Test Definition: THBNG

THBD Gene, Full Gene NGS

Overview

Useful For

Ascertaining a pathogenic alteration in the *THBD* gene in patients with atypical hemolytic uremic syndrome

Ascertaining a pathogenic alteration in the *THBD* gene in patients with reduced thrombin generation and a strong family or personal history of excessive bleeding that is not explained by results of conventional and specialized coagulation testing

This test is **not intended for** prenatal diagnosis

Genetics Test Information

This test detects alterations in the *THBD* gene, which have been associated with one of several clinical phenotypes and hereditary disorders, namely an increased risk for thrombosis, atypical hemolytic uremic syndrome (aHUS), and hereditary excessive bleeding following physical trauma or surgery. A genetic consultation is strongly recommended prior to ordering *THBD* sequencing.

The gene target for this test is:

Gene name (transcript): *THBD* (GRCh37 (hg19) NM_000361)

Chromosomal location: 20p11.21

Testing Algorithm

No screening tests exist for defects in *THBD*.

If thrombotic tendency is a concern, a set of clinical guidelines from the British Society for Haematology on testing for heritable thrombophilia is freely available.(1)

If atypical hemolytic uremic syndrome (aHUS) is a concern, it is strongly recommended an etiological diagnosis of aHUS be attempted prior to genetic testing in order to focus on timely and informed treatment of the patient. Refer to the consensus statement on the identification and diagnosis of thrombotic microangiopathies by the Mayo Clinic Complement Alternative Pathway-Thrombotic Microangiopathy Disease-Oriented Group.(2) Additionally, a recommended list of laboratory investigations for patients identified as having aHUS has been developed by The European Paediatric Study Group.(3)

If a *THBD*-related bleeding disorder is a concern, this disorder has only been recently characterized and no algorithmic testing methodology has been developed that is specific to this disorder. A systematic diagnosis through conventional coagulation testing is recommended prior to considering genetic testing for any suspected bleeding disorder.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)
- Rare Coagulation Disorder Patient Information

Method Name

Custom Sequence Capture and Targeted Next-Generation Sequencing (NGS) Followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing when Appropriate

NY State Available

Yes

Specimen

Specimen Type

Varies

Advisory Information

Routine genetic testing of *THBD* for either a thrombotic or bleeding tendency is not recommended and may be of limited use in the vast majority of cases.

Shipping Instructions

Ambient and refrigerated specimens must arrive within 7 days (168 hours of draw), and frozen specimens must arrive within 14 days (336 hours of draw). Collect and package specimen as close to shipping time as possible.

Necessary Information

Rare Coagulation Disorder Patient Information is required, see Special Instructions. Testing may proceed without the patient information, however, the information aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to fill out the form and send with the specimen.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Peripheral blood

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD) or green top (sodium citrate)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimen in original tube.

Specimen Stability: Ambient (preferred)/Refrigerate/Frozen

Specimen Type: Extracted DNA

Container/Tube: 1.5- to 2-mL tube with indication of volume and concentration of the DNA.

Specimen Volume: Entire specimen

Collection Instructions:

1. Label specimen as extracted DNA and source of specimen.

2. Provide indication of volume and concentration of the DNA.

Specimen Stability: Frozen (preferred)/Refrigerate/Ambient

Forms

1. Rare Coagulation Disorder Patient Information **(T824) is required,** see Special Instructions. Fax the completed form to 507-284-1759.

2. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available in Special Instructions:

-Informed Consent for Genetic Testing (T576)

-Informed Consent for Genetic Testing-Spanish (T826)

3. If not ordering electronically, complete, print, and send a Coagulation Test Request (T753) with the specimen.

Specimen Minimum Volume

Blood: 1 mL
Extracted DNA: 100 mcL at 50 ng/mcL concentration

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)	7 days	
	Frozen	14 days	
	Refrigerated	7 days	

Clinical and Interpretive

Clinical Information

Thrombomodulin (TM) is an endothelial cell membrane protein found mainly in capillary beds that functions as a co-factor to thrombin in the activation of protein C. TM binds to thrombin and switches its substrate specificity from procoagulant substrates fibrinogen, factor V, factor VIII, and platelets to anticoagulant protein C, enhancing activation by 1000-fold. Activated protein C downregulates further thrombin generation, suppressing clot formation. TM also has an anti-inflammatory role as a negative regulator of the complement arm of the innate immune system. TM enhances activation of thrombin activatable fibrinolysis inhibitor (TAFI, also known as procarboxypeptidase B), which inhibits fibrinolysis and inactivates complement-derived anaphlatoxins C3a and C5a. TM, in a thrombin-independent

manner, also interferes with inflammation by suppressing leukocyte trafficking and dampening complement activation through lectin-like domain (4).

