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von Willebrand Disease

Paula D. James, MD and

Associate Professor and Hematologist, Room 2025, Etherington Hall, Queen's University, Kingston, Ontario, Canada K7L 3N6, Tel: 613-533-2946, FAX: 613-533-6855

Anne C. Goodeve, PhD

Reader and Head, Haemostasis Research Group, Department of Cardiovascular, Science, Sheffield University Faculty of Medicine, Dentistry and Health, and Principal Clinical Scientist, Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK

Paula D. James: jamesp@queensu.ca; Anne C. Goodeve: a.goodeve@shef.ac.uk

Abstract

von Willebrand disease (VWD) is a common inherited bleeding disorder characterized by excessive mucocutaneous bleeding. Characteristic bleeding symptoms include epistaxis, easy bruising, oral cavity bleeding, menorrhagia, bleeding after dental extraction, surgery and/or childbirth and in severe cases, bleeding into joints and soft tissues. There are three subtypes: types 1 and 3 represent quantitative variants and type 2 is a group of four qualitative variants: 1) type 2A - characterized by defective von Willebrand factor (VWF) -dependent platelet adhesion because of decreased high molecular weight (HMW) VWF multimers, 2) type 2B - caused by pathologically increased VWF-platelet interactions, 3) type 2M - caused by decreased VWF-platelet interactions not based on the loss of HMW multimers and 4) type 2N - characterized by abnormal binding of VWF to FVIII. The diagnosis of VWD requires specialized assays of von Willebrand factor (VWF) and/or molecular genetic testing of *VWF*. Severe bleeding episodes can be prevented or controlled with intravenous infusions of virally-inactivated plasma-derived clotting factor concentrates containing both VWF and FVIII. Depending on the VWD type, mild bleeding episodes usually respond to intravenous or subcutaneous treatment with desmopressin, a vasopressin analog. Other treatments that can reduce symptoms include fibrinolytic inhibitors and hormones for menorrhagia.

Keywords

genetic analysis; mutation; von Willebrand factor; von Willebrand disease

INTRODUCTION

von Willebrand disease (VWD) was first described in 1926 by a Finnish physician named Dr. Erik von Willebrand.¹ In the original publication he described a severe mucocutaneous bleeding problem in a family living on the Åland archipelago in the Baltic Sea. The index case in this family, a young woman named Hjördis, bled to death during her fourth menstrual period. At least four other family members died from severe bleeding and although the condition was originally referred to as "pseudohemophilia" Dr. von Willebrand

Correspondence to: Paula D. James, jamesp@queensu.ca.

CONFLICT OF INTEREST STATEMENT

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noted that in contrast to hemophilia, both genders were affected. He also noted that affected individuals exhibited prolonged bleeding times despite normal platelet counts.

In the mid 1950s it was recognized that the condition was usually accompanied by a reduced level of coagulation factor VIII (FVIII) activity and that the bleeding phenotype could be corrected by the infusion of normal plasma. In the early 1970s the critical immunologic distinction between FVIII and von Willebrand factor (VWF) was made and since that time significant progress has been made in our understanding of the disorder. Cloning and characterization of the *VWF* gene in the 1980s^{2–5} has facilitated investigation into the genetic basis of VWD. Although the molecular basis of VWD remains the subject of ongoing research, clinical genetic testing has become available for the disease. In this review, clinical and laboratory aspects of VWD will be reviewed, along with indications for genetic testing.

DIAGNOSIS

Clinical diagnosis

von Willebrand disease (VWD) is caused by deficient or defective plasma von Willebrand factor (VWF), a large multimeric glycoprotein that plays a pivotal role in hemostasis by mediating platelet hemostatic function and stabilizing blood coagulation factor VIII (FVIII). It affects 0.1⁶ to 1% of the population;^{7–8} affected individuals registered at a tertiary care centre number up to 1 in 10,000.⁹ The current International Society on Thrombosis and Haemostasis Scientific and Standardisation Committee on VWF (ISTH SSC on VWF) VWD classification comprises three subtypes.¹⁰ Type 1 VWD is a partial quantitative deficiency of essentially normal VWF. Type 2 VWD is characterized by a qualitative deficiency and defective VWF (further subdivided into types 2A, 2B, 2M, 2N). Type 3 VWD is a virtually complete quantitative deficiency of VWF.

VWD should be suspected in persons with excessive mucocutaneous bleeding, such as bruising without recognized trauma, prolonged, recurrent nose bleeds and oral cavity bleeding, including bleeding from the gums after brushing or flossing teeth or prolonged bleeding following dental cleaning or extractions. Prolonged or excessive bleeding following surgery or trauma is often reported. Affected females also frequently experience menorrhagia (usually occurring since menarche)¹¹ and prolonged or excessive bleeding following childbirth.¹² The utility of standard clinical assessment tools to score occurrence of symptoms and their severity as part of VWD diagnosis is becoming recognized.^{13–15} These can determine if there is more bleeding than in the normal population, justifying diagnosis of a bleeding disorder, and can help quantify extent of symptoms, indicating situations requiring clinical intervention.

Laboratory testing

Screening tests for VWD include a complete blood count (CBC), activated partial thromboplastin time (aPTT) and prothrombin time (PT).^{16–17} The CBC might be normal, but could also show a microcytic anemia (if the individual is iron deficient) or a low platelet count (thrombocytopenia), specifically in type 2B VWD.¹⁸ The aPTT is often normal, but may be prolonged when the factor VIII (FVIII) level is reduced to below 30–40 IU/dL (normal range is approximately 50–150 IU/dL) as can be seen in severe type 1 VWD, type 2N VWD, or type 3 VWD. The prothrombin time (PT) is normal in VWD. Although some laboratories may also include a skin bleeding time and platelet function analysis (PFA closure time) in their evaluation of an individual with suspected VWD, these tests lack sensitivity in persons with mild bleeding.^{19–21}

Hemostasis factor assays—The following specific hemostasis factor assays (Table 1) should be performed even if the screening tests are normal.²²

VWF:Ag: Quantity of VWF protein (antigen) in the plasma, measured antigenically using enzyme-linked immunosorbant assay (ELISA) or latex immunoassay (LIA). The normal range, which should be determined independently by each laboratory is approximately 50–200 IU/dL.

