

The Current Understanding of Molecular Pathogenesis of Quantitative von Willebrand Disease, Types 1 and 3

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Abstract

Keywords

- von Willebrand factor
- von Willebrand disease
- bleeding disorder
- gene mutation

Zusammenfassung

Schlüsselwörter

- von-Willebrand-Faktor
- von-Willebrand-Erkrankung
- Blutgerinnungsstörung
- Mutation

Von Willebrand disease (VWD), the most prevalent congenital bleeding disorder, arises from deficiencies in quantity or quality of von Willebrand factor (VWF). The quantitative deficiencies of VWF are considered to be either VWD type 1 (mild/moderate reduction of VWF) or type 3 (virtual absence of VWF). Following cloning of the VWF gene (VWF) in the 1980s, significant progress has been made in our understanding of the pathogenesis of VWD. The genetic basis of type 3 VWD is well defined. VWF causative variations comprising predominantly null alleles have been identified in more than 85% of cases. In contrast, the molecular mechanisms in type 1 disease are only partially characterized. The VWF sequence variations, including mostly missense alterations, are found in only approximately 65% of type 1 VWD patients. It appears that genetic elements outside of VWF may contribute to the pathophysiology of type 1 VWD. This review discusses in detail the current understandings of the genetic basis and molecular mechanisms causing quantitative deficiencies of VWF.

Die Von Willebrand Erkrankung (VWE) ist die häufigsten erbliche Blutungsstörung, die durch Fehler im von Willebrand Faktor (VWF) verursacht wird. Diese Fehler können entweder zu einer Reduktion der VWF-Konzentration führen oder die Funktion des VWF beeinträchtigen. Wenn die VWF-Konzentration leicht bzw. mäßig reduziert ist, spricht man von einer VWE Typ 1, wenn praktisch kein VWF mehr nachweisbar ist von einer VWE Typ 3. Seit der Klonierung des VWF Gens (VWF) innerhalb der achtziger Jahre hat man viel über die Entstehung der VWE herausgefunden. Die genetische Basis des VWE Typ 3 ist relativ gut erklärt. VWF-verursachende Mutationen, die vorwiegend Null-Allele einschließen, wurden in mehr als 85% der Fälle identifiziert. Im Gegensatz dazu sind die genetischen Ursachen des VWE Typ 1 nur teilweise charakterisiert. Die VWF-Sequenzvariationen, die hauptsächlich Missense-Mutationen umfassen, werden nur bei ca. 65% der Patienten mit Typ 1 des VWE gefunden. Die Pathophysiologie der VWE des Typs 1 scheint zusätzlich noch durch genetische Faktoren beeinflusst zu werden, die außerhalb des VWF liegen. In dem hier vorliegenden Review werden ausführlich die genetischen Grundlagen sowie die molekularen Mechanismen diskutiert, die zu einer Reduktion der VWF-Konzentration führen.

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Introduction

Von Willebrand disease (VWD) is the most prevalent inherited bleeding disorder with an autosomal inheritance pattern. It is caused by quantitative and/or functional deficits of von Willebrand factor (VWF), a large multimeric plasma glycoprotein that plays critical roles in hemostasis. VWF mediates platelet adhesion and aggregation in primary hemostasis.^{1,2} Furthermore, VWF carries factor VIII (FVIII) in the circulation, protecting it from rapid proteolytic degradation and delivering it to sites of vascular damage for secondary hemostasis.¹

The estimated prevalence of VWD ranges from 0.1 to 1%, but only approximately 1 in 10,000 individuals has clinically significant bleeding symptoms.² The diagnosis of VWD is based on a personal history of bleeding and laboratory tests assessing abnormalities in VWF including measurement of VWF antigen levels and assays quantifying the binding activity of VWF to platelet GPIb α and collagen, as well as the coagulant activity of FVIII assay.¹ Diagnosis and classification of VWD can be challenging because of considerable heterogeneity in its molecular basis and variability in plasma VWF levels and its clinical manifestations. The mean plasma level of VWF is 100 IU dL⁻¹, but the population distribution is between 50 and 200 IU dL⁻¹.³ Many factors, such as ABO blood group, age, illness, pregnancy, and medication, have an impact on VWF levels, making the diagnosis of VWD difficult.^{3,4} Replacement with plasma-derived VWF concentrates and stimulating the release of VWF from intracellular storages by desmopressin administration are the main therapeutic options for VWD.⁵ However, the effectiveness of the latter treatment is variable between patients and is dependent on the type of genetic defect in VWF. It is expected that most type 1 VWD patients (except patients with accelerated VWF clearance) respond well to desmopressin, whereas type 2 VWD (due to dysfunctional VWF) and type 3 VWD (due to the absence of VWF) do not respond to desmopressin stimulation.⁵ In particular, desmopressin administration is contraindicated in gain-of-function type 2B VWD due to the occurrence of severe thrombocytopenia and a subsequent deficiency of plasma VWF.⁶

In the last three decades, various studies including mutation screening of populations with different ethnic origins, functional analysis of the individual sequence variations in heterologous systems, and genome-wide association studies have made significant progress in our understanding of the genetic etiology and pathogenesis of the VWD. In this review, we will discuss in detail the latest findings on the genetic basis and molecular mechanisms contributing to quantitative VWD types 1 and 3.

Von Willebrand Factor Gene and Protein Structure

The VWF gene (*VWF*) is located on the short arm of chromosome 12 (12p13.31), and it spans approximately 178 kb of genomic DNA. The *VWF* comprises 52 exons ranging from 40 to 342 bp, but exon 28 is exceptionally larger (1.4 kb in size).⁷ The 9-kb *VWF* transcript encodes a pre-pro-VWF protein of 2,813 amino acids (aa).⁸ The pre-pro-VWF precursor has a mosaic

architecture with the following domain structure: D1-D2-D'-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK.^{7,8} The recent studies demonstrated that D domains each contain a VW domain, a C8 fold, a trypsin inhibitor-like (TIL) structure, and an E module. However, D' lacks the VW domain and C8 fold; the D4 domain lacks the E module and encompasses distinctive subdomain D4N.⁸

The domains might be characterized as structural or functional, depending on their role in VWF structure or its interaction with other proteins. Structural domains are involved in the posttranslational processing of VWF, for example, C-terminal domains are required for dimerization of VWF monomers and the D1, D2, and D3 domains for multimerization of VWF dimers.^{9,10} The latter domains contain a CGLC consensus sequence that is highly homologous to the active site of protein disulfide isomerase (PDI) which promotes multimerization through interchain disulfide bonding.¹¹

Functional domains comprise those that enclose cleavage sites for proteolysis (domain A2) and binding sites for collagen (domains A1 and A3), platelets (domain A1 and C1 for platelet glycoprotein Ib [GPIb] and integrin GPIIb/IIIa receptors, respectively), and FVIII (domains D3 and D').¹²⁻¹⁵

VWF Biosynthesis

VWF is produced by endothelial cells and by the platelet precursor, megakaryocytes, accounting for approximately 85 and 15% of the total VWF present in normal platelet-rich plasma, respectively.^{16,17} The primary VWF product (pre-pro-VWF) undergoes systematic posttranslational modifications including dimerization, glycosylation, removal of propeptide domains (D1 and D2 domains), and eventual formation of high molecular-weight multimers containing up to 100 monomers.^{18,19}

