

Assay Summary

von Willebrand Factor Gene (vWF) Mutation Analysis

von Willebrand Disease

Synopsis

von Willebrand's disease (vWD) is the most common inherited bleeding disorder with a prevalence as high as 1 to 2 percent in the general population. The main clinical manifestations include increased or easy bruising, recurrent epistaxis (nosebleeds), excessive bleeding during menstruation and/or childbirth, and postoperative bleeding (particularly after tonsillectomy or dental extractions). The disease is caused by an inherited defect in the von Willebrand factor (vWF), a large multimeric glycoprotein, with essential roles in primary homeostasis and as a carrier of coagulation factor VIII (FVIII) in the circulation¹. The symptoms of vWD are due a quantitative deficiency or qualitative dysfunction of vWF protein. A variety of point mutations, insertions, and deletions in the vWF gene and conversions with the pseudo gene have been described^{2,3}. vWD is classified into three different subtypes⁴. Type 1 accounts for 70 percent of cases with deficiency of vWF, and is typically inherited as an autosomal dominant trait. About 60% of the variation in vWF plasma is due to genetic factors, although a definitive diagnosis is complicated by ABO blood type, ethnic background, reduced penetrance and variable expressivity. Type 2 accounts for 10 to 30 percent of cases and is characterized by qualitative abnormalities of vWF, which is further divided into four subtypes (2A, 2B, 2M, and 2N). Type 2A, 2B, 2M follow autosomal dominant inheritance, while type 2N and a rare type 2A variant follow autosomal recessive inheritance. Type 3 affects approximately 1 to 5 percent of cases and is characterized by very low or undetectable levels of plasma vWF. Type 3 is an autosomal recessive disease with either homozygous or compound heterozygous mutations. Affected individuals have severe bleeding that can be life-threatening if not recognized and treated.

Indications for testing

- Individuals with a clear diagnosis of von Willebrand disease
- Hemophilia patients with symptoms suggestive of VWD complicating hemophilia.
- Individuals with a history of easy bruising, frequent and prolonged nosebleeds, excessive bleeding during menstruation and/or childbirth; excessive and prolonged bleeding after injury, surgery, and dental extractions, but without a clear clinical diagnosis.
- Patients requiring determination of VWF subtype to help direct clinical treatment.
- Individuals with a family history of bleeding or VWD, including pre-symptomatic and prenatal testing for appropriate family members.

Methodology

VWF-SEQ: All coding exons and associated intron junctions of the vWF (52 exons) gene are amplified using vWF specific primer to distinguish from the pseudogene sequence^{5,6,7} followed by direct DNA sequence analysis using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing in the opposite direction. If no mutation is found sequence analysis is performed in both directions. Testing of at-risk family members can be offered by DNA sequence analysis of only the region of the gene with the previously identified mutation.

VWF-ex28: For type 2 VWD, the coding sequence and associated intron junctions of exon 28 amplified using vWF specific primers, followed by direct DNA sequence analysis in both directions using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing an independent PCR amplification in the opposite direction. If no mutation is found, the entire vWF gene is analyzed. The cost is also reflexive.

Limitations

The mutation analysis will not detect mutations located in regions of the genes that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The method also will not detect gross genetic alterations including most duplications, inversions or deletions (in females). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

(a) Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note:

- i) for infants, a minimum of 3 ccs is sufficient
- ii) we accept DNA; at least 10 micrograms

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

Test Request Form (TRF)

- a) A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [von Willebrand's disease Patient Information Form](#): Include a completed von Willebrand's disease Patient Information Form for the proband and a complete pedigree.

Order Codes	CPT Codes	TAT
VWF-SEQ (von Willebrand Disease, full gene sequencing)	83890, 83898(x54), 83904(x54), 83894, 83912	10 wks
VWF-CAS (von Willebrand Disease, targetted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
VWF-EX28 (von Willebrand Disease, exon 28 only)	83890, 83898, 83894, 83904, 83912	4 wks
VWF-PD (von Willebrand Disease, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks

References

1. Mannucci, P.M. (2004) N Engl J Med 351: 683-694
2. Keeney S. (2001). Clin. Lab. Haem. 23: 209 -230
3. Gupta, P.K. (2005). Br J Haematol. 130:752-8.
4. Sadler (1994). Thrombosis and Haemostasis, 71: 520–525.
5. Titani et al (1986) Biochemistry 25: 3171–3184.
6. Ginsburg, D. et al (1985) Science 228:1401-1406.
7. Mancuso et al (1991) Biochemistry 30: 253–269.

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc