD), MutationTaster, and Genomic Evolutionary Rate Profiling (GERP++). Its potential effect on pre-mRNA splicing was evaluated using MaxEntScan and SpliceAI, with a SpliceAI delta-score threshold of ≥ 0.5 considered indicative of a splicing effect. CADD, MutationTaster, and GERP++ were applied using default parameters. All in-silico tools and databases were accessed in July 2026. Evolutionary conservation was assessed using the UCSC 100-way vertebrate Multiz Alignment. Variant classification was performed according to the American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines, with consideration of the ClinGen Sequence Variant Interpretation Working Group recommendations for application of the PVS1 criterion.

Sanger Sequencing and Segregation Analysis

The candidate VWF variant identified by whole-exome sequencing was confirmed by bidirectional Sanger sequencing. The genomic region encompassing the exon 16–intron 16 boundary of VWF was amplified by polymerase chain reaction using specifically designed primers.

The amplified products were purified and sequenced in both the forward and reverse directions using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Segregation analysis was performed in both parents to determine the familial inheritance pattern of the identified variant.

Sequence chromatograms were analysed using Mutation Surveyor software, version 5.1.2 (SoftGenetics, State College, PA, USA), with reference to the MANE Select VWF transcript NM_000552.5.

Ethical Approval and Consent to Participate

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Iran University of Medical Sciences.

Written informed consent for genetic testing and participation in the study was obtained from the patient and her legal guardian. Written informed consent for publication of anonymised clinical information, pedigree data, and genetic findings was also obtained.

Results

Clinical Presentation and Hemostatic Laboratory Findings

A 16-year-old Iranian female was referred to the Iranian Comprehensive Hemophilia Care Center (ICHCC) in Tehran, Iran, for evaluation of recurrent severe bleeding episodes since infancy. She was born to consanguineous parents who were first cousins once removed; specifically, her father was the first cousin of her maternal grandmother. There was no known history of a diagnosed inherited bleeding disorder in previous generations (Figure 1). Her first bleeding manifestation occurred at approximately 9 months of age and consisted of spontaneous soft-tissue haematomas. During childhood, she experienced recurrent epistaxis, gingival bleeding, and soft-tissue haematomas, several of which required hospitalisation and haemostatic treatment. She had received fresh frozen plasma, cryoprecipitate, and plasma-derived

VWF/FVIII concentrate (Haemate P) on multiple occasions, with a satisfactory clinical response.

There was no history of anticoagulant exposure, liver disease, autoimmune disease, malignancy, or other conditions associated with acquired bleeding disorders. Physical examination revealed normal growth and no clinically significant abnormalities.

At diagnostic evaluation, prothrombin time was normal at 10 s, whereas activated partial thromboplastin time was markedly prolonged at 75 s. The activated partial thromboplastin time mixing study corrected into the normal range, supporting a coagulation factor deficiency rather than the presence of an inhibitor. Plasma VWF antigen, VWF ristocetin cofactor activity, and factor VIII activity were markedly reduced to 4%, 3%, and 4%, respectively. Ristocetin-induced platelet aggregation was absent at all tested ristocetin concentrations, whereas aggregation responses to ADP, collagen, and arachidonic acid were preserved. Screening for factor VIII inhibitors was negative at 0 Bethesda units. Concurrent laboratory testing showed microcytic hypochromic anaemia with a serum ferritin concentration of 5.1 ng/mL, consistent with iron deficiency secondary to chronic blood loss. Collectively, the severe bleeding phenotype and haemostatic laboratory findings were strongly consistent with type 3 von Willebrand disease. Detailed laboratory findings are presented in Table 1. Given the early onset and severity of bleeding, markedly reduced VWF levels, parental consanguinity, and absence of a previous molecular diagnosis, genetic testing was performed to identify the underlying disease-causing variant.

