von Willebrand Disease

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3.1 Introduction

von Willebrand disease (VWD) is the most common congenital bleeding disorder (CBD) and is inherited in an autosomal dominant or autosomal recessive manner [1]. The disorder is classified into quantitative deficiency (types 1 and 3) or qualitative defects (type 2) in von Willebrand factor (VWF) [2]. VWD represents a very heterogeneous bleeding disorder with variable bleeding tendency. The bleeding symptoms may be so mild as to not easily distinguish potential sufferers from a normal condition or else may be so severe that it is accompanied by life endangering bleeding (e.g., recurrent occurrence in central nervous system (CNS)). Generally, mucocutaneous bleeds such as epistaxis and menorrhagia are more typical presentations of the disorder, but other rare presentations also can be observed [1, 2]. In addition to the accepted ISTH SSC (International Society on Thrombosis and Hemostasis Scientific Standardization Committee) classification of the disorder, affected individuals are characterized according to clinical manifestations and laboratory findings [3]. In practice, VWD can be classified to mild (VWF:ristocetin cofactor (VWF:RCo): 30–50 U dL^{-1} and/or factor VIII (FVIII) coagulant activity (FVIII:C): 40–60 U dL^{-1}), moderate (VWF:RCo 10–30 U dL^{-1} and/or FVIII:C 20–40 U dL^{-1}), or severe (VWF:RCo <10 U dL^{-1} and/or FVIII:C <20 U dL^{-1}) [4]. Based on such a classification, type 3 VWD, as well as some cases of type 1, type

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2A, type 2M, and type 2N VWD, can be classified to severe VWD [5]. Diagnosis of VWD is a challenge worldwide, and a precise laboratory approach and proper clinical assessment, although mandatory for timely diagnosis of disorder, is sometimes elusive [4]. Although significant progress has occurred in the laboratory diagnosis of VWD in recent years, proper diagnosis of most types of the disorder requires a sophisticated approach, and is thus challenging. This difficulty is due to several issues, including lack of a definitive cutoff between normal and abnormal levels of VWF reflecting normal individuals vs VWD, the effect of different molecular pathogenesis of VWD on VWF level and activity, other factors such as genetic modifiers and physiological factors that can reduce or increase plasma levels of VWF, overlap of bleeding symptomology in normal individuals and those with VWD, and difficulties with performance and interpretation of laboratory tests. Three foremost criteria are proposed for proper diagnosis of VWD: (1) presence of bleeding symptoms, (2) decreased VWF level and/or activity, and (3) the pattern of inheritance [6]. On-demand therapy is the current mainstay of treatment in VWD, but long-term prophylaxis can significantly improve the quality of life in patients with severe hemorrhages, such as hemarthrosis and GI bleeding, and even recurrent epistaxis. Desmopressin is the main therapeutic choice in patients with type 1 and a subset of patients with type 2 VWD, while replacement therapy with VWF/FVIII concentrates represents the main therapeutic option in type 3 and most patients with type 2 VWD, as well as those responsive to desmopressin but with long-term treatment needs [7, 8].

3.2 von Willebrand Factor Synthesis, Structure, and Function

3.2.1 von Willebrand Factor Biosynthesis

The biosynthesis of VWF consists of a series of sequential steps that ultimately leads to incorporation of the protein to storage organelles. These steps include protein production, removal of signal peptide, tail-to-tail dimerization, heat-to-head multimerization, N-linked glycosylation, maturation of N-glycan, O-linked glycosylation, formation of tubules, and incorporation to storage organelles [9].

3.2.2 von Willebrand Factor Structure

VWF is mapped to the tip of short arm of chromosome 12. The *VWF* gene spans 178 kb and consists of 52 exons [10], reflecting a unique gene structure consisting of different repeated sequences [11]. The intron 40 contains 14 Alu repeat and 670-bp repeat of TCTA. In addition the 5′ flanking region has AT repeat resemble to TATA element [12].

VWF is a multimeric protein with molecular weight of 350 kDa, which is coded by 9 kb mRNA and consists of 2813 amino acids, including 22 amino acid signal

peptides, a 741 amino acid propeptide and a 2050 amino acid mature polypeptide [13]. VWF is synthesized as a precursor and processed in endoplasmic reticulum (ER) and Golgi apparatus in endothelial cells. After processing, this protein undergoes dimerization in the ER by disulfide bridging and cleavage into two components, the mature protein and a 97 kDa propeptide. The propeptide, also sometimes referred to as a von Willebrand antigen II, is secreted and circulates independently of the mature VWF and has unknown function [9].

VWF precursor consists of four distinct domains, each of them presenting with two to five tandem copies to arrange as D1-D2-D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK (Fig. 3.1a). The current domain organization recently proposed by Zhou et al. is different to the classical domain structure, with replacement of the B1–B3 and C1–C2 domain regions by six homologous C domains (C1–C6) (Fig. 3.1b) [14]. The D1 and D2 domains correspond to the propeptide, and the remainder represents the mature VWF subunit. The D domains consist of different distinct structures. D1, D2, and D3 domains contain VW domains (VW domain 1, 2, and 3), a C8 fold, a trypsin inhibitor-like (TIL) structure, and an E module. The D4 domain lacks the E module and contains a D4N subunit. In addition, the D′ domain only has TIL and an E module, and the VW and C8 fold are absent [14].

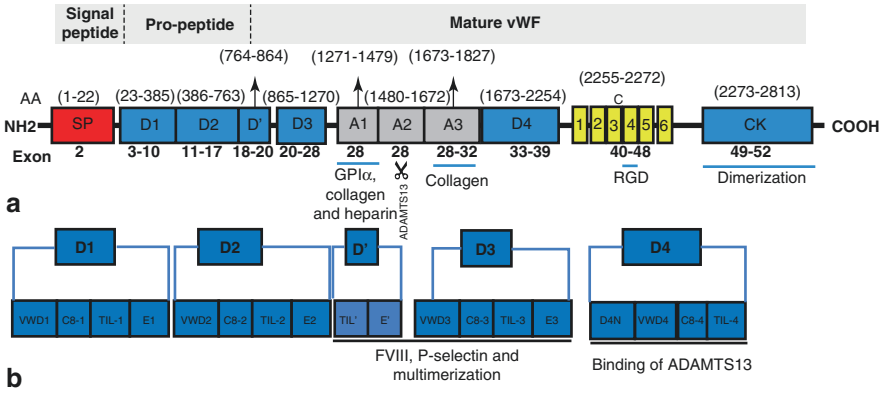

Fig. 3.1 von Willebrand factor (VWF) domain organization. (**a**) The classical structure of VWF. In this organization, VWF is arranged as D1-D2-D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK. The current revised structure of VWF. (**b**) In this structure, as proposed by Zhou et al. in 2012, the B1–B3 domains and C1–C2 domains are replaced by 6 homologous C domains (C1–C6). VWF contains 2813 amino acids and consists of a signal peptide (SP) comprising the first 22 residues, a propeptide comprising the subsequent 750 amino acids (the 23th to 763th amino acid), and a mature subunit (the 764th to 2813th amino acid). The signal sequence is cleaved in the endoplasmic reticulum and separated from VWF. Furin cleaves the propeptide to generate mature VWF. The propeptide consists of two domains (D1 and D2) while mature VWF consist of D′D3 complex, A domains, D4 complex, and stem complex. Stem complex has six domains comprising C1, C2, C3, C4, C5, and C6. Each D domain includes different subdomains. D1, D2, and D3 domains consist of VW domain, a C8 fold, a trypsin inhibitor-like (TIL) structure, and an E module. The D4 domain contains VWD, a C8 fold, TIL, and D4N subunit whereas D′ domain only has TIL and E module

3.2.3 Disulfide Bridging and Multimerization of von Willebrand Factor

About 8.3% of VWF is composed of the amino acid cysteine (234 of the 2813 residues), which if compared to most other human proteins is fourfold higher. In contrast to other domains, the triplicated domain A has only six cysteine residues. The cysteine residues are paired in disulfide bonds in the secreted protein. However, there exist several unpaired cysteines, which are essential for proper folding and secretion of VWF [15]. In ER, the subunits of pro-VWF undergo dimerization by disulfide bonds in C-terminal cysteine knot (CK) domains. This tail-to-tail dimerization needs only the sequence of the last 150 residues. Tail-to-tail pro-VWF is transported to Golgi and forms head-to-head dimerization by further disulfide bonding in D3 domain (Fig. 3.2b). The important point to consider here is the significant role of VWF propeptides (domain D1 and D2) and also D′ in multimerization. These domains are involved in the alignment of the pro-VWF dimer in order to facilitate the interdimeric cross-linking. Although the removal of the propeptide does not prevent the transportation to Golgi, it does inhibit the multimerization of VWF. The D1 and D2 domains contain CxxC sequence (Cys(c) 159-Gly-Leu-Cys(c) 162 and Cys(c) 521-GlyLeu-Cys(c) 524) which resembles the thiol disulfide functional sites and catalyzes disulfide binding in the D3 domain by its disulfide isomerase activity [16]. By adding any extra glycine to the sequences, the dimerization in ER occurs, but the dimers transported to the Golgi are secreted without the formation of multimers [17]. Therefore, D3 and D′ domains are necessary for multimer formation, and any deletion in these domains leads to expansion of only the dimeric form of VWF [18]. After multimerization, the multimers are organized into a helical structure that leads to 100-fold compaction of the protein. In this compacted structure, the D1 and D2 domains (propeptide) and also D′ and D3 domains form the wall of this helical structure, whereas other domains (A1-CK domains) protrudes outward and occupy the space between the tubules. VWF tubules assemble into ministacks that show the initial Weibel-Palade body (WPB)-like structure. In the trans-Golgi network, co-packaging of VWF that contains these ministacks results in maturation and formation of larger WPBs [9].

The regulation of the multimeric size of VWF is mediated by A Disintegrin and Metalloproteinase with ThromboSpondin type 1 motif, member 13 (ADAMTS13) which cleaves VWF at a single site (Tyr1605-Met1606) in the A2 domain [19, 20]. It appears that domain D4, at residues from 1874 to 2813, has a role in binding to ADAMTS13, and this acts as the initial step for proteolysis of VWF by ADAMTS13 (Fig. 3.3) [21].

3.2.4 Post-translational Modifications of von Willebrand Factor

During synthesis, VWF undergoes different post-translational modifications including the removal of the propeptide by the protease furin (Fig. 3.4), completion of N- and O-glycosylation, and sulfation of specific N-oligosaccharides [22]. In ER,

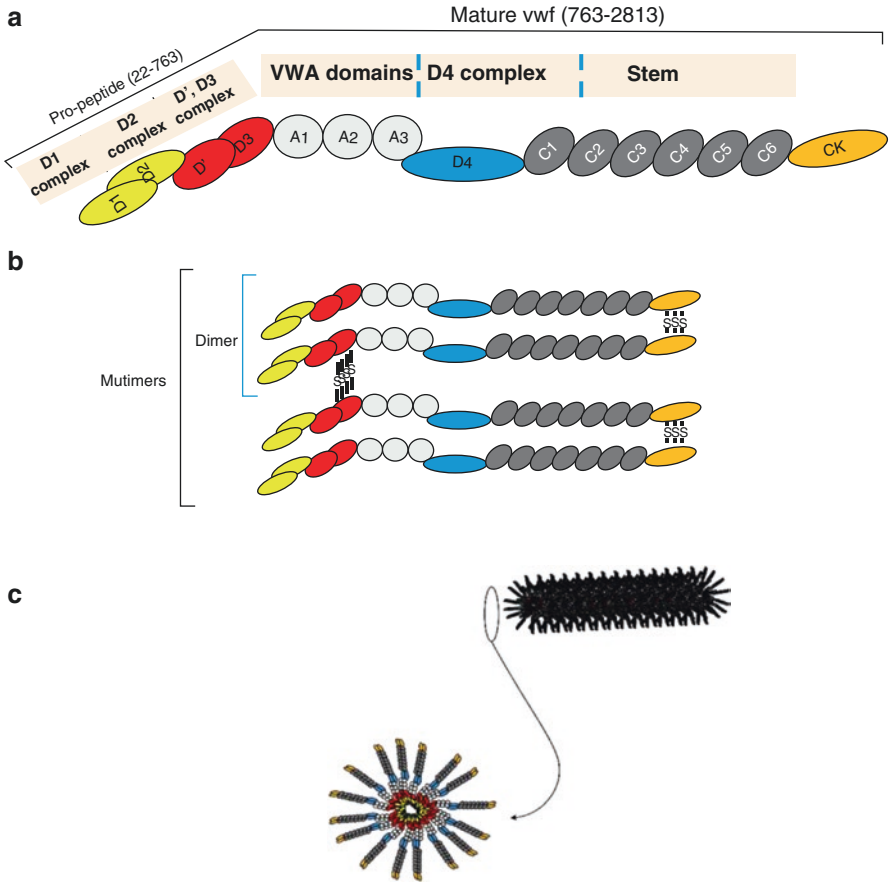

Fig. 3.2 (**a**) The structure of von Willebrand factor (VWF) including both the propeptide and mature VWF). (**b**) Dimer formation occurs via disulfide bonds in the C-terminal (CK) domain. This tail-to-tail dimerization occurs in ER. The tail-to-tail dimers are transported to the Golgi and the multimers formed via disulfide binding in D3 domain. (**c**). The multimers organize in a helical structure. In this right-handed helical structure (tubules), the D1–D2 and D′ and D3 form the wall, while other domains of VWF occupy the spaces of tubules. The tubules assemble in the form of ministacks that show the Weibel-Palade body (WPB)-like structures. Co-packaging of VWF that contains the ministacks leads to large WPB formation

oligosaccharyl tranferase is involved in attachment of 14 saccharide units to asparagine residues. Various studies have shown that pro-VWF has 17 N-linked carbohydrate structures with 4 of them located in the propeptide and 13 in the mature VWF. In addition, along with maturation of N-linked glycan in Golgi, 10 O-linked glycans are added. The N-linked oligosaccharides of this protein are different from other proteins because they contain ABO blood group oligosaccharides [23]. More than 90% of the glycans are capped by sialic acid. In N-linked glycan, there are five

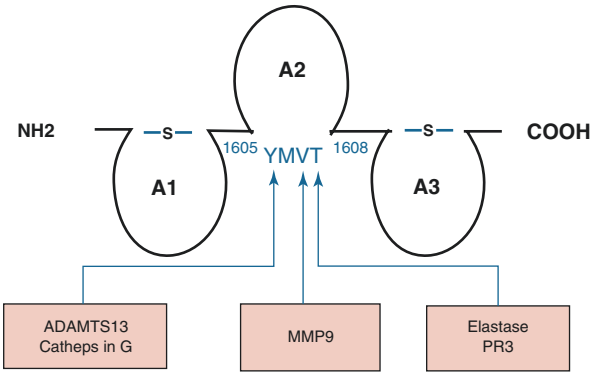

Fig. 3.3 The A domains are organized in loop structures. This structure is formed by disulfide bonds in the A1 and A3 domains. These bonds are between C1272 and C1458 in A1 and C1686 and C1872 in A3 domains. The multimeric size of VWF is regulated by cleavages that occur in the A2 domain. The site of this cleavage is located in Tyr1605-Met1606 and mediated by the metalloproteinase ADAMTS13. *MMP9* matrix metalloproteinase 9

sites for terminal sulfation [23]. The functional role of these sulfated residues remains under investigation [24]. Fig. 3.4 shows the sequential steps for biosynthesis of VWF.