VWF:RCo: The ristocetin cofactor activity assay determines the ability of VWF to agglutinate platelets, initiated by the antibiotic ristocetin. The normal range is approximately 50–200 IU/dL.

Factor VIII:C level: Functional FVIII assay which determines the activity of FVIII in the coagulation cascade. The normal range is approximately 50–150 IU/dL. If abnormalities in the three tests above are identified, specialized coagulation laboratories may also perform specific assays to determine the subtype of VWD.

VWF multimer analysis: SDS-agarose electrophoresis is used to determine the complement of VWF oligomers in the plasma.^{16,22} Normal plasma contains dimers up to >40 mers of VWF. Multimers are classified as high, intermediate, or low molecular weight by counting bands 1–5 as low molecular weight (LMW), 6–10 as intermediate molecular weight (IMW) and >10 as high molecular weight (HMW) forms.²³ HMW multimers are decreased or missing in types 2A and 2B VWD and intermediate MW may also be lost in type 2A. Abnormalities in satellite (“triplet”) band patterns can give clues about pathogenesis and help to sub-classify type 2A VWD using a tertiary classification¹⁰ based on multimeric profile and terminology from an earlier classification,²⁴ in which current type 2A was represented by several subtypes including IIA, IIC, IID and IIE e.g. type 2A(IIE).²²

Ristocetin induced platelet aggregation (RIPA): Ability of VWF to aggregate platelets at 2–3 concentrations of ristocetin. Aggregation at a low concentration (~0.5 mg/ml) is abnormal and may indicate type 2B or its phenocopy, platelet-type pseudo VWD (PT-VWD) resulting from mutations in *GPIBA*, in which enhanced VWF-platelet binding is present.^{17,25}

Binding of FVIII by VWF (VWF:FVIII): This test which determines the ability of VWF to bind FVIII^{26–27} is useful in the identification and differential diagnosis of type 2N VWD.²⁸ There are no standard units for the output of this test.

Collagen binding assay (VWF:CB): This test determines the ability of VWF to bind to collagen (a sub-endothelial matrix component). It is used in some centers to help identify functional VWF discordance (i.e., to help distinguish types 1 and 2 VWD). Collagen binding is dependent on the presence of HMW multimers; reduced collagen binding reflects the loss of HMW multimer forms²⁹ or can reflect a specific collagen-binding deficiency. The normal range is approx 50–200 IU/dL.

MOLECULAR GENETIC TESTING

Molecular genetic pathogenesis

The VWF gene (*VWF*) spans 178 kb of genomic DNA. Its 52 exons encode an 8.8 kb mRNA and a 2813 amino acid protein.^{30–32}

Currently, more than 160 normal variants are known in the exons and closely flanking intronic sequences; amino acid substitutions are reported at 30 residues (Table 2). As non-

Caucasian ethnic groups are being examined, many additional normal variants are being identified. In one study, an average of 17 heterozygous sequence variants were identified in each individual screened for *VWF* mutations.³³ This high degree of polymorphism in the *VWF* gene, along with the large size of the gene and the presence of a partial pseudogene, *VWFP*, replicating exons 23–34 can make full gene sequencing and data interpretation challenging.³¹ For type 1 VWD, about 10–15% of index cases examined in three large multicentre studies had more than one candidate mutation identified; in about half of the cases, the variants were in cis (on the same allele), while the remainder were in trans.^{34–36} Such observations underscore the difficulty of identifying pathologic versus normal allelic variants. Several single nucleotide variants in the *VWF* promoter region have been identified,^{34–36} but their pathogenicity has not yet been confirmed.

Normal allelic variants useful for linkage analysis are summarized in Table 3 and include short tandem repeats in the promoter and intron 40 (rs41402545 and rs36115023),³⁷ plus a large number of common single nucleotide variants.

Normal gene product

The 2813 amino acid (aa) VWF protein is comprised of a 22 aa signal peptide, a 741 aa propeptide and a 2050 aa mature protein.³² Its domain structure from the amino terminus is S-D1-D2-D-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK (Figure 1). During synthesis, tail-to-tail disulphide-linked dimers are formed through the CK domains, followed by head-to-head VWF oligomers. Two disulphide isomerase-like sites in the pro-peptide (CGLC sequences at residues 159–162 and 521–524) facilitate this process.³⁸ VWF has two sites of synthesis; endothelial cells and megakaryocytes, the precursors of platelets. It is stored in endothelial Weible-Palade bodies and platelet alpha granules, being released from alpha granules upon platelet activation. When secreted, VWF consists of multimers up to 40 subunits (dimers) in length. The pro-peptide is cleaved by furin between amino acids 763–764 during multimer production and the pro-peptide (VWFpp) is secreted into the plasma along with VWF. The ratio between VWFpp and mature VWF (VWFpp/VWF:Ag) can be used to estimate relative half life of mature VWF with elevated ratios indicating enhanced clearance.^{39–40}

To render high molecular weight (HMW) VWF less thrombogenic, it is cleaved by ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif)⁴¹ between amino acids p. Tyr1605-Met1606 following secretion.⁴² This multimer proteolysis produces the characteristic “triplet” pattern of satellite bands flanking each main multimer band observed on multimer analysis gels. Abnormalities resulting from enhanced or reduced proteolysis can give clues as to VWD subtype.²²

Abnormal gene product

Abnormalities in VWF are dependent on the type of mutation; the molecular consequences of both the protein and nucleotide abnormality result in different VWD types.

