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
  o Aid in therapeutic recommendations
  o Aid in distinguishing type 2N from mild hemophilia A
  o 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
  o Type 2A
    ▪ Sequences exon 28
    ▪ Exons 11, 12, 14, 15, 16, 24, 25, 51, 52 added when no variant identified
  o Type 2B
    ▪ Sequences exon 28
  o Type 2M
    ▪ Sequences exon 28
    ▪ Exons 30 and 31 added when no variants are identified
  o 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
  o Factor VIII activity
  o VWF antigen
  o 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. 1306del27 (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
- 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

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

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