3.2.5 Intracellular Storage and Secretion of VWF

Following synthesis, VWF is stored in α-granules of megakaryocytes/platelets or in Weibel-Palade bodies (WPBs) of endothelium (Fig. 3.4). α-Granules can still be formed in the absence of VWF, while the formation of WPBs depends on the presence of VWF [25]. About 95% of the VWF formed is constitutively secreted while the remainder is stored. In the WPBs, the VWF contained comprises large multimers, whereas the VWF that is constitutively secreted contains smaller multimers. The largest VWF multimers have a greater number of sites for interaction with platelets and vessel wall. Therefore, the thrombogenic risk of these larger molecules is high [26]. As mentioned, these large multimers are typically present in endothelial cells and platelets and are not normally present in plasma. Different studies suggest that size control of circulating VWF is for prevention of thrombosis [26, 27]. After synthesis and packaging of VWF in WPBs, a complex pathway is initiated that leads to secretion of VWF. The WPBs move in an undirected manner and are located to the cellular periphery. Following their fusion with the plasma membrane, their contents are released into the blood or the subendothelium [28]. Some studies assert that the fusion mechanism is random, whereas in some situations, release of WPB content including interlukin-8 and eotaxin-3 results in fusion of WPBs and plasma membrane. The secretion can occur in different ways. In basal secretion, single WPBs are fused with plasma membrane and

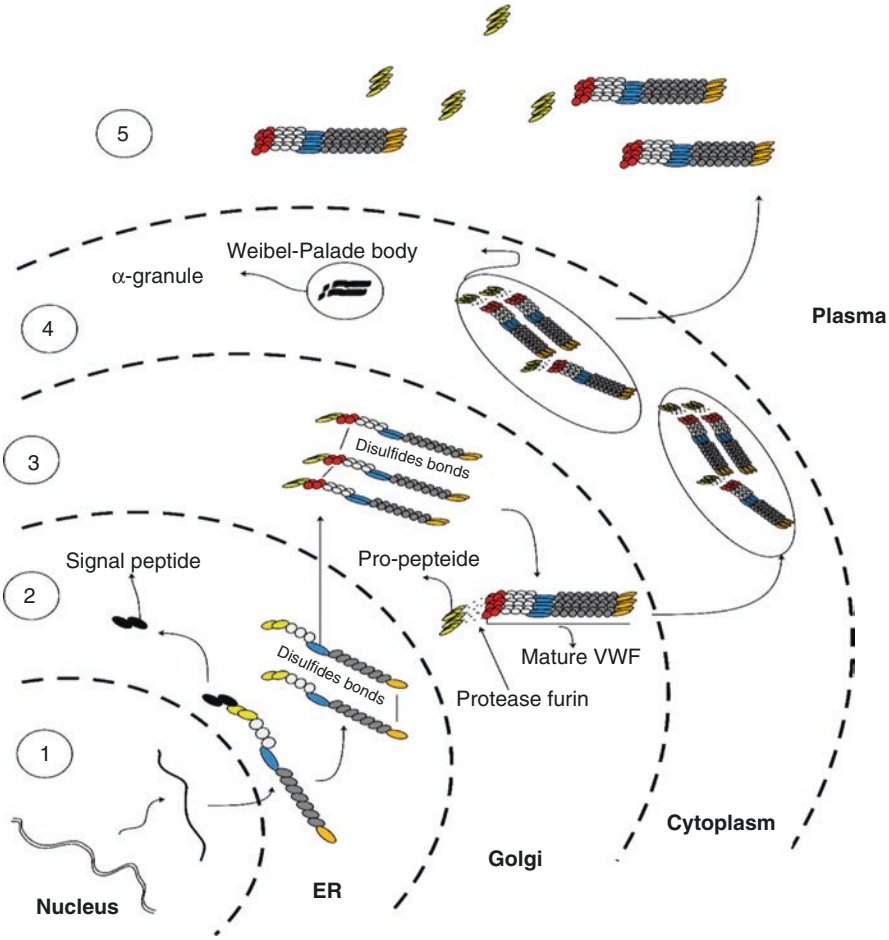

Fig. 3.4 The biosynthesis of von Willebrand factor (VWF). (*1*) The *VWF* gene encodes VWF mRNA which is translated to pre-propeptide VWF. (*2*) The synthesized VWF precursor is transported to the endoplasmic reticulum (ER) and undergoes several processes such as removal of signal peptide, tail-to-tail (C-terminal) dimerization, and addition of N-linked oligosaccharides. (*3*) VWF dimer is transported to Golgi apparatus and undergoes other processes including propeptide cleavage, N-terminal head-to-head multimerization, N-linked glycan maturation, and O-linked glycosylation. (*4*) The mature VWF is stored in storage organelles (Weibel-Palade bodies (WPBs) and α-granules of endothelial cells and megakaryocytes, respectively). (*5*) The contents of secretory granule storage are released into plasma, which thereby contributes to primary hemostasis

release all their contents, including VWF and other proteins. In massive secretion, multiple WPBs aggregate and are fused to secretory vesicles, which results in secretion of massive amounts of VWF multimers. This kind of secretion is potentially highly thrombogenic, therefore necessitating the presence of ADAMTS13 (Fig. 3.5) [9, 28].

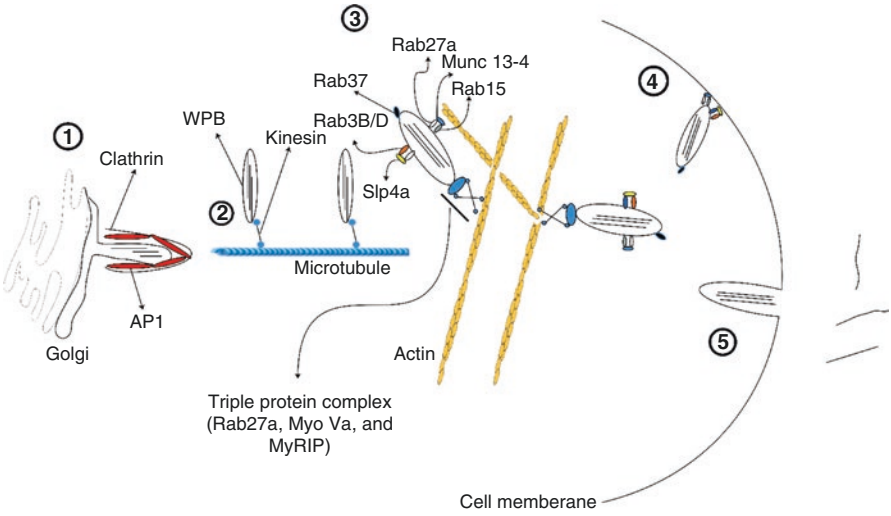

Fig. 3.5 A schematic presentation of VWF secretion. (*1*) The formation of WPBs begins at trans-Golgi network and involves AP1 and clathrin. (*2*) Within the endothelial cell, immature WPBs are transported along microtubules by the kinesin/dynein complex. (*3*) Following secretion, immature WPBs are adhered to the filamentous actin (F-actin) by the tripartite complex Rab27a, MyRIP, and MyoVa. Other proteins such as Rab3, 15, 27a, 37, Munc13-4, and SIp4a are attracted by WPBs. (*4*) Following secretion, F-actin adherence is lost, and three sequential steps tethering, docking, and priming occur, after which WPBs fuse with the cell membrane. A Rab27a and Slp4a-dependent docking step is necessary for the WPBs to release their contents. (*5*) WPBs fuse with the cell membrane and discharge their contents. *VWF* von Willebrand Factor, *WPBs* Weibel-Palade bodies, *AP1* adaptor protein complex 1, *MyRIP* myosin and Rab27a-interacting protein, *MyoVa* myosin Va

3.2.6 Biological Activities of von Willebrand Factor

The mature VWF consists of different functional sites that are capable of binding to other molecules and which exert various biological activities. These sites in each subunit act independent of multimer assembly; indeed, after proteolysis of the large native molecules, the isolated monomeric fragments still have substrate recognition specificity [27].

3.2.7 Stabilization and Transport of Coagulation Factor VIII

In patients affected by hemophilia A, due to defects in the *F8* gene, the FVIII level is decreased and the VWF level is normal. In contrast, in most of the patients with VWD, largely due to defects in the *VWF* gene, concomitant reduction of both FVIII and VWF occurs, because the survival of FVIII is dependent on its interaction with VWF [29]. The location of VWF that binds to FVIII is located with the first 272 amino acids of mature VWF, with the amino acids 78–96 having the substantive role

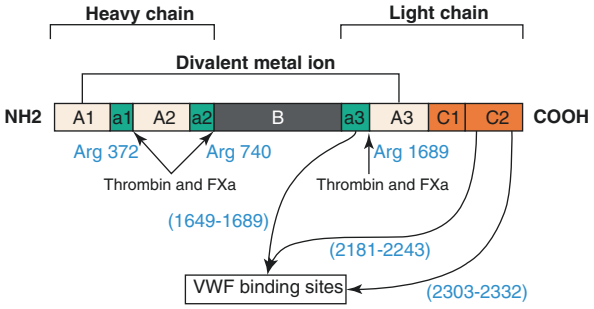

Fig. 3.6 Factor VIII (FVIII) structure and major binding sites for von Willebrand factor (VWF). FVIII consists of two chains including heavy and light chain. Heavy chain consists of A1, A2, and B domains while the light chain contains A3, C1, and C2 domains. Each A domain flanked by short segments (a1, a2, and a3) which have a high content of acidic amino acids. FVIII has different binding sites for different proteins. This factor is activated by thrombin and activated factor X (FXa) via proteolysis which occurs at Arg372, Arg740, and Arg1689 residues. This proteolysis leads to the release of B domain. FVIII has three binding sites for VWF, located at residues 1649–1689, 2181–2243, and 2303–2333. Following the activation of FVIII, thrombin cleaves FVIII at the Arg1689 residue and effectively destroys the binding site for VWF

in binding of VWF to FVIII [30]. In addition, the disulfide bond in the N-terminal of VWF has a crucial role in binding of FVIII. The respective binding sites in FVIII are located in the terminus of the light chain in the segment between 1669 and 1689 and also N-terminal and C-terminal of C2 domain including residues 2181–2243 and residues 2303–2332 [31, 32]. After transport of the FVIII to the location of hemostatic need, and the activation of FVIII, thrombin cleaves this coagulation factor at Arg1689. Thus, the binding site of VWF is destroyed and activated FVIII (FVIIIa) is released. In this way, VWF at the site of thrombus formation may deliver FVIIIa for coagulation reactions (Fig. 3.6) [9].

3.3 Platelet Adhesion

Following injury to the endothelium, VWF binds to different components of subendothelium as well as to platelets in order to sequester them to the injury site [33].

3.3.1 Interaction of von Willebrand Factor with Extracellular Protein

VWF is capable of binding to different types of collagen including type I, II, III, IV, V, and VI. Domains A1 and A3 are responsible for interaction with the main components of extracellular matrix (especially collagen). Each domain is capable of binding to different types of collagen [34] [35]. The A1 domain, which covers residues 497 to 716, binds to collagen type VI while the A3 domain, comprising

residues 910 to 1111, binds to collagen types I and III [36, 37]. Collagen types I and III support VWF-dependent platelet adhesion in high shear rate, whereas collagen type IV mostly supports platelet adhesion at lower shear rates. The more important collagen binding site of VWF is therefore the A3 domain [38].

3.3.2 Interaction of von Willebrand Factor with Glycoprotein Ibα

The A1 domain has a site of interaction with glycoprotein (GP) Ibα, which in turn is a component of the platelet GPIb-V-IX receptor complex [39]. This interaction has a significant role in platelet activation, platelet adhesion, and finally platelet aggregation. In addition, binding of VWF to this receptor is crucial for the formation of thrombus. Initial platelet adhesion results from interaction between VWF and GpIbα under high fluid shear. In laboratory tests, the bacterial glycopeptide antibiotic ristocetin can bind to VWF and facilitate platelet GPIbα binding under low shear, and thereafter provokes platelet aggregation. This property of ristocetin is exploited in several VWF tests, including the ristocetin-induced platelet aggregation (RIPA) assay and VWF the ristocetin cofactor (VWF:RCo) assay, both of which permit laboratory estimation of the binding of VWF to GpIbα [40]. Botrocetin is a venom derived from viper *Bothrops jararaca* and can also activate platelets via GpIbα binding to cause VWF-dependent platelet aggregation [38]. The botrocetin-induced platelet aggregation (BIPA) is used more selectively in laboratory diagnostics [41].