Type 1— *VWF* mutations have been identified in up to approximately 65% of patients diagnosed with type 1 VWD in multicentre studies.^{34–36} Missense mutations predominate but may affect VWF through different mechanisms. A common heterozygous in-frame large deletion of exons 4–5 has recently been reported in a cohort of UK type 1 VWD patients⁴³ and this along with similar large deletions may also contribute to the mutation spectrum in this disease type. As molecular genetic testing has only been undertaken in type 1 VWD relatively recently, pathogenic mechanisms have not yet been ascertained in many cases.

Missense mutations mostly in the D3 domain^{39,44} reduce the residence time of VWF in plasma by up to fifteen-fold. p.Arg1205His, the so-called “Vicenza” variant^{45–47} is the best

characterised and most common of these mutations.^{34–36} Such mutations have been referred to as type 1 clearance (1C),⁴⁰ although this is not a VWD category that has been recognized in the ISTH SSC on VWF classification.¹⁰ Intracellular retention appears to be a common mechanism for type 1 VWD pathogenicity and has recently been demonstrated for missense mutations in a number of VWF domains.⁴⁸ Haploinsufficiency resulting from a heterozygous null allele results in reduced VWF expression in a small proportion of cases.^{34–36}

Type 2A—This subtype is the most common among type 2 VWD variants and encompasses missense mutations in several locations which act through a variety of mechanisms to result in a loss of HMW and sometimes IMW multimers: 1) impaired dimer assembly (CK domain); 2) impaired multimer assembly (D3 and D2 domains);⁴⁹ 3) enhanced susceptibility to ADAMTS13 (A2 and A1 domains);⁵⁰ 4) intracellular retention (D3, A1 and A2 domains).⁴⁹ More than one mechanism can contribute to the pathogenesis of a number of mutations. All result in VWF with fewer GpIb binding sites and less effective platelet clot formation. The mutations are classified as a group as clinically affected individuals are treated similarly.¹⁰

Type 2B—Missense mutations enhance the ability of VWF to bind platelet glycoprotein GpIb, such that binding occurs spontaneously without the requirement for VWF to bind to collagen following subendothelial damage. The resulting platelet-VWF complex is removed from circulation and can result in thrombocytopenia. The largest multimers bind platelets preferentially, so are lost to a greater extent. VWF binding to platelets can also enhance its' susceptibility to ADAMTS13 cleavage, which also contributes to the loss of HMW multimers. Whilst all of these features may be present to varying degrees in the majority of patients, not all cases demonstrate the “classic” 2B VWD presentation. For example, mutations affecting p.Pro1266Leu may only demonstrate enhanced GpIb binding (RIPA) but no thrombocytopenia or HMW multimer loss,¹⁸ and in some cases all parameters other than RIPA may be normal.^{51–52}

Type 2M—VWF is poor at binding GpIb, often due to mutations in the A1 domain that alter protein conformation and reduce binding affinity (Figure 1), but without the loss of HMW multimers seen in type 2A.⁵³ Mutations in the A3 domain that affect binding to collagen in the subendothelium^{54–55} could also be classified as 2M VWD,¹⁰ but unlike most 2M VWD patients, individuals with these mutations do respond well to desmopressin and may thus warrant a different classification.

Type 2N—Affinity of VWF for FVIII is reduced due to alteration of key amino acids in the FVIII binding site or to conformational changes having an indirect effect on VWF-FVIII binding. The majority of patients have a normal multimeric profile, but in some cases supranormal multimers are present, whereas others may demonstrate a loss of HMW multimers and can have a 2A(IIE) appearance.⁵⁶

Type 3—Both alleles are affected by mutations (null and missense) that result in lack of VWF secretion from the cell. The majority of type 3 VWD patients have two null alleles and produce no significant quantity of VWF. About 20% of alleles carry missense mutations predominantly located in the D1–D2 (exons 3–11) domains and D4–CK (exons 37–52) domains (Figure 1). These may impair dimerisation or multimerisation of VWF, resulting in intracellular retention and lack of VWF secretion into plasma.⁵⁷ The remaining 80% are null alleles located throughout *VWF*. Null alleles can result from many different types of mutations. These mutations do not result in a functional protein product as the mutation results in either the complete absence/instability of mRNA or protein or the expression of a

non-functional gene product (e.g., a protein that cannot be secreted). Large deletions predominantly resulting in frameshift mutations affecting one or more exon also contribute to type 3 VWD mutation spectrum.⁵⁸

Currently, *VWF* is the only gene known to be associated with VWD. However, the ISTH SSC on *VWF* classification scheme¹⁰ does not restrict VWD to being caused by mutations in the *VWF* gene. Evaluation of the entire protein coding and flanking intronic regions of the *VWF* gene fails to identify a *VWF* mutation in some cases of “apparent” VWD. Failure to identify a causative mutation in the *VWF* gene does not exclude ‘VWD’. Platelet-type pseudo VWD (PT-VWD) results from mutations in the *GPIBA* gene, but presents phenotypically like type 2B VWD.

CLINICAL TESTING

The domain structure and exons encoding each VWF domain are shown in Figure 1. Sequence analysis of the entire coding region and flanking exon/intron junctions of *VWF* is available, although it is technically challenging due to the gene structure.

Heterozygous large deletion mutations are not detected by PCR and DNA sequencing, but require either a deletion specific gap-PCR⁴³ or a dosage analysis technique that can detect any exon deletion/duplication such as multiplex ligation-dependent probe amplification (MLPA).^{59–60} Linkage analysis can be useful in type 3 VWD to facilitate prenatal diagnosis if time is insufficient for full mutation analysis, or if two mutations cannot be identified in an affected individual.⁶¹

In those individuals with types 2A and 2B VWD in whom specific VWD hemostasis factor assays can provide a clear diagnosis, molecular genetic testing may not be warranted.⁶² Molecular genetic testing for VWD may be indicated in the following circumstances.