Despite its role in coagulation, it is unclear whether thrombomodulin gene alterations play a significant role in venous thromboembolism (5). The *THBD* gene encodes thrombomodulin and Â pathogenic alterations in the gene appear rare among thrombophilic patients, even those with severe thrombophilia (4). No severe TM deficiencies have been identified in patients with thrombosis, indicating that thrombomodulin alterations, in the absence of protein C deficiency, might not be associated with large vessel thrombosis (5). Since strong genotype-phenotype correlation has yet to be demonstrated between these alterations and venous thrombosis, indiscriminate testing for alterations in *THBD* or other genes related to coagulation in unselected patients presenting with a first episode of venous thrombosis is not recommended. A set of clinical guidelines from the British Society for Haematology on testing for heritable thrombophilia (1) is freely available. There is somewhat stronger evidence of an association between thrombomodulin gene polymorphisms and a moderately increased risk for arterial thrombosis, although more studies including a larger number of patients are needed to more firmly establish this risk (5).

Some alterations in *THBD* are associated with atypical hemolytic uremic syndrome (aHUS), which is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. An estimated 5% of patients with aHUS have alterations in THBD (4). However, it appears that an alteration of a single *THBD* allele is not sufficient by itself to cause aHUS and additional factors are probably required, such as virus-like illness (4). A method to screen patients of aHUS based on presentation and a recommended list of investigations has been developed by The European Paediatric Study Group for HUS (3).

Finally, a specific alteration in *THBD*, c.1611C>A (p.Cys537*), is associated with an autosomal dominant hereditary bleeding disorder characterized by excessive bleeding following physical trauma or surgery and extremely elevated levels of soluble TM (6,7,8). As of January 2019, this is the only alteration in *THBD* associated with a bleeding disorder reported in the Human Gene Mutation Database (HGMD Professional 2018.4). A systematic diagnosis through conventional coagulation testing is recommended prior to considering genetic testing for any suspected bleeding disorder. *THBD*-related bleeding is associated with normal results for these tests (i.e., normal prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen assays) (7). Individuals with *THBD*-related bleeding have been observed to have normal results for the following assays as well: Coagulation factors II, V, VII, VIII, IX, X, XI, XII, and XIII, von Willebrand factor antigen, von Willebrand cofactor activity, plasma antithrombin, protein C and S levels, and activated protein C ratio (7). Additionally, there should be no evidence of platelet dysfunction. In individuals with *THBD*-related bleeding, prothrombin consumption index has been observed to be elevated (7) and levels of soluble TM are extremely elevated (6). If an assessment of thrombin generation and measurement of plasma TM levels finds reduced thrombin generation or there are extremely high levels of soluble TM and no other explanation for bleeding can be found, a diagnosis of *THBD*-related bleeding should be considered and molecular testing is clinically indicated (7,9).

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided.

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics and Genomics recommendations as a guideline. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Consultations with the Mayo Clinic Special Coagulation Clinic, Molecular Hematopathology Laboratory, or Thrombophilia Center are available for DNA diagnosis cases. This may be especially helpful in complex cases or in situations where the diagnosis is atypical or uncertain.

Cautions

Clinical:

Some individuals may have a mutation that is not identified by the methods performed. The absence of a mutation, therefore, does not eliminate the possibility of thrombophilia or atypical hemolytic uremic syndrome (aHUS). This assay does not distinguish between germline and somatic alterations, particularly with variant allele frequencies (VAF) significantly lower than 50%. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If there is a family history of thrombophilia or atypical hemolytic uremic syndrome, it is often useful to test first-degree family members to help establish the clinical significance of variants of unknown significance.