The VWF binding site for GPIb is located in 293 residues of N-terminal and for optimal binding requires the sulfation of amino acid tyrosine at positions of 276, 278, and 279 [42]. Several studies have shown that the amino acids located in the boundary region between domain D3 and A1 (474 to 488 residues) and also the region in A1 domain (694–708 residues) are probably involved in this binding [43, 44]. Other studies have shown that another region in the A1 domain (residues 514 to 542) mediates the binding of VWF to GPIbα. In addition, carbohydrate chain has a role in binding of VWF to GPIbα. Although glycosylation of VWF is not necessarily needed for this binding, the O-linked sugars in each side of the A1 domain enhance this function [45–47].

3.3.3 Interaction of von Willebrand Factor with Integrin αIIbβ3 (GPIIb/IIIa)

Platelet GPIIb-IIIa, also known as the integrin αIIbβ3, is a surface receptor that, after platelet activation, can bind to different ligands, including fibrinogen, fibronectin, and VWF [48]. In domain C1 of VWF, the tetra peptide motif Arg-Gly-Asp-Ser (located in the C-terminal of the molecule) is a binding site for αIIbβ3 (GPIIb/IIIa). Although the major interaction for platelet adhesion is GbIb binding, inhibition of integrin αIIbβ3 (GPIIb/IIIa) also impairs platelet adhesion [38, 49]. In fact, VWF-integrin αIIbβ3 (GPIIb/IIIa) interaction leads to further platelet

adhesion and facilitation of platelet aggregation initiated through the binding of GpIb to VWF [50].

3.4 von Willebrand Disease

VWD, with an estimated incidence of ~1%, is the most common CBD and is most often inherited in an autosomal dominant pattern. The disorder was first described by Erik von Willebrand in 1926, naming it "hereditary pseudohemophilia." VWD is caused by defects and/or deficiencies in VWF concentration, structure, or function. Patients with VWD present with variable bleeding episodes, mostly mucocutaneous bleeds. Epistaxis and bruising are the most common presentations in children, while menorrhagia and hematoma are common in adults, somewhat dependent on gender and type of VWD [2]. Generally, the severity and frequency of bleeding also depends on the type of disease, severity of defects, or deficiency in VWF and sometimes also the age and gender of the individual. Plasma levels of VWF gradually increase with age, and this may ameliorate the diagnosis of the disorder or its effect in some cases. VWD is classified into three main types: types 1 and 3 represent partial and complete deficiency of VWF, respectively, while type 2 reflects qualitative defects further characterized into four subgroups. These comprise (i) type 2A VWD, with loss of high- and sometimes intermediate-molecular-weight VWF multimers; (ii) type 2B VWD, with increased affinity of VWF for GPIb; (iii) type 2M VWD, with defects in VWF function such as platelet adhesion but a relatively normal pattern of (i.e., no substantial decrease in high molecular weight) VWF multimers; and (iv) type 2N, with markedly decreased VWF binding affinity to FVIII (Table 3.1) [1, 51].

Table 3.1 Classification of von Willebrand disease

Disorder		Definition	Prevalence	Inheritance
Type 1 VWD		Partial deficiency of VWF	1 in 1000 (40–80%)	AD
Type 2 VWD	2A	Qualitative defect with loss of high and sometimes intermediate-molecular-weight VWF multimers	~20%[a]	AD or AR
	2B	Qualitative defect with increased affinity to platelet GPIb	–	AD
	2M	Qualitative defect with decreased platelet adhesion property but relatively normal pattern of VWF multimers	–	AD
	2N	Qualitative defect with markedly decreased affinity of VWF to factor VIII	–	AR
Type 3 VWD		Complete deficiency of VWF	1 in 1 million (<1%)	AR

VWD von Willebrand disease, *VWF* von Willebrand factor, *AD* autosomal dominant, *AR* autosomal recessive

[a]Overall incidence of all type 2 VWD, with types 2A, 2M, 2B, and 2N each representing up to 25% or more of type 2 VWD cases, depending on the geography and the local study population

Table 3.2 Frequency of different types of von Willebrand disease

VWD type	London (*n*: 134)	German Democratic Republic (*n*: 111)	Swedish (*n*: 106)	UK (*n*: 116)	Jordan (*n*: 65)	Italy (*n*: 1286)
Type 1 VWD (%)	75	76	70	71	59	73
Type 2 VWD (%)[a]	19	12	10	23	29.5	21
Type 3 VWD (%)	6	12	20	6	11.5	6

VWD von Willebrand disease
[a]Overall incidence for type 2 VWD

Type 3 VWD, with an estimated incidence of 1 per 1 million in the general population in developed countries, is the rarest and the most severe form of disorder, while type 1 is the most commonly diagnosed form of VWD (Table 3.2). Relative frequency depends on the geographical location and the team making the diagnosis. For example, the diagnosis of type 3 VWD in some locations may include patients that would otherwise be identified as severe type 1 in other locations [52].

Types 1 and 2 VWD are highly heterogeneous disorders, and bleeding tendency is related to circulating level of functional VWF. Triad diagnostic criteria have been proposed for VWD: (1) bleeding symptoms, (2) decreased VWF activity measured (e.g., as measured by VWF:RCo), and (3) pattern of inheritance (autosomal dominant or recessive). Among these criteria, bleeding history is perhaps the most important criteria, although this is often elusive in young children due to lack of exposure to bleeding risk situations [53]. In respect to bleeding history, it is always important to determine whether the bleeding tendency is "lifelong" or of recent onset, the latter potentially indicating an acquired abnormality rather than an inherited one [53].

Diagnosis of VWD is a challenge worldwide, especially in the mildest form of the disease, as well as in complex types, and misdiagnosis is common and can occur due to use of the wrong methods or tests or ineffective test panels. Correct diagnosis and classification of VWD is crucial as it can influence patient management. However, in practice, typing of the disorder may be difficult because the patient's phenotype might vary over the time, the patient's genotype may not show a mutation or may show complex patterns, and some laboratory tests have low sensitivity and specificity. Moreover, the boundary between normal and abnormal phenotypes is not clearly defined [54].

3.4.1 Type 1 von Willebrand Disease

Type 1 VWD with an estimated incidence of 1 per 1000 is not only the most common form of VWD but also the most often diagnosed CBD. This disorder is inherited in autosomal dominant manner with variable penetrance and a highly variable phenotype. Type 1 VWD accounts for 40–80% of all cases with VWD, and based on molecular pathogenesis, VWF level is between 5 and 40%. A considerable number of factors are responsible for the highly variable clinical and laboratory phenotype in type 1 VWD. In about 30% of patients with type 1 VWD, a mutation cannot be

detected in the *VWF* gene. Genetic modifiers and physiological factors are major factors that can reduce plasma level of VWF, with one well-known genetic modifier outside the *VWF* gene being ABO blood group [55].

Plasma VWF level is ~25% lower in individuals with O blood group than non-O blood group. Other genetic modifiers that can help explain the low level of VWF in type 1 VWD with undetectable mutation in *VWF* gene are CLEC4M (C-type lectin domain family 4, member M) and STXBP5 (syntaxin-binding protein 5), which are probably involved in clearance and exocytosis of VWF, respectively [56, 57]. The severity of bleeding episodes in type 1 VWD depends on severity of the VWF deficiency in plasma; therefore, risk of severe bleeds is higher in patients with lower plasma levels of VWF. Plasma FVIII level is also reduced in parallel with VWF but normally is at ~1.5× the level of VWF and is usually between 5 and 50% (5 to 50 IU/dL). Since type 1 VWD phenotypically defines an inherited bleeding disorder with partial quantitative VWF deficiency, both VWF antigen (VWF: Ag) and VWF activity fall in parallel, and functional abnormality can be excluded if all VWF activity/VWF:Ag ratios are around unity (generally >0.6) [58]. With use of sensitive assays, a considerable number of patients with historical diagnosis of type 1 VWD may have mild abnormalities of multimer structure or distribution; however, bleeding in this form of the disorder is generally attributed to the VWF concentration and not to any selective decrease in large VWF multimers or specific abnormalities in ligand binding sites [59]. Increased susceptibility of VWF to proteolytic cleavage may also contribute to the severity of type 1 VWD. The Tyr1584Cys mutation, for example, increases the susceptibility of VWF to cleavage by ADAMTS13. Desmopressin is generally suitable as the therapeutic choice in most patients with type 1 VWD, especially for short duration or minor treatments [59].

3.4.2 Type 2 von Willebrand Disease

Type 2 VWD is characterized by qualitative defects in structure and function of VWF and is classified in four types: 2A, 2B, 2M, and 2N. Classically, type 2A has been considered the most common form, while 2N and 2B represent the rarest forms. However, the relative frequency of type 2 VWD depends in part on the geography in which the diagnosis is made, as well as the diagnostic team. For example, type 2N VWD is relatively more frequent in some parts of France and Italy, and type 2B diagnosis requires performance of RIPA, which if omitted may lead to diagnosis of such patients as 2A VWD [60, 61]. Type 2 VWD is mostly transmitted in an autosomal dominant manner, except for type 2N, which has an autosomal recessive pattern of inheritance. Type 2 VWD is less common than type 1 VWD and represents ~20% of all cases of VWD. Type 2 VWD is usually attributable to mutations that either impair specific functional domains of VWF or which affect VWF multimer assembly or proteolysis. Generally, mutations responsible for type 2 VWD are highly penetrant, and bleeding episodes are highly reproducible in a given family. Type 2 VWD is diagnostically the most challenging form of VWD [62]. The hallmark of this type of disorder is low-functional VWF/VWF:Ag ratio (<0.7).

Functional VWF is usually assessed as VWF:RCo, although other assays such as collagen binding (VWF:CB) may be utilized. Lack of large multimers is evident in types 2A and 2B VWD, with loss of intermediate multimers also often seen in type 2A VWD. Impaired RIPA in platelet-rich plasma (PRP) or in whole blood is evident in types 2A and 2M VWD, whereas an increased RIPA responsiveness is seen in 2B VWD [41].

3.4.2.1 Type 2A von Willebrand Disease

Type 2A is classically considered the most frequent type 2 VWD, comprising up to 20% of all cases with VWD and typically >50% of all type 2 VWD. Impaired VWF multimerization or increased susceptibility of multimers to degradation with ADAMTS13 is the key impairment evident in this disorder, causing selective reduction of high-molecular-weight multimers (HMWM) and sometimes intermediate-molecular-weight multimers (IMWM), leading to diminished activity of the binding domains for GPIb and probably GPIIb/IIIa [61].

3.4.2.2 Type 2B von Willebrand Disease

In type 2B VWD, gain-of-function mutations, usually in the A2 domain of VWF, cause an increased affinity of VWF for platelet GpIb. This is often identified in laboratory testing by elevated RIPA responsiveness. In 2B VWD, the platelet-VWF complex is removed from the plasma circulation, often leading to loss of HMWM also (mild) thrombocytopenia. Although these features are seen with varying degree in the majority of patients, not all cases present with the "classic" 2B VWD presentation. Additional features of 2B VWD include slightly decreased to normal levels of VWF:Ag and FVIII and relatively decreased VWF:RCo and VWF:CB (ratios of VWF:RCo/Ag and VWF:CB/Ag both <0.7). VWF multimeric pattern is normal in some patients with ("atypical") type 2B, and in such patients, the functional VWF/VWF:Ag ratio may also be normal. In 2B VWD patients in their second and third trimester of pregnancy, the pregnancy associated with increased level of VWF may worsen the thrombocytopenia due to an increase of abnormal endogenous VWF and increased clearance. A similar situation can occur if desmopressin is administrated in patients with 2B VWD [63].

3.4.2.3 Type 2M von Willebrand Disease

Type 2M is mostly due to mutations in the A1 domain of VWF, leading to conformational changes in VWF protein and decreased affinity to GpIbα. VWF multimeric pattern is essentially normal, but platelet-dependent VWF activities are decreased. Rarely, these mutations can occur in the A3 domain, and thus also affect collagen binding. Generally, type 2M VWD cases are identified by low VWF:RCo/Ag ratios without lack of HMWM or low VWF:RCo/Ag ratio and normal VWF:CB/Ag ratio. Misdiagnosis is an important and largely under-recognized issue in type 2M VWD, and a considerable number of clinicians or laboratories identify this type "incorrectly" as type 1 or type 2A VWD. Patients with type 2M present with mild to moderate bleeding tendency but severe bleeds also may occur [64].

3.4.2.4 Type 2N von Willebrand Disease

Type 2N VWD (VWD "Normandy") was originally described in patients from the Normandy region of France, in 1989, as a variant of VWD caused by defects in the ability of VWF to bind FVIII. This defect causes decreased plasma level of FVIII, thereby resembling hemophilia A. However, the inheritance pattern of 2N VWD is autosomal recessive (whereas hemophilia A is sex-linked, being carried on the X chromosome), and the defect is present on VWF (whereas in hemophilia A, the defect is in FVIII). Symptoms of type 2N VWD are similar to that of (mild) hemophilia A [65]. Phenotypically, VWF:Ag, VWF:RCo, and multimeric pattern are normal in type 2N VWD, but the level of FVIII is decreased due to the increased clearance of FVIII (since the VWF does not bind FVIII, the FVIII is easily degraded in circulation). Sometimes 2N VWD may arise as a duplex defect. For example, patients with a 2N VWD mutation on one *VWF* gene and another defect in the other *VWF* gene may show more complex phenotypes and more severe/complex bleeding patterns. For example, if the second *VWF* gene carries a null mutation, essentially mimicking a "heterozygous type 3 VWD," then the resulting phenotype will express with lowered VWF levels. Although the majority of patients with type 2N VWD have normal multimeric patterns, occasional patients may show loss of HMWM should a complex genotype be evident [60].