1) To establish the VWD subtype in those individuals in whom specific VWD hemostasis factor assays suggest VWD, but in whom genetic analysis may provide a more definitive diagnosis; 2) to distinguish between type 2N VWD, mild hemophilia A (males), or (symptomatic) carriers of hemophilia A (females) with reduced FVIII:C levels, where phenotypic testing remains inconclusive (e.g., VWF:FVIII:B assay is unavailable or unhelpful); 3) families with type 3 VWD requesting carrier analysis and prenatal diagnosis. A recent guideline on VWD genetic testing has been published by the UK Haemophilia Centre Doctors Organisation.⁶¹

Type 1 VWD

Point mutations have been identified in ~65% of individuals with type 1 VWD and occur throughout the *VWF* gene.^{34–36} Fully penetrant, dominantly inherited missense mutations are more often identified when VWF:Ag and VWF:RCO levels are <25 IU/dL. In contrast, incompletely penetrant dominantly inherited missense mutations, such as p.Tyr1584Cys⁶³ and p.Arg924Gln^{64–65} are identified in ~50% of individuals whose VWF:Ag and VWF:RCO levels are above 25 IU/dL.

The extent to which incompletely penetrant *VWF* mutations contribute to bleeding phenotype in individuals with VWF levels of around 50 IU/dL is not clear and genetic analysis in such cases may not be easy to interpret.⁶¹

Type 2 VWD

Many mutations seen in types 2A and 2M, and all missense mutations in type 2B are located in exon 28; thus, this exon should be examined first when any of these three VWD subtypes is suspected.

Type 2A VWD—This subtype includes missense mutations acting through a variety of mechanisms giving rise to similar phenotypes. The majority of type 2A VWD is inherited in an autosomal dominant fashion. The major mutation location is exon 28, where mutations predominantly affect the A2 domain. Missense mutations have also been reported very recently in the D3 and A1 domains (exons 22 and 25–28), resulting in the subtype 2A(IIE). This variant, which is reported to make up about 30% of all 2A cases includes many missense mutations affecting cysteine residue.⁴⁹ Less common missense mutations responsible for dimerisation (CK domain, exon 52, 2A(IID))⁵⁸ and multimerisation defects (D2 domain, exons 11–17, 2A(IIC))^{58,66} can be sought subsequently. In the rare, recessive form of the disorder (2A(IIC)), affected individuals are either homozygous for the same missense mutation (often seen in consanguineous families) or compound heterozygous for a missense mutation and a null allele.⁶⁶ Presence of a second null mutation may be inferred, unless the full gene is analysed.⁵⁸

Type 2B VWD—Missense mutations are located in exon 28 in or close to the A1 domain.¹⁸

Type 2M VWD—A small number of missense mutations are reported in exon 28, with case reports in exons 27, 30–31 and 52.^{54–55,58} These additional locations can be analysed following exon 28.

Type 2N VWD—The majority (~80%) of missense mutations are located in exons 18–20 which can be analysed first with a much lower proportion of mutations in exons 17 and 24–27.^{28,58} Type 2N has autosomal recessive inheritance and affected individuals are either homozygous or compound heterozygous for missense mutations, or compound heterozygous for a missense mutation and a mutation resulting in lack of VWF expression. The latter mutations are found throughout VWF. p.Arg854Gln occurs in 2% of Caucasian populations^{66–67} and frequently contributes to the phenotype. In an individual referred for genetic testing, lack of a missense mutation in the above exons can exclude VWD 2N.

Type 3 VWD—Mutations associated with type 3 VWD are found throughout the entire coding region of *VWF* (i.e., exons 2–52). Sequence analysis of this region identifies mutations in 80–90% of type 3 VWD patients. Deletion/duplication (dosage) analysis has recently become available using MLPA,⁵⁹ but the extent of contribution of large deletions or duplications has not been established within type 3 VWD patient cohorts and large heterozygous mutations have likely been overlooked. Large deletions currently comprise 9% of mutation reports in type 3 VWD patients on VWFdb.⁵⁸ Linkage analysis can be useful in type 3 VWD to facilitate prenatal diagnosis if time is insufficient for full mutation analysis, or if two mutations cannot be identified in an affected individual.⁶¹

CLINICAL DESCRIPTION

Natural history

VWD is a congenital bleeding disorder; however, symptoms may only become apparent on hemostatic challenge and bleeding history may become more obvious with increasing age. Thus, it may take some time before an abnormal bleeding history is recognized. Bleeding history is also dependent on disease severity; type 3 VWD is often diagnosed early in life, but in families with no previous disease history, non-accidental injury may be suspected,

whereas mild type 1 VWD may not be diagnosed until midlife, despite a history of bleeding episodes. Individuals with VWD primarily manifest excessive mucocutaneous bleeding (bruising, epistaxis, menorrhagia etc) and do not tend to experience musculoskeletal bleeding unless their FVIII:C level is <10 IU/dL, as can be seen in type 2N or type 3 VWD.

Type 1 VWD—Type 1 VWD accounts for up to 70% of all VWD. It typically manifests as mild mucocutaneous bleeding; however, symptoms can be more severe when VWF levels are less than 15 IU/dL. Epistaxis and bruising are common symptoms among children. Menorrhagia is the most common finding in women of reproductive age.^{68–69}

Type 2 VWD—**Type 2 VWD** accounts for about 25% of all VWD. The relative frequency of the subtypes is 2A>2N>2M>2B in European populations.

Individuals with type 2A VWD usually present with mild to moderate mucocutaneous bleeding.