Technical Limitations:

Next generation sequencing (NGS) may not detect all types of genetic variants. Additionally, rare polymorphisms may be present that could lead to false negative or positive results. Therefore test results should be interpreted in the context of activity and antigen measurements, clinical findings, family history, and other laboratory data. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/duplication analysis. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If multiple alterations are identified, NGS is not able to distinguish between alterations that are found in the same allele ("in cis") and alterations found on different alleles ("in trans"). This limitation may complicate diagnosis or classification and has implications for inheritance and genetic counseling. To resolve these cases, molecular results must be correlated with clinical history, activity and antigen measurements, and/or family studies.

Unless reported or predicted to cause disease, alterations found deep in the intron or alterations that do not result in an amino acid substitution are not reported. These and common polymorphisms identified for this patient are available upon request.

Reclassification of Variants Policy: At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

Contact the laboratory if additional information is required regarding the transcript or human genome assembly used for the analysis of this patient's results.

Clinical Reference

1. Baglin T, Gray E, Greaves M, et al: Clinical guidelines for testing for heritable thrombophilia. Br J Haematol 2010 Apr;149(2):209-220

2. Go RS, Winters JL, Leung N, et al: Thrombotic Microangiopathy Care Pathway: A Consensus Statement for the Mayo Clinic Complement Alternative Pathway-Thrombotic Microangiopathy (CAP-TMA) Disease-Oriented Group. Mayo Clin Proc. 2016; Sep;91(9):1189-1211. doi: 10.1016/j.mayocp.2016.05.015

3. Ariceta G, Besbas N, Johnson S, et al: Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatr Nephrol 2009 Apr;24(4):687-696. doi: 10.1007/s00467-008-0964-1

4. Delvaeye M, Noris M, De Vriese A, et al: Thrombomodulin mutations in atypical hemolytic-uremic syndrome. N

Engl J Med. 2009 Jul 23;361(4):345-357

5. Anastasiou G, Gialeraki A, Merkouri E, et al: Thrombomodulin as a regulator of the anticoagulant pathway: implication in the development of thrombosis. Blood Coagul Fibrinolysis. 2012 Jan;23(1):1-10. doi: 10.1097/MBC.0b013e32834cb271

6. Dargaud Y, Scoazec JY, Wielders SJ, et al: Characterization of an autosomal dominant bleeding disorder caused by a thrombomodulin mutation. Blood. 2015;125(9):1497-1501. doi: 10.1182/blood-2014-10-604553

7. Langdown J, Luddington RJ, Huntington JA, Baglin TP: A hereditary bleeding disorder resulting from a premature stop codon in thrombomodulin (p.Cys537Stop). Blood 2014 Sep 18;124(12):1951-1956

8. Maclachlan A, Dolan G, Grimley C, el al: On Behalf of the UK GAPP Study Group: Whole exome sequencing identifies a mutation in thrombomodulin as the genetic cause of a suspected platelet disorder in a family with normal platelet function. Platelets 2017 Sep;28(6):611-613. doi: 10.1080/09537104.2017.1283011

9. Philippou H: Unexplained bleeding: another player to look out for! Blood 2014 Sep 18;124(12);1850-1851

10. Faioni EM, Franchi F, Castaman G, et al: Mutations in the thrombomodulin gene are rare in patients with severe thrombophilia. Br J Haematol. 2002 Aug;118(2):595-599

11. Kunz G, Ireland HA, Stubbs PJ, et al: Identification and characterization of a thrombomodulin gene mutation coding for an elongated protein with reduced expression in a kindred with myocardial infarction. Blood 2000 Jan 15;95(2):569-576

Performance

Method Description

Next generation sequencing (NGS) and/or Sanger sequencing are performed.

Regions of homology, high guanine-cytosine (GC)-rich content, and repetitive sequences may not provide accurate sequence. Therefore, all reported alterations detected by NGS in these regions are confirmed by an independent reference method. However, this does not rule out the possibility of a false-negative result in these regions.

Sanger sequencing is used to confirm alterations detected by NGS when appropriate.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Performed weekly, Varies

Analytic Time

21 days

Maximum Laboratory Time

28 days

Specimen Retention Time

Whole Blood: 2 weeks; DNA: Indefinitely

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

81479

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
THBNG	THBD Gene, Full Gene NGS	92995-0

Result ID	Test Result Name	Result LOINC Value
113108	THBNG Result	50397-9
113102	Alterations Detected	82939-0
113101	Interpretation	69047-9
113103	Additional Information	48767-8
113104	Method	85069-3
113105	Disclaimer	62364-5
113106	Panel Gene List	48018-6
113107	Reviewed By	18771-6