3.4.3 Type 3 von Willebrand Disease

Type 3 VWD is the most severe form of the disorder, although fortunately also the rarest type of VWD (1 in 1 million in the general population); however, frequency is higher in developing countries due to high rates of consanguinity [66].

By definition, type 3 VWD is a severe bleeding disorder with undetectable plasma and platelet level of VWF and also a low level of FVIII:C (<20 U dL^{-1}). The clinical presentations and bleeding episodes are generally more severe than other types of VWD and are often similar to those of moderately severe hemophilia A. Due to concomitant decrease of VWF and FVIII, these patients not only present mucocutaneous hemorrhages but also with hemarthrosis and hematomas, as associated to decreased FVIII level [52].

3.5 Clinical Manifestations of von Willebrand Disease

Clinical manifestations and bleeding tendency among patients with VWD are highly variable and range from quite mild conditions to severe bleeding diathesis sufficient to require urgent medical intervention. Mucocutaneous bleeding such as epistaxis and menorrhagia is the typical presentation of VWD, and post-dental extraction bleeding is the most common post-surgical bleeding event. Since VWF also binds to FVIII and facilitates platelet function, VWD may cause bleeding symptoms that are typical of platelet function disorders or mild to moderately severe hemophilia A or both [3, 67].

In some patients, especially in males, surgery may be the first hemostatic challenge that leads to "abnormal bleeding" and therefore facilitates the diagnosis in previously unrecognized cases.

A wide overlap can be observed between the bleeding diathesis of patients with mild VWD and the normal population; therefore, a proper bleeding history is a critical and crucial component in the diagnosis of VWD and should be done carefully. Women with mild VWD are often more symptomatic than men, because they are subject to greater hemostatic challenges (menstruation and childbirth). In children with VWD, the pattern of hemorrhagic symptoms are different from children with more severe congenital bleeding disorders, with life-threatening bleeds such as intracranial hemorrhage (ICH) and umbilical cord bleeding being more rare among young VWD patients. In children with VWD, the most common clinical presentations are bruising and epistaxis, although these presentations are also frequent in normal healthy children, further challenging their diagnosis [68]. Some of standard clinical presentations in VWD, such as menorrhagia and post-surgical bleeding, are not clearly prevalent or evaluable in the pediatric population. Standard bleeding assessment tools (BATs) and scoring systems may be useful for correct assessment of bleeding episodes among these patients because significant bleeding diathesis may be overlooked while minimal bleeding symptoms may be over emphasized [69].

In adults, hematoma, menorrhagia, and bleeding from minor wounds are the most frequent symptoms, depending on VWD type, disease severity, and patient gender. Post-dental extraction and post-surgical bleeds are common and occur in about two-thirds of VWD patients. Gastrointestinal bleeding (GI) is also reported in VWD, predominantly among adults, and this can sometimes be severe [70]. In some patients, especially those with severe VWD, epistaxis can be so severe as to require medical intervention with clotting factor concentrates or blood transfusion. This diathesis can be life-threatening in some patients and may require long-time secondary prophylaxis [66].

Post-partum hemorrhage (PPH) can also be observed among women with VWD, but with lower frequency than may be expected because of increasing VWF levels during pregnancy. Delayed PPH can occur due to gradual decrease of VWF level to baseline level post-delivery. Prolonged vaginal bleeding following normal vaginal delivery is a common presentation of women with VWD. Menorrhagia (>80 mL of blood loss per menstrual period) is a common and important bleeding symptom of women with VWD and ~15% of women with menorrhagia have VWD. Therefore, menorrhagia is a sensitive but nonspecific presentation of VWD in women. This symptom can be accompanied by anemia and iron deficiency. Therefore, careful gynecological assessment of women with VWD is crucial [52, 69].

In general, bleeding symptoms are mild in type 1 VWD and more severe in type 2 and type 3 VWD. Since, FVIII level is only slightly reduced in most types of VWD, spontaneous hemarthrosis or hematoma are rare in type 1, 2A, and 2B VWD, while in type 3 VWD the severity of diathesis often resembles that of hemophilia.

Table 3.3 Clinical manifestations of patients with von Willebrand disease

	Scandinavian (*n*: 264)	Iranian (*n*: 385)	Italian		
Bleeding	VWD	Type 3	Type 1 (*n*: 671)	Type 2 (*n*: 497)	Type 3 (*n*: 66)
Epistaxis (%)	62	77	61	63	66
Menorrhagia (%)	60	69	32	32	56
Post-dental extraction bleeding (%)	51	–	31	39	53
Hematoma (%)	49	52	13	14	33
Bleeding from minor wounds (%)	36	–	36	40	50
Gum bleeding (%)	35	70[a]	31	35	56
Post-surgical bleeding (%)	28	41	20	23	41
Post-partum bleeding (%)	23	15	17	18	26
Gastrointestinal bleeding (%)	14	20	5	8	20
Hemarthrosis (%)	8	37	3	4	45
Hematuria (%)	7	1	2	5	12
CNS bleeding (%)	–	2	1	2	9

CNS central nervous system
[a]Oral cavity bleeding

Type 3 VWD mostly is accompanied with severe bleeds, while type 1 and type 2 VWD are very heterogeneous, and the severity of clinical presentations is associated with circulation level of functional VWF (e.g., as measured by VWF:RCo). Severe life-threatening bleeding can occur in type 3 VWD and sometimes in patients with type 2 VWD but is a rare presentation of type 1 VWD. ICH is a rare presentation of VWD that usually is only reported in type 3 VWD. An interesting recent finding is the reduced risk of cardiovascular disease and ischemic stroke among patients with VWD (Table 3.3).

3.5.1 Type 1 von Willebrand Disease

Although the bleeding symptoms are variable in type 1 VWD, bleeding tendency in these patients is usually mild, and major bleeds are rare. The heterogeneity in bleeding symptoms in Type 1 VWD may be partially explained by presence of genetic variations [71].

Due to the physiological rise in plasma VWF level throughout life, patients with type 1 VWD may have normal levels of VWD later in life; however, the severity and frequency of bleeding symptoms in older patients may still be significant, especially if there is an increase in hemostatic challenges (e.g., older patients more likely to be candidates for surgery). The most common clinical presentations in children with type 1 VWD are easy bruising and epistaxis, while menorrhagia, oral cavity bleeding, prolonged bleeding from minor lacerations, GI bleeding, and bleeding after tooth extractions or oral surgery are other presentations [72].

Clinical manifestations and bleeding episodes are an important part of the diagnosis of type 1 VWD. Out of three main criteria for diagnosis of VWD, clinical symptoms assessment is perhaps the most important part of the diagnosis [53]. A patient that meets all three diagnostic criteria may be diagnosed as type 1 VWD, while an asymptomatic patient with low level of VWF and positive family history of VWD without personal history of VWD and without personal history of clinical symptoms can be assigned to a category of "possible type 1 VWD." To fulfill this definition, an asymptomatic patient with low level of VWF should have at least two relatives with definitive VWD [58].

Based on a multicenter study on sensitivity and specificity of bleeding symptoms in the diagnosis of type 1 VWD, it was shown that menorrhagia and epistaxis are not good predictors of type 1 VWD, while cutaneous bleeding and post-dental extraction bleeding should be considered as the most sensitive symptoms. It was proposed that laboratory investigation for VWD should be done for those with at least three minor bleeds or those with at least two major bleeds if they include cutaneous bleeding or post-dental extraction bleeding [73].

3.5.2 Type 2 von Willebrand Disease

Patients with type 2 VWD have variable bleeding tendency with moderate to severe hemorrhagic tendency. The severity and incidence of hemorrhagic symptoms vary between different subtypes of type 2 VWD, often explainable by differences in levels of FVIII, VWF, and/or HMWM VWF. The bleeding tendency in type 2A VWD is generally greater that in type 2M; this cannot easily be explained by differences in residual FVIII or VWF levels but may be attributed to higher rate of GI bleeding in type 2A VWD. The higher relative risk of type 2A VWD can also be attributed to a lesser extent to a higher rate of menorrhagia. In type 2B VWD, thrombocytopenia is a contributory factor that can worsen the hemorrhage risk in affected patients. Intra-articular bleeding (hemarthrosis) is a common presentation of hemophilia that in addition to type 3 VWD can also be observed in type 2N. The risk of hemarthrosis is strongly dependent on residual plasma FVIII levels as well as the severity of VWD [60].

GI bleeding due to angiodysplasia is a well-known complication of VWD, notably in types 2A and 2B VWD. Two main reasons for the occurrence of this kind of bleeding are older age and lack of HMWM in plasma. It was shown in a long term prospective study that GI bleeding due to angiodysplasia was not observed in VWD Vicenza, which is accompanied by presence of ultra-large VWF multimers. The development of angiodysplasia in VWD is attributed to the role of VWF in regulation of angiogenesis, and enhanced angiogenesis is observed with reduced VWF level. It appears that some forms of type 2A VWD expressing the S1506L mutation are associated with higher risk of angiodysplasia [74]. GI bleeding due to angiodysplasia with an incidence of ~2–6 is a common cause of digestive tract bleeding in elderly people (Table 3.4).

Table 3.4 Clinical manifestations of patients with type 2 von Willebrand disease

Disorder	Clinical manifestations
Type 2A VWD	Gastrointestinal bleeding (36.9%)
	Post-surgical bleeding (80%)
	Post-dental extraction bleeding (73%)
	Menorrhagia (50%)
	Epistaxis (34%)
	Prolonged bleeding from minor wounds (33%)
	Post-circumcision bleeding (32%)
	Bruising (24%)
	Cephalohematoma (~9%)
	Hematuria (4%)
	Central nervous system bleeding (3%)
	Umbilical stump bleeding (6%)
Type 2B VWD	Easy bruising (59%)
	Epistaxis (52%)
	Menorrhagia (25%)
	Oral cavity bleeding (23%)
	Prolonged bleeding from minor wounds (~27%)
	Gastrointestinal bleeding (14%)
	Umbilical stump bleeding (10%)
	Hemorrhage (~2%)
Type 2M VWD	Gastrointestinal bleeding (3.3%)
	Cephalohematoma (~9%)
	Bruising after birth (~7%)
	Prolonged bleeding from minor wounds (~32%)
Type 2N VWD	Cephalohematoma (~11%)
	Hemarthrosis
	Bruising after birth (~11%)
	Prolonged bleeding from minor wounds (~33%)

VWD von Willebrand disease

3.5.3 Type 3 von Willebrand Disease

Type 3 is the most severe form of VWD that from a clinical aspect is similar to some extent to hemophilia. Although hemophilia-like hemarthrosis or recurrent spontaneous mucosal bleeding is more frequent in type 3 VWD than in type 1 or 2 VWD, the rate of these bleeds is significantly lower than hemophilia A. The bleeding symptoms in type 3 are due to concomitant reduction of FVIII and VWF, leading to occurrence of both primary and secondary hemostasis presentations. Life-endangering hemorrhage in CNS is a rare presentation of VWD that is usually only reported in type 3 VWD [52]. Although the rate of GI bleeding is relatively high in type 3 VWD patients, the incidence of other life-threatening bleeds such as umbilical cord bleeding is relatively low in this disorder, and therefore the pattern of bleeding in type 3 VWD is different from other severe congenital bleeding disorders such

as factor XIII deficiency. Since type 3 VWD represents an autosomal recessive congenital bleeding disorder, patients are homozygote or double heterozygotes. Carriers of type 3 VWD, with a single defective *VWF* gene, are typically asymptomatic, similar to most of other congenital bleeding disorders [66].

3.6 Diagnosis of von Willebrand Disease

VWD is diagnosed on clinical features, comprising personal and family history of bleeding or bruising, and confirmed by laboratory testing. As VWD is due to deficiencies or defects in the plasma protein VWF, a large adhesive protein with multiple activities, laboratory testing therefore centers on assessment of VWF by means of a panel of assays [6]. The more comprehensive the assay panel is, the more likely it is to achieve a correct diagnosis or exclusion of VWD. Vice versa, the less comprehensive the assay panel is, the more likely it is to achieve an incorrect diagnosis. The minimum recommended test panel comprises Factor VIII coagulant (FVIII:C), VWF antigen (VWF:Ag), and VWF "activity" using several other assays, generally including evaluation of platelet Glycoprotein (GP) Ib binding and collagen binding (VWF:CB), with factor VIII (FVIII) binding (VWF:FVIIIB) performed more selectively. Decreases in VWF:Ag and the various VWF activities, as well as the pattern of such changes, help define VWD and its type and the need (if any) for further testing. The most often used assay for measuring GPIb binding activity is the ristocetin cofactor assay (VWF:RCo), which historically measured the agglutination by VWF of fixed human platelets in the presence of ristocetin [75]. This assay is now often replaced or supplemented with other assays based on binding of VWF to recombinant GPIb, generally without the use of platelets and with or without ristocetin [75–77].

Because of the large number of different laboratory tests now available and the many different methodologies in use, associate terminology for tests involving "platelet-dependent" function has recently been updated by the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) [6, 77]. The main elements of the recommended nomenclature are summarized in Table 3.5.

Nevertheless, the assay group reflecting assays of GPIb binding (i.e., classical VWF:RCo, as well as methodologies now defined as "VWF:GPIbR" and "VWF:GPIbM") would be expected to broadly derive similar test results for VWD patients and are thus essentially recognized to be "interchangeable" in VWD diagnostics.