Patients with type 2B VWD typically present with mild to moderate mucocutaneous bleeding, although in some cases it can be severe.⁷⁰ In many patients with type 2B VWD, the development or worsening of thrombocytopenia may occur during stressful situations, such as severe infection, during surgery or pregnancy, or if treated with desmopressin.¹⁸

In type 2M VWD, individuals typically present with mild-moderate mucocutaneous bleeding symptoms, but bleeding episodes can be severe, particularly in the presence of very low or absent VWF:RCo.

Symptoms of type 2N VWD are essentially the same as those seen in mild hemophilia A and include excessive bleeding at the time of surgery or procedures as both disorders result from reduced FVIII:C.

Type 3 VWD—Type 3 VWD accounts for <5% of VWD. It manifests with severe bleeding including both excessive mucocutaneous bleeding and musculoskeletal bleeding. The latter may be more pronounced where FVIII:C levels are lowest.

Penetrance

In autosomal dominant type 1 VWD, mutations resulting in plasma VWF level <25 IU/dL are often fully penetrant whereas those resulting in higher VWF levels are often incompletely penetrant. Mutations causal for other autosomal dominant VWD types; 2A, 2B and 2M are often fully penetrant.

DIFFERENTIAL DIAGNOSIS

Both type 2N VWD and mild hemophilia A (caused by mutations in *F8*) result in reduced levels of FVIII:C (~5–40 IU/dL) with normal or borderline low levels of VWF. Although the VWF:FVIII:B test which determines the ability of VWF to bind FVIII is a useful way to distinguish between the two disorders,⁷¹ its availability may be limited and the test may sometimes yield equivocal results.⁷²

In families with reduced FVIII:C, an X-linked pattern of inheritance can help identify those with mild hemophilia A. When family history is uninformative, it may be preferable to perform sequence analysis of *F8* before *VWF*, even in symptomatic females who are simplex cases (i.e., a single occurrence in a family), because a *F8* mutation plus skewed X-chromosome inactivation (Lyonisation) are often responsible for symptoms. In these cases, *F8* intrachromosomal inversions may be sought and DNA sequence analysis or mutation

scanning of *F8* exons 1–26 undertaken.^{73–74} In females, dosage analysis using MLPA can also be used to identify heterozygous partial or complete gene deletions or duplications.^{75–76} *F8* mutations may be detected in >50% of cases referred for “possible 2N VWD or hemophilia A”.⁷² When *F8* mutations are absent, *VWF* can be analysed.

Platelet-type pseudo VWD (PT-VWD, also called pseudo VWD) mimics type 2B VWD but is caused by mutations in *GPIBA*. The disorders can be distinguished by mixing patient or control plasma and platelets to determine which component is defective using platelet aggregation or flow cytometry, but these assays are technically challenging.^{25,52,77–78} When mutations are absent from exon 28 of *VWF*, mutations in exon 2 of *GPIBA* may be identified. To date, missense mutations reported to affect GpIb amino acids p.Gly249 and p.Met255 plus a 27bp in-frame deletion p.Pro449_Ser457del (c.1345_1371del27)^{79–80} have been reported (following Human Genome Variation Society nomenclature;⁸¹ reference sequences NP_000164.4 and NM_000173.4, legacy numbering G233, M239, P433–441 deletion).

PT-VWD is probably under diagnosed.⁷⁹ Misdiagnosis of PT-VWD may result in ineffective treatment of patients. VWF concentrate is needed to correct reduced VWF level, but platelet transfusion may also be required if there is significant thrombocytopenia.²⁵ The half-life of replaced VWF is reduced in PT-VWD due to binding to abnormal GpIb, so VWF concentrate has to be administered more frequently than in VWD. Molecular genetic testing of *GPIBA* may identify missense or in-frame mutations^{80,82} in approximately 10% of persons diagnosed with 2B VWD.

Acquired von Willebrand syndrome (AVWS)

This mild to moderate bleeding disorder can occur due to a variety of conditions,^{83–85} but is not caused by *VWF* mutation. It is most often seen in persons over age 40 years with no prior bleeding history. AVWS has diverse pathology and may result from; 1) lymphoproliferative or plasma cell proliferative disorders, paraproteinemias (monoclonal gammopathy of unknown significance, MGUS), multiple myeloma and Waldenstrom macroglobulinemia. Antibodies against VWF have been detected in some of these cases; 2) autoimmune disorders including systemic lupus erythematosus (SLE), scleroderma and antiphospholipid antibody syndrome; 3) shear-induced VWF conformational changes leading to increased VWF proteolysis (such as aortic valvular stenosis and ventricular septal defect; 4) markedly increased blood platelet count (including essential thrombocythemia or other myeloproliferative disorders); 5) removal of VWF from circulation by aberrant binding to tumor cells (e.g., Wilm tumor or certain lymphoproliferative disorders); 6) decreased VWF synthesis (for example, resulting from hypothyroidism); 7) use of certain drugs (including valproic acid, ciprofloxacin, griseofulvin, hydroxyethyl starch).

MANAGEMENT

Evaluations following initial diagnosis

To establish the extent of disease in an individual diagnosed with VWD, the following evaluations are recommended; 1) a personal and family history of bleeding to help predict severity and tailor treatment. Use of a standardised bleeding assessment tool can be helpful.^{13–14,86} 2) A joint and muscle evaluation for those with type 3 VWD (musculoskeletal bleeding is rare in types 1 and 2 VWD). 3) Screening for hepatitis B and C as well as HIV if the diagnosis is type 3 VWD, or if the individual received blood products or plasma-derived clotting factor concentrates before 1985. 4) Baseline serum concentration of iron and ferritin (to assess iron stores), as many individuals with VWD are iron deficient, particularly women with menorrhagia. 5) A gynecological evaluation for women with menorrhagia.⁸⁷

Treatment of manifestations

Individuals with VWD benefit from referral to a comprehensive bleeding disorders, program for education, treatment, and genetic counseling.

