Diagnosing von Willebrand disease: Genetic analysis

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Key Points: Mutations causing quantitative types 1 and 3 von Willebrand disease are seen throughout VWF, but those causing qualitative type 2 affect discrete functional domains.

Genetic analysis can aid determination of VWD type and thus relevant treatment when phenotypic analysis is inconclusive.

Learning Objectives: VWD displays a wide range of different phenotypes resulting from mutation to its several different domains.

VWD inheritance pattern can be dominant, co-dominant or recessive dependent on the contribution of different mutations to phenotype.

Abstract

Investigation of a patient with possible von Willebrand disease includes a range of phenotypic analyses. Often, this is sufficient to discern disease type, and this will suggest relevant treatment. However for some patients, phenotypic analysis does not sufficiently explain the patient's disorder and for this group, genetic analysis can aid diagnosis of disease type. PCR and Sanger sequencing have been mainstays of genetic analysis for several years. More recently, next generation sequencing has become available, with the advantage that several genes can be simultaneously analysed where necessary, e.g. for discrimination of possible type 2N von Willebrand disease or mild haemophilia A. Additionally, several techniques can now identify deletions/duplications of an exon or more that result in von Willebrand disease including multiplex ligation-dependent probe amplification and micro-array analysis. Algorithms based on next generation sequencing data can also identify missing or duplicated regions. These newer techniques enable causative VWF defects to be identified in more patients than previously, aiding a specific VWD diagnosis. Genetic analysis can also be helpful in the discrimination between type 2B and platelet-type von Willebrand disease and in prenatal diagnosis for families with type 3.

Introduction

von Willebrand disease (VWD) is the most commonly diagnosed autosomally inherited bleeding disorder. It affects approximately one in 10,000 individuals sufficiently to be referred to a tertiary care centre.[1] von Willebrand factor (VWF) is a very large multimeric glycoprotein[2] that performs two essential haemostatic functions; it provides a means to bind platelets at sites of vascular damage and at high shear stress supports platelet aggregation. It also carries and protects coagulation factor VIII (FVIII) in the circulation, delivering it to sites of vascular injury.

Initial analysis for possible VWD examines bleeding symptoms in the patient and their family and this typically includes epistaxis, easy bruising and other haemorrhagic symptoms. A bleeding score (BS) system has been defined and can help predict outcomes and replacement therapy.[3] Laboratory testing of quantity and activity parameters for VWF then determine whether they deviate from the normal range. VWD is divided into three main categories; type 1 VWD (VWD1) is a partial quantitative deficiency, and VWD3 an almost complete quantitative deficiency of VWF whilst VWD2 is categorised into 4 types; 2A, 2B, 2M and 2N dependent on VWF function perturbed by mutation.

Recently, guidelines on the diagnosis and management of VWD have recommended that cut-off values are used in the diagnostic pathway. These suggest that patients having VWF activity levels ≥30-35 IU/dL should be classified as having "low VWF" in recognition that this can be a risk factor for bleeding, rather than being given a VWD diagnosis.[4,5]

For many patients diagnosed with VWD, laboratory phenotypic assays readily suggest VWD type in the individual, enabling the clinical team to select the most relevant management for that disease type. However, where this is not the case, genetic analysis can help understand the patient's disorder through identification of the causative mutation(s). For many years, PCR and Sanger sequencing were utilised to identify mutations in the VWF gene (*VWF*), but more recently, next generation sequencing (NGS) has been introduced to diagnostic and research institutions. This technology enables simultaneous parallel sequencing of many genes. Large deletions and duplications of an exon or more can be sought using multiplex ligation-dependent probe

amplification (MLPA) or microarray specific for *VWF,* or through analysis of NGS data using an algorithm to seek missing or duplicated regions.

This article summarises the mutation classes and their inheritance patterns associated with the different VWD types and how this information can be useful in provision of the most relevant patient management.

