

# Platelet Disorders Gene Sequencing Panel

ABCG5	ABCG8	ACBD5	ACTN1	ANKRD26	ANO6 (TMEM16F)
AP3B1	AP3D1	ARPC1B	BLOC1S3	BLOC1S6	CD36
CYCS	DIAPH1	DTNBP1	ETV6	FERMT3	FLI1
FLNA	FYB1	GATA1	GF1B	GP1BA	GP1BB
GP6	GP9	HOXA11	HPS1	HPS3	HPS4
HPS5	HPS6	ITGA2	ITGA2B	ITGB3	LYST (CHS1)
MASTL	MECOM	MPIG6B	MPL	MYH9	NBEA
NBEAL2	ORAI1	P2RX1	P2RY1	P2RY12	PLA2G4A
PRKACG	PTGS1	RAB27A	RASGRP2	RBM8A	RUNX1
SLFN14	STIM1	STX11	STXBP2	TBXA2R	TBXAS1
THPO	TUBB1	UNC13D	VIPAS39	VPS33B	VPS45 WAS

## Description:

The Platelet Disorders Gene Sequencing Panel utilizes Exome Sequencing (ES) technology to identify inherited forms of platelet dysfunction. Utilizing a predefined list of 67 clinically significant genes, this panel analyzes mutations related to adhesion and activation receptor genes, secretion and membrane regulation genes, and platelet production genes related to genetically inherited platelet disorders. Compared to ES, this targeted approach results in fewer sequence changes identified: allowing for a shorter turnaround time and decreased cost of testing. This test will be performed on the proband only and will not include the identification of incidental findings.

## Indications:

- Platelet dysfunction/defect
- Abnormal bleeding
- Unexplained thrombocytopenia
- Easy bruising/spontaneous ecchymoses
- Positive family history of bleeding disorders or platelet function disorders

## What Is Reported?

### Variants that will be discussed in detail in the report:

- **Pathogenic/likely pathogenic variants:** Variants that are known to be pathogenic or for which the laboratory has sufficient evidence suggesting pathogenicity.

### Variants that will be listed in the report:

- Variants of uncertain clinical significance.

### What is not reported?

- Variants in genes not included in the predefined gene list
- Variants where there is currently no evidence of association with the disease and that are identified in healthy individuals (benign or likely benign variants)
- Variants that predict an increased risk of diseases, but do not cause a disease by themselves (risk alleles).

**Note:** Platelet Disorders Panel cases with negative or uncertain findings can be reflexed to Whole Exome Sequencing (WES). A separate test order and a signed consent form is required for all WES testing. In addition, including biological parental samples is strongly encouraged to assist with the analysis of WES and to increase test yield. Reflex to WES orders can either be placed simultaneously or separately. Separate reflex to WES orders are subject to review prior to the initiation of testing. Please see our website at [www.cincinnatichildrens.org/exome](http://www.cincinnatichildrens.org/exome) to obtain a WES test requisition and consent form.

## Genetic Conditions Commonly Associated with Platelet Disorders

Gene	Inheritance	Condition
<i>ABCG5</i>	AR	Macrothrombocytopenia and sitosterolemia
<i>ABCG8</i>	AR	Macrothrombocytopenia and sitosterolemia
<i>ACBD5</i>	AD	Autosomal Dominant Thrombocytopenias
<i>ACTN1</i>	AD	Congenital Macrothrombocytopenia
<i>ANKRD26</i>	AD	Autosomal Dominant Thrombocytopenias
<i>ANO6 (TMEM16F)</i>	AR	Scott Syndrome
<i>AP3B1</i>	AR	Hermansky-Pudlak syndrome
<i>AP3D1</i>	AR	Hermansky-Pudlak syndrome 10
<i>ARPC1B</i>	AR	Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease
<i>BLOC1S3</i>	AR	Hermansky-Pudlak syndrome
<i>BLOC1S6</i>	AR	Hermansky-Pudlak syndrome
<i>CD36</i>	AR	Platelet glycoprotein IV deficiency
<i>CYCS</i>	AD	Autosomal Dominant Thrombocytopenias
<i>DIAPH1</i>	AD	Macrothrombocytopenia and hearing loss
<i>DTNBP1</i>	AR	Hermansky-Pudlak syndrome
<i>ETV6</i>	AD	Thrombocytopenia and cancer susceptibility
<i>FERMT3</i>	AR	Leukocyte adhesion deficiency, type III
<i>FLI1</i>	AD/AR	Paris-Trousseau (Jacobson) Syndrome, bleeding disorder
<i>FLNA</i>	X linked	X-linked thrombocytopenia with PVNH
<i>FYB1</i>	AR	Thrombocytopenia 3
<i>GATA1</i>	X linked	X-linked thrombocytopenia
<i>GF11B</i>	AD/AR	Gray platelet syndrome, bleeding disorder
<i>GP1BA</i>	AD/AR	Bernard-Soulier syndrome, Platelet-type von Willebrand's disease
<i>GP1BB</i>	AR	Bernard-Soulier syndrome, giant platelet disorder
<i>GP6</i>	AR	GPVI deficiency
<i>GP9</i>	AR	Bernard-Soulier syndrome
<i>HOXA11</i>	AD	Amegakaryocytic thrombocytopenia radio-ulnar synostosis
<i>HPS1</i>	AR	Hermansky-Pudlak syndrome
<i>HPS3</i>	AR	Hermansky-Pudlak syndrome
<i>HPS4</i>	AR	Hermansky-Pudlak syndrome
<i>HPS5</i>	AR	Hermansky-Pudlak syndrome
<i>HPS6</i>	AR	Hermansky-Pudlak syndrome