In the endoplasmic reticulum (ER), pre-pro-VWF dimerizes "tail-to-tail" over three disulfide bonds formed between the cysteine knot (CK) domains, catalyzed by a family member of PDI (PDIA1).^{20,21} Subsequently, VWF dimers are moved to the Golgi complex, where multimerization and the proteolytic removal of the VWF propeptide occur. Multimerization occurs through additional interchain disulfide bridging between D3 domains.^{11,19,22} Propeptide and D' domain support proper alignment of the pro-VWF dimers, which is crucial for the multimerization process.²³ Along with the multimer elongation, VWF is packed in storage organelles, which are named Weibel-Palade bodies (WPBs) in endothelial cells and α -granules in platelets.²⁴ The VWF is secreted from endothelial cells through either a constitutive or a regulated pathway, whereas VWF stored in α -granules will be released upon platelet activation.^{25,26}

The VWF is extensively glycosylated with 17 N-linked and 10 O-linked oligosaccharide chains, which make up approximately 20% of the mass of the protein.^{27,28}

VWD Classification

VWD is classified as quantitative types 1 and 3, and qualitative type 2. Type 1 VWD, which is due to a mild to moderate reduction in functionally normal VWF, is the most prevalent of

the disease constituting 65 to 80% of VWD cases.⁴ Type 3 is characterized by the virtually complete absence of VWF, and it affects 0.1 to 5.3 per million people depending upon the rate of consanguinity.²⁹ Type 2 disease, characterized by functionally abnormal VWF, occurs in 20 to 35% of patients and is further classified into the four types: 2A, 2B, 2M, and 2N. The functional defects include enhanced (2B) or loss of (2A and 2M) affinity to platelet receptors or impaired binding to FVIII (2N).⁴

VWD Genetic Basis

A review of the current mutation databases indicates that genetic etiology and molecular pathogenesis of VWD are extremely heterogeneous. The VWD mutation listings can be found in the online Human Gene Mutation Database (HGMD) (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=VWF>) and the Coagulation Factor Variant Databases portal supported by the European Association for Haemophilia and Allied Disorders (EAHAD) (https://grenada.lumc.nl/LOVD2/VWF/home.php?select_db=VWF). In HGMD, 1,027 VWD mutations encompassing 673 missense/nonsense substitutions, 110 splicing variations, 20 regulatory substitutions, 136 small deletions/insertions/indels, 50 large deletions, 9 large insertions/duplications, 28 complex rearrangements, and 1 repeat variation are recorded (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=VWF>; accessed in March 2019).

The genetic investigations have provided extensive insights into genetic etiology of type 2 and type 3 VWD. Pathologic variants have been identified in more than 85% of type 2 and type 3 VWD populations.^{29–31} In contrast, the genetic pathology of type 1 disease remains incompletely defined in many cases, and pathogenic sequence variations are found in only approximately two-thirds of type 1 VWD patients. Likely, other genetic determinants outside of VWF are involved.^{30,32–34}

Although previous studies have largely elucidated the genetic basis of VWD, they may have missed mutations associated with VWD by conventional polymerase chain reaction and Sanger DNA sequencing. For instance, these techniques are incompetent to detect large heterozygous deletions (or duplications) because of the presence of the normal VWF allele which can mask the failure in amplification of the deleted allele. Many previous reports, missing methods analyzing gene dosage such as multiplex ligation-dependent probe amplification (MLPA), have failed to identify large deletions (or duplications) in heterozygous type 1 and 2 and in compound heterozygous type 3 patients. It is therefore expected that the contribution of copy number variations in the pathogenesis of VWD is underestimated. Furthermore, the former cohort studies have mainly focused on the sequencing of only coding exons of VWF and the immediate surrounding intronic region, and then potential causative mutations in the promoter region (particularly in mild-moderate type 1 VWD) and deep intronic region are overlooked. Additionally, the synonymous sequence alterations affecting splicing might have been passed over, since their potential impact had been misinterpreted in the first place. In some instances, the deleterious impact of the

previously overlooked VWF silent mutations was highlighted later following further RNA transcript studies and reanalysis of genomic DNA.^{35,36}

Pathogenic Mechanisms Underlying Type 1 VWD

Type 1 VWD, which is defined by a quantitative deficiency of VWF with plasma VWF levels ranging from 5 to 50 IU/dL, has complex pathogenesis and demonstrates variability in phenotypic penetrance and expressivity.^{30,32,33}

Type 1 VWD is a multifactorial trait in which more than one gene and various environmental factors influence plasma levels of VWF.³⁷ It is now confirmed that the ABO blood group is the most imperative genetic modifier of plasma VWF levels, such that plasma VWF levels in individuals with blood group O are approximately 25% lower compared with those in non-O blood group individuals.³⁷ This is likely due to glycan structures presented on group O-VWF, which lead to accelerated clearance of the protein.³⁸ Coinheritance of blood group O has an important pathogenic role in type 1 VWD. It is demonstrated that blood group O is overrepresented in type 1 VWD subjects (73% of type 1 VWD cases vs. 45% of the general population).^{32,39}

Over the last decade, several groups have studied the genetic basis of type 1 VWD cohorts of the European and North American populations. Remarkably, the results from all of these studies have steadily reported that VWF candidate mutations have been identified in approximately 65% of index cases.^{30,32–34} Furthermore, data from these studies indicated that the likelihood of detecting a VWF mutation rises with lower VWF levels (VWF:Ag < 30 IU/dL). The large multicenter studies of type 1 VWD cases from the United States (Zimmerman program), European (MCMDM-1 VWD study), and Canadian cohorts reported that overall approximately 82% of cases had VWF mutations when the VWF:Ag was ≤30 IU/dL, whereas only approximately 50% of cases with VWF:Ag > 30 IU/dL had VWF mutations.^{32–34}

Genetic Variants within VWF

To date, more than 250 different candidate mutations have been documented in HGMD for type 1 VWD which comprise candidate missense variations (accounting for the majority of pathogenic variants: ~64%), splice-site variations (~15%), promoter sequence variations (~5%), small deletions (~5%), small insertions (~3%), nonsense mutations (~4%), gene conversions (~2%), and large deletions (~2%) (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=VWF>, accessed in March 2019; ▶Fig. 1A). Although type 1 VWD is mainly due to only one affected allele and inherited in an autosomal-dominant manner, approximately 15% of patients have more than one candidate mutation (homozygous and compound heterozygous).³³ Notably, some assigned type 1 VWD mutations were later considered to be reclassified as VWD type 2 (2A or 2M) due to displaying subtle abnormal multimers following completion of the MCMDM-1 VWD study.⁴⁰ In ▶Table 1, the mutations listed in databases for both type 1 and type 2 VWD are marked by an asterisk. Furthermore, in some patients, VWD type 1 is caused by

