Von Willebrand Disease Genetic Subtyping – Type 2 and Platelet Type

Indications for Ordering

- Confirm a phenotypic diagnosis of von Willebrand disease (VWD) types 2A, 2B, 2M, or 2N, or platelet type
  - Aid in therapeutic recommendations
  - Aid in distinguishing type 2N from mild hemophilia A
  - Aid in distinguishing type 2B from platelet type von Willebrand disease (PT-VWD)
- Evaluate family members of individuals with known variants

Test Description

- Type 2 – polymerase chain reaction (PCR) followed by bidirectional sequencing of selected *VWF* exons
  - Type 2A
    - Sequences exon 28
      - Exons 11, 12, 14, 15, 16, 24, 25, 51, 52 added when no variant identified
  - Type 2B
    - Sequences exon 28
  - Type 2M
    - Sequences exon 28
      - Exons 30 and 31 added when no variants are identified
  - Type 2N
    - Sequences exons 4, 9, 17, 18, 19, 20, 21, 24, 25, 27
- PT-VWD – PCR of the *GPIBA* gene followed by targeted variant analysis

Tests to Consider

Primary tests

von Willebrand Disease, Type 2A (*VWF*) Sequencing Exon 28 with Reflex to 9 Exons 2005480
- Molecular test to confirm a phenotypic diagnosis of vWD type 2A

von Willebrand Disease, Type 2B (*VWF*) Sequencing 2005486
- Molecular test to distinguish VWD type 2B from PT-VWD

von Willebrand Disease, Type 2M (*VWF*) Sequencing 2005490
- Molecular test to confirm a phenotypic diagnosis of VWD type 2M

von Willebrand Disease, Type 2N (*VWF*) Sequencing 2005494
- Molecular test to distinguish VWD type 2N from hemophilia A

von Willebrand Disease, Platelet Type (*GP1BA*) 4 Mutations 2005476
- Molecular test to distinguish VWD type 2B from PT-VWD

Related tests

Familial Mutation, Targeted Sequencing 2001961
- Useful when a pathogenic familial variant identifiable by sequencing is known
- Initial work up of suspected vWD

von Willebrand Panel 0030125
- Recommended panel for the initial workup of suspected VWD
- Panel contains the 3 recommended tests for the diagnosis of VWD
  - Factor VIII activity
  - VWF antigen
  - VWF activity (ristocetin cofactor)

Factor VIII, Activity 0030095
- Alternate test for the workup of VWD
- Lacks VWF Activity (ristocetin cofactor) test

von Willebrand Factor Antigen 0030285
- Do not use as a standalone test to diagnose VWD

von Willebrand Factor Activity (Ristocetin Cofactor) 0030250
- Do not use as a standalone test to diagnose VWD

von Willebrand Modified Panel 0030284
- Alternate test for the workup of VWD
- Lacks factor VIII activity test
- Subclassify established VWD to assist with therapeutic decisions

von Willebrand Panel with Reflex to von Willebrand Multimeric Analysis 2003387
- Recommended panel to subclassify VWD when high suspicion for VWD exists
- Contains VWF multimers, factor VIII activity, VWF antigen, and VWF activity (ristocetin cofactor)
- Multimeric testing is performed when ristocetin cofactor, VWF antigen, or factor VIII activity is low or if the ratio of ristocetin cofactor to VWF antigen is <0.7

von Willebrand Factor Multimers 0092281
- Subclassify VWD after initial testing is positive

[von Willebrand Multimeric Panel 0030002]

- Not recommended except in suspected cases of acquired VWD or high suspicion of VWD
- Contains VW multimeric, factor VIII activity, VWF antigen, VWF activity (ristocetin cofactor)

Disease Overview

Incidence – 1/100-1,000 individuals

Symptoms

- Mucocutaneous bleeding after brushing or flossing teeth
- Unexplained bruising
- Prolonged repeated nosebleeds
- Menorrhagia
- Prolonged bleeding following childbirth, trauma, or surgery
- VWD is classified by VWF status (deficiency versus abnormal)
- See table

Genetics

Genes

- Type 2 – *VWF*
- Platelet – *GP1BA*

Inheritance

- Autosomal dominant – types 2B, 2M, most of 2A, and PT-VWD
- Autosomal recessive – 2N, and 20% of 2A

Penetrance

Autosomal dominant types 2A, 2B, and 2M

- Incomplete penetrance when VWF:Ag and VWF:RCo levels are 25-50 IU/dL
- Full penetrance is expected when VWF:Ag and VWF:RCo levels are <25 IU/dl

