von Willebrand Factor (VWF) Exon 28 Genotyping

Background

von Willebrand disease (VWD) is the most common inherited human bleeding disorder. Its prevalence has been estimated to be up to 1% in some populations. Mucocutaneous bleeding, including epistaxis and menorrhagia, and prolonged bleeding after trauma and surgery are common manifestations of this condition. Not all individuals with genetic mutations will develop VWD and the severity of symptoms is variable. Multiple environmental and genetic variables can affect the levels of VWF.

VWF is a large, complex, high molecular weight (HMW) multimeric glycoprotein that mediates initial adhesion of platelets to collagen in injured sub-endothelium and to the platelet surface receptor glycoprotein (GP) Ib and also serves as the carrier protein for coagulation factor VIII (FVIII) in plasma, protecting it from rapid proteolytic degradation in blood. It also plays a major role in primary hemostasis and coagulation.

VWD is classified into two major categories, quantitative and qualitative defects. Quantitative VWF defects include type 1 (partial deficiency of VWF) and type 3 (complete absence of VWF) in plasma and/or platelets. Qualitative VWF defects include type 2, which is further classified into four subtypes, type 2A, 2B, 2M and 2N, by different pathophysiologic mechanisms. Correct diagnosis of VWD is critical for selection of an appropriate treatment and better prognosis.

The VWF gene is located in chromosome 12p13.3, comprising 52 exons and spanning approximately 180kb with 8439 base pairs of coding sequence. The VWF gene is highly polymorphic. So far, there are at least 2,728 single-nucleotide polymorphisms and 91 insertions/deletions in the VWF gene, with the highest ethnic variability in Africans/African Americans and followed closely by Asians. Exon 28 is the largest of the VWF exons with frequent mutations, and encodes several sites essential for ligand-binding and cleavage functions like the A1 and A2 domains of VWF where platelet GPIb, collagen or heparin binds. It has the highest number of deleterious mutations. Compared to VWD type 1 where mutations may occur throughout the VWF gene, VWF mutations in VWD type 2A, 2B and 2M are primarily located in exon 28.

Classification of VWD:

1. **Type 1 VWD** (OMIM#193400): There is mild quantitative deficiency of VWF in type 1 VWD, which has an autosomal dominant pattern of inheritance. It represents approximately 65-80% of all VWD cases. Candidate mutations within the coding region, promoter or splice sites of the VWF gene have been identified in 65% of cases. Missense mutations predominate (70%) followed by splicing (9%) and transcriptional (8%). Candidate mutations have been identified in almost all exons; exon 28 is the most frequently involved. Candidate VWF mutations are more likely to be found with VWF plasma level of <30%. Pathogenic mechanisms have not yet been ascertained in all cases and a genetic cause is not clear in 35% of patients.

2. **Type 3 VWD** (OMIM#277480): Severe quantitative deficiency of VWF occurs in type 3 VWD, which has an autosomal recessive inheritance pattern. VWF plasma levels are very low (1-5%) in type 3 VWD. It represents less than 5% of all VWD cases and 85-90% of type 3 patients have identified VWF gene mutations. The most common types of mutations include nonsense (31%), missense (18%) and small deletions (18%). The most common nonsense mutation, R1659X, is in exon 28. The mutations may be homozygous or compound heterozygous for two different null alleles. Desmopressin infusion is not clinically useful in type 3 VWD patients.

3. **Type 2 VWD** (OMIM#613554): The qualitative deficiency of VWF in type 2 VWD represents approximately 20-35% of all VWD cases.

Type 2A VWD (autosomal dominant) is characterized by defective platelet-dependent binding mainly due to the absence of HMW VWF and represents 20-25% of all VWD cases. Almost 73% of these mutations are located within exon 28 (D1/D2/D'D3, A2, CRCK domains) and 90% of mutations in this exon are missense mutations. Other mutations include deletion, insertion and frameshift

mutations. Phenotypic testing is not always reliable in differentiating type 2A from type 2M.