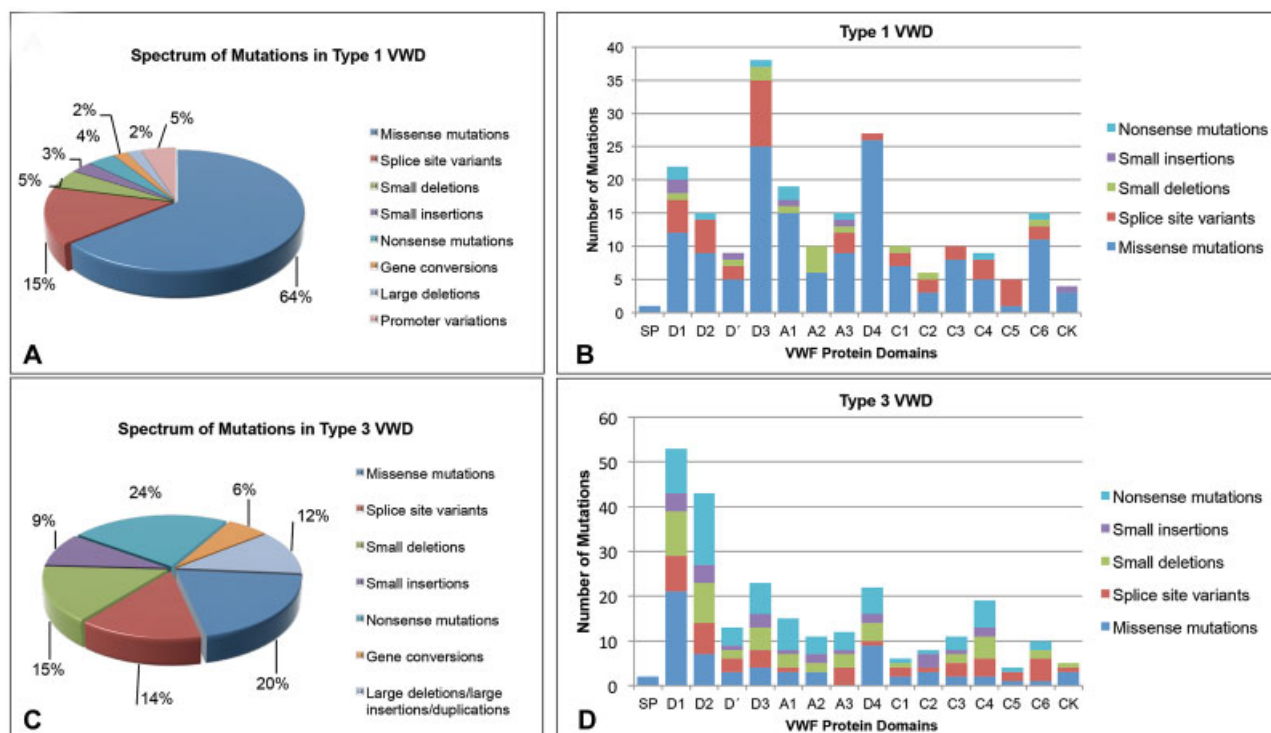


Fig. 1 Spectrum and distribution of mutations in type 1 and type 3 VWD. Panels (A) and (C) demonstrate spectrum and frequency (%) of mutations in type 1 and type 3 VWD, respectively. As it is shown, in type 1 VWD missense mutations are predominant (64%), while in type 3 VWD null alleles (splice site variants, small deletions/insertions, nonsense mutations, large deletions, large insertions, duplications, and gene conversions) account for the majority of the mutations. Panels (B) and (D) show the distribution of mutations on VWF domains in type 1 and type 3 VWD, respectively. VWD, von Willebrand disease.

dominant-negative mutations, such as p.Cys1130Phe and p.Cys1149Arg. Heterodimerization of variant and normal VWF subunits leads to intracellular retention of both variant and normal VWF and consequently results in a dominant-negative effect on the secretion of VWF.^{4,41,42} Until now, the pathogenic mechanisms causing quantitative type 1 VWD, in general, include decreased VWF synthesis, impaired VWF storage and secretion, and accelerated VWF clearance (→Fig. 2). The decreased VWF synthesis might be due to promoter mutations disrupting binding of transcription factors as well as null mutations (nonsense, deletion, and splice-site variations) resulting in no stable VWF mRNA production or truncated proteins retained in the ER. In the HGMD registry, several sequence variations in the promoter region of the VWF are recorded for type 1 VWD, but the pathogenicity of these variations has not been yet verified. More than 30 putative splicing mutations are reported for type 1 VWD of which some are confirmed to be leading to aberrantly spliced VWF transcripts. The RNA transcript studies done by several groups showed that these splicing variants might lead to either exon skipping, cryptic splice-site activation, or intron retention, thus resulting in truncated proteins.^{35,43–46} An interesting consensus splice-site mutation is c.1534–3C > A, because it is identified as both homozygous and heterozygous in type 1 VWD cases. This splice-site mutation impairs but does not abolish normal mRNA splicing. It simultaneously induces skipping of the exon 14, activation of a cryptic splice site, and a normal VWF transcription. Interestingly, the cases which are heterozygous

for the c.1534–3C > A mutation always had low platelet VWF levels, but sometimes circulating VWF levels within the normal range.⁴³ This indicates the possibility of a discrepant splicing procedure in endothelial cells and platelet precursors, megakaryocytes, which should be taken into consideration in future studies.

Missense variations in type 1 VWD either impair VWF storage and secretion or cause accelerated VWF clearance from the plasma circulation (→Table 1; →Fig. 2). The missense mutations affecting storage and secretion of the VWF are spread throughout all VWF domains and the molecular mechanisms by which these mutations cause defective intracellular packaging and secretion of VWF are not completely characterized (→Fig. 1B). It is believed that some of these mutations cause substantial structural changes in VWF protein, such that the ER quality control system prevents trafficking to the Golgi compartment. The retained VWF protein either aggregates in the ER or undergoes intracellular degradation that eventually leads to less WPB storage and less production of VWF^{47–56} (→Table 1). Wang et al suggested that all cysteine mutations involved in intrachain disulfide bonds (e.g., p.Cys1060Tyr, p.Cys1130Phe, and p.Cys1149Arg) may disrupt the conformation of VWF, causing ER retention.⁵⁴ Alternatively, some of the mutations (with mild ER retention or only dilated ER) mainly disturb tubule formation and normal storage of VWF, leading to diminished exocytosis of WPBs and consequently resulting in less VWF secretion (e.g., p.Cys2190Tyr, p.Ala1716Pro, and p.Tyr1584Cys; →Table 1).^{48,50,55,57}

Table 1 List of missense mutations detected in type 1 VWD and their impact on VWF biosynthesis based on in vitro and in vivo functional studies

aa Exchange	Domain	Functional characterization/ impact on splicing
p.Gly19Arg ³³	SP	No impact on the expression of VWF/Putative impact on splicing ⁴⁹
p.Ser49Arg ¹⁰⁷	D1/VWD	n/a
p.Leu60Pro ¹⁰⁸	D1/VWD	n/a
p.Asn99Ser ³¹	D1/VWD	n/a
p.Leu129Met ³⁴	D1/VWD	n/a
p.Ala138Asp ³¹	D1/VWD	n/a
p.Asp141Gly ⁵⁰	D1/VWD	Intracellular retention; impaired secretion ⁴⁹
p.Gly160Trp ³³	D1/VWD	Intracellular retention; impaired secretion ⁴⁹
p.Cys162Arg ¹⁰⁹	D1/VWD	n/a
p.Asn166Ile ³³	D1/VWD	n/a
p.Cys237Trp ¹⁰⁹	D1/C8	n/a
p.Arg324Pro ¹⁰⁷	D1/TIL	n/a
p.Cys325Phe ³¹	D1/TIL	n/a
p.Arg491Pro ¹⁰¹	D2/C8	n/a
p.Asp558Tyr ⁸⁸	D2/C8	n/a
p.Met576Ile ³⁴	D2/C8	n/a
p.Ala594Gly ¹¹⁰	D2/C8	n/a
p.Ala641Val ³³	D2/C8	n/a
p.Cys707Arg ¹⁰¹	D2/TIL	n/a
p.Met736Thr ³¹	D2/E	n/a
p.Met740Ile ⁴²	D2/E	n/a
p.Lys762Glu ³⁴	D2/E	n/a
p.Arg768Gln ⁶⁰	D'/TIL	n/a
p.Met771Ile ³³	D'/TIL	A combination of retention in ER along with impaired storage in WPBs and secretion ⁵⁶
p.Met771Val ⁵⁶	D'/TIL	A combination of ER retention along with impaired storage, multimerization, and secretion ⁵⁶
p.Arg782Gln ⁵⁶	D'/TIL	A combination of retention in ER along with impaired storage in WPBs and secretion ⁵⁶
p.Asn857Ser ³⁰	D'/E	n/a
p.Leu881Arg ³³	D3/VWD	Decreased secretion (only in single expression but not for co-expression), normal storage ⁴⁷
p.Arg924Trp ³⁴	D3/VWD	No impact on expression and function of VWF ⁵⁶
p.Phe1021Cys ³¹	D3/VWD	n/a
p.Cys1060Tyr ³⁶	D3/VWD	n/a
p.Ala1108Pro ¹⁰⁹	D3/VWD	n/a
p.Cys1111Tyr ³⁴	D3/C8	n/a
p.Cys1130Phe ⁴⁰	D3/TIL	Associated with accelerated VWF clearance ⁶⁴ ; ER retention, reduced storage, and secretion ⁵³