In respect to an "ideal" diagnostic test panel, then, (i) VWF:Ag assays quantify the level of VWF protein without reference to its functional activity; (ii) "GPIb-binding assays," as defined above, define platelet binding; (iii) VWF:CB assays quantify the activity of VWF binding to subendothelial matrix components (in this case collagen); (iv) FVIII coagulant function quantifies the activity of FVIII; (v)

Table 3.5 Recommended nomenclature of the ISTH VWF SSC for VWF test parameters

Abbreviation for assay	Description of assay	Comments
VWF:Ag	von Willebrand factor antigen	All assays that provide a quantitative level of VWF protein, be it by ELISA, LIA, or other methodology
VWF:CB	von Willebrand factor collagen-binding capacity	All assays that provide a quantitative level of VWF collagen-binding capacity, be it by ELISA or other methodology
VWF:RCo	von Willebrand factor ristocetin cofactor activity	Historically, this referred to assays that used ristocetin to facilitate VWF binding to GPIb, where the only such assay type available was that based on platelet agglutination. This has changed with the advent of non-platelet-based methods, which incorporated rGPIb. The updated recommendations place these new assays into new categories, namely, VWF:GPIbR
VWF:GPIbR	All assays that are based on the ristocetin-induced binding of von Willebrand factor to a recombinant wild-type GPIb fragment	Essentially, these are VWF:RCo assays that do not use platelets and which currently comprise several IL Werfen assays, as performed by either CLIA or LIA technology. These assays essentially generate test results that are very similar to those generated using "standard" VWF:RCo assays that utilize platelets
VWF:GPIbM	All assays that are based on the spontaneous binding of von Willebrand factor to a gain-of-function mutant GPIb fragment	Essentially a GPIb-binding assay that does not utilize platelets or ristocetin and which currently comprises the Siemens Innovance VWF Ac assay (by LIA), as well as non-commercialized ELISA-based assays. These assays essentially generate test results that are very similar to those generated using VWF:GPIbR or classical VWF:RCo assays, despite the lack of ristocetin in the assay
VWF:Ab	All assays that are based on the binding of a monoclonal antibody (MAB) to a von Willebrand factor A1 domain epitope	Essentially a VWF-binding assay that utilizes a monoclonal antibody; this currently comprises the IL Werfen "VWF Activity" assay (LIA), as well as ELISA-based assays. Like VWF:GPIbM assays, VWF:Ab assays do not use ristocetin. VWF:Ab assays provide results that may or may not match VWF:GPIbM, VWF:GPIbR, or classical VWF:RCo assays
VWF:FVIIIB	von Willebrand factor: factor VIII binding capacity	All assays that provide a quantitative level of VWF—factor VIII binding capacity, irrespective of specific methodology. Generally performed by ELISA

CLIA chemiluminescent immuno-assay, *ELISA* enzyme-linked immunosorbent assay, *GPIb* (platelet) glycoprotein Ib, *LIA* Latex immunoassay, *ISTH* International Society on Thrombosis and Haemostasis, *rGPIb* recombinant (platelet) glycoprotein Ib, *VWF* von Willebrand factor, *SSC* Scientific and Standardization Committee

VWF:FVIIIB (as required for 2N VWD) quantifies the activity of VWF binding to FVIII; and (v) VWF multimers (as selectively required) assess the multimeric profile or structure of VWF.

For the purpose of this section, the VWD classification scheme proposed by the ISTH SSC, and last updated in 2006, will be utilized [3], and provides the simplest and most clinically relevant classification scheme, separating VWD into six types, as summarized previously (Table 3.1).

The anticipated test patterns in different types of VWD are summarized in Table 3.6, with greater detail provided in Table 3.7. Type 1 VWD represents a partial quantitative deficiency of (functionally normal) VWF, so there is concordant decrease in VWF measured by any VWF assay (be it VWF:Ag, VWF:CB, or GPIb binding), and the ratio of any one VWF assay to any other is close to unity (in practice, >0.7). Type 3 VWD represents a total loss of VWF, and all VWF test results will be close to 0 U/dL, albeit recognizing that lower limit VWF sensitivity issues means that some assays cannot detect to these low levels.

In contrast, type 2 VWD represents qualitative VWF defects/deficiencies, such that VWF activity is proportionally decreased below that of VWF:Ag; furthermore, the VWD type can be defined by the type of activity reduced. In type 2A VWD, defining a loss of HMW VWF multimers, patients express a relative reduction of all VWF activities sensitive to this loss (this includes both GPIb binding and VWF:CB assays). In practice, this is expressed as the ratio of VWF activity/VWF:Ag assays being lower than ~0.7. Type 2B VWD defines an increased affinity of VWF for GPIb which often leads to loss of HMW VWF multimers and similar VWF test patterns to type 2A VWD. Type 2M VWD defines decreased VWF-dependent platelet adhesion without a selective deficiency of HMW VWF multimers. In type 2M VWD, there are specific changes in VWF function related to specific *VWF* mutations. In practice, most type 2M mutations affect GPIb binding, and less so collagen binding; thus, there is usually a low GPIb binding/VWF:Ag ratio, but the CB/Ag ratio may be normal. Type 2N VWD defines a decreased binding affinity for FVIII, as identified by the specific test VWF:FVIIIB. Phenotypically, however, these patients present similarly to those with hemophilia A, showing relatively lower FVIII:C to VWF:Ag ratios.

The impetus for testing of FVIII:C as part of a VWD diagnostic profile is manyfold. As VWF protects FVIII, lower levels of VWF (viz., VWD), in general, also mean lower levels of FVIII:C. In type 3 VWD, for example, levels of FVIII:C are generally <10 U/dL. In type 1 VWD, the FVIII:C is generally proportional to VWF:Ag. In type 2N VWD, FVIII:C is proportionally lower than VWF:Ag.

In summary, a recommended approach to diagnosis or exclusion of VWD is shown as an algorithm in Fig. 3.7. All patients being screened for VWD should be tested with the 4-test panel of VWF:Ag, VWF:CB, VWF GPIb binding, and FVIII:C. This recommendation is made based on decades of experience with this panel, and the issues arising with diagnostic errors when a different or smaller first line testing panel is employed [6, 78–82].Which tests within each category should be employed by laboratories is to some extent dependent on local instrumentation and test availability. For example, VWF:Ag by ELISA may be preferred if VWF:CB

Table 3.6 Classification scheme for von Willebrand disease

VWD type	Description	Phenotypic diagnosis	Incidence/comments
1	Partial quantitative deficiency of VWF	Low levels of VWF, with VWF functional concordance (i.e., ratio of functional VWF/VWF:Ag approximates unity)	Most common presentation of "VWD" to most laboratories, with most patients presenting with mildly reduced levels of VWF
2A	Decreased VWF-dependent platelet adhesion and a selective deficiency of high-molecular-weight (HMW) VWF multimers	Loss of HMW VWF. Usually low levels of VWF, with VWF functional discordance (i.e., ratios of GPIb binding/Ag *and* CB/Ag typically <0.7)	Globally considered to be the most common presentation of type 2 VWD
2B	Increased affinity of VWF for platelet glycoprotein Ib	Low to normal levels of VWF, typically with VWF functional discordance (i.e., ratios of GPIb binding/Ag *and* CB/Ag generally <0.7), loss of HMW VWF and (mild) thrombocytopenia. Atypical cases may not show this pattern	Rare form (generally 10–20%) of type 2 VWD (= ~1–5 cases per million population). Defined by enhanced responsiveness in a RIPA assay
2M	Decreased VWF-dependent platelet adhesion without a selective deficiency of high-molecular-weight (HMW) VWF multimers	Low to normal levels of VWF, usually with VWF functional discordance detected by GPIb binding/Ag generally <0.7, but relatively normal CB/Ag ratio. HMW VWF present, but multimers may show other abnormalities	Under-recognized form of type 2 VWD. Probably as common as 2A VWD. Some (rare) 2M cases show low CB/Ag ratio, with normal GPIb binding/Ag. Some 2M cases show low CB/Ag ratio and low GPIb binding/Ag
2N	Markedly decreased binding affinity for factor VIII	Defined by VWF:FVIIIB assay, with low FVIIIB/VWF ratios (~0.5 for heterozygous mutations, and <0.3 for more severe genetic changes)	Rare form (generally <10%) of type 2 VWD (= ~1–5 cases per million population)
3	Virtually complete deficiency of VWF	Typically defined by VWF levels <2 U/dL and FVIII:C <10 U/dL	Rare form of VWD in developed countries (~1–5 cases per million population) but disproportionately more common in developing countries

testing is also being performed by ELISA. Alternatively, the availability of latex-based technology may drive the combined use of latex-based VWF:Ag and GPIb-binding assays, or availability of an AcuStar instrument may drive the utility of chemiluminescence test procedures [78]. Also, problems with classical VWF:RCo assays may drive usage of more modern GPIb binding assay alternatives such as VWF:GPIbR or VWF:GPIbM.

Table 3.7 Anticipated test patterns in different types of von Willebrand disease (VWD) and VWD type

VWD type	VWF:Ag	VWF:GPIb binding[a]	VWF:CB	FVIII:C	Multimers	GPIb binding / Ag[b]	CB/Ag[b]	FVIII/ VWF[b]	Comments/additional testing
1	↓ to ↓↓	↓ to ↓↓	↓ to ↓↓	N to ↓↓	Normal pattern but reduced intensity	>(0.5–0.7)	>(0.5–0.7)	>(0.5–0.7)	VWF levels between ~30 and 50 U/dL will generally not be associated with *VWF* mutations and can be considered as representing "low" VWF as a risk factor for bleeding. VWF levels below ~30 U/dL will often be associated with *VWF* mutations and can be considered as representing "true" VWD
2A	N to ↓↓	↓↓ to ↓↓↓	↓↓ to ↓↓↓	↓ to ↓↓	Loss of HMW VWF	<(0.5–0.7)	<(0.5–0.7)	>(0.5–0.7)	2A and 2B VWD can only be distinguished by means of RIPA. Platelet-type (PT-) VWD phenotypically resembles 2B VWD; these can be distinguished by means of RIPA mixing studies, or by genetic analysis of *VWF* and/or platelet *GPIb* genes
2B	N to ↓↓	↓ to ↓↓↓	↓ to ↓↓↓	N to ↓↓	Loss of HMW VWF	<(0.5–0.7)	<(0.5–0.7)	>(0.5–0.7)	
2N	N to ↓↓	N to ↓↓	N to ↓↓	↓↓to ↓↓↓	Normal pattern	>(0.5–0.7)	>(0.5–0.7)	<(0.5–0.7)	Phenotypically similar to hemophilia A; distinguish using VWF:FVIII-binding assay or genetic analysis of *FVIII* and/or *VWF* genes

2M	N to ↓↓	(↓ to ↓↓↓)	(↓ to ↓↓↓)	↓ to ↓↓	No loss of HMW VWF; some multimer defects may however be observed	<(0.5–0.7) (platelet-binding defect) or >(0.5–0.7) (collagen-binding defect)	<(0.5–0.7) (collagen-binding defect) or >(0.5–0.7) (platelet-binding defect)	>(0.5–0.7)	2A and 2M VWD can only be distinguished by comprehensive or composite panel testing, including VWF:Ag, GPIb binding assay[a], *plus* VWF:CB or multimer analysis. Platelet binding dysfunction 2M VWD is far more common than collagen binding defect variants
3	↓↓↓ (absent)	↓↓↓ (absent)	↓↓↓ (absent)	↓↓↓	No VWF present	NA	NA	NA	Type 3 VWD can only be identified when VWF tests are performed, and these are sensitive to very low levels of VWF. The parents of affected patients should also be tested for VWF levels

Ag antigen, *CB* collagen binding, *FVIII* factor VIIII, *HMW* high-molecular-weight (VWF), *GPIb* glycoprotein Ib (the platelet VWF receptor), *N* normal, *NA* not applicable, *RCo* ristocetin cofactor, *RIPA* ristocetin-induced platelet aggregation, *VWF* von Willebrand factor, *VWD* von Willebrand disease

[a]For the purpose of this review, VWF GPIb-binding assays here include classical VWF:RCo assays plus "VWF:GPIbM" assays (such as the Siemens VWF Ac assay) and latex or CLIA-based "VWF:GPIbR" assays. Monoclonal antibody-based "activity" assays ("VWF:Ab" or "VWF:Act") will also often match, but there will be some significant discrepancies

[b]Assay ratios used as cutoff for type 1 vs 2 VWD discrimination generally range in the region of 0.5–0.7 (viz., 0.5, 0.6, or 0.7). Different assays and different laboratories will use different cutoffs based on local evaluation. Type 2N VWD patients yield FVIIIB/VWF:Ag ratios around 0.5 (0.3–0.7) for heterozygous mutations and <0.3 for more severe genetic changes (including homozygous, double heterozygous, or combined heterozygous 2N mutation with second null allele)

Once the methodology has been selected by laboratories, the recommended basic VWD test panel should be able to diagnose or exclude VWD with additional investigations selected on a case-by-case basis, dependent on the results from the 4-test panel. The basic caveat always remains that diagnosis or exclusion of VWD requires, at the very least, repeat testing using a fresh sample, due to pre-analytical and also analytical limitations.

If all VWF tests are normal, confirmed on repeat testing, then either the patient does not have VWD, or else has a form of VWD that is not able to be defined with current testing. Additional assays to define the bleeding disorder, inclusive of platelet function, may be required. If all VWF tests are low, confirmed on repeat testing, but all VWF values are concordant (ratios of GPIb binding/Ag and CB/Ag both >0.7), then the patient has type 1 VWD. In this case, an assessment of severity based on absolute VWF level can ensure; however, unlike the case for hemophilia [83], there is no available consensus for cutoff values defining severity of VWD, and the values presented in Fig. 3.7 are only meant to provide a guide.