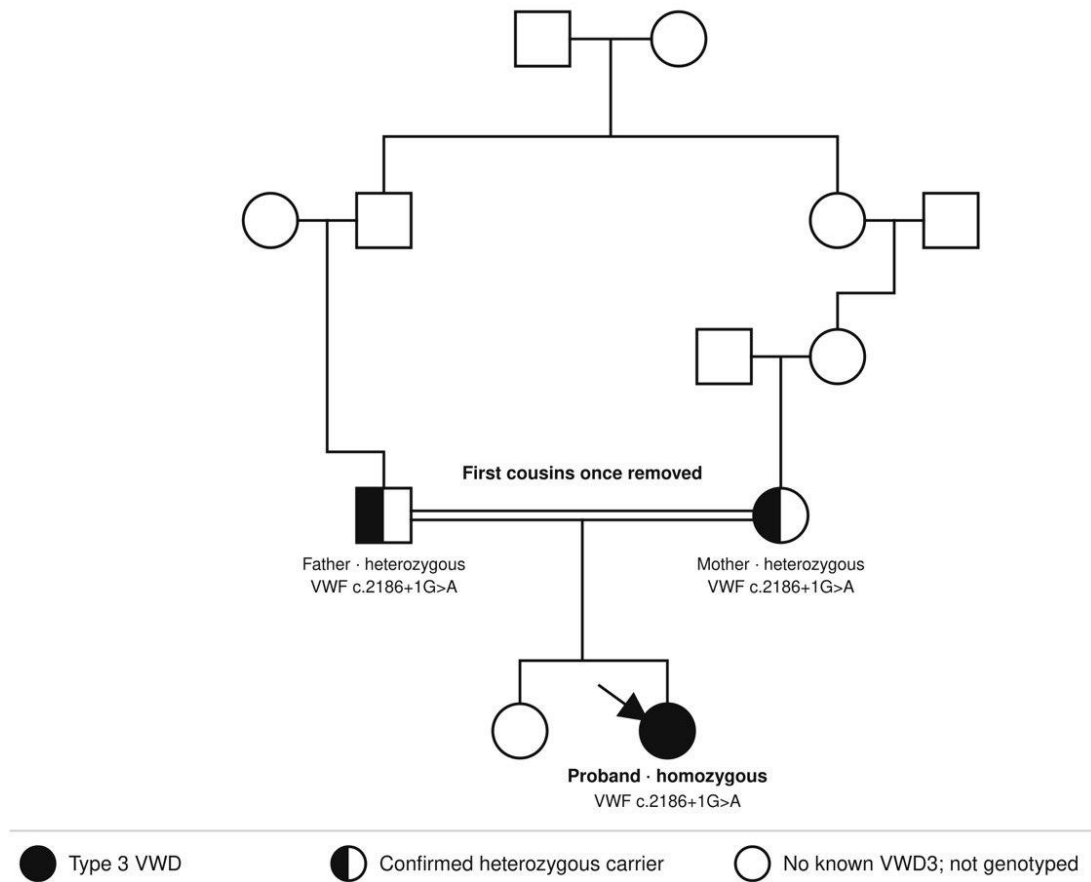


Figure 1. Pedigree and segregation of the VWF c.2186+1G>A variant. The proband, indicated by an arrow, is affected with type 3 von Willebrand disease and is homozygous for the VWF NM_000552.5:c.2186+1G>A variant. Both parents are confirmed heterozygous carriers. The parents are related as first cousins once removed; specifically, the father is the first cousin of the maternal grandmother. Filled symbols indicate affected individuals, half-filled symbols indicate confirmed heterozygous carriers, and open symbols indicate individuals without a known diagnosis of type 3 von Willebrand disease who were not genetically tested. The double horizontal line denotes consanguinity.

Table 1. Laboratory findings of the proband at diagnosis

Test	Result	Reference range
VWF antigen (VWF:Ag)	4%	50–150%

Test	Result	Reference range
VWF activity (VWF:RCo)	3%	50–150%
Factor VIII activity	4%	50–150%
Prothrombin time (PT)	10 s	10–13 s
Activated partial thromboplastin time (aPTT)	75 s	28–38 s
aPTT mixing test	38 s (corrected)	30–40 s
Bleeding time (Ivy)	>20 min	3–7 min
Fibrinogen (Clauss)	2.28 g/L	1.5–4.5 g/L
FXIII screening	Normal	Normal
Platelet count	$672 \times 10^9/L$	$150\text{--}450 \times 10^9/L$
FVIII inhibitor (Bethesda)	0 BU	0 BU
VWF inhibitor (Bethesda)	0 BU	0 BU
Ristocetin-induced platelet aggregation	Absent	—
ADP / collagen / arachidonic acid aggregation	Normal	—
Blood group	O, Rh positive	—
Hemoglobin	11.2 g/dL	12.5–16 g/dL
Mean corpuscular volume (MCV)	86 fL	79–96 fL
Serum ferritin	5.1 ng/mL	10–124 ng/mL

