

von Willebrand Disease, Type 2 (VWF) Sequencing, Selected Exons or Platelet-Type von Willebrand Disease (GP1BA), 4 Mutations

TO CONFIRM A DIAGNOSIS OF VON WILLEBRAND DISEASE (VWD), TYPES 2A, 2B, 2M, OR 2N, OR PLATELET-TYPE VWD

Disease Overview

- von Willebrand factor (VWF) is a large multimeric glycoprotein that plays a critical role in primary hemostasis.
- The main functions of VWF are binding factor VIII to protect it from premature proteolytic degradation and binding sub-endothelial collagen at the site of vascular damage, causing platelet recruitment via the platelet *GP1BA* receptor, and facilitating clot formation.
- Common symptoms of von Willebrand disease (VWD) include mucocutaneous bleeding after brushing or flossing teeth, unexplained bruising, prolonged repeated nosebleeds, menorrhagia, and prolonged bleeding following childbirth, trauma, or surgery.
- Initial evaluation for VWD includes a panel of tests to evaluate VWF antigen (VWF:Ag) and activity (ristocetin cofactor activity: VWF:RCo), as well as factor VIII activity. Additional tests may also be necessary for diagnosis and subtyping.
- Treatment for VWD is best achieved at a comprehensive bleeding disorder program. Two common treatments include desmopressin, which releases stored VWF, and clotting factor concentrates containing both VWF and factor VIII. In patients who are intolerant or do not respond to desmopressin, clotting factor concentrate is required. Patients often benefit from fibrinolytic inhibitors (to treat or prevent bleeding episodes) and hormonal treatments (to decrease menorrhagia).
- VWD is subclassified by whether disease is caused by a decreased amount of VWF or the presence of structurally or functionally abnormal VWF.
 - Type 1, caused by a partial deficiency of VWF, accounts for 70 percent of cases and is associated with mild mucocutaneous bleeding.
 - Type 2, caused by structurally or functionally abnormal VWF, accounts for 25 percent of cases, and its clinical presentation is highly variable.
 - Type 3, caused by complete absence of VWF, accounts for <5 percent of cases and is associated with severe mucocutaneous and musculoskeletal bleeding.
- VWD type 2 is further subdivided into 2A, 2B, 2M, and 2N subtypes. It is useful to distinguish these subtypes, as therapeutic recommendations vary among the groups. Subtype frequency in the Caucasian population is 2A>2N>2M>2B.

- 2A causes mild to moderate mucocutaneous bleeding and is variably responsive to desmopressin.
- 2B causes mild to moderate mucocutaneous bleeding. Thrombocytopenia may be present due to the enhanced ability of VWF to bind platelet receptor *GP1BA*, causing removal of the platelet/VWF complex. Symptoms may worsen with severe infection, surgery, pregnancy, or desmopressin treatment.
- 2M causes mild to moderate mucocutaneous bleeding and rarely responds to desmopressin.
- 2N causes symptoms similar to mild hemophilia A as both disorders result from reduced factor VIII activity; mutations affect the ability of VWF to bind and protect factor VIII. Desmopressin only treats minor bleeding; severe bleeding requires concentrate containing both VWF and factor VIII.
- Platelet type VWD (PT-VWD or pseudo-VWD) is caused by *GP1BA* mutations and is not considered a type of VWD. Clinical presentation is often indistinguishable from VWD type 2B, but VWD type 2B is caused by mutations in *VWF*.

Prevalence

VWD affects one in 100 to one in 1,000 individuals.

Genetics

- *VWF* is the only gene known to cause VWD, but other conditions may have an indistinguishable phenotype.
 - PT-VWD is phenotypically indistinguishable from type 2B but is caused by *GP1BA* mutations. In fact, both PT-VWD and VWD type 2B are caused by gain-of-function mutations that lead to enhanced binding between plasma VWF and *GP1BA*.
- Both autosomal dominant and recessive forms of VWD exist.
 - Autosomal dominant: all cases of types 2B and 2M and most cases of types 1 and 2A
 - Autosomal recessive: all cases of type 2N and 3; occasional cases of type 1 and 2A.
 - Most affected individuals carrying a dominant mutation also have an affected parent; the percentage of cases that occur due to de novo mutations is unknown.
 - Typically, dominant mutations are incompletely penetrant when VWF:Ag and VWF:RCo levels are 25–50 IU/dL. Full penetrance can be expected from dominant mutations when VWF:Ag and VWF:RCo levels are <25 IU/dL.

- Eighty percent of *VWF* mutations causing autosomal dominant VWD type 2A, 2B, and 2M are located in exon 28; up to 20 percent of mutations causing autosomal recessive type 2A can be detected by sequencing *VWF* exons 11, 12, 14, 15, 16, 24, 25, 51, and 52.
- Although mutations causing VWD type 2N have been identified in *VWF* exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27, the sensitivity of such targeted sequencing is unknown.
- Assays for large gene deletions/duplications in *VWF* are not clinically available; furthermore, the percentage of VWD caused by deletions/duplications is unknown.
- Four different mutations in *GPIBA* are responsible for the majority of PT-VWD.

Indications for Ordering

- To confirm a phenotypic diagnosis of VWD type 2A, 2B, 2M, 2N, or PT-VWD.
- To distinguish VWD type 2B from PT-VWD.
- To distinguish VWD type 2N from mild hemophilia A.
- Testing for family members of individuals with known mutations.