Type 1 VWD

This is the most prevalent VWD type in most populations, with proportions of patients up to 70% in some studies. Type 1 VWD (VWD1) patients have von Willebrand factor antigen (VWF:Ag) and VWF activity assays with activity/antigen ratio >0.7 and normal VWF multimers. VWF activity can be determined using ristocetin co-factor (VWF:RCo), that measures binding to GpIb, whilst VWF:CB measures binding to collagen. New assays assess binding to engineered GpIb fragments genetic (VWF activity).[6] Recent recommendations of using lower cut-off values for inclusion of these patients amongst those with VWD (30 or 35 IU/dL) will reduce the proportion of individuals classified as affected by VWD1.[7,8] For those within this borderline group, strongest predictors of VWD diagnosis are reduced VWF:RCo in platelet aggregation , especially in those with non-O blood-group and female sex.[9]

Mutations are located throughout *VWF* from the promoter region[10] to exon 52,[11,12] the majority are missense mutations (75%), while splice, deletion, nonsense, insertion, duplication and large in-frame deletions mutations comprise minor proportions.[13] Most patients have dominantly inherited disease resulting from a single mutation whilst a smaller proportion (~5-10%) have more than one mutation contributing to their symptoms, some with recessive inheritance.[14-16] Blood-group O is present in up to ~65% of VWD1.[11]

The predominant mechanism responsible is intracellular retention of mutant VWF, resulting in reduced secretion into plasma.[17] Rapid VWF clearance from the circulation also contributes,[18] with VWF half-life in unaffected individuals being approximately 8-12 hours and individuals with ABO

blood group O having shorter half-lives. The ratio between VWF propeptide (VWFpp) and VWF:Ag can be exploited to highlight rapid clearance, with the "Vicenza" mutation p.Arg1205His exemplifying this phenotype. The VWF half-life following desmopressin administration was ~10% that of Haemate P. Modelling mathematically the synthesis, clearance and cleavage of p.Arg1205His using a one tenth half-life relative to normal VWF replicated the mutation features of low VWF level, ultralarge multimers and reduced satellite bands.[19] Rapid clearance can result in desmopressin not providing sufficiently sustained VWF elevation for longer clinical procedures; VWFpp/VWF:Ag measurement or genetic analysis can identify the cause.

In contrast to VWD types 2A, 2B and 2M where most heterozygous mutations are fully penetrant (all individuals with the mutation have disease symptoms), approximately half of VWD1 families have incomplete penetrance (only some individuals with a mutation have symptoms). ABO blood group contributes to reduced VWF:Ag level and this is exemplified by the common variant p.Tyr1584Cys.[20] The main use for genetic analysis in VWD1 is to help understand the cause of the more severe presentations and inheritance risk for family members.

Other genes that contribute to variation in VWF level

The Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) Consortium combined analysis of 5 community-based studies (23,608 participants with European ancestry) to seek genome-wide single nucleotide variant associations with *F7, F8* and *VWF* genes. In addition to *VWF* and *ABO*, variants in 5 further genes were identified that influenced both VWF and FVIII levels; *STXBP5, SCARA5, STAB2, TC2N* and *CLEC4M.* Type 1, but not type 2 patient analysis in the Willebrand in Netherlands study demonstrated association of *STXBP5* with VWF:Ag level reduction of -3.0 IU/dL per allele and *CLEC4M* with VWF:Ag level of -4.3 IU/dL and VWF activity of -5.75 IU/dL per allele.[21] ABO blood group is associated with up to ~25% variation in VWF levels between blood-group O and non-O.