The two main treatments are desmopressin (1-deamino-8-D-arginine vasopressin (DDAVP)) and clotting factor concentrates containing both VWF and FVIII (VWF/FVIII concentrate). Individuals with VWD should receive prompt treatment for severe bleeding episodes.

Desmopressin—Most individuals with type 1 VWD and some with type 2 VWD respond to intravenous or subcutaneous treatment with desmopressin,^{88–89} which promotes release of stored VWF and raises levels 3–10 fold. Intranasal preparations are also available. Following VWD diagnosis, a desmopressin challenge is advisable to assess VWF response.¹⁷ Desmopressin is the treatment of choice for acute bleeding episodes or to prevent bleeding during surgery. In persons who are intolerant to desmopressin or have a poor VWF response, clotting factor concentrate is required. Desmopressin is contraindicated in individuals with arteriovascular disease and in those over age 70 years for whom VWF/FVIII concentrate is required. Because desmopressin can cause hyponatremia (imbalance of water to salt, which can lead to seizures and coma), fluid intake should be restricted for 24 hours following its administration to minimize this risk.

Intravenous infusion of VWF/FVIII clotting factor concentrates—In those who are non-responsive to desmopressin (i.e., where the VWF deficiency is not sufficiently corrected) and for those in whom desmopressin is contra-indicated (below), bleeding episodes can be prevented or controlled with intravenous infusion of virally-inactivated plasma-derived clotting factor concentrates containing both VWF and FVIII.⁹⁰ Such concentrates are prepared from pooled blood donations from many donors. Virus inactivation procedures eliminate potential pathogens.

Indirect treatments—In addition to treatments that directly increase VWF levels, individuals with VWD often benefit from indirect hemostatic treatments. Fibrinolytic inhibitors (i.e., tranexamic acid) can be helpful for treatment or prevention of bleeding episodes.⁹¹ Hormonal treatments (i.e., the combined oral contraceptive pill) can be particularly effective for the treatment of menorrhagia.⁹² Non-medical treatments including the levonorgestrel-releasing intrauterine system⁹³ or endometrial ablation⁹⁴ may be useful in selected patients with VWD. A consensus document on the management of women with VWD was recently published.⁹⁵

Obstetric issues—VWF levels increase throughout pregnancy with the peak occurring in the third trimester.⁹⁶ Despite this, pregnant women with VWD are at increased risk for bleeding complications and care should be provided in centers with experience in perinatal management of bleeding disorders.^{12,97}

Although deliveries should occur based on obstetric indications, instrumentation should be minimized.⁸⁷ Delayed, secondary post-partum bleeding may be a problem as VWF level rapidly returns to pre-pregnancy level following delivery.

Pediatric issues—Special considerations should be taken for the care of infants and children with VWD. Infant males should only be circumcised after consultation with a pediatric hemostasis specialist. Desmopressin should be used with caution, particularly in those under the age two years, because of the potential difficulty in restricting fluids in this age group. Additionally, VWF levels are higher in the neonatal period and so phenotypic testing for milder forms of VWD should be delayed until later in childhood.

Prevention of primary manifestations

Individuals with type 3 VWD may be given prophylactic infusions of VWF/FVIII concentrates to prevent musculoskeletal bleeding and subsequent joint damage.⁹⁸

Prevention of secondary complications

Desmopressin should be used with caution, particularly in those under two years old. Individuals with VWD should be vaccinated for hepatitis A and B.¹⁷ Prevention of chronic joint disease is a concern for individuals with type 3 VWD; however, controversy exists about the specific schedule and dosing of prophylactic regimens.⁹⁸ This is the subject of an ongoing international trial, with prophylactic treatment being studied for joint bleeding, nosebleeds, and menorrhagia.

Surveillance

Individuals with milder forms of VWD can benefit from being followed by treatment centers with experience in the management of bleeding disorders. Individuals with type 3 VWD should be followed in experienced centers and should have periodic evaluations by a physiotherapist to monitor joint mobility.

Agents/circumstances to avoid

Activities with a high risk of trauma, particularly head injury should be avoided. Medications with effects on platelet function (aspirin (acetylsalicylic acid, ASA), clopidogrel, or non-steroidal anti-inflammatory drugs, NSAIDs) should be avoided as they can worsen bleeding symptoms. Infant males should only be circumcised following consultation with a pediatric hemostasis specialist.

THERAPIES UNDER INVESTIGATION

Recombinant VWF is in clinical trials⁹⁹ and is expected to be available for patient use shortly.

GENETIC COUNSELING

Mode of inheritance

Most cases of VWD type 1, along with types 2A, 2B and 2M are inherited in an autosomal dominant manner, whereas types 2N, 3 and rarer occurrences of type 1 and 2A are inherited in an autosomal recessive manner.

Risk to family members - autosomal dominant inheritance

Parents of a proband—Most individuals diagnosed with one of the autosomal dominant (AD) types of VWD have an affected parent. However, a proband with AD VWD may have the disorder as the result of a new gene mutation. The proportion of cases caused by *de novo* mutations has not been well studied, but recent multicentre studies on type 1 VWD identified *de novo* mutations in at least 2–4% of probands, that were absent from parents.^{34,100} Where the mutation causing AD VWD found in the proband cannot be detected in the DNA of either parent, two possible explanations are germline mosaicism in a parent or a *de novo* mutation in the proband. Neither possibility has been sufficiently investigated to comment on relative likelihood of occurrence. Evaluation of parents of a proband with AD VWD may determine that one is affected but has escaped previous diagnosis because of failure by health care professionals to recognize the symptoms and/or a milder phenotypic presentation. Therefore, an apparently negative family history cannot be confirmed until appropriate evaluations have been performed.