If there is a low ratio of GPIb binding/Ag and/or CB/Ag, and this pattern is confirmed on repeat testing with a fresh sample, then the patient may have type 2A, 2B, or 2M VWD (or even possibly platelet type VWD). Here further studies are also indicated. Generally, this means either ristocetin-induced platelet aggregation (RIPA) assessment and/or multimer assessment, depending on test findings and

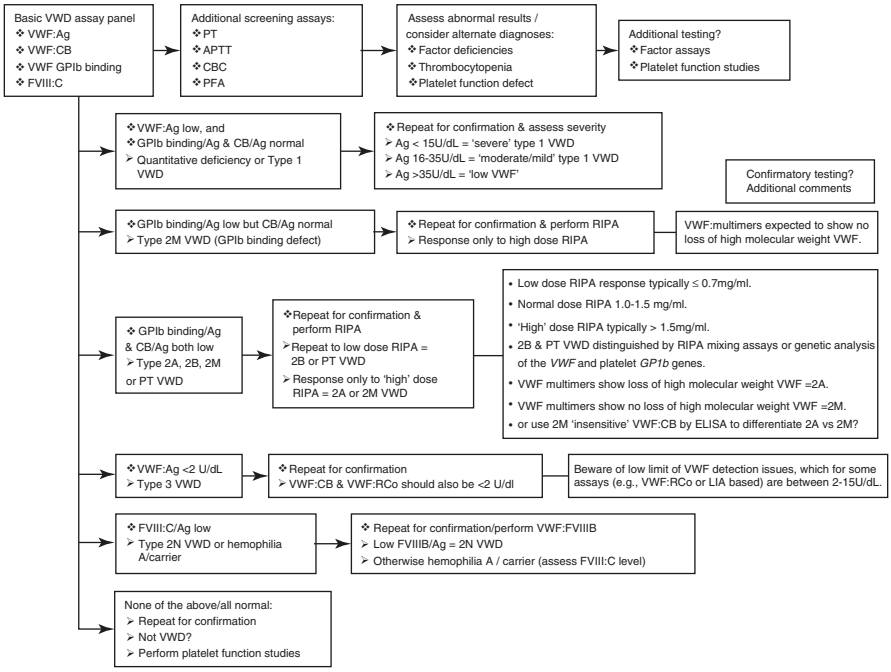

Fig. 3.7 A recommended approach for diagnosis or exclusion of von Willebrand disease

local availability. In our experience, RIPA analysis is usually more important than multimer analysis, and indeed, selection of the right test methodologies for VWF:Ag, VWF:CB, and GPIb-binding assays will often enable prediction of the mutimer pattern negating the need for its performance. Thus, a low ratio of GPIb binding/Ag plus a low ratio of CB/Ag usually points to a loss of HMW VWF, and thus likely 2A, 2B, or platelet-type VWD; instead, a low ratio of GPIb binding/Ag ***or*** CB/Ag (but not both) usually discounts a loss of HMW VWF and instead points to a type 2M VWD.

If all VWF test results are below the measuring range of the assays used, then this will create problems with clear diagnosis, but this usually means severe type 1 VWD or else type 3 VWD; therapy is similar in both cases, although clinical severity is often worse in type 3 VWD.

Finally, if the ratio of FVIII:C/VWF:Ag is low, this suggests either hemophilia A or 2N VWD. Hemophilia A is more common, and being sex-linked affects males more than females; however, misdiagnoses of both hemophilia A and 2N VWD, where the correct diagnosis was the other, do occur. Thus, testing by performance of a VWF:FVIIIB assay is recommended and, after repeat testing for confirmation, could include genetic analysis of *FVIII* and/or *VWF* for final definitive verification.

Genetic analysis may also be useful where patients have been defined to be type 2A, 2B, 2M, or platelet-type VWD and is also typically successful when performed on such patients. Genetic analysis is useful in some type 3 VWD investigations, but generally not useful in type 1 VWD [84].

3.7 Molecular Basis of von Willebrand Disease

VWD is an autosomal hemorrhagic disorder that is defined by dysfunction or deficiency of VWF, which has a critical role in the initiation of platelet adhesion at the site of vascular injury and which can also bind to and stabilize FVIII [85]. VWF is encoded by the *VWF* gene, which is located on the short arm of chromosome 12 (12p13.3), spans 180 kb, and consists of 52 exons, of which exon 50 (40 kb) and exon 28 (1/3 kb) are considered the longest and smallest exons, respectively. The intron 40 contains 14 Alu repeat and 670 bp repeat of TCTA. In addition, the 5′ flanking region has AT repeat resemble to TATA element. The *VWF pseudogene 1* (*VWFP1*), which is located on the long arm of chromosome 22 (22q11–13), spans ~21–29 kb and shows 97% homology with 23–34 exons of the *VWF* gene but encodes no functional transcript. The *VWF* gene is transcribed to an 8.8 kb mRNA which translates to a 2813-amino acid pre-pro-VWF protein. Pre-pro-VWF comprises of a signal peptide (pre) with 22 amino acids, a pro-peptide (pro) with 741 amino acids and a 2050-amino acid mature protein. Therefore, any mutation that leads to qualitative and/or quantitative abnormalities in VWF can be associated with VWD. Different biosynthetic events such as gene expression (transcription, translation), post-translational processing, dimerization/multimerization mechanisms, proteolytic processing, storage, secretion processing, structure, clearance, and function of VWF can be affected by these mutations. According to the ISTH-SSC VWF

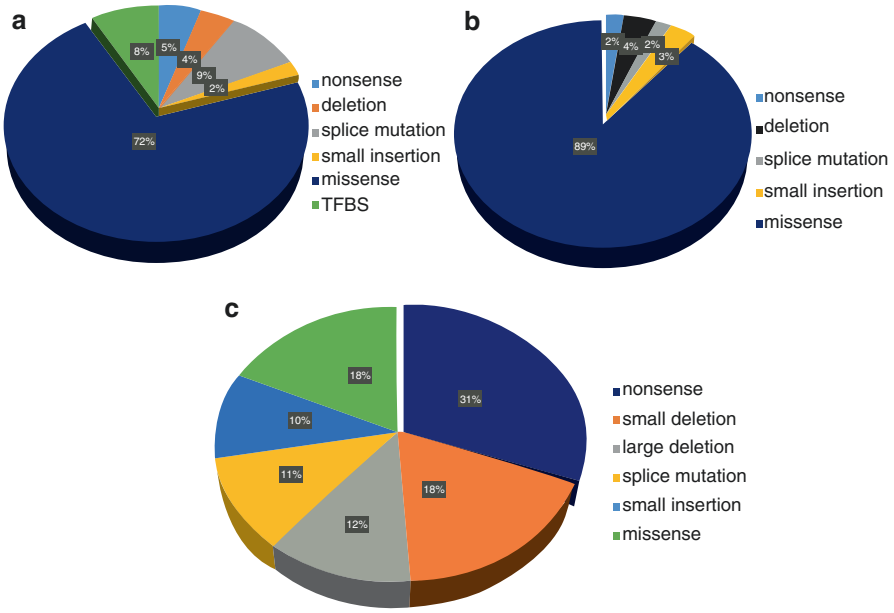

Fig. 3.8 Mutation distribution in patients with von Willebrand disease. (**a**) Patients with type 1 von Willebrand disease, (**b**) patients with type 2 von Willebrand disease, and (**c**) patients with type 3 von Willebrand disease. *TFBS* transcription factor binding sites

Online database (http://ragtimedesign.com/vwf/mutation/) and other studies, over 400 separate mutations have currently been reported in patients with VWD. VWD based on mutation locations and type of the nucleotides and protein abnormalities is divided into three hereditary types, comprising types 1, 2, and 3. Patients with acquired VWD do not have hereditary *VWF* mutations [85]. Different types of mutations associated with congenital VWD include (1) mutations that involve transcription factor binding sites (TFBS) and which lead to absent or reduced RNA transcription, (2) splice site mutations that disrupt the splice donor site (GT) and splice acceptor site (AG) of each intron and leading to exon skipping and production of shortened RNA and protein, (3) nonsense mutations, (4) small deletions, (5) insertions, (6) duplications, (7) large deletions, and (8) missense mutations (Fig. 3.8).

3.7.1 Type 1 von Willebrand Disease

Type 1 VWD is the most common type of VWD, is inherited in an autosomal dominant manner, and accounts for 40–80% of all VWD. Based on the ISTH-SSC VWF online database (http://ragtimedesign.com/vwf/mutation/), more than 130 different mutations have currently been identified in patients with type 1 VWD. About 65%

of these mutations occur within the splice site, promoter, or coding region of the *VWF* gene. In addition, about 70% of all mutations are missense substitutions, which may increase VWF clearance or interrupt the intracellular traffic, storage, and secretion of VWF. Approximately 15–20% of patients with type 1 VWD show more than one *VWF* mutation, and 30% of type 1 VWD patients show no evident mutations (Fig. 3.7). The Vicenza mutation (c.3614G>A, p.Arg1205His) in exon 27 of *VWF* gene is associated with VWD type 1C (clearance). The common laboratory findings of this variant includes decreased levels of VWF:Ag, VWF:RCo, and FVIII to ~0.15 U/mL, ~0.20 U/mL, and <0.30 U/mL of normal, respectively. The Vicenza variant often occurs with another variant of type 1 VWD (c. 2220G>A, p.Met740Leu). Another variant of type 1 VWD occurs following a c.4751A>G mutation in exon 28 of *VWF* gene leading to p.Tyr1584Cys and is associated with mild phenotype [86].

3.7.2 Type 2 von Willebrand Disease

Type 2 VWD is inherited in an autosomal dominant or recessive manner and is characterized by a qualitative deficiency that affects the function of VWF. Type 2 VWD accounts for ~20% of all VWD. Missense, frame shift mutations, duplications, and insertions are considered as the mutations mostly causing type 2 VWD. More than 160 different mutations have currently been identified in the *VWF* gene in type 2 VWD [85, 87, 88].

3.7.2.1 Type 2A von Willebrand Disease

Type 2A is an autosomal dominant or autosomal recessive disorder characterized by a qualitative defect of VWF and associated with an absence of large multimers VWF and reduction of VWF-mediated platelet adhesion. Mutations in the *VWF* gene encoding the A1 and A2 domains of VWF lead to two groups (I and II) of type 2A VWD. Misfolded VWF in group I leads to increased intracellular retention of VWF. The examples of this group are c.4513G>C and c.4820T>A mutations in exon 28 of *VWF* gene that lead to p.Gly1505Arg and p.Val1607Asp amino acid substitution (AAS), respectively. In group II, the biosynthesis, multimerization, and secretion of VWF is normal, but the sensitivity of synthesized VWF to ADAMTS13 cleavage is increased; the c.4789C>T mutation in exon 28 of *VWF* gene that leads to p.Arg1597Trp AAS is an example of group II. Multimerization of VWF can be affected by different mutations that involved D2 and D3 domains of VWF. Type 2A has been classified into the subtypes IIA, IIC, IID, and IIE. Subtype IIA is related to mutations affecting the A2 domain, which leads to enhanced proteolysis of the mutant VWF by ADAMTS13. Subtype IIC is associated with mutations affecting the D1 and D2 domains (pro-VWF), which leads to impaired multimerization in Golgi complex. The mutant VWF in subtype IID shows mutations affecting the CK domain and is associated with impaired dimerization in ER. Finally, subtype IIE is related to the mutations that impair disulfide bond formation of inter subunits in the Golgi complex [85, 89, 90].

3.7.2.2 Type 2B von Willebrand Disease

Type 2B is an autosomal dominant disorder that accounts for ~20% of all type 2 VWD. The GpIb-, heparin-, and collagen-binding sites are located within the A1 domain, and more than 20 different missense gain-of-function mutations that occur within *VWF* gene (exon 28) affecting this domain have been identified that lead to increased VWF affinity to GPIb. About 90% of type 2B VWD occur due to four mutations in exon 28 of *VWF* gene: (1) c.3916C>T mutation that leads to p.Arg1306Trp AAS, (2) c.4022G>A mutation that leads to p.Arg1341Gln AAS, (3) c.3946G>A mutation that leads to p.Val1316Met AAS, and (4) c.3922C>T mutation that leads to p.Arg1308Cys AAS [91, 92].

3.7.2.3 Type 2M von Willebrand Disease

Type 2M VWD, like type 2B, is an autosomal dominant disorder, but unlike type 2B, the disorder is due to loss-of-function mutations (missense, deletion, and frame shift) affecting the A1 domain (exon 28 of *VWF*), which prevent the interaction of VWF with GpIb. Type 2M VWD less frequently can occur following mutations that affect the A3 domain. Based on the ISTH-SSC VWF online database (http://ragtimedesign.com/vwf/mutation/) and other studies, about 30 different type 2M causing mutations have currently been identified. The c.5356C>G mutation in exon 31 of *VWF* gene leads to p.His1786Asp AAS with defective binding site for both types (I and III) of collagen [85, 91, 93].

3.7.2.4 Type 2N von Willebrand Disease

Type 2N VWD is a qualitative VWF abnormality that in contrast to other subtypes of type 2 VWD is inherited in an autosomal recessive manner and caused by defective VWF-FVIII interaction. The FVIII binding site spans the D′ and D3 domains and lies between amino acid Ser764 and Arg1035 of pre-pro-VWF, which is encoded by exons 18–28 of the *VWF* gene. Therefore, mutations within these domains can cause type 2N VWD. The majority of these mutations occur in exons 18–28, but some rare variants have been identified within exons 17 and 21–27. These mutations impair binding of VWF to FVIII, which leads to reduction of VWF-FVIII binding capacity and therefore causes a reduction in the level of plasma FVIII. About 30 different mutations have been reported to date, with the c.2561G>A mutation in exon 20 of *VWF* gene leading to p.Arg854Gln AAS being most frequent. Some of the mutations causing type 2N VWD are 1) c.2363G>A and c.2362T>C mutations in exon 18 of *VWF* gene that lead to p.Cys788Tyr and p.Cys788Arg AASs and 2) c.2635G>A and c.2573G>T mutations in exon 20 of *VWF* gene that lead to p.Asp879Asn and p.Cys858Phe AASs and which not only decrease the VWF-FVIII binding but also lead to reduction of HMWM VWF [89, 92, 94].

3.7.3 Type 3 von Willebrand Disease

Type 3 VWD is the most severe type of VWD and is inherited in an autosomal recessive manner. A wide range of mutations is detected in these patients including

deletions, missense, frame shift, nonsense, and splice site. These mutations lead to absence or severe quantitative defects in VWF. Null mutations comprise ~80% of all mutation causing type 3 VWD. Some nonsense mutations such as p.Arg1659Stop and p.Arg2535Stop occur at CpG sequences. According to the ISTH-SSC VWF online database (http://ragtimedesign.com/vwf/mutation/), about 120 different mutations have been reported to date. Mutation distribution in patients with type 3 VWD is shown in Fig. 3.9.