Structure/function

VWF – large multimeric glycoprotein

- Plays a critical role in hemostasis
- VWF binds factor VIII to protect it from premature degradation
- Causes platelet recruitment via GP1BA receptor
- Facilitates clot formation

Variants

GP1BA variants

- c.746 G>T, (p.Gly249Val)
- c.746 G>A (p.Gly249Ser)
- c. 763A>G (p.Met255Val)
- c. 1306del 27 (p.436-444 del 9)

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity
  - 80% for VWD types, 2B, and 2M
  - 99% for 2A and unknown for other VWD subtypes
- Analytical specificity and sensitivity – 99% for type 2

Results

Type 2A (*VWF*) Sequencing

- Positive – 1 pathogenic type 2A *VWF* gene variant was detected
  - Individual may be affected if the variant is dominant
  - If the variant is recessive, individual is at least a carrier of VWD
- Uncertain – 1 variant of uncertain significance was detected
- Negative – no pathogenic *VWF* gene variant detected

Type 2B (*VWF*) Sequencing

- Positive – 1 pathogenic variant detected
  - Individual is at risk to be affected with type 2B VWD
- Uncertain – 1 variant of uncertain significance was detected
- Negative – no pathogenic variants detected
  - Individual may still be affected with VWD if an undetected pathogenic variant is present

Type 2M (*VWF*) Sequencing

- Positive – 1 pathogenic variant detected
  - Individual is at risk to be affected with type 2M VWD
- Uncertain – 1 variant of uncertain significance was detected
- Negative – no pathogenic variants detected
  - Individual may still be affected with VWD if an undetected pathogenic variant is present

Type 2N (*VWF*) Sequencing

- Positive
  - 2 pathogenic variants detected
    - Individual is predicted to be affected with VWD
  - 1 pathogenic *VWF* gene variant detected
    - Individual is at least a carrier and may be affected if an undetected VWF variant is present
- Negative – no pathogenic variants detected
  - Individual appears to be neither a carrier of or affected with type 2N VWD

GP1BA Variant Detection

- Positive – 1 pathogenic variant detected
  - Individual predicted to be affected with PT-VWD
- Negative – negative for 4 variants tested in the *GP1BA* gene
  - Risk for PT-VWD is reduced, but not eliminated
  - Individual may have a rare *GP1BA* variant

Limitations

- A negative result does not eliminate the possibility of VWD as undetected pathogenic variant(s) may be present in 1 of the unsequenced exons, a noncoding region, or the promoter
- *VWF* sequencing may identify sequence variants with uncertain clinical significance
- *VWF* variants, other than those in exons tested, will not be detected
- Large *VWF* deletions/duplications will not be detected

- No *GP1BA* variants, other than the 4 targeted, are detected by analysis for PT-VWD
- Rare diagnostic errors may occur due to primer-site variants

Clinical Characteristics of Subtypes			
Type	**Defect**	**Clinical Presentation**	**Treatment**
Type 1 – 70-80% of cases	Partial deficiency of VWF	Mild mucocutaneous bleeding	Desmopressin is usually only needed for surgery, dental extractions, childbirth, or injuries
Type 2 – 15-30% of cases • Subtype frequency in the Caucasian population – 2A>2N>2M/2B	Structurally or functionally abnormal VWF	Highly variable • 2A – mild to moderate mucocutaneous bleeding o May have thrombocytopenia • 2B – mild to moderate mucocutaneous bleeding o Thrombocytopenia may be present o Enhanced ability of VWF to bind platelet receptor *GP1BA*, causes removal of the platelet/VWF complex • 2N – symptoms are similar to hemophilia A	• 2A – desmopressin therapy may worsen the thrombocytopenia; treatment for severe bleeding episodes may require clotting factor concentrates • 2B – desmopressin therapy may worsen the thrombocytopenia; clotting factor concentrates may be required • 2M – response to desmopressin is usually very poor; may require clotting factor concentrates • 2N – desmopressin for minor bleeding; severe bleeding requires concentrate with both VWF and factor VIII
Type 3 – rare	Complete absence of VWF	Severe mucocutaneous and musculoskeletal bleeding	Requires clotting factor concentrates containing both VWF and factor VIII
Platelet-type (PT-VWD or pseudo-VWD)	Abnormal high-affinity interaction between platelet glycoprotein Ib/V/IX complex and vWF Caused by *GPIBA* variants	Often indistinguishable from VWD type 2B	