Siblings of a proband—The risk to the siblings (sibs) of the proband depends upon the genetic status of the proband's parents. Where the parent of the proband is affected, the risk to the sibs is 50%. The sibs of a proband with clinically unaffected parents are still at increased risk for VWD because of the possibility of reduced penetrance in a parent. If the disease-causing mutation found in the proband cannot be detected in the DNA of either parent, then the risk to sibs is low, but greater than that of the general population, because of the possibility of germline mosaicism.

Offspring of a proband—Each child of an individual with AD VWD has a 50% chance of inheriting the mutation.

Other family members of a proband—The risk to other family members depends upon the status of the proband's parents. If a parent is found to be affected, his or her family members may be at risk.

Risk to family members – autosomal recessive inheritance

Parents of a proband—The parents of an individual with autosomal recessive (AR) VWD are obligate heterozygotes (i.e., carriers of one mutant allele). Heterozygotes (carriers) are generally asymptomatic. For type 3 VWD, approximately 15% show some mild bleeding symptoms, whereas more than 50% have VWF levels less than 50 IU/dL.¹⁷ Reduced FVIII level correlates with increased bleeding tendency and individuals with low levels may be more at risk of bleeding associated with surgery.¹⁰¹

Siblings of a proband—At conception, each sib of an individual with AR VWD has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Once an at-risk sib is known to be unaffected, the risk of his/her being a carrier is 2/3. Heterozygotes (carriers) are generally asymptomatic.

Offspring of a proband—The offspring of an individual with autosomal recessive VWD are obligate heterozygotes (carriers) for a disease-causing mutation in the VWF gene.

Other family members of a proband—Each sib of the proband's parents is at 50% risk of being a carrier.

Carrier detection

Carrier testing for at-risk family members is possible once the disease-causing mutations have been identified in the family.

Prenatal testing

Prenatal diagnosis for pregnancies at increased risk (generally only for type 3 VWD) is possible by analysis of DNA extracted from fetal cells obtained by chorionic villus sampling (CVS) at approximately 11 to 13 weeks gestation or amniocentesis usually performed at about 15–18 weeks gestation. The disease-causing allele(s) of an affected family member must be identified before prenatal testing can be performed. Pre-implantation genetic diagnosis (PGD) may become available for families in which the disease-causing mutation(s) have been identified.^{102–103}

GENOTYPE-PHENOTYPE CORRELATIONS

Response to desmopressin

Correlation of mutation with the response to treatment has been investigated in type 1 and 2 VWD. Most of 77 EU type 1 VWD patients demonstrated clinically useful rises in VWF and FVIII levels following desmopressin infusion, those that did not largely had mutations in the D3 and A1–A3 domains.⁸⁸ In type 2 VWD, many patients have a clinically useful response to desmopressin, although it may be short-lived in some cases. In type 2B cases however, thrombocytopenia may be exacerbated.^{18,104} However, individuals with 2B mutations p.Pro1266Leu and p.Arg1308Leu do not experience thrombocytopenia and can be treated with desmopressin.¹⁸ Utility of desmopressin for management of delivery in types 1 and 2 VWD has recently been correlated with causative mutations, with prevention of significant bleeding by 2–3 administrations in most cases.⁹⁶

Inhibitory antibodies

A small proportion of type 3 VWD patients develop antibodies in response to replacement therapy (7.5–9.5%).^{105–106} Systematic surveys of mutation correlation with inhibitors have not been conducted but large gene deletions and nonsense mutations have been reported in this patient group. Anaphylaxis following replacement therapy may also be experienced by the same individuals.¹⁰⁵

ABO blood group

Blood group contributes about 25% of the variance in plasma VWF level; ABO glycosylation of VWF influences its rate of clearance.¹⁰⁷ Individuals with non-O blood groups have higher VWF levels than those with O blood group, those with group AB have the highest levels. Blood group O is significantly more common in type 1 VWD than in the general population or in type 2 VWD¹⁰⁸ and is particularly common in cases with no mutation identified.³⁵ ABO blood group appears to be an important contributor to penetrance and reduced VWF level in type 1 VWD^{34–35} as has been observed with the common mutation p.Tyr1584Cys.^{63,109}

Nomenclature

Current and previously used nomenclature for VWD and VWF is detailed in Table 4.

Concluding Comments

Significant progress has been made in our understanding of VWD since its original description in 1926 by Dr. Erik von Willebrand. The identification of VWF, and subsequently, the identification, cloning and characterization of the *VWF* gene has led to the availability of genetic testing, which when rationally applied can aid in the management of patients.

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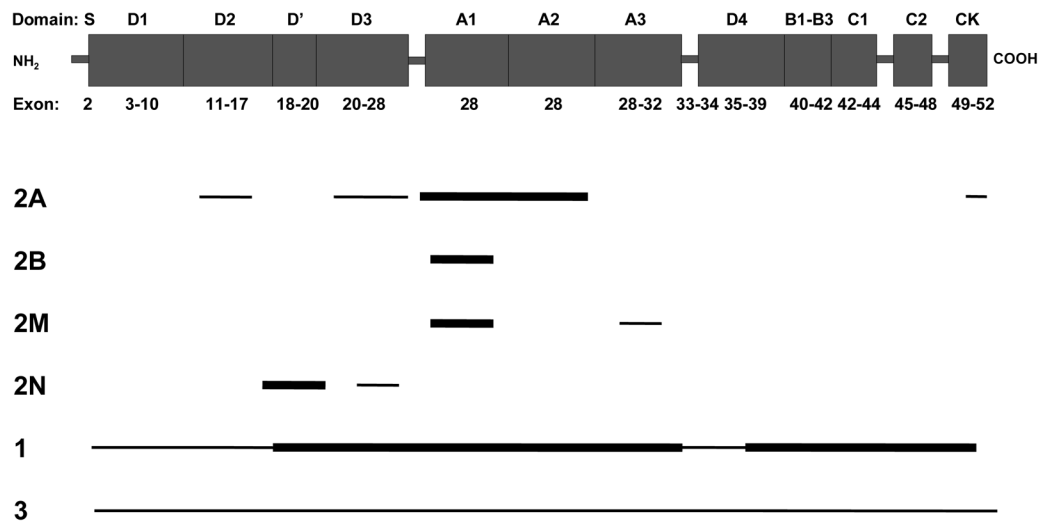


Figure 1. Location of VWF mutations by VWD type. Thick horizontal lines indicate the approximate position of exons where mutations are most prevalent; thinner lines indicate exons with mutations of lower frequency. Mutations that result in type 2 VWD affect VWF function and cluster in domains primarily disrupted by missense mutations.