Type 2 VWD

Type 2A VWD

This is the largest grouping amongst VWD2, and includes several different disease mechanisms resulting from mutations in diverse VWF domains (Figure 1). All have high molecular weight multimer (HMWM) loss, but demonstrate differing multimer profiles.[22] Most patients have reduced VWF activity/VWF:Ag ratios. A tertiary category for VWD2A includes mutations in the most common locations; the A2 domain (IIA), D3 domain (IIE), D2 domain (IIC) and CK domains (IID).[23]

VWD2A/IIA

Classic VWD2A/IIA mutations are located in the A2 domain (amino acids (AA) 1500-1672) and comprise half of 2A mutations listed on the VWF database.[24] Mutation mechanisms include impaired VWF secretion and enhanced sensitivity to the cleaving protease, ADAMTS13 that occurs under high shear stress.[25]

A1 domain

20% of VWD2A mutations lie in the adjacent A1 domain (AA 1271-1458) where they impact binding to glycoprotein IB (GPIb) and include the common variants p.Arg1315Cys, p.Arg1374Cys and p.Arg1374His. These mutations have been variously classified as resulting in VWD types 2A, 2M, and 1 as multimer abnormalities do not always appear to be detectable.[26,27]

D3 domain

The VWD2A/IIE phenotype was described many years ago, but mutations responsible were only described as a single VWD category in 2010.[28] Mutations result in intracellular retention, relative loss of HWMW and reduced ADAMTS13-mediated proteolysis. Missense mutations are located in ex22 and 25-28, many introducing/substituting cysteine residues; replacement of p.Cys1130 is the most common change.[29] These mutations, like p.Arg1205His result in rapid VWF clearance.

D2 domain

VWD2A/IIC propeptide mutations have a multimerisation defect resulting in reduced HMWM. Unlike most VWD2A, mutations are recessively inherited and are located in ex11-16 (AA 404-768). In addition to homozygous missense mutations, patients also have small in-frame deletions/insertions with some being compound heterozygous.

CK domain

VWD2A/IID mutations are located in the cysteine knot domain and affect ex51-52 (AA 2730-2801). Mutations impair VWF dimerisation leading to odd numbered satellite bands on multimer analysis.[30]

Mutation analysis can aid understanding of disease process in VWD2A either through analysis of exon 28 alone or preferably of the entire gene.

Type 2B VWD

VWD2B results from an unusual gain-of-function mutation type.[31] Dominantly inherited A1 domain missense mutations (AA 1266-1461) alter VWF confirmation, facilitating spontaneous interaction with GPIb. This can result in circulating aggregates of VWF-platelets that are cleared from the circulation, resulting in thrombocytopenia. Platelet-type VWD (PT-VWD) results from dominantly inherited gain-of-function missense mutations in *GP1BA,* leading to a similar phenotype.[32] Treatment may differ between these two disorders; platelet transfusions can result in platelet clearance in VWD2B,[33] where they may be helpful in PT-VWD. Genetic analysis is straightforward for these disorders, often using Sanger sequencing to examine the *VWF* A1 domain and *GP1BA* region encoding the beta hairpin loop (AA 246-255).[34]

Type 2M VWD

Type 2M mutations reduce VWF-GPIb or VWF-collagen binding to sub-endothelium, with no HMWM loss. There is often a marked difference between VWF activity and VWF:Ag, with activity considerably lower than VWF:Ag. The A1 domain is the predominant mutation location (exon 28, AA 1229-1439), where missense mutations impair GPIb interaction, reducing platelet activation. Mutations affecting collagen-binding are not always sought through phenotypic analysis; they may impair binding to the more commonly used collagen types I/III or the less commonly investigated types IV/VI.[35-37] These mutations are located in the A1 or A3 domains (ex28-32).[24]

Type 2N VWD

VWD2N missense mutations in the D'-D3 domains (ex17-25) impair binding of FVIII to VWF (VWF:FVIIIB), resulting in reduced FVIII half-life in the circulation.[38] Typically, FVIII:C levels mimic those seen in mild haemophilia A (range 5-40 IU/dL), whilst VWF:Ag levels are normal or mildly reduced.[38] Inheritance is recessive; at least one of the two mutations affects VWF:FVIIIB. The second mutation can be a further copy of the same missense mutation, a different VWF:FVIIIB missense mutation or commonly a null allele (nonsense, splice-site, deletion/insertion). The most frequent mutation in the European populations is p.Arg854Gln, for which ~1% individuals are heterozygous. Unless there is an obvious family history indicating inheritance pattern, it can be difficult to distinguish mild haemophilia A from 2N VWD. A phenotypic VWF:FVIIIB assay or genetic analysis to discriminate these two disorders can be used. Desmopressin response may be curtailed in 2N patients due to shortened FVIII half-life.