3.8 Treatment of von Willebrand Disease

Management of patients with VWD includes prevention or treatment of bleeding diathesis by correction of dual hemostatic defects of primary hemostasis (due to lack or decrease of VWF) and secondary hemostasis (due to FVIII deficiency). These corrections can be performed by raising endogenous VWF (usually using desmopressin) or in unresponsive patients, by infusion of exogenous VWF/FVIII (typically as plasma concentrates) [7, 95, 96].

Several therapeutic choices are available for management of patients with VWD. Fresh frozen plasma (FFP) and cryoprecipitate represent older or traditional therapies. Cryoprecipitate was actually the main therapeutic choice for many years in the past, and even today it remains a major therapeutic option in a considerable number of developing countries where commercial VWF/FVIII concentrates may be unavailable or unaffordable; however, the risk of virus transmission and the need to transfuse a high volume of product reflect substantive obstacles for its continued use. More commonly, desmopressin, plasma-derived VWF/FVIII concentrates, purified plasma VWF, and recombinant VWF (rVWF) reflect the main therapeutic choices. In addition, adjuvant therapies can also be used for management of hemorrhagic symptoms in patients with VWD. Such agents include antifibrinolytic therapy with tranexamic acid or epsilon aminocaproic acid, which can improve hemostasis in patients without changing their plasma level of VWF [7, 96].

In summary, the different therapeutic choices that can be used for the management of patients with VWD are:

1. Desmopressin
2. Adjuvant agents
3. Cryoprecipitate
4. FFP
5. Intermediate purity factor VIII/VWF concentrate
6. High-purity factor VIII/VWF concentrate
7. Recombinant VWF
8. Platelet concentrate

Choice of treatment depends on several factors including type of VWD, severity of disorder, severity of bleeding episodes, and type and duration of surgery. In patients with less severe forms of VWD, such as type 1 VWD and some patients

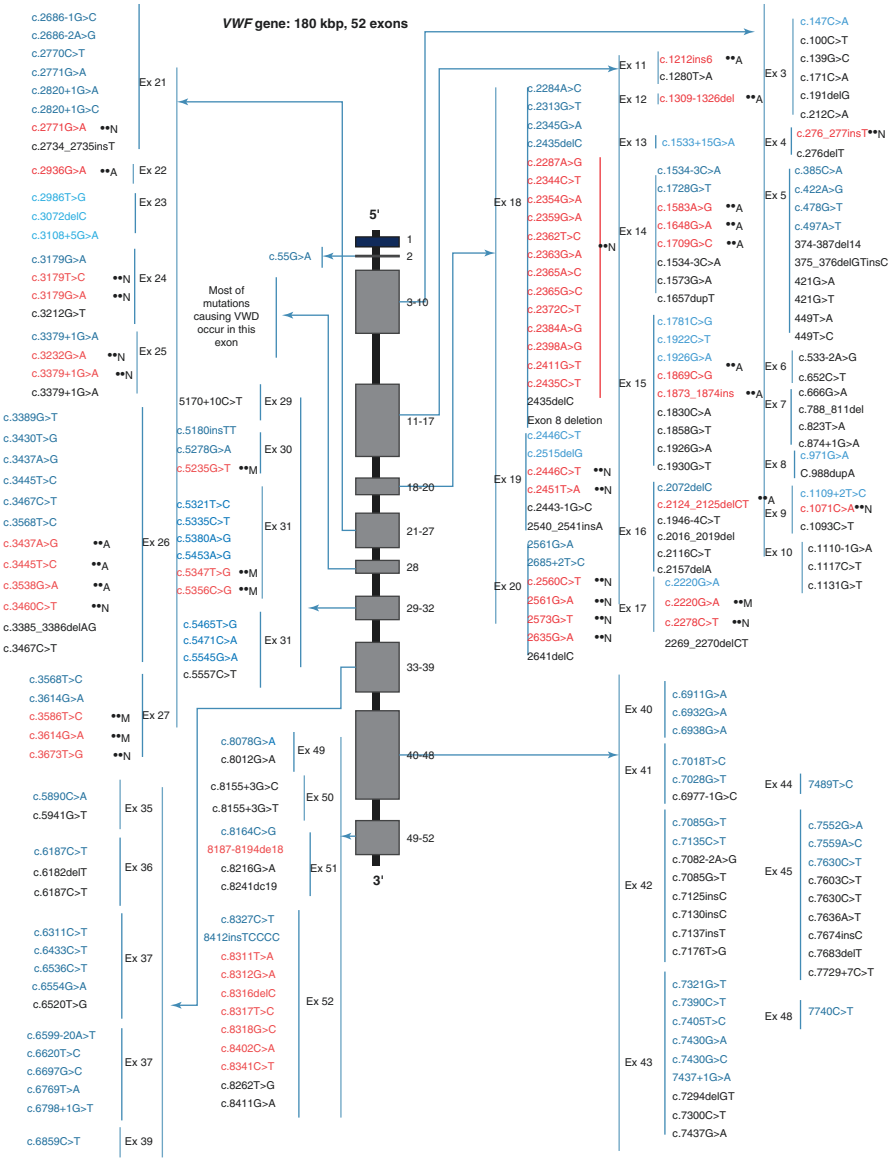

Fig. 3.9 The schematic presentation of *von Willebrand factor (vWF)* gene structure, which comprises of 52 exons with genetic abnormalities that lead to von Willebrand disease (VWD). Based on ISTH-SSC VWF online database (http://ragtimedesign.com/vwf/mutation/), approximately 400 different mutations have currently been reported and include mutations causing type 1 VWD (blue mutations), mutations causing type 2 VWD (red mutations), and mutations causing type 3 VWD (black mutations). *inv* inversion, *del* deletion, *ins* insertion, *dup* duplication

with type 2 VWD, desmopressin is a suitable choice, while in those patients with severe VWD (including type 3VWD and most of the patients with type 2 VWD), replacement therapy comprises the main therapeutic choice. Although on-demand therapy (meaning treatment of hemorrhage as soon as possible after onset of bleeding) is the mainstay of treatment of patients with VWD, long-term prophylaxis for those patients with severe hemorrhages (e.g., type 3 VWD) can significantly improve the quality of life [7].

3.8.1 Desmopressin

Desmopressin (1-deamino-8-D-arginine vasopressin (DDAVP)) is a synthetic analogue of vasopressin that (in a lower concentration) was primarily used for the treatment of diabetes insipidus. This synthetic drug causes an increase of endogenous FVIII and VWF by their release from storage sites into plasma. VWF is released from Weibel-Palade bodies of endothelial cells, while the source of FVIII is not well-known. However the exact cellular mechanism of DDAVP has not been fully elucidated. It seems that DDAVP activates the endothelial vasopressin V2 receptor (VR2), which results in the activation of cAMP-mediated signaling pathway. This leads to exocytosis of VWF and t-PA from WPB and also production of nitric oxide (NO) by NO synthase (NOS) activation in endothelial cells (Fig. 3.10) [7, 97].

Patients with baseline FVIII and functional VWF levels of 10–20 IU/dL or greater are more likely to show sufficient response to DDAVP to attain hemostasis. Therefore, this agent is useful in the management of patients with mild hemophilia or mild VWD, and if required, patients can receive repeated doses of drug in 12–24 h intervals. However, repeated doses of desmopressin cause less effective responses, and eventual depletion of FVIII and VWF stores, with this tachyphylaxis affecting patients with hemophilia more than those with VWD.

Desmopressin can be used for most patients with type 1 VWD, possible type 1 VWD, and some patients with type 2 VWD and can increase FVIII and VWF levels three to five times within 30 min (Table 3.8) [7, 95, 96].

Most patients with type 1 VWD with available ("releasable") VWF in storage sites are responsive to desmopressin, while those with low levels of releasable VWF are less responsive or their response is short-lasting. In the former group, FVIII, VWF, and bleeding time (BT) are usually corrected after administration of desmopressin. Although desmopressin is effective in some patients with type 2 VWD, mostly in types 2A and 2M, it's ineffective in the majority of patients, and in patients with type 2B, it is considered contradicted because it may lead to release of abnormal VWF that may worsen thrombocytopenia and may increase the risk of bleeding. Due to the lack of releasable stores of VWF in type 3 VWD, these patients are typically unresponsive to DDAVP [7, 98].

Desmopressin can be given intravenously, intranasally, or subcutaneously. Intravenous administration of DDAVP is preferred for acute hemorrhages and for surgical procedures. Stimate (CSL Behring, LLC, Kiel, Germany) nasal spray contains 1.5 mg/mL desmopressin acetate contains 150 μg DDAVP per puff [99].

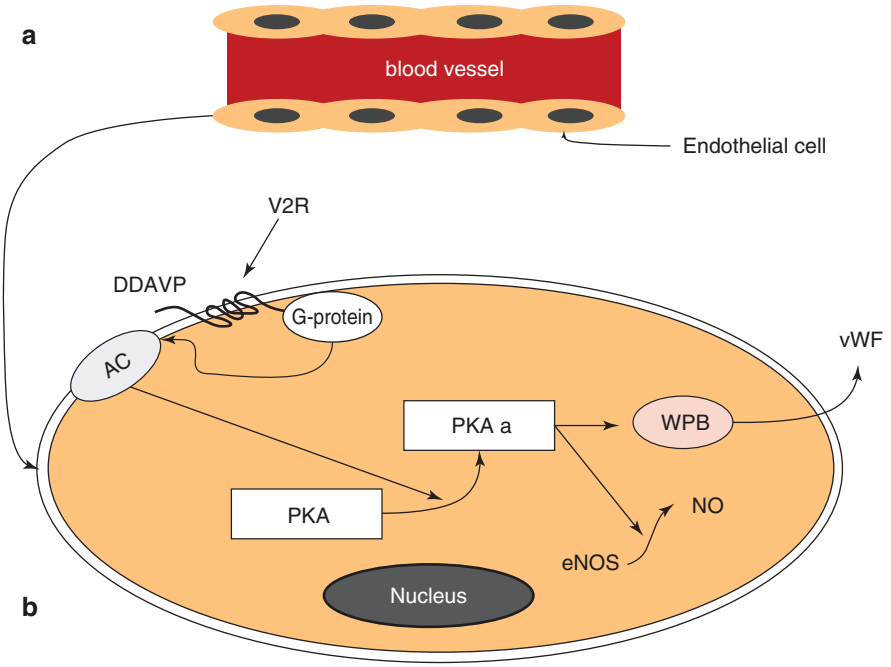

Fig. 3.10 Mechanism of DDAVP-induced VWF secretion. (a) the schematic presentation of a blood vessel. (b) DDAVP binds to V2 receptor on the endothelial cells and result in G-protein activation, adenylyl cyclase (AC) activation, cAMP production, and protein kinase A (PKA) activation. PKA stimulates the secretion of VWF from Weibel-Palade bodies (WPBs). In addition PKA phosphorylate the Ser1177 residue of nitric oxide synthase (NOS) and leads to NO production. *V2R* V2 receptor, *DDAVP* 1-deamino-8-D-arginine vasopressin (DDAVP) or desmopressin, *PKA* protein kinase A, *eNOS* endothelial nitric oxide synthase, *NO* nitric oxide

Table 3.8 Therapeutic response to desmopressin in von Willebrand disease

Disorder	Response
Possible type 1 VWD	Usually effective
Type 1 VWD	Usually effective
Type 2A VWD	Occasionally effective
Type 2B VWD	May be contraindicated
Type 2M VWD	Occasionally effective
Type 2N VWD	Rarely effective
Type 3 VWD	Ineffective

VWD von Willebrand disease

Desmopressin may have some side effects in some patients such as hypotension, cardiovascular complications, flushing, and hyponatremia. Hyponatremia can be prevented by limited fluid intake to 1500 mL for 24 h after desmopressin administration, while other side effects are mostly due to vasodilating effects of the DDAVP and can usually be attenuated by slowing the infusion rate. Since genotype and phenotype of VWD can affect the effectiveness of desmopressin response, a test

dose is recommended before establishment of the magnitude and duration of the drug response. In fact, determination of desmopressin responsiveness in a non-bleeding state is a requirement in patients with moderate type 1, 2A, and 2M ahead of such treatment for bleeds [98].

For this purpose, 0.3 μg/kg in 50 mL is administrated intravenously over 30 min. FVIII:C, VWF:Ag, and functional VWF (e.g., VWF:RCo) should be assessed pre-infusion and at 1, 2, and/or 4 and 24 h post-infusion. Patients with sufficient response have a two to five times increase from baseline levels and have FVIII and VWF levels above 50 U/dL at 1 hour post-infusion. The levels remain above 30 U/dL at 4 h post-infusion but generally return to baseline levels by 24 h [56, 98, 100].

3.8.2 Concentrates

Transfusion therapy with human blood products containing FVIII/VWF is the main therapeutic choice in patients unresponsive to desmopressin, or for long-term therapy. Early studies revealed successfully the management of VWD patients with cryoprecipitate administration every 12–24 h, and subsequently cryoprecipitate was used for many years as the main treatment option in VWD. Although the risk of transmission of blood-borne diseases is the main concern for continued use of cryoprecipitate, this product may still reflect the main therapeutic choice in a considerable number of patients, especially in developing countries, due to low cost and easy production process [8, 56, 98, 100].

Virus-inactivated VWF/FVIII concentrates that were initially developed for the management of hemophilia are considered safer and more suitable therapeutic option for patients unresponsive to desmopressin. VWF/FVIII concentrates can be used for on-demand therapy (to stop bleeding when they occur) and to prevent bleeding in surgery or for long-term secondary prophylaxis [101].