(Continued)

Table 1 (Continued)

aa Exchange	Domain	Functional characterization/ impact on splicing
p.Cys1130Gly ⁴⁰	D3/TIL	Associated with accelerated VWF clearance ⁶⁴ ; intracellular retention, impaired secretion ⁵¹
p.Cys1130Arg ⁴⁰	D3/TIL	Associated with accelerated VWF clearance ⁶⁴ ; intracellular retention, impaired secretion ⁵¹
p.Trp1144Gly ⁵⁹	D3/TIL	Associated with accelerated VWF clearance ⁵⁹ ; intracellular retention, impaired secretion ⁵¹
p.Arg1145Cys ¹¹¹	D3/TIL	n/a
p.Tyr1146Cys ³⁴	D3/TIL	Impaired pseudo-WPBs formation and secretion, reduction in large multimers ⁵⁶
p.Cys1149Arg ⁵³	D3/TIL	Associated with accelerated VWF clearance ⁵⁹ ; ER retention, impaired storage and secretion ⁵³
p.Thr1156Met ⁵⁶	D3/TIL	ER retention, impaired pseudo-WPB formation and secretion, loss of large multimers ⁵⁶
p.Glu1161Lys ³¹	D3/TIL	n/a
p.Cys1165Phe ¹⁰³	D3/TIL	n/a
p.Cys1190Arg ³⁴	D3/TIL	n/a
p.Arg1205Cys ⁶²	D3/E	Associated with accelerated VWF clearance ⁶⁰ ; macrophage-mediated clearance of VWF in vivo ⁶²
p.Arg1205Ser ⁶²	D3/E	Associated with accelerated VWF clearance ⁶⁰ ; macrophage-mediated clearance of VWF in vivo ⁶²
p.Arg1205His ⁶²	D3/E	Associated with accelerated VWF clearance ⁶⁰ ; macrophage-mediated clearance of VWF in vivo ⁶²
p.Cys1227Arg ¹¹²	D3/E	n/a
p.Val1229Gly ³⁴	D3/E	n/a
p.Asn1231Thr ³⁴	D3/E	n/a
p.Asn1231His ³¹	D3/E	n/a
p.Pro1266Leu ¹¹³	D3/E	n/a
p.Val1279Ile ²	A1	n/a
p.Ser1285Pro ³³	A1	ER retention, shorter and rounder WPBs, and impaired secretion ^{50,55}
p.Leu1307Pro ⁴⁰	A1	ER retention, less number of WPBs, shorter and rounder WPBs, impaired secretion ⁵⁰
p.Arg1315Cys ⁴⁰	A1	Associated with accelerated VWF clearance ⁵⁸
p.A1 g1315His ⁴⁰	A1	n/a
p.Arg1315Leu ⁴⁰	A1	n/a
p.Arg1342Cys ³³	A1	n/a
p.Leu1361Ser ³³	A1	Associated with accelerated VWF clearance ⁵⁸
p.Arg1374Cys ⁵⁸	A1	Associated with accelerated VWF clearance ⁵⁸
p.Ser1378Phe ⁴⁰	A1	n/a
p.Arg1379Cys ¹¹²	A1	n/a
p.Pro1413Leu ³³	A1	n/a

(Continued)

Table 1 (Continued)

aa Exchange	Domain	Functional characterization/ impact on splicing
p.Gly1415Asp ³³	A1	Associated with accelerated VWF clearance ⁵⁸
p.Ile1416Asn ⁴⁰	A1	Associated with accelerated VWF clearance ⁶⁰
p.Glu1447Gln ¹¹⁴	A1	n/a
p.Arg1564Trp ³¹	A2	n/a
p.Arg1583Trp ³³	A2	Not pathologic: normal WPBs, normal secretion ⁵⁰
p.Tyr1584Cys ⁵⁰	A2	Normal secretion ⁵⁰ ; increased susceptibility to ADAMTS13 ⁵⁵
p.Lys1617Arg ¹⁰⁹	A2	n/a
p.asn1635Thr ¹¹⁴	A2	n/a
p.Arg1668Ser ³⁴	A2	n/a
p.Ala1716Pro ⁴⁸	A3	A combination of ER retention along with impaired storage and secretion ⁴⁸
p.Val1758Ala ¹⁰⁹	A3	n/a
p.Val1760Ile ³⁴	A3	Associated with accelerated VWF clearance ⁵⁸
p.Leu1774Ser ³³	A3	n/a
p.Arg1779Leu ³¹	A3	n/a
p.Lys1794Glu ³³	A3	Associated with accelerated VWF clearance ⁵⁸
p.Asn1818Ser ³⁴	A3	n/a
p.Val1822Gly ³³	A3	Associated with accelerated VWF clearance, ⁵⁸ ER retention, shorter and less number WPBs ⁵⁰
p.Arg1837Trp ¹¹⁵	A3	n/a
p.Cys1879Phe ¹¹⁵	D4/D4N	n/a
p.Lys1887Asn ¹¹⁵	D4/D4N	n/a
p.Cys1899Arg ¹⁰¹	D4/D4N	n/a
p.His1900Pro ³¹	D4/D4N	n/a
p.Pro1933Leu ¹¹⁵	D4/D4N	n/a
p.Glu1938Asp ¹¹⁵	D4/D4N	n/a
p.Thr1951Ala ¹¹⁵	D4/VWD	n/a
p.Pro2063Ser ³⁴	D4/VWD	No impact on in vitro VWF expression ⁴⁹
p.Gly2083Asp ¹⁰¹	D4/VWD	n/a
p.Cys2085Phe ¹⁰¹	D4/VWD	n/a
p.Thr2104Ile ³⁴	D4/VWD	n/a
p.Pro2145Ser ³³	D4/C8	n/a
p.Cys2150Tyr ³¹	D4/C8	n/a
p.Val2153Phe ³¹	D4/C8	n/a
p.Ser2179Phe ⁴	D4/C8	Associated with accelerated VWF clearance ⁵⁹
p.Cys2184Tyr ³¹	D4/C8	n/a
p.Arg2185Trp ³⁴	D4/C8	n/a
p.Arg2185Gln ³⁴	D4/C8	n/a
p.Cys2190Tyr ⁴⁸	D4/C8	

