Bernard Soulier Syndrome Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 3 genes known to cause Bernard Soulier Syndrome. This panel can be ordered as:

- **Next Generation Sequencing (NGS) only;**
- **NGS with reflex to Array Comparative Genomic Hybridization (aCGH) Deletion/Duplication if biallelic clinically significant variants are not detected by sequencing ;**
- **NGS with concurrent aCGH Deletion/ Duplication (both testing methodologies performed simultaneously); or Deletion/Duplication by aCGH only.**

Bernard Soulier syndrome (BSS) is an autosomal recessive bleeding disorder characterized by macrothrombocytopenia and platelet dysfunction due to quantitative or qualitative defects in the platelet glycoprotein (GP) receptor Ib/V/IX. The GPIb/V/IX complex is a platelet receptor for von Willebrand factor, enabling platelet adhesion at sites of vascular injury. GPIbα, GPIbβ and GPIX, encoded by the *GP1BA, GP1BB* and *GP9* genes, are all required for efficient expression of the complex on the platelet surface, while absence of GPV does not appear to affect receptor expression or VWF binding.

Autosomal recessive Bernard Soulier syndrome is a rare disorder with an estimated incidence of 1 in one million births. Patients typically present with moderate to severe bleeding, thrombocytopenia with increased platelet size and normal granularity, and abnormal platelet function with absent or markedly reduced aggregation response to ristocetin. In cases of quantitative GPIb/V/IX deficiency, flow cytometric analysis demonstrates decreased or absent expression of GPIb (CD 42) on the platelet surface.

Unlike many autosomal recessive disorders, in which heterozygous carriers of pathogenic variants are not expected to be affected, carriers of pathogenic heterozygous *GP1BA, GP1BB* and *GP9* variants can present with mild macrothrombocytopenia with minimal bleeding; certain heterozygous variants are specifically known to cause autosomal dominant macrothrombocytopenia, such as the *GP1BA* (c.515C>T, p.Ala156Val) variant, also known as the Bolzano variant. There is intra- and inter-familial variability in heterozygous phenotypes, with some having normal platelet count and size and others having more significant macrothrombocytopenia, suggesting the disorders associated with pathogenic variants in these genes encompass a continuum in terms of clinical presentation. Pathogenic variants in *GP1BA* are also associated with platelet type von Willebrand disease. *GP1BB* is within the DiGeorge syndrome critical region and can be partially or fully deleted in patients with 22q11.2 deletion syndrome.

The NGS panel evaluates for single nucleotide variants and small deletions and duplications, which are most commonly responsible for genetic disease. However, large deletions and duplications, also referred to as copy number variations (CNVs), are a known cause of genetic disorders, but can escape detection by next generation sequence analysis. Additional testing with aCGH Deletion/Duplication analysis is available for all genes on this panel to evaluate for large deletions and duplications encompassing one or more exons, or affecting an entire gene.

Refer to the table for further information about each gene in the Bernard Soulier Syndrome Panel, including the clinical phenotype and inheritance pattern.

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Bernard Soulier Syndrome Panel: gene, clinical phenotype and inheritance pattern.		
Gene	Clinical Phenotype	Inheritance
GP1BA	**Bernard Soulier syndrome (BSS):** macrothrombocytopenia with normal platelet granularity and moderate to severe bleeding due to decreased/absent/dysfunctional platelet GPIb/IX expression with decreased/absent platelet aggregation with ristocetin.	Autosomal Recessive
	***GP1BA*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Autosomal Dominant
	Platelet type von Willebrand disease: thrombocytopenia with mild bleeding due to loss of VWF high molecular weight multimers from increased binding of platelets and VWF	Autosomal Dominant
GP1BB	**Bernard Soulier syndrome (BSS):** macrothrombocytopenia with normal platelet granularity and moderate to severe bleeding due to decreased/absent/dysfunctional platelet GPIb/IX expression with decreased/absent platelet aggregation with ristocetin.	Autosomal Recessive
	***GP1BB*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Autosomal Dominant
GP9	**Bernard Soulier syndrome (BSS):** macrothrombocytopenia with normal platelet granularity and moderate to severe bleeding due to decreased/absent/dysfunctional platelet GPIb/IX expression with decreased/absent platelet aggregation with ristocetin.	Autosomal Recessive
	***GP9*-related macrothrombocytopenia:** mild to moderate thrombocytopenia with absent/mild bleeding	Autosomal Dominant