Abbreviations: VWF, von Willebrand factor; VWF:Ag, VWF antigen; VWF:RCo, VWF ristocetin cofactor activity; PT, prothrombin time; aPTT, activated partial thromboplastin time; FXIII, coagulation factor XIII; BU, Bethesda units; MCV, mean corpuscular volume. VWF:Ag was measured by ELISA and VWF:RCo by the ristocetin cofactor assay.

Whole-Exome Sequencing and Variant Identification

Whole-exome sequencing identified a homozygous canonical splice-donor variant in the VWF gene (NM_000552.5:c.2186+1G>A). The variant affects the invariant guanine at the +1 position

of intron 16, immediately downstream of exon 16. The variant was detected at a sequencing depth of 65×, with all reads supporting the alternative allele and none supporting the reference allele (allele depth, 0/65), consistent with a homozygous genotype. Visual inspection of the aligned reads using Integrative Genomics Viewer, version 2.19.4, confirmed high-quality read support without apparent sequencing or alignment artefacts. No other pathogenic or likely pathogenic single-nucleotide variants or small insertions/deletions relevant to the patient's bleeding phenotype were identified within the analysed regions. At the time of analysis, the c.2186+1G>A variant was absent from gnomAD version 4.1 and had not been reported in ClinVar, HGMD Professional, the EAHAD VWF Variant Database, LOVD, or the published literature.

Variant Annotation and In Silico Assessment

The NM_000552.5:c.2186+1G>A variant was annotated as a canonical splice-donor variant affecting the invariant +1 guanine of the donor splice site of intron 16. On the GRCh38 reference genome, the variant corresponds to chr12:6,052,542C>T (NC_000012.12:g.6052542C>T). The difference in nucleotide representation reflects the reverse-strand orientation of the VWF gene. MutationTaster predicted a disease-causing effect, with a reported probability of 1.0. The variant had a CADD Phred score of 35 and a GERP++ score of 4.77, supporting predicted deleteriousness and strong evolutionary constraint at the affected nucleotide. Both MaxEntScan and SpliceAI predicted marked disruption of the native donor splice site, supporting an adverse effect on normal VWF pre-mRNA splicing. However, because RNA-based functional studies were not performed, the precise transcript consequence could not be established. The molecular annotation and in silico findings are summarised in Table 2.

Table 2. Molecular annotation and in silico assessment of the *VWF* NM_000552.5:c.2186+1G>A variant

Feature	Result	Interpretation
Gene	<i>VWF</i>	Associated with von Willebrand disease
Reference transcript	NM_000552.5	MANE Select transcript
Transcript-level variant	c.2186+1G>A	Affects the invariant +1 nucleotide

Feature	Result	Interpretation
Genomic variant	GRCh38: chr12:6,052,542C>T (NC_000012.12:g.6052542C>T)	Reverse-strand representation
Molecular consequence	Canonical splice-donor variant	Predicted disruption of normal splicing
Zygosity	Homozygous	Consistent with autosomal recessive VWD3
Read depth	65×	Adequate sequencing coverage
Allele depth	0/65 (reference/alternative)	All reads supported the alternative allele
gnomAD allele frequency	Absent	Supports rarity
ClinVar/HGMD status	Not reported	Previously unreported variant at the time of analysis
CADD Phred score	35	Strong predicted deleteriousness
MutationTaster	Disease-causing (probability 1.0)	Supports a damaging effect
GERP++ score	4.77	Strong evolutionary constraint
Splice-site prediction	Disruption of the canonical donor site	Supports an abnormal splicing effect

Abbreviations: AD, allele depth; CADD, Combined Annotation Dependent Depletion; GERP++, Genomic Evolutionary Rate Profiling; gnomAD, Genome Aggregation Database; HGMD, Human Gene Mutation Database; VWD3, type 3 von Willebrand disease.

