



CONGENITAL THROMBOCYTOPENIA MPL, WAS, RUNX1 AND MYH9 SEQUENCE ANALYSIS

BloodCenter of Wisconsin offers DNA sequencing of the MPL, MYH9, RUNX1, and WAS genes for diagnosis of congenital thrombocytopenia.

BACKGROUND:

Congenital Amegakaryocytic Thrombocytopenia (CAMT) is a rare autosomal recessive bone marrow failure syndrome characterized by thrombocytopenia in neonatal period. CAMT is not associated with other physical abnormalities. Many patients develop pancytopenia in childhood. CAMT is caused by mutations in the MPL gene that encodes the thrombopoietin receptor (TPOR). MPL mutations are the only known cause of CAMT. Over 95% of CAMT patients have homozygous MPL mutations. In contrast, rare autosomal dominant germline mutations in MPL have been shown to cause thrombocythemia.

RUNX1 is a transcription factor and a key regulator of hematopoiesis and myeloid differentiation. Germline mutations in the RUNX1 gene result in a rare autosomal dominant familial platelet disorder (FPD) characterized by mild to moderate thrombocytopenia, qualitative platelet defects and a predisposition to development of myeloid malignancies. RUNX1 mutations are found in the majority (>90%) of pedigrees with FPD.

The WAS-related disorders include Wiskott-Aldrich syndrome (WAS), X-linked thrombocytopenia (XLT) and X-linked neutropenia (XLN). These disorders predominantly affect platelets and lymphocytes and are caused by mutations in the WAS gene. WAS and XLT are characterized by thrombocytopenia and small platelets. Other features of WAS include, recurrent infections, eczema, and an autoimmune disorder. WAS-related disorders exhibit X-linked recessive inheritance. Most affected individuals will be male and female carriers are usually asymptomatic except in cases of skewed X-inactivation. DNA sequence analysis detects approximately 97% of mutations in these disorders.

MYH9-related disorders (MYH9-RD) are a group of disorders characterized by autosomal dominant inheritance, large platelets, thrombocytopenia and mutations in the MYH9 gene. The group includes May-Hegglin Anomaly, Sebastian, Fechtner and Epstein syndromes and DNFA17. Other clinical symptoms associated with MYH9-RD include hearing loss, glomerulonephritis and presenile cataract. DNA sequence analysis of MYH9 detects approximately 99% of reported mutations in MYH9-related disorders. Approximately, 35% of cases are sporadic; half of the cases have a documented *de novo* mutation. Some genotype/phenotype correlations predict severity of clinical symptoms.

REASONS FOR REFERRAL:

- Confirmation of diagnosis
- Evaluation of family members
- Prenatal diagnosis

METHOD:

PCR amplification and bi-directional DNA sequence analysis is performed. The complete coding region and splice junction of each exon is compared to the reference sequence and the functional implications of sequence variations are characterized using data in the Human Gene Mutation Database (HGMD).

LIMITATIONS:

Large deletions or duplications and mutations that are outside the regions sequenced will not be detected by these assays. Large deletions or duplications are detected using the aCGH deletion/duplication Analysis (test code 4800) Rare polymorphisms within primer or probe regions may interfere with detection of gene variants. Sensitivity will be highest in patients with the classic clinical symptoms defining these disorders with the expected inheritance pattern.

MPL mutations are the only known cause of CAMT. The sensitivity of MPL sequence analysis is >97%. A promoter mutation has been described that is not detected by this assay.

The exact clinical sensitivity of RUNX1 sequence analysis is unknown. The majority (>90%) of FPD pedigrees have RUNX1 mutations. Large deletions and duplications have been described that are not detected by sequencing.

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LIMITATIONS CONT:

The sensitivity of WAS sequence analysis is approximately 98% for patients with WAS-related disorders. Large deletions, insertions and rearrangements (2% of cases) are not detected by this assay.

One large MYH9 deletion/duplication has been reported in MYH9-RD that is not detected by sequencing.

REPORTING OF RESULTS:

All hematologic disorder related variants predicted to be pathogenic, likely pathogenic, variant of unknown significance and likely benign will be reported. Variants classified as benign are not to be report but are available upon request. Results are reported in accordance with ACMG next-generation sequencing standards.

A comprehensive database of gene-phenotype relationships listed by gene name can be found at <http://www.omim.org>.

SPECIMEN REQUIREMENTS:

5 ml EDTA (lavender top) whole blood OR high quality DNA (≥ 500 ng at 25 ng/uL)
Contact the laboratory to discuss prenatal sample requirements.

TURNAROUND TIME: 14 days

CPT CODES:

MPL Sequence Analysis
CPT Code: 81479

MYH9 Sequence Analysis
CPT Code: 81479

RUNX1 Sequence Analysis
CPT Code: 81479

WAS Sequence Analysis
CPT Code: 81406

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, 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/Platelet Neutrophil Immunology Laboratory
BloodCenter of Wisconsin
638 N. 18th St.
Milwaukee, WI 53233
Phone: 800-245-3117, ext. 6250

REFERENCES:

- Ballmaier M and Germeshausen M, Advances in the understanding of congenital amegakaryocytic thrombocytopenia (2009) BJH 146: 3-16.
- Owen C et al, Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy (2008) Blood 112, 4639-4645.
- Filipovich, A et al, WAS-Related Disorders (2007) Gene Reviews <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=was>
- Balduini et al, Recent advances in the understanding and management of MYH9-related inherited thrombocytopenias. (2011) BJH 154:161-174
- Savoia, A et al, Heavy chain myosin -9 related disease (MYH9-RD): neutrophil inclusions of myosin -9 as a pathognomonic sign of the disorder. Thrombosis and Haemostasis 103.4/2010
- Rehm, H.L., et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med 2013; 15: 734-737. doi:10.1038/gim.2013.92

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