Table 1

Classification of VWD based on specific VWF tests

VWD Type	VWF:RC ₀ ^a	VWF:Ag ^a	RC ₀ /Ag	FVIII:C IU/dL ^a	Multimer pattern ^b	Other
1	Low	Low	Equivalent	~1.5x VWF:Ag	Normal	
2A	Low	Low	VWF:RC ₀ < VWF:Ag	Low or normal	Abnormal	HMW
2B	Low	Low	VWF:RC ₀ < VWF:Ag	Low or normal	Abnormal	HMW RIPA ^c (platelet count)
2M	Low	Low	VWF:RC ₀	Low or normal	Normal	
2N	Normal/low	Normal/low	Equivalent	<30	Normal	VWF:FVIII ^d
3	Absent	Absent	NA	<10	Absent	

^aRelative to the reference range (approximate values); VWF:RC₀ (50–200 IU/dL); VWF:Ag (50–200 IU/dL); FVIII:C (50–150 IU/dL).

^bHMW, high molecular weight multimers

^cIncreased agglutination at low concentrations of ristocetin

^dThe ability of VWF to bind and protect FVIII is reduced. VWF and FVIII levels can look exactly like those in males with mild haemophilia A or in symptomatic hemophilia A carrier females.

Table 2

VWF Normal Allelic Variants

DNA Nucleotide Change	Protein Amino Acid Change	VWF Exon/Intron	rs# ^a	Restriction Site/Type of Polymorphism	Reference Sequence
c.1451A>G ^b	p.His484Arg	Exon 13	rs1800378	Rsa I	
c.1946-19_1946-17dupCTT ^b	none	Intron 15	rs106222288	3 bp insertion/deletion	
c.2365A>G ^b	p.Thr789Ala	Exon 18	rs1063856	Rsa I	
c.2555A>G	p.Gln852Arg	Exon 20	rs216321	Nla IV	
c.4141A>G ^b	p.Thr1381Ala	Exon 28	rs216311	Hph I	
c.4414G>C	p.Asp1472His	Exon 28	rs1800383	RleA I	NM_000552.3 NP_000543.2
c.4641C>T ^b	p.Thr1547Thr	Exon 28	rs216310	BstE II	
c.6187C>T	p.Pro2063Ser	Exon 36	NA		
c.6977-542_6977-541ins24	none	Intron 40	rs36115023	deletion/insertion polymorphism	
c.6977-715_6977-714ins16	none	Intron 40	rs41402545	deletion/insertion polymorphism	
c.8113G>A	p.Gly2705Arg	Exon 49	rs7962217		

Only a small proportion of common non-synonymous variants are listed.

^a rs#, reference SNP number.

^b Normal variants particularly useful for linkage analysis because they are common in several ethnic groups and/or affect the cleavage site of a well-behaved restriction enzyme.

Table 3

VWF Pathologic Allelic Variants

Class of Variant Allele	VWD Type	DNA Nucleotide Change	Protein Amino Acid Change	VWF exon	Reference Sequence
	1	c.3614G>A	p.Arg1205His	27	
	1	c.4751A>G	p.Tyr1584Cys	28	
	2A	c.4517C>T	p.Ser1506Leu	28	
	2A	c.4789C>T	p.Arg1597T ^{ip}	28	
	2B	c.3797C>T	p.Pro1266Leu	28	
	2B	c.3916C>T	p.Arg1306T ^{ip}	28	
	2B	c.3946G>A	p.Val1316Met	28	
	2B	c.4022G>A	p.Arg1341Gln	28	NM_000552.3 NP_000543.2
Pathologic ^a	2M	c.3835G>A	p.Val1279Ile	28	
	2M	c.4273A>T	p.Ile1425Phe	28	
	2N	c.2372C>T	p.Thr791Met	18	
	2N	c.2446C>T	p.Arg816T ^{ip}	19	
	2N	c.2561G>A	p.Arg854Gln	20	
	3	c.2435delC	p.Pro812ArgfsX31	18	
	3	c.4975C>T	p.Arg1659X	28	
	3	c.7603C>T	p.Arg2535X	45	

Nomenclature follows the standard naming conventions of the Human Genome Variation Society⁸¹

^aExamples of the most frequent variants identified in each VWD type are shown. ⁵⁸

Table 4

Previous and Current Nomenclature

Previous nomenclature	Current nomenclature
von Willebrand's disease	von Willebrand disease
vWF	VWF
vWD	VWD
RiCof (ristocetin cofactor activity)	VWF:RCo ^a
FVIII RA _g (FVIII related antigen)	VWF:Ag ^a
Platelet-type VWD	Platelet-type pseudo-von Willebrand disease (PT- VWD), caused by mutations in <i>GPIBA</i> and thus not a form of VWD
Acquired VWD	Acquired von Willebrand syndrome (AVWS) ¹⁷ is the preferred terminology for defects in VWF concentration, structure or function that are neither inherited nor reflective of mutations in the <i>VWF</i> gene, but which arise as consequences of other medical conditions

^aRecommended abbreviations for VWF and its activities are detailed in Mazurier and Rodegheiro¹¹⁰