Evolutionary Conservation Analysis

Evolutionary conservation analysis using the UCSC 100-way vertebrate Multiz Alignment showed that the nucleotide corresponding to NM_000552.5:c.2186+1 is highly conserved across vertebrate species.

The high degree of conservation, together with the high GERP++ score, supports strong functional constraint at the canonical donor splice site and is consistent with a deleterious effect on normal pre-mRNA splicing.

ACMG/AMP Classification of the Variant

The homozygous NM_000552.5 (VWF):c.2186+1G>A variant was classified according to the ACMG/AMP framework, with consideration of the ClinGen Sequence Variant Interpretation Working Group recommendations for application of the PVS1 criterion.

The variant met the PVS1, PM2_Supporting, and PP4 criteria, supporting a final classification of Pathogenic.

PVS1 was applied at a very strong level because the variant affects the invariant +1 nucleotide of the canonical donor splice site of intron 16. Loss of function is an established disease mechanism for type 3 von Willebrand disease, and canonical ± 1 or ± 2 splice-site variants are expected to disrupt normal pre-mRNA splicing. Exon 16 is present in the MANE Select transcript NM_000552.5 and is not known to undergo biologically relevant alternative splicing. If the variant results in complete exon 16 skipping, the resulting transcript is predicted to be out of frame and susceptible to nonsense-mediated decay. However, in the absence of RNA-based functional analysis, the precise aberrant transcript consequence could not be established, and other abnormal splicing outcomes cannot be excluded.

PM2_Supporting was applied because the variant was absent from all populations represented in gnomAD version 4.1.

PP4 was applied because the patient exhibited a highly specific phenotype consistent with type 3 von Willebrand disease, including severe bleeding beginning in infancy, profoundly reduced VWF antigen and activity, and markedly decreased factor VIII activity.

Sanger sequencing confirmed homozygosity in the proband and demonstrated that both parents were heterozygous carriers, establishing biparental inheritance consistent with an autosomal recessive pattern. Based on the combination of PVS1, PM2_Supporting, and PP4, the NM_000552.5(VWF):c.2186+1G>A variant was classified as Pathogenic.

Sanger Sequencing Confirmation and Segregation Analysis

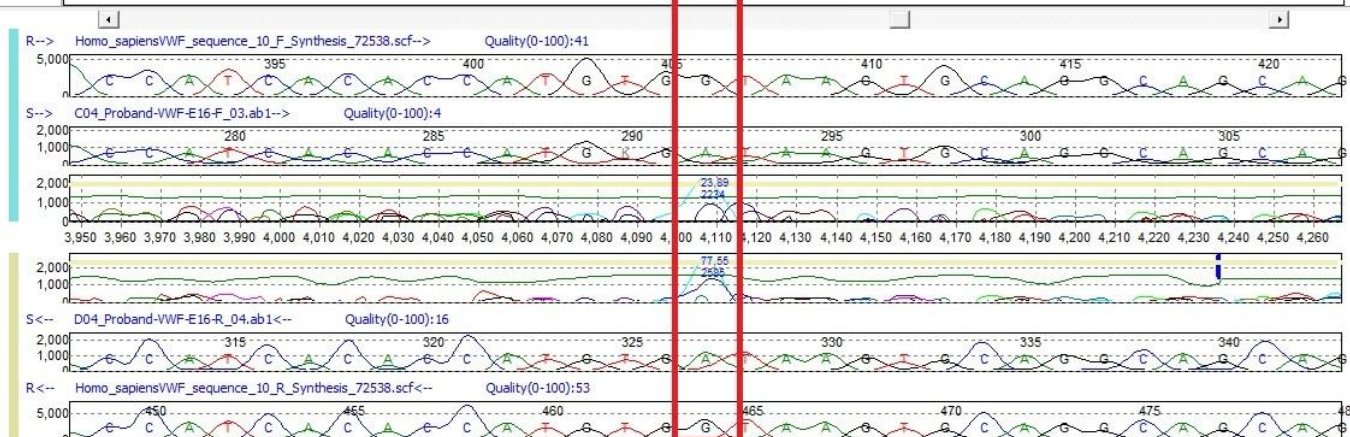
Bidirectional Sanger sequencing confirmed the NM_000552.5:c.2186+1G>A variant identified by whole-exome sequencing. The proband was homozygous for the G>A substitution at the canonical donor splice site of intron 16, whereas both parents were heterozygous carriers.