Indications for testing:

Bernard Soulier Syndrome Panel (NGS and/or aCGH), order code 4880:

The Bernard Soulier Panel should be considered:

- In patients presenting with the specific phenotype of lifelong moderate/severe bleeding, macrothrombocytopenia with normal platelet granularity, decreased/absent platelet aggregation with ristocetin and/or decreased GPIb expression on platelet flow cytometry.
- To clarify possible carrier status in individuals who have a family history of a Bernard Soulier syndrome, but the causal familial variant(s) is unknown.

Single Gene Analysis (order code 4855) or Custom Blood Disorder Panel (Order Code 4850), (NGS and/or aCGH):

Analysis of genes included in this panel may also be ordered as a standalone Single Gene Analysis or as a Custom Blood Disorder Panel (2-10 genes), by NGS and/or by aCGH, as dictated by the patient's clinical and laboratory phenotype, as well as their ancestry, or to supplement previous genetic testing.

Targeted Familial Variant Analysis (order code 4970):

Targeted variant analysis for clinical diagnosis, carrier identification, or prenatal diagnosis can also be performed on any gene in the panel when the pathogenic variant(s) is known in the family. If the proband was not tested at Versiti, a control sample is preferred and may be required (please call the laboratory to discuss). If the familial variant is a large deletion or duplication, aCGH for the involved gene is required.

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, to be directed to our genetic counselors and clinical support team.

Test method:

NGS: This next-generation sequencing assay analyzes the complete coding region of 3 genes plus a minimum 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 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.

aCGH: The specific genes are analyzed for copy number variations due to deletion or duplication by high density gene-focused array Comparative Genomic Hybridization. Probes are approximately 60bp in length and density of coverage in exonic regions is a minimum of 4 probes per 500 bp. Genomic DNA for the samples and gender-matched references are denatured, labeled with fluorescent dye and hybridized, the array is washed and scanned, and analysis is performed for the specific genes requested.

Assay sensitivity and limitations:

NGS: 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.

aCGH: Balanced chromosomal rearrangements (i.e., translocations, inversions) or point mutations that may be the cause of the clinical phenotype cannot be detected via aCGH. Any exonic deletion or duplication smaller than 500bp may not be detected. Low level of mosaicism will not be detected by aCGH. Probe performance could be affected by multiple SNPs in a given region. Breakpoints occurring outside the targeted gene(s) will not be defined.

Clinical Sensitivity

The clinical sensitivity of comprehensive genetic testing (NGS and aCGH) of the 3 genes known to be associated with BSS is highest in patients with the classic presentation of bleeding, macrothrombocytopenia with normal granularity, decreased/absent platelet aggregation with ristocetin and decreased GPIb expression on platelet flow cytometry. The autosomal dominant macrothrombocytopenia phenotype is not specific and cannot be easily clinically distinguished from other inherited thrombocytopenias with normal/large platelet size. In that specific context, the clinical diagnostic yield of this panel is expected to be low and the Inherited Thrombocytopenia Panel or Autosomal Dominant Thrombocytopenia Panel is recommended, as it would have higher clinical sensitivity.

Reporting of Results

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards and guidelines. 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); copy number variants are described in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).

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 St
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

Test code: 4880

For suggested CPT codes, visit the 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, Option 1, or LabInfo@versiti.org

References

Bernard Soulier syndrome references

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Variant interpretation references

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