VWD Platelet-Type Sequence Analysis (GP1BA)

Versiti offers sequencing of the full coding region of the GP1BA gene for diagnosis of platelet-type von Willebrand disease.

von Willebrand disease (VWD) is the most common inherited bleeding disorder and is characterized by either quantitative or qualitative defects of von Willebrand factor (VWF). Platelet-type VWD (PT-VWD) is a rare autosomal dominant disorder caused by heterozygous pathogenic gain-of-function variants in GP1BA (the gene that encodes the platelet glycoprotein Ibα subunit), and the disorder shares an identical clinical presentation to qualitative type 2B VWD. Patients present with variable degrees of thrombocytopenia (which can fluctuate) and mild to moderate bleeding.

The mechanism of disease in PT-VWD is increased affinity of platelet glycoprotein Ib alpha (GP1Bα) for normal VWF, while type 2B VWD is caused by an abnormality of VWF that results in increased binding of high molecular weight VWF multimers to normal platelets. PT-VWD is therefore commonly referred to as "pseudo-VWD" to acknowledge that it is caused by a platelet abnormality, not a VWF defect.

Determination of treatment requires accurate diagnosis of PT-VWD versus type 2B VWD to appropriately target the underlying defect of the enhanced interaction of platelets with VWF with platelet-based versus plasma-based therapy. Standard low-dose ristocetin-induced platelet aggregation may not distinguish between these two disorders. The identification of clinically significant variants in GP1BA can be used to diagnose PT-VWD and distinguish it from type 2B VWD. VWF Exon 28 Sequence Analysis can be used to establish the diagnosis of type 2B VWD.

While specific pathogenic variants in GP1BA cause PT-VWD, other pathogenic variants in GP1BA are associated with autosomal recessive Bernard-Soulier syndrome and autosomal dominant macrothrombocytopenia, and may be identified with this assay; see test description for Bernard-Soulier syndrome panel (order code 4880) for more information.

Indications for testing:

- Confirmation of Platelet-Type VWD in patients with differential diagnosis of PT-VWD versus type 2B VWD
- Diagnosis of individuals with a family history of Platelet-Type VWD

Informed Consent

It is recommended that healthcare providers obtain informed consent from the patient when genetic testing is ordered, consistent with any applicable state laws and regulations, documenting that the patient has been advised of and understands the indications for and implications of the genetic test. If needed, an informed consent form for Versiti Hematology Genetics testing can be found at http://www.versiti.org/hg under *forms*.

Test method:

30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence. These targeted 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 coverage 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 directors, 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 the NGS 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.

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Reporting of Results

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards. Sequence variants and large deletions and duplications 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. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container, seal and place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Diagnostic Laboratory
Versiti
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: 1289

CPT Code: For suggested CPT code information, visit Versiti.org/testmenu.

Turnaround Time: 21 days

References:

Platelet-type VWD references

1. James PD, Connell NT, Ameer B, Di Paola J, Eikenboom J, Giraud N, Haberichter S, Jacobs-Pratt V, Konkle B, McLintock C, McRae S, R Montgomery R, O'Donnell JS, Scappe N, Sidonio R, Flood VH, Husainat N, Kalot MA, Mustafa RA. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv. 2021 Jan 12;5(1):280-300. doi: 10.1182/bloodadvances.2020003265
2. Othman M. Platelet-type von Willebrand disease: a rare, often misdiagnosed and underdiagnosed bleeding disorder. Semin Thromb Hemost. 2011 Jul;37(5):464-9. doi: 10.1055/s-0031-1281030. Epub 2011 Nov 18
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4. Weiss HJ, Meyer D, Rabinowitz R, Pietu G, Girma JP, Vicic WJ, Rogers J. Pseudo-von Willebrand's disease. An intrinsic platelet defect with aggregation by unmodified human factor VIII/von Willebrand factor and enhanced adsorption of its high-molecular-weight multimers. N Engl J Med. 1982 Feb 11;306(6):326-33. doi:

Variant interpretation references

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6. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 2013;15(9):733- 747. doi:10.1038/gim.2013.92
7. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405- 424. doi:10.1038/gim.2015.30
8. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020;22(2):245- 257. doi:10.1038/s41436-019-0686-8.

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