Type 3 VWD

In populations where consanguineous partnerships are common, the prevalence of VWD3 may be 10-20 fold greater than in outbred populations. In European populations, ~one individual/10^6 is affected. Patients have undetectable VWF levels (<5 IU/dL) and reduced FVIII (<10 IU/dL) resulting from lack of VWF to protect FVIII from proteolysis. 15-20% of mutations are missense substitutions

and these can result in a less severe phenotype, with reduced VWFpp/VWF:Ag indicating enhanced clearance from plasma, and reduced BS compared with patients having two null mutations. Missense mutations often result in VWF being retained within the endoplasmic reticulum as VWF is likely misfolded. Whereas VWD3 patients with null alleles in the Willebrand in the Netherlands (WiN) study produced no detectable VWF, those with measurable VWFpp (indicating some VWF production) demonstrated higher VWF:Ag (0 v 4 IU/dL), activity (0 v 1 IU/dL) and FVIII levels (2 v 9 IU/dL).[18] The disorder is recessively inherited with most patients having two null alleles. A very small proportion may develop anti-VWF antibodies, with only a rare patient where the antibody is inhibitory for one of the VWF activities. Genetic analysis can identify causative mutation(s) in nearly all VWD3 affected individual so that inheritance risks in relatives can be determined. Prenatal diagnosis for a possible VWD3 affected pregnancy can be undertaken using chorionic villus or amniocentesis samples from ~11-18 weeks gestation, dependent on sample type.

Heterozygous carriers of a null mutation may have normal or reduced VWF plasma levels. From 15-48% have been diagnosed with VWD1 that can be classified as having "co-dominant inheritance."[39,40]

Clinical utility of VWF genetic testing

Molecular testing for VWD is justified in individuals in whom phenotypic assays suggest VWD, but genetic analysis may provide a more specific diagnosis to aid appropriate management. Examples are; to distinguish between VWD2N, mild hemophilia A in males, or symptomatic hemophilia carrier status in females; to discriminate between VWD2B and platelet-type pseudo VWD (PT-VWD), to help understand the patient's disorder where more than one mutation leads to a more complex phenotype and to provide prenatal diagnosis for families with VWD3. For VWD1, patients where rapid VWF clearance occurs following desmopressin administration or when inheritance pattern is unclear and mutation determination can explain disease risk in relatives, genetic analysis can be useful.

Conclusion

VWF mutations are located throughout the VWF gene, resulting in a wide range of mutation types that include quantitative and qualitative disorders. VWF protein is involved in several processes that can be damaged by mutation and the varying phenotypes in VWD illustrate the processes impaired.

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Conflict of Interest Disclosures