## Genetic Conditions Commonly Associated with Platelet Disorders (continued)

Gene	Inheritance	Condition
<i>ITGA2</i>	AD	Glycoprotein Ia deficiency
<i>ITGA2B</i>	AD/AR	Glanzmann's thrombasthenia, bleeding disorder
<i>ITGB3</i>	AD/AR	Glanzmann's thrombasthenia, bleeding disorder
<i>LYST (CHS1)</i>	AR	Chediak-Higashi syndrome
<i>MASTL</i>	AD	Autosomal Dominant Thrombocytopenias
<i>MECOM</i>	AD	Radioulnar synostosis with amegakaryocytic thrombocytopenia 2
<i>MPIG6B</i>	AR	Thrombocytopenia, anemia, and myelofibrosis
<i>MPL</i>	AD/AR	Congenital amegakaryocytic thrombocytopenia
<i>MYH9</i>	AD	MYH9 Disorders
<i>NBEA</i>	AD	Autism and dense granule deficiency
<i>NBEAL2</i>	AR	Gray platelet syndrome
<i>ORAI1</i>	AD/AR	Stormorken Syndrome
<i>P2RX1</i>	n/a	ADP receptor defects
<i>P2RY1</i>	AR?	Moderate platelet-related bleeding phenotype with diminished platelet responsiveness to thrombin and thrombin-mimetic peptides in vitro
<i>P2RY12</i>	AR	ADP receptor defects, bleeding disorder
<i>PLA2G4A</i>	AR	Cytosolic phospholipase A2, Deficiency of phospholipase A2 group IVA
<i>PRKACG</i>	AR	Congenital Macrothrombocytopenia
<i>PTGS1</i>	AD/AR	Platelet-type bleeding disorder 12; Prostaglandin-endoperoxide synthase 1 deficiency
<i>RAB27A</i>	AR	Griscelli syndrome, type 2
<i>RASGRP2</i>	AR	Impaired RAP1 activation and $\alpha_{IIb}\beta_3$ signaling, bleeding disorder
<i>RBM8A</i>	AR	Thrombocytopenia absent radius (TAR) syndrome
<i>RUNX1</i>	AD	Thrombocytopenia and AML susceptibility
<i>SLFN14</i>	AD	Bleeding disorder, platelet-type, 20
<i>STIM1</i>	AD/AR	Stormorken Syndrome
<i>STX11</i>	AR	Familial HLH types 4
<i>STXBP2</i>	AR	Familial HLH types 5
<i>TBXA2R</i>	AD	Thromboxane A2 receptor deficiency
<i>TBXAS1</i>	AD/AR	Thromboxane A synthase (Ghosal syndrome), Thromboxane Synthase deficiency
<i>THPO</i>	AD	Cyclic Thrombocytopenia
<i>TUBB1</i>	AD	Congenital Macrothrombocytopenia
<i>UNC13D</i>	AR	Familial HLH types 3
<i>VIPAS39</i>	AR	ARC Syndrome, Arthrogyrosis-renal dysfunction-cholestasis syndrome
<i>VPS33B</i>	AR	Arthrogyrosis-renal dysfunction-cholestasis syndrome
<i>VPS45</i>	AR	Congenital neutropenia & platelet a granule defect
<i>WAS</i>	X linked	Wiskott-Aldrich syndrome

