von Willebrand Disease Type 2N Sequence Analysis

Versiti offers DNA sequencing of *VWF* exons 17-21 and 24-27 (order code 1288) for detection of germline variants associated with type 2N von Willebrand disease (VWD).

Von Willebrand disease (VWD) is a common inherited bleeding disorder with a reported incidence ranging from 0.01% to 1%. VWD is classified into subtypes of quantitative (types 1 and 3) and qualitative (type 2) defects, caused by pathogenic variants in *VWF*. Type 2N VWD is a rare autosomal recessive type of VWD characterized by a qualitative defect in VWF in which it does not bind factor VIII (FVIII) adequately, resulting in a shortened plasma half-life of FVIII and a clinical phenotype similar to mild hemophilia A. Unlike hemophilia A, type 2N VWD does not show an X-linked pattern of inheritance, as it results from homozygous or compound heterozygous pathogenic variants of the *VWF* gene affecting the factor VIII binding domain of VWF (2N/2N) or resulting from a combined qualitative 2N defect of one allele and a quantitative defect on the other allele (2N/type 1). In patients with type 2N VWD, factor VIII levels are disproportionately reduced compared to VWF levels (which are either normal or modestly depressed). Genetic testing of *VWF* exons 17-21 and 24-27, the exons encoding the factor VIII binding domain of VWF, offers clinical utility in confirming a diagnosis of type 2N VWD (2N/2N) to facilitate selection of appropriate medical therapy, and accurately determine recurrence risk. Note that patients with low VWF levels are more likely to have compound heterozygosity for qualitative and quantitative defects (2N/1) and that both variants may be not detected within the limited *VWF* exons included in the Type 2N analysis; ordering *VWF* Sequence Analysis (all exons; order code 1395) may be more appropriate in those cases.

Plasma assays (factor VIII-VWF binding assay, FVIII Chromogenic and VWF antigen/activity) may help to distinguish between type2 N VWD (2N/2N or 2N/1) and heterozygous carriers versus a diagnosis of hemophilia (affected male or carrier female) and guide the selection of optimal genetic testing. For patients in whom a single heterozygous pathogenic or likely pathogenic variant is identified by VWD type 2N sequence analysis, the factor VIII-VWF binding assay is recommended in order to phenotypically distinguish type 2N/1 disease from a simple type 2N carrier status. The factor VIII-VWF binding assay can be ordered (VWD Type 2N Binding assay-order code 1089), and is part of the VWD Type 2N Binding Profile (order code 1088) and included as appropriate in the reflexive algorithmic VWD Diagnostic Evaluation (order code 1800).

Other subtypes of VWD vary in clinical and laboratory phenotype as well as inheritance pattern. For genetic evaluation of these other types of VWD, see *VWF* Sequence Analysis (order code 1395) and *VWF* Exon 28 Sequence Analysis (order code 1284).

Indications for testing:

- Confirm diagnosis of type 2N von Willebrand disease
- Distinguish type 2N VWD from mild hemophilia A
- Evaluate abnormal Factor VIII-VWF binding results
- Facilitate selection of appropriate medical therapy
- Identification of pathogenic variants to allow for familial testing or prenatal diagnosis

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services (414) 937-6396 or 800-245-3117 (ext. 6250) to be directed to genetic counselors or clinical support team.

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Test method:

This next-generation sequencing assay analyzes the complete coding region of VWF exons 17-21 and 24-27 plus a minimum of 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence (VWF, NM_000552.3). These regions are captured by hybridization, amplified and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low quality are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination.

Assay sensitivity and limitations:

The analytical sensitivity of this test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp), or variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology.

Clinical sensitivity

The clinical sensitivity for detecting pathogenic variants in individuals with either homozygous or compound heterozygous type 2N von Willebrand disease is greater than 99%. Complex and sometimes severe phenotypes resulting from compound heterozygosity for qualitative and quantitative defects, such as 2N/1 may be not detected by this assay and VWF Sequence Analysis (exons 1-52; order code 1395) can be considered.

Reporting of results:

Results are classified and reported in accordance with ACMG next-generation sequencing standards. Sequence variants predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org).

Specimen requirements:

Parental/Patient/Pediatric: 3-5 mL Whole blood (EDTA tube, lavender top), 2-5 mL Bone marrow (EDTA tube, lavender top), 3-4 Buccal swabs, or ≥1ug of DNA at ≥50ng/uL of High Quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; back up culture of amniocytes or chorionic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL Whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell contamination studies. For questions please contact the laboratory to discuss sample requirements.

SHIP

Shipping requirements:

Ship on an ice pack or at room temperature. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container; place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines.

Send to:
Versiti Client Services
Diagnostic Laboratories
638 N. 18th Street
Milwaukee, WI 53233

ORDER

Required forms:

Please complete all pages of the requisition form. Clinical history (including patient's ethnicity, clinical diagnosis, family history and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround time:

Order code: 1288

For suggested CPT codes, visit Versiti.org/test menu

Turnaround time: 21 days

The CPT codes provided are subject to change as more information becomes available. CPT codes are provided only as guidance to assist clients with billing.

For additional information related to shipping, billing or pricing, please contact, Versiti Client Services (414) 937-6396 or 800-245-3117, (ext. 6250), or Labinfo@versiti.org

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von Willebrand disease type 2N references

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Variant interpretation references

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