Table 1 (Continued)

aa Exchange	Domain	Functional characterization/ impact on splicing
		A combination of slightly ER colocalization along with drastically impaired storage and secretion ⁴⁸
p.Cys2190Trp ³¹	D4/C8	n/a
p.Leu2207Pro ³³	D4/TIL	Intracellular retention, impaired secretion ⁴⁹
p.Glu2233Gly ³⁴	D4/TIL	n/a
p.Glu2233Lys ³¹	D4/TIL	n/a
p.Glu2233Val ³¹	D4/TIL	n/a
p.Cys2237Phe ¹⁰⁸	D4/TIL	n/a
p.Cys2254Tyr ³¹	D4/TIL	n/a
p.Cys2257Ser ³³	C1	Intracellular retention, impaired secretion ⁴⁹
p.Arg2287Trp ¹¹⁶	C1	Pathologic mild reduction in secretion ⁴⁹
p.Asn2290Tyr ⁵	C1	n/a
p.Cys2304Tyr ³³	C1	Marked intracellular retention, impaired secretion ⁴⁹
p.Arg2311His ¹¹⁰	C1	n/a
p.Arg2313His ³³	C1	n/a
p.Cys2327Tyr ¹¹⁵	C1	n/a
p.Cys2340Arg ³⁴	C2	n/a
p.Gly2343Val ³⁴	C2	n/a
p.Arg2379Cys ³⁴	C2	n/a
p.Gly2441Cys ³³	C3	Associated with accelerated VWF clearance ⁵⁸ ; marked intracellular retention, impaired secretion ⁴⁹
p.Cys2448Tyr ¹¹⁵	C3	n/a
p.Cas2451Tyr ³¹	C3	n/a
p.Arg2464Cys ³³	C3	Mild reduction in secretion, faster-running multimer bands ⁴⁹
p.Cys2468Arg ³¹	C3	n/a
p.Ser2469Pro ³³	C3	n/a
p.Cys2477Ser ³³	C3	Intracellular retention, impaired secretion ⁴⁹
p.Cys2477Tyr ³³	C3	Intracellular retention, impaired secretion ⁴⁹
p.Ser2497Pro ³³	C4	n/a
p.Gly2518Ser ³³	C4	Mild reduction in secretion, faster-running multimer bands, no impact on VWF clearance ⁴⁹
p.Gln2520Pro ³³	C4	Associated with accelerated VWF clearance, ⁵⁹ reduction in secretion, faster-running multimers ⁴⁹
p.Arg2535Pro ³¹	C4	n/a
p.Gly2560Val ¹¹⁵	C4	n/a
p.Cys2619Tyr ³⁰	C5	Disrupting intrachain disulfide bond, intracellular retention, impaired secretion ⁵⁷
p.Thr2647Met ³⁴	C6	n/a
p.Lys2662Arg ²⁹	C6	n/a

Table 1 (Continued)

aa Exchange	Domain	Functional characterization/ impact on splicing
p.Arg2663Pro ³³	C6	n/a
p.Arg2663Cys ⁴⁸	C6	ER retention, impaired secretion ⁴⁸
p.Cys2671Gly ³¹	C6	n/a
p.Cys2676Tyr ³¹	C6	n/a
p.Cys2676Phe ³⁰	C6	Disrupting intrachain disulfide bond, intracellular retention, impaired secretion ⁵⁷
p.Asn2679Ser ³¹	C6	n/a
p.Cys2693Tyr ³³	C6	ER retention, abnormal storage in WPBs, and impaired secretion ⁵⁷
p.Pro2695Arg ¹¹⁷	C6	n/a
p.Pro2722Ala ³³	C6	n/a
p.Pro2776Leu ³¹	CK	n/a
p.Leu2786Pro ³¹	CK	n/a
p.Cys2804Tyr ⁹¹	CK	n/a

Abbreviations: ER, endoplasmic reticulum; n/a: no answer; TIL, trypsin inhibitor-like; WPBs, Weibel–Palade bodies; VWD, von Willebrand disease; VWF, von Willebrand factor.

Notes: List of type 1 missense mutations published in the literature with references. The location of the mutations on VWF domains and subdomains is based on updated domain designations.⁸ The impact of these missense variations on VWF biosynthesis (if reported) is given with references. The symbol “*” indicates type 1 VWD mutations which are also reported as type 2 (2A and/or 2M) VWD. The gray lines indicate mutations associated with accelerated VWF clearance.

On the other hand, type 1 mutations associated with accelerated VWF clearance, causing reduction in half-life of VWF (to 1–3 hours, compared with 8–12 hours for normal VWF), lead to severe reduction in plasma levels of VWF:Ag (~10–15 IU/dL) in the affected patients.^{58,59} Type 1 VWD patients with increased VWF clearance, which comprise approximately 15 to 20% of type 1 VWD cases, are characterized by an elevated ratio of VWFpp to VWF:Ag (a ratio of >2.2) and a rapid elimination of VWF from circulation following desmopressin administration.^{58–61} Using the VWFpp/VWF:Ag ratio, more than 20 different missense substitutions are associated with the elevated clearance phenotype (▶ **Table 1**, lines marked in gray).^{58,62,63} Remarkably, these mutations are not only associated with VWD type 1 but can also be linked to VWD types 2A, 2B, and 3.⁵⁸ The type 1 mutations provoking accelerated VWF clearance are mostly located in the VWF D3 domain (missense variations p.Arg1205His/Cys/Ser, p.Cys1130Phe/Gly/Arg, p.Trp1144Gly, and p.Cys1149Arg).^{31,59,64} It is believed that the D3 domain may contain regulatory or recognition sites for VWF survival and clearance.⁵⁹ The half-life of VWF is modulated by several different mechanisms including VWF glycans, shear force exposure which alters the tertiary conformation of the protein as well as receptors expressed on the sinusoidal endothelial cells and on the hepatic and splenic macrophages.⁶⁵ The recent investigations demonstrated that carbohydrate determinants presented on VWF protein modulate VWF clearance through both hepatic asialoglycoprotein receptor and macrophage-dependent pathways. In addition, it has been shown that variations in VWF glycosylation may play a role in the pathophysiology of type 1 VWD with accelerated VWF clearance.⁶⁶ The p.Arg1205His variant (known as the Vicenza variant) is the classical mutation associated with accelerated VWF clearance which is characterized by a

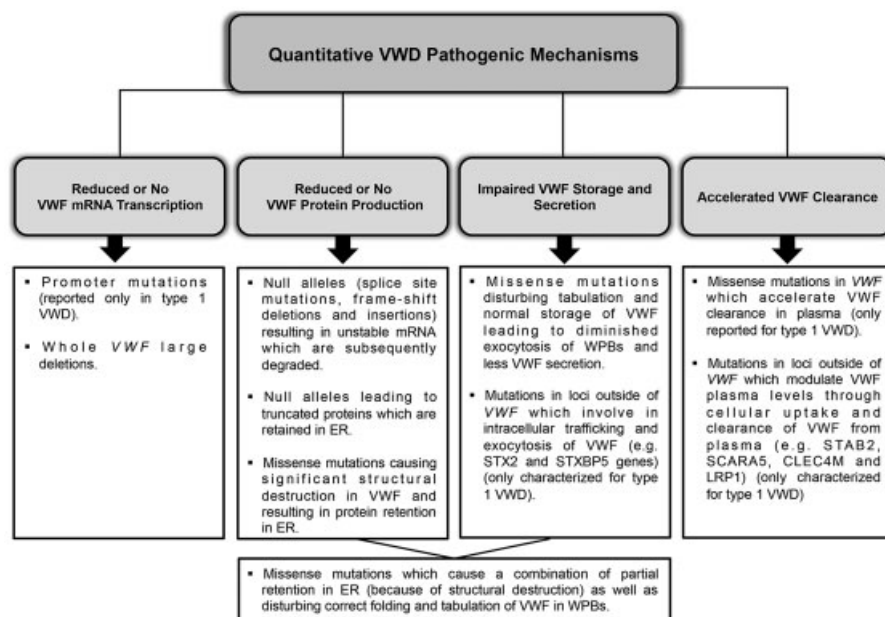


Fig. 2 Molecular mechanisms underlying quantitative VWF deficiencies. This diagram illustrates the main mechanisms through which production and biosynthesis of VWF are impaired. These mechanisms are described for both type 1 and type 3 VWD. Exceptions are VWF promoter mutations, mutations outside VWF involving intracellular trafficking of VWF and mutations associated with accelerated VWF clearance, which are only characterized for type 1 VWD. VWD, von Willebrand disease.