These findings confirmed the WES findings and demonstrated biparental inheritance of the variant, consistent with an autosomal recessive inheritance pattern (Figure 2).

	72616	72621	72626	72631	72636	72641																							
*nce_106.fa	C	C	A	T	C	A	C	C	A	T	G	T	G	G	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G
*72538.scf	C	C	A	T	C	A	C	C	A	T	G	K	G	G	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G
*6-R_04.ab1	C	C	A	T	C	A	C	C	A	T	G	T	G	G	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G
*_72538.scf	C	C	A	T	C	A	C	C	A	T	G	T	G	G	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G

*nce_106.fa
*6-F_03.ab1

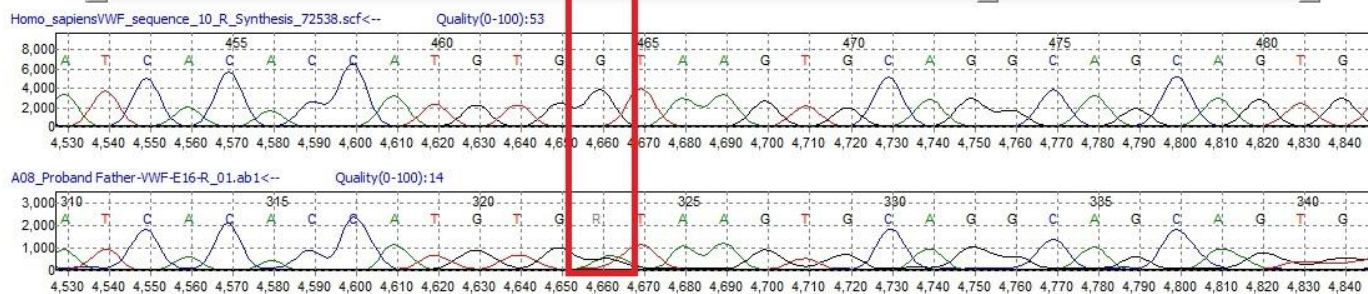
A. Proband-homozygous



	72616	72621	72626	72631	72636	72641	72646																						
*nce_106.fa	A	T	C	A	C	C	A	T	G	T	G	G	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G	T	G
*72538.scf	A	T	C	A	C	C	A	T	G	T	G	R	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G	T	G
*6-R_01.ab1	A	T	C	A	C	C	A	T	G	T	G	R	A	A	G	T	G	C	A	G	G	C	A	G	C	A	G	T	G