## Methodology:

**Procedure:** Platelet disorders gene sequencing panel uses Human Comprehensive Exome kit from Twist Bioscience to capture the exonic regions of genes from the genomic DNA extracted from the patient. Targeted regions are sequenced using an Illumina sequencing system with paired-end reads. Sequence reads are aligned to the human reference genome (build UCSC hg19). Variants within exons and flanking sequences are identified and evaluated by a validated in-house developed bioinformatics analysis pipeline that includes the usage of GATK and Fabric Genomic Analysis platform. Mutations in the promoter region of ANKRD26 are analyzed; allele specific analysis for the 253kb inversion as well as targeted analysis of the c.118-308 region in UNC13D are performed. Data quality is assessed to confirm it has a minimum coverage of 20X for >95% of targets of interest.

### Technical Limitations:

- Pathogenic variants may be present in a portion of the genes not covered by this test or in regions with suboptimal data due to homologous issue, polynucleotides, or nucleotide repeats, and therefore may not be identified. Thus, the absence of identified pathogenic variants does not exclude the possibility of a genetic etiology for the patient's symptoms.
- Certain types of mutations are not detected. Only single base pair changes or small insertions or deletions of DNA are detected. Large deletions, duplications, or rearrangements, mitochondrial genome mutations, repeat expansions, low level mosaicism and many epigenetic defects may not be detected by this test.

### Regions of Homology

These gene regions with homology may generate suboptimal data with potential false negative results.

GENE	TRANSCRIPT	EXON	CHROM	EXON_START	EXON_END
RBMSA	NM_005105	6	1	145509165	145509211

### Low coverage (<20X) regions

GENE	TRANSCRIPT	EXON	CHROM	EXON_START	EXON_END
GP6	NM_001083899	8	19	55525449	55526533
VPS45	NM_001279353	13	1	150115015	150115109

**Please note:** These regions represent the low coverage (<20X) regions identified during our test validation. For specific patient cases, these regions may vary.

Note: Targeted deletion and duplication analysis of every gene on this panel except *FERMT3*, *GP6*, *GP9*, *HPS5*, *HPS6*, *MPIG6B*, *P2RY1*, and *PTGSI* is clinically available at an additional charge.

## Turn-Around Time:

56 days (8 weeks)

## Specimen:

At least 3 mls whole blood in a lavender top (EDTA) tube or saliva in an Oragene saliva kit. Please call the lab at 513-636-4474 for a free saliva collection kit. Label the tube with the patient's name, birth date, and date of collection. Alternatively, 10 mcg of DNA may be submitted.

**We are unable to accept blood samples collected within two (2) weeks of a transfusion.**

## CPT Codes:

- **Platelet Disorders Gene Sequencing Panel:** 81443
- **Deletion and duplication analysis of any single Gene on the Platelet Disorders Gene Sequencing Panel except *GPIBB* and *WAS*:** 81479
- **Deletion and duplication analysis of *GPIBB*:** 81404
- **Deletion and duplication analysis of *WAS*:** 81406

## Shipping Instructions:

Please enclose **test requisition** with sample. **All information must be completed before sample can be processed.**

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Saturday.

## Ship to:

Genetics and Genomics Diagnostic Laboratory  
3333 Burnet Avenue NRB 1042  
Cincinnati, OH 45229  
513-636-4474

## References:

Bolton-Maggs, P.H.B., E.A. Chalmers, et al. (2006) *A Review of Inherited Platelet Disorders with Guidelines for Their Management on Behalf of the UKHCDO*. *British Journal of Haematology* 135(5): 603–33.

Dixon-Salazar TJ, Silhavy JL, et al. (2012) *Exome sequencing can improve diagnosis and alter patient management*. *Sci Transl Med*. 4(138):138ra78. Handin, R.I. (2005) *Inherited Platelet Disorders*. *Hematology. American Society of Hematology. Education Program*: 396–402.

Freson, K. and Turro, E. (2017) *High-throughput sequencing approaches for diagnosing hereditary bleeding and platelet disorders*. *Journal of Thrombosis and Haemostasis* 15(7): 1262–72.

Nurden, A.T. and P. Nurden (2014) *Congenital Platelet Disorders and Understanding of Platelet Function*. *British Journal of Haematology* 165(2): 165–78.

Romasko, E.J., B. Devkota, et al. (2018) *Utility and Limitations of Exome Sequencing in the Molecular Diagnosis of Pediatric Inherited Platelet Disorders*. *American Journal of Hematology* 93(1):8-16.