significantly elevated VWFpp/VWF:Ag ratio (often >10) and slightly abnormal VWF multimer patterns. Recent studies have shown that enhanced clearance of the p.Arg1205His/Ser/Cys variants can be mediated through elevated binding to the receptors of hepatic and splenic macrophages.⁶² However, the pathogenic mechanisms of the other mutations involved in the etiology of the accelerated VWF phenotype are not yet clearly understood.

The most frequently occurring type 1 VWD mutation is p.Tyr1584Cys substitution (in domain A2 VWF); it is usually detected as heterozygous in approximately 15% of index patients. The p.Tyr1584Cys variant is associated with VWF:Ag levels of approximately 40 IU/dL, and it has demonstrated incomplete penetrance and variable bleeding severity.^{30,33,34} The *in vitro* studies and animal models have shown that p.Tyr1584Cys resulted in impaired secretion and enhanced ADAMTS13 cleavage.⁶⁷

A VWF Mutation or a Functional Polymorphism?

It is sometimes challenging to determine whether a certain sequence variant is disease-associated or is an interindividual difference with functional significance but no direct clinical relevance. The VWF locus is highly polymorphic with a large number of single nucleotide polymorphisms (SNPs) in VWF coding sequences and promoter region (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?genelid=7450), some of which, in association with pathologic mutations or in specific haplotypes together with other variants, have an additive influence on VWF:Ag levels and affect VWD severity.^{68–70} A good example is the established association of linked polymorphic variations C/T at -1234, A/G at -1185, and G/A at -1051 in the promoter region of VWF, which are segregated as two distinct haplotypes: -1234C/-1185A/-1051G (haplotype 1) and -1234T/-1185G/-1051A (haplotype 2), with plasma VWF levels. Keightley et al showed that the homozygous individuals for haplotype 1 had the highest mean VWF:Ag levels (0.962 U/mL), intermediate values of VWF:Ag (0.867 U/mL) were found for heterozygotes, and homozygous subjects for haplotype 2 had the lowest mean plasma VWF:Ag levels (0.776 U/mL).⁶⁴ This association might be due to differential binding of transcription proteins regulating VWF expression to these haplotypes.⁷¹

The Arg924Gln missense substitution is another frequent VWF coding region variant which is associated with low VWF:Ag levels in type 1 VWD cohorts. It is recorded as type 1/type 2N VWD mutation in variant databases. However, an investigation including *in vitro* gene expression study and RNA transcript analysis of BOECs (blood outgrowth endothelial cells) isolated from a p.Arg924Gln carrier patient demonstrated that this substitution does not result in a major effect on VWF biosynthesis, but this polymorphism might be a polymorphic marker for an unknown intronic splicing variant, leading to activation of the 5' cryptic splice-site within exon 28 of the VWF.⁷²

Genetic Determinants Outside VWF Contributing to Variability in Plasma VWF Levels

In the remaining 35% of type 1 VWD patients, for whom no causative mutation is identified in the coding region or proximal

promoter of VWF, the genetic determinants might be found in deep intronic regions, a distant regulatory sequence of VWF or at loci external to the VWF contributing to the VWF biosynthesis pathway. In recent years, genome-wide association studies, linkage analyses, and hypothesis-based *in vitro* and *in vivo* experiments have identified more than 10 novel loci (in addition to previously recognized ABO blood group genes and VWF) that are associated with variations in VWF plasma levels. These new gene determinants of VWF levels include genes coding STXBP5 (syntaxin-binding protein 5), SCARA5 (scavenger receptor class A—member 5), STAB2 (Stabilin-2), STX2 (syntaxin 2), TC2N (tandem C2 nuclear domains), CLEC4M (C-type lectin domain family 4—member M), UFM1 (ubiquitin-fold modifier 1), GPCR (platelet G-protein coupled receptor), AVPR2 (arginine vasopressin-2 receptor), LRP1 (lipoprotein receptor-related protein-1), ACE (angiotensin-converting enzyme), and Siglec-5 (sialic acid binding Ig-like lectin 5).^{58,73–78} These loci may modulate VWF plasma levels either by involvement in intracellular trafficking and exocytosis of VWF (e.g., STX2 and STXBP5 genes) or through cellular uptake and clearance of VWF from plasma (e.g., STAB2, SCARA5, CLEC4M, and LRP1).^{78,79} Further *ex vivo* gene expression and *in vivo* mouse studies of CLEC4M provided additional evidence that a variable number of tandem repeat (VNTR) polymorphisms in CLEC4M gene influence VWF levels in plasma. The CLEC4M protein is a lectin receptor possessing an N-terminal cytoplasmic domain, a transmembrane domain and an extracellular neck region which is highly polymorphic possessing three to nine VNTRs of a conserved 23-aa sequence. It has been demonstrated that CLEC4M functions as a VWF clearance receptor by binding and internalizing VWF and targeting it to early endosome. Furthermore, it was shown certain VNTR allele changes the affinity of CLEC4M binding, contributing to the variability of plasma VWF levels.⁸⁰

Pathogenic Mechanisms Underlying Type 3 VWD

The genetic etiology of type 3 VWD is almost well defined, with a broad spectrum of mutations spread throughout the entire VWF which cause almost no production of VWF (VWF:Ag < 5 IU/dL). Type 3 VWD, which is inherited as a recessive disease, is caused due to either homozygous or compound heterozygous VWF mutations. However, it appears that 40 to 50% of type 3 VWD patients demonstrate codominant inheritance rather than a typical recessive inheritance pattern, which is acknowledged by heterozygous carriers having a mild type 1 VWD phenotype in their families.^{29,81} Type 3 VWD demonstrates variability in the severity of clinically relevant bleeding episodes, although VWF is virtually absent in all type 3 VWDs consistently. Assessment of the bleeding score (BS) in Canadian type 3 VWD cohorts demonstrated that patients with mutations within VWFpp exhibited more severe bleeding (BS = 22) than those with mutations elsewhere in VWF (BS = 13).²⁹ Consistent with the aforementioned finding, it was recently shown that BS was significantly higher in type 3 patients with undetectable VWFpp (BS = 19.5) than in those with detectable VWFpp levels (BS = 14).⁶¹

Genetic analysis of VWD type 3 cohorts from several populations has demonstrated that two variant *VWF* alleles (homozygous or compound heterozygous mutations) have been identified on average in approximately 85% of cases.^{29,30,82–86} In the remaining 15% of the cases, either no causative variation was detected or only one *VWF* mutation was identified, which does not explain the severe phenotype of type 3 VWD.