*nce_106.fa
*6-R_01.ab1

B. Father-Heterozygous



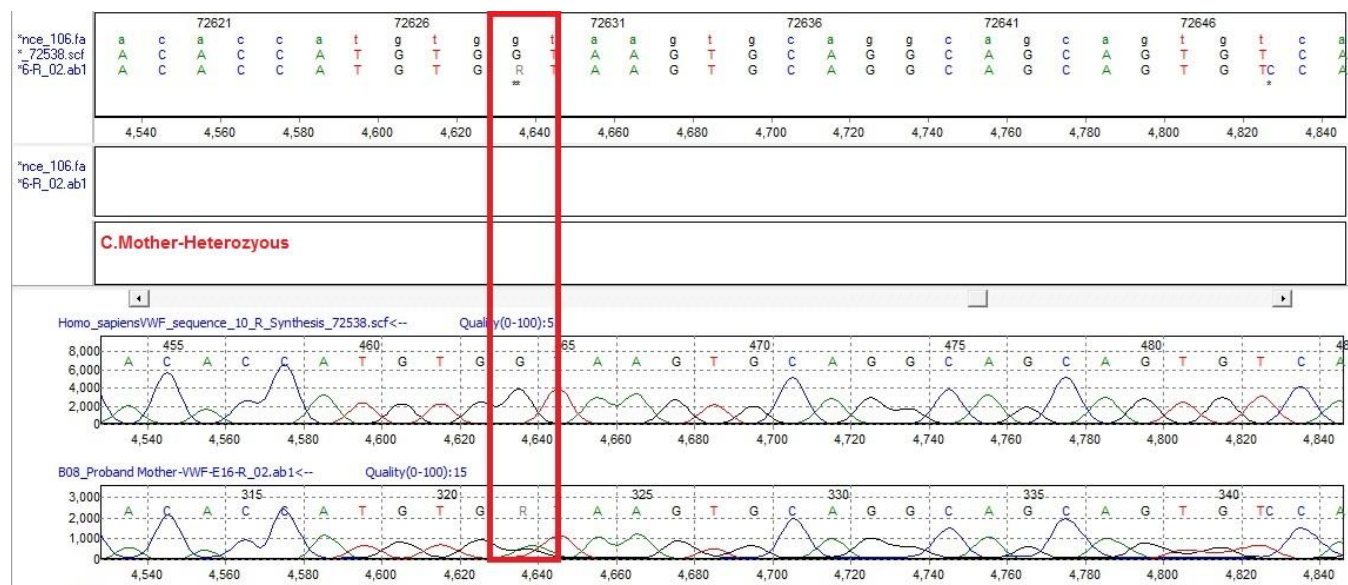


Figure 2. Sanger sequencing confirmation and familial segregation of the VWF NM_000552.5:c.2186+1G>A variant. Representative Sanger sequencing chromatograms from the proband (A), father (B), and mother (C). The proband is homozygous for the c.2186+1G>A splice-donor variant, whereas both parents are heterozygous carriers, confirming biparental inheritance consistent with autosomal recessive transmission. The variant position is indicated by the red boxes.

Discussion

We report a previously unreported homozygous canonical splice-donor variant, NM_000552.5:c.2186+1G>A, identified in an Iranian patient with a clinical and laboratory phenotype strongly consistent with type 3 von Willebrand disease. The variant affects the invariant guanine at the +1 position of the donor splice site of intron 16. This nucleotide forms part of the highly conserved GT dinucleotide required for normal recognition of the exon–intron boundary by the spliceosome. Single-nucleotide substitutions affecting canonical donor or acceptor splice sites

frequently disrupt normal pre-mRNA processing and represent an established loss-of-function mechanism in inherited human disorders (9).

Disruption of the native donor splice site may result in several aberrant transcript outcomes, including exon skipping, intron retention, partial exon deletion, or activation of a cryptic splice site (9). In the present case, MaxEntScan and SpliceAI predicted marked disruption of the native donor splice site. If complete skipping of exon 16 occurs, the resulting transcript is predicted to be out of frame and susceptible to nonsense-mediated decay. However, because RNA-based functional analysis was not performed, the precise transcript consequence of the c.2186+1G>A variant could not be established, and alternative abnormal splicing outcomes cannot be excluded. The affected donor splice site lies within the region of VWF encoding the propeptide (VWFpp), which is essential for normal multimerisation of von Willebrand factor (11). Although the c.2186+1G>A variant does not directly alter the coding sequence, disruption of the donor splice site immediately downstream of exon 16 would be expected to interfere with normal VWF transcript processing and ultimately impair the production of functional VWF. Notably, exon 16 of VWF has previously been examined in Iranian patients with type 3 von Willebrand disease, although no pathogenic variant in this region was identified in that cohort (12). The present case therefore adds a pathogenic splice-donor variant at the exon 16/intron 16 boundary in this population (12).

The molecular findings in this patient are consistent with the known genetic architecture of type 3 von Willebrand disease. Biallelic loss-of-function variants, including nonsense, frameshift, canonical splice-site, and large deletion variants, account for a substantial proportion of genetically characterised cases (7–10,13). In the 3WINTERS-IPS study, Baronciani et al. demonstrated marked allelic heterogeneity among European and Iranian patients with type 3 von Willebrand disease, including numerous homozygous and previously unreported VWF variants (13). The relatively high proportion of homozygous variants among Iranian patients is consistent with the higher prevalence of consanguineous unions in this population (13).