In patients with type 3, most patients with type 2 VWD, and a number of patients with type 1 VWD, this treatment type reflects the current treatment of choice (Table 3.9) [8, 56].

The three main requirements for VWF/FVIII concentrates are as follows: (1) they should contain enough biologically active VWF to correct the platelet adhesion defect otherwise due to the low level of plasma VWF and enable stabilization of endogenous FVIII; (2) their efficacy and pharmacokinetics should be assessed in clinical trials prior to clinical use; and (3) they should be efficacy-virus inactivated [102].

A number of concentrates have been used for the treatment of VWD. These concentrates were primarily produced as “FVIII concentrates” for the treatment of hemophilia, but since they also contained VWF, they also were then also used for the treatment of VWD. The amount of HMWM of these products differs, and those without HMWM are less effective in the management of mucosal hemorrhages. Today, most “FVIII concentrates” actually lack VWF, and only those with high amounts of functional VWF are useful for the treatment of VWD. Although many concentrates are now available for this purpose, there is considerable difference

Table 3.9 Therapeutic options for management of patients with von Willebrand disease

Disorder	Therapeutic choice	Additional treatment
Type 1 VWD	Desmopressin: (1) Intravenously: 0.3 μg/kg or (2) Subcutaneously: 0.3 μg/kg or (3) Intranasally: 300 μg (150 μg per nostril)[a]	Tranexamic acid: 3 or 4 times daily
Type 2 VWD	(1) Desmopressin: (1) Intravenously: 0.3 μg/kg[a] or (2) Subcutaneously: 0.3 μg/kg or (3) Intranasally: 300 μg (150 μg per nostril)[b] or (2) VWF-FVIII concentrate or (3) VWF concentrate	Tranexamic acid: 3 or 4 times daily
Type 3 VWD	(1) VWF-FVIII concentrate or (2) VWF concentrate	Tranexamic acid: 3 or 4 times daily

VWD von Willebrand disease, *VWF* von Willebrand factor, *FVIII* Factor VIII
[a]For indication of desmopressin use, refer to Table 3.1
[b]For those with body weight <50 kg, only one dose of 150 μg is sufficient

among concentrates in the amount and activity of VWF and FVIII. Specific activity is crucial to determine the degree of relative purity of FVIII/VWF concentrates. VWF:RCo/Ag ratio can be used as a surrogate marker for determination of VWF activity while VWF:RCo/FVIII:C ratio gives data on the amount of FVIII:C protein associated with VWF in the VWF/FVIII concentrate. It is important to avoid excessive concentrations of FVIII:C with injection of repeated doses in patients with VWD [56, 100].

In patients with VWD, a VWF:RCo/VWF:Ag ratio <0.7 is considered as representative of dysfunctional VWF (and thus a type 2 VWD). In a study of six concentrates, only three had a ratio of >0.7, with Immunate (Baxter Bioscience, Austria) having the lowest ratio. Immunate is therefore not approved for treatment of VWD in some countries, including Sweden. HMWM VWF was largely preserved in Haemate-P/Humate-P (Aventis Behring, Marburg, Germany), Innobrand and Facteur Willebrand (LFB, Lille, France) concentrates, with well-preserved multimeric composition being preferred for management of mucosal bleeding (Table 3.10) [56, 98, 100, 103].

For proper management of VWD, the correct dose of concentrate should be used. Several factors should be considered in defining a correct dose, including type and severity of bleeding episodes, type of concentrate, baseline FVIII and VWF levels and VWD type, as well as bleeding history and therapeutic response. The type of hemorrhage is an important issue and this should receive particular attention. Some bleeding, such as mucosal hemorrhage (especially GI bleeding), is difficult to stop, while management of post-traumatic hemorrhage and post-surgical bleeding from soft tissues can be managed more easily. For estimation of VWF

Table 3.10 Main characteristics of VWF-FVIII concentrates and recombinant VWF

Concentrate	Company	Country	Purity	Virus inactivation	Contents	VWF:RCo/ FVIII:C	VWF:RCo/ VWF:Ag
Immunate	Baxter Bioscience, Vienna	Austria	IEX	S/D + vapor heat	FVIII:C 1000 IU $10\ mL^{-1}$	0.67	0.15
Alphanate	Grifols, Los Angeles	USA	HLCT	S/D + dry heat		0.9	0.47
Fanhdi	Grifols, Cambridgeshire	UK	Precipitation, HLCT	S/D + dry heat		1	0.47
Wilate	Octapharma Hoboken, NJ	USA	Affinity CT, size exclusion	S/D + dry heat		0.9	0.9
Biostate	CSL Behring, Melbourne	Australia	Precipitation, HLCT	S/D + dry heat		2	0.8
Facteur Willebrand	LFB, Lille	France		Solvent/detergent	VWF:RCo 1000 IU $20\ mL^{-1}$		0.72
Wilfactin	LFB, Les Ulis	France	Ion exchange, affinity CTs	S/D + nanofiltration + dry heat		>10	0.95
Innobrand	LFB	France	Ion exchange CT	S/D	VWF:RCo 1100 IU $20\ mL^{-1}$		0.89
Haemate-P[a] Humate-P[b]	Aventis Behring	Germany	Polyelectrolyte precipitation	Pasteurization	FVIII:C 250 IU $10\ mL^{-1}$	2	0.91
Factor 8Y	Bio Products Laboratory, Hertfordshire	UK	Heparin/glycine precipitation	Dry heat	FVIII:C 315 IU $10\ mL^{-1}$	0.81	0.29
Koate-DVI	Bayer Corp	USA	Precipitation, size exclusion	S/D + dry heat	FVIII:C 1050 IU $10\ mL^{-1}$		0.48
Vonicog alfa (Vonvendi)[c]	Baxalta, Bannockburn	USA	–	S/D		No FVIII:C	

[a]In Europe
[b]In the USA, *IEX* ion-exchange chromatography, *HLCT* heparin ligand chromatography
[c]Recombinant VWF, *S/D* Solvent/detergent

loading dose, it should be considered that 1 IU/kg of FVIII usually increases plasma FVIII:C 2 U/dL, whereas 1 IU/kg VWF:RCo usually increases the VWF:RCo 1.5 U/dL; therefore the following formulas can be used for the determination of proper dose [56, 95].

$$\text{Factor VIII : C : 1 U / kg} = 2\%\text{rise in plasma dose} = \%\text{desired FVIII rise} \times \text{wt}(\text{kg}) \times 0.5$$

$$\text{VWF : RCo : 1 U / kg} = 1.5\%\text{rise in plasma dose} = \%\text{desired VWF : RCo rise} \times \text{wt}(\text{kg}) \times 0.67$$

Different VWF/FVIII concentrate doses are recommended for different situations in patients with VWD (Table 3.11).

A high plasma level of FVIII is a risk factor for venous thromboembolism (VTE), and patients with VWD who received repeated dose of concentrates containing both FVIII and VWF may experience VTE. When a patient with VWD receives repeated dose of these concentrates, plasma FVIII:C should be monitored. With use of pure VWF, this risk can be avoided, but plasma level of FVIII increases very slowly, and therefore in acute situations, these concentrates should be used in combination with FVIII concentrate [56, 95].

Table 3.11 Recommended dose of factor VIII/von Willebrand factor concentrate for management of patients with von Willebrand disease (VWD) unresponsive to desmopressin

	Dose (IU/kg)		Number of infusion		Objective	
Bleeding type	Italian	Netherlands	Italian	Netherlands	Italian	Netherlands
Major surgery	50	50	Once a day (days 1–3), then every other day	Twice daily 25 IU FVIII/kg, based on FVIII:C levels	Maintain FVIII:C >50 U/dL for at least 7–14 days	FVIII:C 50 IU/dL for 7–10 days
Minor surgery	30	30–50	Once a day (days 1–3), then every other day	Twice daily 15–25 IU FVIII/kg, based on FVIII:C levels	Maintain FVIII:C >30 U/dL for at least 5–7 days	FVIII:C >50 IU/dL for 3 days and 30 IU/dL for additional 4–7 days
Dental extraction	20–40	20–40	Single	Single dose + tranexamic acid	Maintain FVIII:C >30 U/dL for up to 6 h	FVIII:C and VWF:RCo >50 IU/dL
Spontaneous or post-traumatic bleeding	20–40	20–40	Single	Usually single	Maintain FVIII >30 U/dL	–

3.8.3 Recombinant von Willebrand Factor

RVWF (Vonicog alfa (Vonvend)) is a new product for the management of VWD that is synthesized within genetically engineered Chinese hamster ovary (CHO) cell lines. This engineered cell line co-expresses both *VWF* and *FVIII* genes. The produced VWF is purified by immune-affinity chromatography that yields a rVWF with 99% purity. Since the product is not exposed to ADAMTS13, it retains all sizes of VWF multimers including HMWM and ultra-large multimers. In USA, rVWF is licensed for on-demand treatment of patients with VWD. This product should be given under supervision, and the proper dose of product should be used (Table 3.12) [104].

3.8.4 Prophylaxis

Since the bleeding tendency is less severe in VWD, prophylaxis treatment is not as common as in hemophilia A and B. Prophylaxis should be considered for patients with type 3 VWD and recurrent hemarthrosis. Patients with recurrent GI bleeding and those with frequent epistaxis can also benefit from prophylaxis treatment. Therefore, in such patients with frequent bleeding, long-term secondary prophylaxis should be considered. In fact, hemarthrosis, GI bleeding, menorrhagia, and recurrent epistaxis are the most common indications for long-term secondary prophylaxis. Although overall use of prophylaxis in VWD is low and is around 1.5%, this percentage is higher in type 3 VWD, and about one-fifth of patients undergo prophylaxis. Regular prophylaxis decreases the number of bleeds, decreases the severity of hemorrhages, prevents arthropathy, and improves the quality of life in

Table 3.12 Recommended dose of intravenous recombinant von Willebrand factor (Vonvendi) for the management of patients with von Willebrand disease in the USA

Bleeding type	Initial dose	Subsequent doses
Minor bleeding	40–50 IU/kg (adjust dose based on the extent and location of bleeding; should achieve VWF levels >60%, based on VWF:RCo >0.6 IU/mL)	40–50 IU/kg every 8–24 h (as clinically required)
Major bleeding	50–80 IU/kg (adjust dose based on the extent and location of bleeding; should achieve VWF levels >60%, based on VWF:RCo >0.6 IU/mL)	40–60 IU/kg every 8–24 h for ~2–3 days (as clinically required; maintain trough VWF:RCo levels >50% as long as deemed necessary)
When a rapid increase in FVIII is needed and baseline FVIII values are <40% of normal activity or unknown	Administer the first dose of rVWF with an approved rFVIII (one that does not contain VWF) within 10 min of completing rVWF infusion at a ratio of 1.3:1 (i.e., 30% more rVWF than rFVIII; calculate by dividing rVWF dose by 1.3)	

patients with VWD. It should be noted that all patients with VWD are candidates for regular long-term prophylaxis; however, most patients with type 3 VWD, and some other entities of VWD, including type 2A and type 2B with GI bleeding, are candidates of long-term prophylaxis [102, 105].

Different therapeutic doses are used for long-term prophylaxis, and 50 IU of VWF:RCo/kg two or three times a week reflects a commonly used dose [56, 95].

3.8.5 Surgery

Surgical procedures represent an important hemostatic challenge in patients with VWD, and the majority of patients with major surgery should be managed by replacement therapy, as therapy with DDAVP is precarious owing to the frequent onset of poor responsiveness following repeated doses. For successful management of these patients, a strict cooperation between the patient's surgeon and hematologist is mandatory [56, 106].

Strategies for preoperative management of patients with VWD depends on several important factors including the type of VWD, kind of surgery, baseline levels of VWF and FVIII, and therapeutic response to desmopressin. The type of hemorrhagic problem, including mucosal or soft-tissue bleeding, also can affect treatment strategies. A strict follow-up is recommended for the first 10–15 days post-surgery, especially in those patients who undergo major surgery, since delayed hemorrhage may occur until complete wound healing is achieved. Although FVIII is the main determinant of post-surgical hemorrhage in patients with VWD, it is not sufficient to only substitute FVIII in these patients, because the half-life of FVIII is very low in the absence of VWF. Since the ability of infused VWF in raising and maintaining plasma FVIII is not dependent on VWF multimeric composition of concentrates, those concentrates with loss of HMWM may be effective in some surgeries. However, when surgery involves mucous membranes, then concentrates that contain HMWM VWF should be used [56].

A characteristic of VWF concentrates that are almost devoid of FVIII:C is delay in the plasma increase in FVIII:C. This is an important issue especially in patients with type 3 VWD who undergo emergency surgical procedures representing acute life-threatening bleeds. In patients with scheduled surgical procedure, FVIII should be administrated 12 h before surgery. In elective surgery, a pure VWF should be given 12–24 h prior to the surgical procedure in order to increase FVIII levels sufficiently [106].

3.8.6 Pregnancy

VWF and FVIII levels increase two- to threefold during the second and third trimesters in patients with types 1 and 2 VWD and fall to baseline by 7–21 days postpartum; however, this increase is not observed in type 3 VWD, and thus, management of pregnant women with VWF/FVIII is required. A rapid decrease in plasma VWF

and FVIII can occur post-partum, and subsequently PPH, including delayed PPH with a risk of 30%, can occur [107].

In type 2B VWD, this increase in VWF reflects an "abnormal VWF" which can worsen thrombocytopenia. Generally, patients VWD should be monitored for functional VWF (e.g., VWF:RCo) and FVIII:C once during the third trimester and within 10 days of expected date of delivery. In pregnant women with VWD, the risk of hemorrhage is minimum when the VWF:RCo and FVIII levels are >30 U/dL. In women with type 1 VWD with FVIII:C level <30 U/dL, it may be necessary to administer desmopressin at parturition and for 3–4 days thereafter. To prevent late hemorrhage, VWF:RCo and FVIII:C levels should be monitored for at least 2 weeks post-partum [56, 108].

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