Type 2B VWD (autosomal dominant) is characterized by "gain of function" phenotype as the result of missense mutations, which causes spontaneous binding of platelet receptor GPIbα to HMW VWF, and thrombocytopenia. Unlike other VWD types, desmopressin therapy is contraindicated in these patients because of worsening thrombocytopenia. All mutations causing type 2B are found in exon 28 (A1 domain) and 96% of them are missense. H1268D, R1306W, R1308C, I1309V, V1316M and R1341Q/W are especially prone to the development of thrombocytopenia. Type 2B VWD is phenotypically indistinguishable from platelet-type VWD that is due to mutations in the gene encoding for GPIbα (OMIM#177820).

Type 2M VWD (autosomal dominant) is caused by the alteration of protein conformation that reduces GPIbα binding affinity to HMW VWF. 75% of the identified mutations are located in exon 28 (A1 domain) Of these, 93% are missense mutations and the remainder are in-frame small deletions. Missense mutations, including S1731T, W1745C and S1783A in the A3 domain are reported to interfere with VWF binding to collagen and cause mild bleeding. Phenotypically it can be very difficult to differentiate between type 2M and types 2A, 2B and even type 1 VWD. These patients do not respond to desmopressin infusion.

Type 2N (autosomal recessive) is characterized by reduced affinity to Factor VIII. Symptoms largely result from reduced factor VIII levels (5-30%) and this can mimic hemophilia A (OMIM#306700). About 85% of mutations occur in exons 18-27 (D'D3 domain).

Comprehensive coagulation tests including VWF antigen and functional activities (ristocetin cofactor and collagen binding activity), ristocetin induced platelet aggregation and multimer analysis are used in the laboratory diagnosis of VWD. However, an accurate diagnosis of VWD is often difficult due to its heterogenous nature and high variation or detection limit of assay. VWF exon 28 sequencing can facilitate an accurate diagnosis, particularly in type 2 VWD variants. It aides in the selection of an appropriate therapy by subtype of VWD and is useful for prenatal testing and genetic counseling. However, the VWF gene is highly polymorphic and has a high degree of ethnic variability. Some genetic variants are known to affect VWF antigen or ristocetin cofactor levels. For example, the c.4414G>C (p.D1472) sequence variant in exon 28 in healthy individuals is reported to associate with isolated decreased ristocetin cofactor activity and can be misdiagnosed as type 2M VWD. Therefore, the exon 28 genotyping is very useful but limited, due in part to, heterogeneity of symptoms in VWD. Careful interpretation is required for the evaluation of pathogenesis of any novel sequence variation found in patients with VWD or workup for possible VWD.

Clinical Indications

This test can be ordered as a single test by clinicians or as a reflex test by pathologists.

1. **Diagnostic Testing**
   The test is especially useful in patients with clinical phenotype of type 2 VWD, as it is highlighted by the following consideration:

   a) Confirming the correct diagnosis of type 2 VWD variant.

   b) Establishing the correct diagnosis in those individuals in whom phenotype suggests type 2 VWD but phenotypic data is insufficient to further discriminate type 2A, type 2B and type 2M variants. This is crucial in patients with type 2B VWD where desmopressin is contraindicated as it can worsen thrombocytopenia and lead to bleeding-related complications. Type 2M frequently does not respond to desmopressin but type 2A generally responds to this treatment.

   c) Differentiating type 2B VWD from platelet-type-VWD. All known mutations associated with type 2B VWD are in exon 28.

2. **Carrier and prenatal testing**
   This test is useful for families with known sequence variants associated with type 2 VWD and also for type 3 where the most common nonsense mutation R1659X is in exon 28.

Methodology

Genomic DNA (gDNA) extracted from the peripheral blood are used for sequencing von Willebrand factor (VWF) gene exon 28. The assay involves PCR amplification and bi-directional Sanger sequencing of exon 28 (c.3675-5053). The reference sequence NM_000552.3 is used and variants are compared with the VWD database (http://www.vwf.group.shef.ac.uk/). Other existing mutation databases including the Human Gene Mutation Database (HGMD) and dbSNP are queried. Newly identified variants are assessed based on evolutionary conservation, location within the functional domain, and *in silico* predictions.