The pathogenicity of the c.2186+1G>A variant is supported by several independent lines of evidence. First, the variant affects the invariant +1 nucleotide of a canonical donor splice site in a gene for which loss of function is an established mechanism of type 3 von Willebrand disease, supporting application of the PVS1 criterion at a very strong level (8,14,15). Second, the variant was absent from all populations represented in gnomAD version 4.1, supporting application of

PM2_Supporting. Third, the patient exhibited a highly specific phenotype characterised by severe bleeding beginning in infancy, profoundly reduced VWF antigen and activity, and markedly decreased factor VIII activity, supporting application of PP4 (6,14,16). Sanger sequencing confirmed homozygosity in the proband and demonstrated that both parents were heterozygous carriers, establishing biparental inheritance consistent with an autosomal recessive pattern. Collectively, these findings support classification of the variant as Pathogenic according to the ACMG/AMP framework (14–16).

Parental consanguinity is an important feature of this case. Consanguineous unions increase the likelihood that both parents carry the same rare recessive allele and consequently increase the probability of homozygosity in their offspring (17,18). This pattern contributes to the increased burden of severe autosomal recessive disorders in some populations of the Greater Middle East (17). Molecular confirmation is therefore particularly valuable for carrier detection, cascade testing of at-risk relatives, genetic counselling, and discussion of reproductive options.

This case also illustrates the clinical value of molecular genetic testing in severe inherited bleeding disorders. Although type 3 von Willebrand disease is primarily diagnosed through clinical assessment and haemostatic laboratory testing, identification of the underlying genetic defect can confirm the molecular diagnosis, clarify the inheritance pattern, support family studies, and assist in distinguishing VWD from other inherited bleeding disorders with overlapping phenotypes (7,19). Whole-exome sequencing enables simultaneous assessment of the coding regions and adjacent splice junctions of multiple haemostasis-related genes. Nevertheless, it may not reliably detect all deep-intronic variants, complex structural rearrangements, or copy-number abnormalities, and negative findings should therefore be interpreted in the context of the technical limitations of the assay (7).

The principal limitation of this report is the absence of RNA analysis or another functional splicing assay. Consequently, the exact effect of the c.2186+1G>A variant on the VWF transcript remains unknown. In addition, VWF multimer analysis and VWF propeptide measurement were not performed, although at a VWF antigen level of 4% multimer analysis is of limited additional diagnostic value; VWF ristocetin cofactor activity at this very low level may also approach the lower limit of detection of the assay. Finally, the findings are based on a single family. Despite these limitations, the canonical splice-site location, absence from population databases, severe and

highly specific phenotype, and confirmed biparental inheritance provide strong and concordant evidence supporting a pathogenic role for this variant.

Conclusion

We identified a previously unreported homozygous canonical splice-donor variant, NM_000552.5:c.2186+1G>A, in an Iranian patient with type 3 von Willebrand disease. The variant affects the invariant +1 nucleotide of the donor splice site of intron 16 and is predicted to disrupt normal VWF pre-mRNA splicing.

The severe and highly specific clinical phenotype, absence of the variant from population databases, and confirmation of biparental inheritance support its classification as pathogenic according to the ACMG/AMP framework. This finding expands the mutational spectrum of VWF and underscores the value of molecular testing for diagnostic confirmation, family studies, carrier detection, and genetic counselling in severe inherited bleeding disorders. Future RNA-based or functional splicing studies would help confirm the precise consequence of this variant on VWF transcript processing.

Acknowledgements

The authors thank the patient and her family for their cooperation and willingness to participate in this study. The authors also acknowledge the clinical and laboratory personnel of the Comprehensive Hemophilia Care Center for their assistance with the clinical evaluation and molecular investigations.

Funding

The authors received no specific funding for this work.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Patient-level data are not publicly available because of privacy and ethical restrictions.

Declaration of Generative AI Use

The authors used a generative AI-based large language model solely for language editing and improvement of readability. The tool was not used to generate, analyse, or interpret clinical, laboratory, or genetic data. All authors reviewed and edited the manuscript and take full responsibility for its content.