Up to date, more than 320 sequence variations have been documented in HGMD (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=VWF>, accessed in March 2019), of which the majority (~80%) are null alleles comprising a miscellany of nonsense mutations (~24%), small deletions (~15%), splice-site variations (~14%), small insertions (~9%), partial and total *VWF* deletions (~11%), large insertions/duplications (~1%) as well as gene conversions (~6%; **–Fig. 1C, D**). An average of approximately 70% of the all sequence variations identified in cohort studies of type 3 VWD were reported for the first time, indicating the high susceptibility of *VWF* for sequence variations and mutational heterogeneity in type 3.^{29,30,82,87,88} Alloantibody development against *VWF* has been reported rarely, approximately 5 to 10% of type 3 VWD patients, and occurs most often in patients with nonsense mutations or partial/complete *VWF* deletions on both alleles.^{89–92}

Nonsense mutations: More than 70 nonsense mutations are documented in HGMD which constitute the majority of null alleles in type 3 VWD. Nonsense mutations are scattered over the whole *VWF* from D1 to C domains of *VWF* protein with a high incidence at the CpG mutational hot spot (**–Fig. 1D**).

Small deletions and insertions: Several small deletions (about 50) and insertions (more than 50) resulting in a shift of the reading frame and consequently a premature translational stop codon have been documented for type 3 VWD. These small deletions and insertions were mainly located in a stretch of the same nucleotide (e.g., c.2435delC in a stretch of cytosine) or within a string of a dinucleotide (e.g., c.4092delAC in a stretch of ACACAC).^{30,83} The frameshift deletion of single cytosine in exon 18 of the *VWF* (c.2435delC, p.Pro812Argfs*31), which had been identified in the original VWD family from the Åland Islands, was found frequently in type 3 cohorts of European populations (German, Hungarian, Finish, Swedish, and Italian populations).^{30,83,87,93} However, the small deletion c.2435delC was not detected in investigated Canadian and Indian populations,^{29,84,92} whereas the small insertion c.8418_8419ins-TCCC (p.Pro2807Leufs*24) and the small deletion c.2908delC (p.Leu970Serfs*9) are the most frequent type 3 frameshift mutation in Canadian and Indian populations, respectively.^{29,92}

Splicing site variations: A notable number of putative splice site substitutions (more than 40 core splice-site variants) affecting consensus 3' acceptor and 5' donor splice sites have been recorded for type 3 VWD. However, the pathogenicity of only a few of them has been evaluated in detail by RNA transcript analyses. Using total RNA isolated from platelets and leukocytes, it is confirmed that the donor

splice-site variant 533–2A > G in intron 5 of *VWF* and the acceptor splice-site substitution 8155 + 3G > C in intron 50 impair recognition of consensus splicing signals and eventually resulted in skipping of exons 6 and 50, respectively. The acceptor splice site 7730–1G > C in intron 45 of *VWF* activates a cryptic acceptor splice site in exon 46, two nucleotides downstream of the original splice.⁴ Another splice-site variant G > T at position 7770 + 1 in intron 46 caused a combination of different splicing defects including activation of a cryptic splice site in intron 46, 25 nucleotides downstream of the original donor core splice site, as well as skipping of exon 46.⁹⁴ Eventually, these splicing defects change the reading frame of the protein leading to premature stop codons and consequently result in truncated proteins with no function.⁴⁴ Alternatively, the splicing defects may lead to nonstable mRNA and subsequently nonsense-mediated mRNA decay (NMD). An example for this incident is the core acceptor splicing variant c.7082–2A > C whose deleterious effect was hidden by NMD (no aberrant mRNA). Homozygosity of mRNA SNPs (which were detected as heterozygous at the genomic level) confirmed the absence of the transcript from the second allele and degradation of the abnormal mRNA (**–Fig. 2**).⁴⁴

Large deletions: About 35 *VWF* large deletions encompassing (homozygous or compound heterozygous) deletions of either only one exon (exon 6 and exon 42 including some intronic regions), longer segments of the *VWF* affecting several exons (exons 1–3, 1–5, 4–5, 4–34, 4–49, 5–16, 6–16, 11–16, 14–15, 17–18, 14–52, 16–43, 16–52, 17–18, 19–20, 17–25, 22–43, 23–52, 26–34, 33–34, 33–38, and 48–52), or even complete *VWF* are described (several reports).^{29,82,92,95} The deletion of the entire *VWF* and deletion of exons 4–5 have been frequently reported in different populations.^{29,30,96,97} Of interest, these two large deletions have also been detected in type 3 heterozygous family members (diagnosed as mild type 1 VWD). This suggests a higher incidence of large deletions in type 1 VWD than it is thought. Genomic structure analysis of breakpoints has shown that deletion junctions of three large deletions of exons 4–5, 1–3, and 5–16 of *VWF* were flanked by homologous Alu repetitive elements, promoting unequal homologous recombination and Alu-mediated deletions.^{87,96} On the other hand, it seemed that complete *VWF* deletion detected in German and Italian populations was not driven by an Alu-mediated mechanism but rather by a nonhomologous-end-joining-dependent DNA repair mechanism.⁹⁷ The large deletions may lead to either no mRNA synthesis, changing the reading frame of a codon (out of frame) and retention of truncated protein in ER, or in-frame amino acids missing proteins which can interfere with the correct folding and biosynthesis of *VWF*.⁹⁶ Using BOECs derived from a patient with deletion of exons 4–5 *VWF*, it has been shown that a combination of reduced mRNA synthesis with ER retention of deleted *VWF* protein impairs *VWF* biosynthesis.⁹⁸

Gene conversions: An interlocus gene conversion event between *VWF* on chromosome 12 (12p13.31) and *VWF* pseudogene on chromosome 22 (22q11.22 to 22q11.23) has been demonstrated as the cause of VWD type 3.^{29,83,84}

Gene conversions result from high homology between a pseudogene and the corresponding sequences in *VWF* (exons 23–34). The reported gene conversions (more than 20) are characterized by multiple missense and nonsense substitutions within exons 23 to 34 of *VWF* (domains D3–A1) which are distributed in varying lengths between <100 to 3,000 bp.^{82,84,92} Gene conversions are particularly prevalent in Indian populations.

Missense substitutions: Missense variations comprise approximately 20% of sequence variations which are spread over the entire *VWF*, within SP and D1–CK domains (Table 2).^{29–31,82,92,99–101} Nonetheless, they are mainly accumulated within domains D1 and D2 of *VWF*pp (~45% of missense substitutions; Fig. 1D). The *VWF*pp missense mutations disrupt the conformation of *VWF*pp, which interrupt proper *VWF*pp/mature *VWF* alignment required for disulfide-linked multimers.^{102,103} Consequently, they cause significant intracellular retention of the protein and impaired secretion.^{104–106}

Conclusion

The genetic basis of type 1 and type 3 VWD differs from each other. The characterized genetic *VWF* defects in both types 1 and 3 VWD are very heterogeneous, including point mutations, frameshift variations, transcriptional aberrations, and chromosomal rearrangements. Furthermore, these genetic variations are not confined to a specific region of the *VWF*, but they are spread throughout the whole *VWF*. The diagnostic genetic analysis of VWD, therefore, requires sequencing of all *VWF* exons and their surrounding intronic regions as well as analysis tests for gene rearrangement screening like MLPA.