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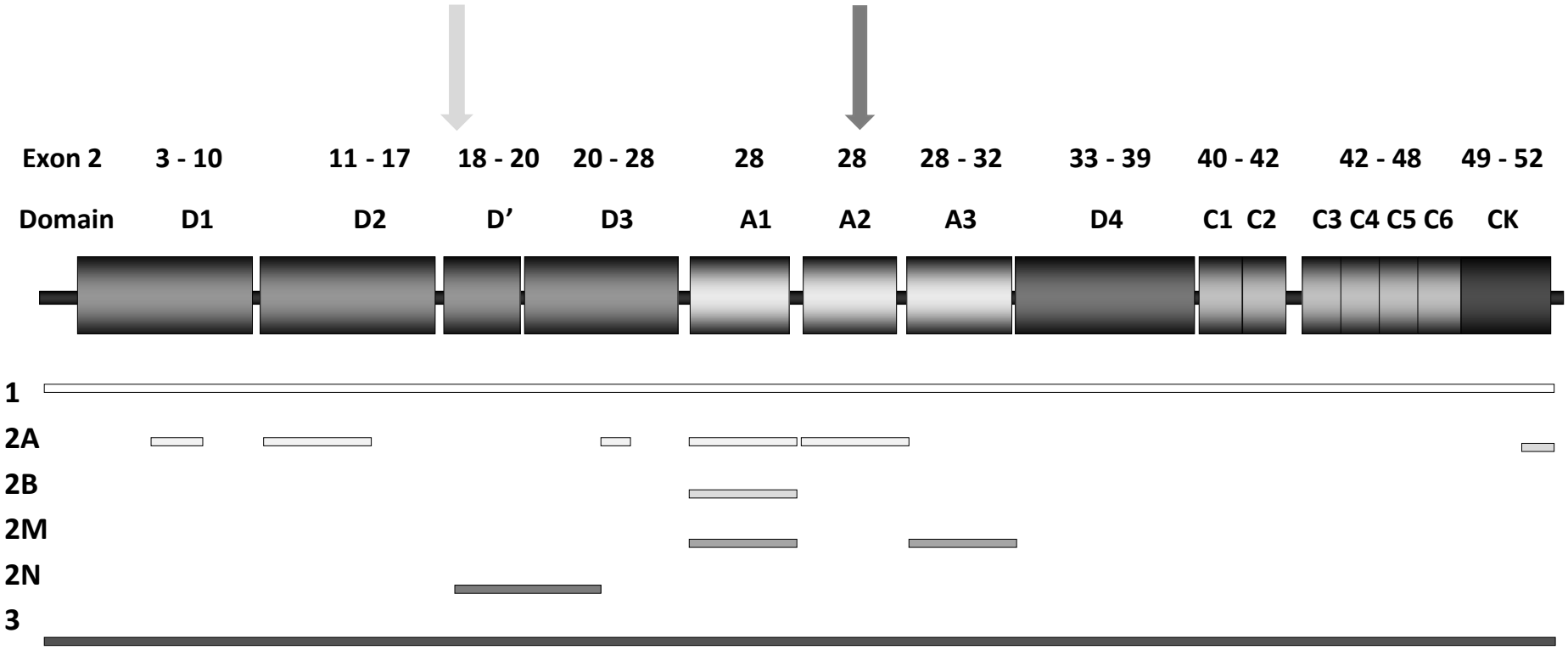
Table 1. Summary of mutation location for VWD patients reported on the VWF mutation database

VWD Type	VWF domain	Mutation location	Inheritance pattern	Phenotype
1	All	Promoter-ex52	Majority dominant, minority >1 mutation or recessive	VWF activity/VWF:Ag >0.7
2A/IIC	D2	Ex11-16	Recessive	Mostly very discrepant VWF activity/VWF:Ag
2A/IIE	D3	Ex22 & 25-28	Dominant	Discrepant VWF activity/VWF:Ag, most <0.6
2A	A1	Ex28	Dominant	Discrepant VWF activity/VWF:Ag
2A/IIA	A2	Ex28	Dominant	Discrepant VWF activity/VWF:Ag
2A/IID	CK	Ex51-52	Dominant	Discrepant VWF activity/VWF:Ag
2B	A1	Ex28	Dominant	Wide range of VWF activity/VWF:Ag possible
2M	A1 A3	Ex28 Ex29-32	Dominant	Discrepant VWF activity/VWF:Ag and/or VWF:CB/VWF:Ag
2N	D'-D3	Ex17-20 Ex24-25	Recessive	FVIII:C 5-40 IU/dL VWF:Ag normal/reduced
3	All	5' VWF-Ex52	Recessive	VWF:Ag <5 IU/dL FVIII:C <10 IU/dL

VWF mutation database - https://grenada.lumc.nl/LOVD2/VWF/variants.php

Figure 1. Structure of von Willebrand factor, highlighting mutation location



Exons encoding specific VWF domains are highlighted above the VWF domain structure. Shaded rectangles indicate VWF domains. Mutation locations are denoted by shaded bars indicating regions associated with each VWD type. Furin (light grey) and ADAMTS13 cleavage sites (dark grey) are indicated by arrows.