Author Contributions

All authors contributed to the conception and design of the study. Clinical evaluation and data collection were performed by Shadi Tabibian. Laboratory and molecular analyses were performed by Morvarid Ataebi. Variant interpretation was performed by Narjes Bakhtari. The initial draft of the manuscript was prepared by Azam Bolhassani. All authors critically reviewed and revised the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

References:

1. Seidizadeh O, Eikenboom JCJ, Denis CV, Flood VH, James P, Lenting PJ, et al. von Willebrand disease. *Nat Rev Dis Primers*. 2024;10(1):51.
2. Turecek PL, Johnsen JM, Pipe SW, O'Donnell JS. Biological mechanisms underlying inter-individual variation in factor VIII clearance in haemophilia. *Haemophilia*. 2020;26(4):575-83.
3. Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. *Blood*. 2016;128(16):2007-16.
4. Dorgalaleh A, Tabibian S, Shiravand Y, Favaloro EJ. von Willebrand disease. In: Dorgalaleh A, editor. *Congenital Bleeding Disorders*. Cham: Springer; 2018. p. 57-102.
5. Sadler JE, Budde U, Eikenboom JC, Favaloro EJ, Hill FG, Holmberg L, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. *J Thromb Haemost*. 2006;4(10):2103-14.
6. Tosetto A, Badiie Z, Baghaipour MR, Baronciani L, Battle J, Berntorp E, et al. Bleeding symptoms in patients diagnosed as type 3 von Willebrand disease: Results from 3WINTERS-IPS, an international and collaborative cross-sectional study. *J Thromb Haemost*. 2020;18(9):2145-54.
7. Seidizadeh O, Lillicrap D. Molecular genetic testing in von Willebrand disease: past, present, and beyond. *Haematologica*. 2026;111(1):44-53.
8. Christopherson PA, Haberichter SL, Flood VH, Perry CL, Sadler BE, et al. Molecular pathogenesis and heterogeneity in type 3 VWD families in U.S. Zimmerman program. *Journal of Thrombosis and Haemostasis*. 2022;20(7):1576-88.
9. Krawczak M, Reiss J, Cooper DN. The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. *Hum Genet*. 1992;90(1-2):41-54.
10. Eikenboom JCJ. Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. *Best Pract Res Clin Haematol*. 2001;14(2):365-379.
11. Haberichter SL, O'Donnell JS. Structure and multiple functions of von Willebrand factor. *Haematologica* 2026; 111(1):15-25

12. Nasiri M, Galehdari H, Darbouy M, Yavarian M, Keikhaee B. The role of exon 45 and 16 in the pathogenesis of von Willebrand disease in Iranian patients. *Iran J Ped Hematol Oncol*. 2012;2(4):164–170.
13. Baronciani L, Peake I, Schneppenheim R, Goodeve A, Ahmadinejad M, Badiie Z, et al. Genotypes of European and Iranian patients with type 3 von Willebrand disease enrolled in 3WINTERS-IPS. *Blood Adv*. 2021;5(15):2987–3001.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants. *Genet Med*. 2015;17(5):405–424.
15. Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, et al. Recommendations for interpreting the loss-of-function PVS1 ACMG/AMP variant criterion. *Hum Mutat*. 2018;39(11):1517–1524.
16. Biesecker LG, Byrne AB, Harrison SM, Pesaran T, Schäffer AA, Shirts BH, et al. ClinGen guidance for use of the PP1/BS4 co-segregation and PP4 phenotype specificity criteria. *Am J Hum Genet*. 2024;111(1):24–38.
17. Chekroun I, Shenbagam S, Almarri MA, Mokrab Y, Uddin M, Alkhnbashi OS, et al. Genomics of rare diseases in the Greater Middle East. *Nat Genet*. 2025;57(3):505–514.
18. Chouery E, Ibrahim JN, Deeb ME, Sobh A, Ghanem M, Sobh J, et al. Consanguinity in the Lebanese population: knowledge, attitudes and practices. *J Community Genet*. 2026;17(2).
19. Dorgalaleh A, Tabibian S, Shams M, Ala F, Bahoush G, Jazebi M, et al. Von Willebrand disease in Iran: diagnosis and management. *Ann Blood*. 2018;3:4.