Despite significant progress in our understanding of molecular mechanisms underlying quantitative *VWF* deficiencies, questions regarding genetic determinants affecting *VWF* levels in plasma remain to be answered, and genetic diagnosis of quantitative VWD is still challenging. The *VWF* is highly polymorphic, with a long list of reported SNPs detected in healthy populations, indicating difficulty in ascertaining the pathologic nature of the novel identified sequence substitutions and correlating them with the manifesting phenotype. Hence, further functional evaluations including in vitro gene expression studies, RNA transcript analysis, investigation biosynthesis and storage of *VWF* in BOECs isolated from the patients, and in vivo animal studies are required to confirm the contribution of these genetic variations to disease pathogenesis. About 35% of type 1 and 15% of type 3 VWD cases remain without clear causative genetic defect within *VWF*. For the majority of type 3 mutation-negative cases, the causative mutations might be located within deep intronic regions or distant regulatory sequences, whereas in type 1 negative-mutation cases, the causative defects are expected outside of the *VWF* locus. However, additional studies are required to entirely clarify the contribution of genetic loci outside of *VWF* to type 1 VWD.

Table 2 List of missense mutations identified in type 3 VWD and their impact on *VWF* biosynthesis based on in vitro functional studies

aa Exchange	Domain	Functional characterization/ impact on splicing
p.Met1Val ²⁹	SP	n/a
p.Gly19Arg ³³	SP	No impact on the expression of <i>VWF</i> /putative impact on splicing ⁴⁹
p.Arg34Gly ¹⁰⁷	D1/VWD	n/a
p.Cys35Arg ⁸⁷	D1/VWD	n/a
p.Asp47His ⁸³	D1/VWD	n/a
p.Gly79Val ³¹	D1/VWD	n/a
p.Arg81Gly ⁸⁷	D1/VWD	n/a
p.Ser85Pro ⁸³	D1/VWD	n/a
p.Tyr87Ser ¹⁰⁶	D1/VWD	Multimerization deficiency ¹⁰⁶
p.Asp141Asn ⁸³	D1/VWD	n/a
p.Asp141Tyr ⁸³	D1/VWD	Multimerization deficiency ¹⁰³
p.Gly142Asp ¹⁰¹	D1/VWD	n/a
p.Leu150Pro ¹¹⁰	D1/VWD	n/a
p.Leu150Gln ⁹⁹	D1/VWD	n/a
p.Gly160Arg ⁹²	D1/VWD	n/a
p.Arg273Trp ¹⁰⁴	D1/C8	Multimerization deficiency ¹⁰⁴
p.Arg273Pro ¹⁰¹	D1/C8	n/a
p.Cys275Ser ⁹¹	D1/C8	Multimerization deficiency ¹⁰²
p.Cys295Ser ⁸⁷	D1/TIL	n/a
p.Tyr301Cys ³¹	D1/TIL	n/a
p.Asn318Lys ⁹²	D1/TIL	n/a
p.Lys355Arg ⁸²	D1/E	n/a
p.Trp377Cys ¹¹⁸	D1/E	n/a
p.Asp437Tyr ⁸²	D2/VWD	n/a
p.Arg447Trp ³¹	D2/VWD	n/a
p.Cys584Phe ¹⁰⁵	D2/C8	Impairing storage and secretion ¹⁰⁵
p.His596Asn ⁸⁷	D2/C8	n/a
p.Cys623Tyr ⁸⁷	D2/C8	n/a
p.Cys633Arg ⁸²	D2/C8	n/a
p.Met736Ile ⁹²	D2/E	n/a
p.Met814Ile ⁸²	D'/IL	n/a
p.cys827Tyr ¹⁰⁰	D'/IL	n/a
p.Arg854Trp ⁹²	D'/E	n/a
p.Gly967Val ¹⁰¹	D3/VWD	n/a
p.Cys1071Phe ⁸³	D3/C8	n/a
p.Tyr1107His ¹⁰¹	D3/C8	n/a
p.Cys1165Arg ¹⁰³	D3/C8	n/a
p.Val1279Ile ⁹²	A1	n/a
p.Leu1288Val ⁹²	A1	n/a
p.Arg1315Cys ⁹⁹	A1	A combination of retention in ER along with defect in storage in WPBs and secretion ⁹⁸
p.Ile1509Val ⁸²	A2	n/a

Table 2 (Continued)

aa Exchange	Domain	Functional characterization/ impact on splicing
p.Gln1667His ⁹²	A2-A3	n/a
p.Glu1673Asp ⁹²	A2-A3	n/a
p.Gln1931His ⁹²	D4/D4N	n/a
p.Cys1946Phe ¹⁰¹	D4/D4N	n/a
p.Gly2044Asp ⁸²	D4/VWD	n/a
p.Asn2066Ser ¹¹⁰	D4/VWD	n/a
p.Cys2174Gly ⁸³	D4/C8	n/a
p.Cys2184Ser ⁹⁰	D4/C8	Intracellular retention, impaired secretion ⁹⁰
p.Cys2184Phe ³¹	D4/C8	n/a
p.Cys2212Arg ⁹⁰	D4/TIL	Intracellular retention, impaired secretion ⁹⁰
p.Cys2237Arg ²⁹	D4/TIL	n/a
p.Cys2283Arg ³⁰	C1	n/a
p.Cys2325Ser ⁹⁰	C1	Intracellular retention, impaired secretion ⁹⁰
p.Cys2362Phe ⁸⁶	C2	Intracellular retention and impaired secretion ⁸⁶
p.Pro2373Leu ¹⁰¹	C2	n/a
p.Cys2394Trp ⁹⁶	C2	n/a
p.Thr2454Asn ¹⁰⁹	C3	n/a
p.Cys2491Arg ⁸²	C3	Retention in ER and a strong reduction in secretion ⁵²
p.Arg2535Gln ⁹²	C4	n/a
p.Asn2546Tyr ⁶¹	C4	n/a
p.Asn2636Tyr ²⁹	C5	n/a
p.Cys2671Tyr ⁵⁴	C6	ER retention and reduced storage in WPBs, impaired secretion ⁵⁴
p.Cys2739Tyr ⁹⁹	CK	ER retention; disorganized storage of VWF, impaired secretion ⁵³
p.Cys2754Trp ⁵³	CK	ER retention; disorganized storage of VWF, impaired secretion ⁵³
p.Cys2804Tyr ⁸³	CK	n/a

Abbreviations: ER, endoplasmic reticulum; n/a: no answer; WPBs, Weibel–Palade bodies; VWF, von Willebrand factor.

Notes: List of type 3 missense mutations reported in the published literature with references. The location of the mutations on VWF domains and subdomains is based on updated domain designations.⁸ The impact of these missense variations on VWF biosynthesis (if reported) is given with references.

Lastly, improving our understanding of the pathogenesis mechanisms contributing to low VWF levels in plasma provides information to establish complementary molecular diagnostic tests and develop new treatment regimens for the quantitative trait of VWD.

Recipient of the Günter Landbeck Award, 2016.

Conflict of Interest

Dr. Oldenburg reports grants and personal fees from Bayer, grants and personal fees from Biotest, personal fees from Chugai, grants and personal fees from CSL

Behring, personal fees from Grifols, grants and personal fees from Novo Nordisk, grants and personal fees from Octapharma, personal fees from Pfizer, personal fees from Roche, personal fees from SOBI, grants and personal fees from Shire, outside the submitted work. Personal fees were received for travel support, participation in advisory boards, and participating in symposia as chair or speaker.

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