Assay Limitations

Sequence variant changes in other exons, introns, large deletions and splice sites may not be identified in this sequencing assay. Rare/unidentified variants in the PCR primer annealing sites may result in allele drop-out. Synonymous sequence changes and common benign variants or single nucleotide polymorphisms (SNPs) are not routinely reported but can be made available upon request.

Interpretation

Negative: No genetic variant is identified in VWF exon 28.

Positive: A mutation or pathogenic variant, either homozygous or heterozygous, is identified in VWF exon 28.

When a mutation or pathogenic variant is identified, the type of VWD it is associated with will be determined using the STH-SSC VWF Online Database (VWFdb) of the University of Sheffield (UK); http://vWF.group.shef.ac.uk/

When variant is not in the VWFdb, other existing databases including the Human Gene Mutation Database (HGMD) and dbSNP will be queried. Newly identified variants are assessed based on evolutionary conservation, location within the functional domain, and in silico predictions.

Examples of reported common mutations in exon 28 by various type of VWD are listed in below.

VWD classification	Nucleotide substitution	Amino acid substitution
Type 1	c.4751A>G	p.Y1584C
Type 2B	c.3916C>T	p.R1306W
Type 2B	c.3922C>T	p.R1308C
Type 2B	c.3946G>A	p.V1316M
Type 2B	c.4022G>A	p.R1341Q
Type 2A	c.4517C>T	p.S1506L)
Type 2A	c.4790G>A	p.R1597Q
Type 2A	c.4789C>T	p.R1597W
Type 2A	c.4825G>A	p.G1609R
Type 3	c.4975C>T	p.R1659X

Detection of sequence variants may provide additional insight into the patient's phenotype. Careful interpretation is required in determining pathogenicity of any novel sequence variant found in patients with VWD or possible VWD. Correlation with clinical findings, and laboratory results for VW antigen and functional activity, ristocetin induced platelet aggregation VWF and VWF multimer analysis is always recommended for correct interpretation.

References

Berber E. The molecular genetics of von Willebrand disease. *Turk J Haematol.* 2012 Dec; 29(4):313-24.

Gadisseur A et al. Laboratory diagnosis and molecular classification of von Willebrand disease. *Acta Haematol* 2009;121:71-84.

Goodeve AC. The genetic basis of von Willebrand disease. *Blood Rev.* 2010 May; 24(3):123-34.

Hampshire DJ et al. The International Society on Thrombosis and Haematosis von Willebrand disease database: an update. *Semin Thromb Haemostasis* 2011:37(5):470-9.

ISTH-SSC VWF Online Database [homepage on the internet] ©2007 The University of Sheffield [Updated 2010 August 07: cited October 2010] Available from: http://vWF.group.shef.ac.uk/

James PD, Goodeve AC. von Willebrand disease. *Genet Med.* 2011 May; 13(5):365-76.

Lillicrap D. von Willebrand disease: advances in pathogenetic understanding, diagnosis and therapy. H*ematology Am Soc Hematol Educ Program*. 2013;2013:254-60

Wang QY et al. Characterizing polymorphisms and allelic diversity of von Willebrand factor gene in the 1000 genomes. *J Thromb haemost* 2013;11(2):261-9.

Test Overview

Test Name	von Willebrand Factor (VWF) Exon 28 Genotyping
Reference Range	See interpretation
Patient Preparation	No special preparation needed.
Acceptable Specimen Types	Peripheral Blood: 5ml collected in an EDTA (Lavender) tube.
Stability	Ambient: 24 hours; Refrigerated: 5 days; Frozen: unacceptable
Processing and Storage	Transport blood at room temperature and store at room temperature for no more than 24 hours. If specimen is to be stored for longer than 24 hours it should be placed at 4°C. Extracted DNA is stored at 4°C.
Criteria for Rejection	Clotted, frozen, hemolyzed peripheral blood Improperly labeled specimens Samples collected in incorrect anticoagulant Blood collected in heparin tubes (possible PCR inhibition)
Billing Code	90426
CPT Code	80403, G0452

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