Additional Ordering Notes

- If there is a known family history of VWD, please provide the patient's symptoms, the relationship between the patient and the affected family member(s), and the specific *VWF* mutation, if known.
- For familial mutation testing, please order Familial Mutation, Targeted Sequencing (ARUP test code 2001961) and provide a copy of the affected relative's test result detailing the specific mutation identified.

Contraindications

- Testing asymptomatic individuals who have affected relatives without a known pathogenic *VWF* mutation.
- Targeted testing for a familial *VWF* mutation in any exon other than 4, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 27, 28, 30, 31, 51, and 52.

Interpretation

- The VWD subtype and inheritance pattern will be provided for pathogenic mutations identified, if known.
- Heterozygotes for known dominant pathogenic *VWF* mutations are at risk for symptoms of VWD. Since reduced penetrance is observed in VWD, individuals may or may not be symptomatic.
- Heterozygotes for known recessive pathogenic *VWF* mutations are predicted to be carriers of VWD. Approximately 10 percent of carriers may develop mild symptoms of VWD.
- Compound heterozygotes for known recessive pathogenic mutations are predicted to be affected with VWD.
- If no mutations are detected in an individual with a phenotype consistent with VWD type 2B, consideration should be given to *GPIBA* sequencing.
- If no mutations are detected in *VWF* exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27 in an individual with a phenotype consistent with VWD type 2N, factor VIII gene sequencing is recommended.
- If no mutations are identified, this does not eliminate the possibility of VWD as undetected pathogenic mutation(s) may be present in one of the unsequenced exons, a non-coding region, or the promoter.
- *VWF* sequencing may identify sequence variants with uncertain clinical significance.

Methodology

- Polymerase chain reaction (PCR) followed by bidirectional sequencing of *VWF* exons 4, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 27, 28, 30, 31, 51, and 52 depending on the specific VWD test ordered.
 - Analysis of VWD type 2A begins with sequencing exon 28. If no mutations are identified, exons 11, 12, 14, 15, 16, 24, 25, 51, and 52 are subsequently sequenced.
 - Analysis of VWD type 2B involves sequencing exon 28.
 - Analysis of VWD type 2M begins with sequencing exon 28. If no mutations are identified, exons 30 and 31 are subsequently sequenced.
 - Analysis of VWD type 2N involves sequencing *VWF* exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27.
 - Analysis of PT-VWD involves PCR of the *GPIBA* gene followed by targeted mutation analysis of four common mutations: c.746 G>T, (p. Gly249Val), c.746 G>A (p. Gly249Ser), c. 763A>G (p. Met255Val), and c. 1306del 27 (p. 436-444 del 9).
- Analytical sensitivity and specificity are 99 percent.
- Clinical sensitivity is approximately 80 percent for VWD types 2B and 2M, 99 percent for VWD type 2A, and unknown for type 2N and PT-VWD using the tests as described above. The sensitivity of these tests for other types of VWD is unknown.

Limitations

- *VWF* mutations, other than those in the exons tested, will not be detected.
- Large *VWF* deletions/duplications will not be detected.
- No *GPIBA* mutations, other than the four targeted, are detected by analysis for PT-VWD.
- Rare diagnostic errors may occur due to primer-site mutations.

Related Tests

- von Willebrand Factor Activity Ristocetin Cofactor (vWF:RCO) ([0030250](#))
- von Willebrand Factor Antigen (vWF:Ag) ([0030285](#))
- Factor VIII Activity ([0030095](#))
- von Willebrand Modified Panel (vWF:Ag and vWF:RCO) ([0030284](#))
- von Willebrand Panel (vWF:Ag, vWF:RCO and FVIII activity) ([0030125](#))
- von Willebrand Multimeric Panel (vWF:Ag, vWF:RCO, FVIII activity, and multimers) ([0030002](#))
- von Willebrand Factor Multimers ([0092281](#))
- von Willebrand Panel with Reflex to von Willebrand Multimeric Analysis ([2003387](#))

References

1. Federici AB. The use of desmopressin in von Willebrand disease: the experience of the first 30 years (1977–2007). *Haemophilia* 2008;14 Suppl 1:5–14.
2. Federici AB, et al. Clinical and molecular predictors of thrombocytopenia and risk of bleeding in patients with von Willebrand disease type 2B: a cohort study of 67 patients. *Blood* 2009;113:526–34.
3. James P, Lillicrap D. Genetic testing for von Willebrand disease: the Canadian experience. *Semin Thromb Hemost* 2006;32:546–52.
4. Keeney S, et al. The molecular analysis of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organisation Haemophilia Genetics Laboratory Network. *Haemophilia* 2008;14:1099–111.

Test Information

2005480	von Willebrand Disease, Type 2A (<i>VWF</i>) Sequencing Exon 28 with Reflex to Exons 11, 12, 14, 15, 16, 24, 25, 51 & 52
2005486	von Willebrand Disease, Type 2B (<i>VWF</i>) Sequencing Exon 28
2005490	von Willebrand Disease, Type 2M (<i>VWF</i>) Sequencing Exons 28, 30 and 31
2005494	von Willebrand Disease, Type 2N (<i>VWF</i>) Sequencing Exons 4, 9, 17, 18, 19, 20, 21, 24, 25, and 27
2005476	von Willebrand Disease, Platelet Type (<i>GPIBA</i>) 4 Mutations

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

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