Test Definition: KCNN4

KCNN4 Full Gene Sequencing, V

Overview

Useful For

Genetic confirmation of a dehydrated hereditary stomatocytosis (DHSt) 2/hereditary xerocytosis diagnosis with the identification of an alteration known or suspected to cause disease in the *KCNN4* gene

Second-tier testing for patients in whom previous targeted gene variant analyses were negative for a specific RBC membrane disorder

Establishing a diagnosis of a hereditary RBC membrane disorder, allowing for appropriate management and surveillance of disease features based on the gene involved, especially if splenectomy is a consideration

Genetics Test Information

This test detects pathogenic alterations within the *KCNN4* gene which are associated with hereditary stomatocytosis.

The gene target for this test includes the following:

Gene name (transcript): *KCNN4* (GRCh37 (hg19) NM_002250)

Chromosomal location: chr19q13.31

Highlights

This test should be used as an adjunct to abnormal RBC membrane studies.

-Variants in *KCNN4* are associated with rare red cell membrane disorder dehydrated hereditary stomatocytosis, which causes chronic hemolytic anemia.

-Informative protein studies (eg, osmotic fragility, ektacytometry) and peripheral blood findings, correlated with the patient clinical history, should be performed prior to genetic testing.

Special Instructions

- Informed Consent for Genetic Testing
- Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information
- Informed Consent for Genetic Testing (Spanish)

Method Name

Polymerase Chain Reaction (PCR) Amplification /Sanger Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Necessary Information

The following information is required on patient information or test request form:

1. Clinical diagnosis

2. Pertinent clinical history (submit CBC results and relevant clinical notes)

3. Differentials based on clinical or morphologic presentation

4. Date of collection

5. Specimen type, whole blood or extracted DNA

Specimen Required

Submit only 1 of the following specimens:

Preferred:

Specimen Type: Peripheral blood

Container/Tube:

Preferred: Lavender top (EDTA) or yellow top (ACD)

Acceptable: Green top (heparin)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimen in original tube.

3. Label specimen as blood.

Specimen Stability Information: Ambient 14 days (preferred) or Refrigerated < or =30 days

Acceptable:

Specimen Type: Extracted DNA from whole blood

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

Specimen Volume: Entire specimen

Collection Instructions: Label specimen as extracted DNA from blood and provide indication of volume and concentration of the DNA

Specimen Stability Information: Frozen/Refrigerate/Ambient < or =30 days

Forms

1. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information **is required**, see Special Instructions.

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 Benign Hematology Test Request (T755) with the specimen

Specimen Minimum Volume

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

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Bone marrow biopsies Paraffin-embedded tissue Frozen Tissue Slides Paraffin-embedded bone marrow aspirate clot Methanol-acetic acid (MAA)-fixed pellets Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical and Interpretive

Clinical Information

Dehydrated hereditary stomatocytosis (DHSt) 2 (also called hereditary xerocytosis) is an autosomal dominant hereditary hemolytic disorder caused by an abnormal cation leak in the red cell membrane, resulting in loss of a potassium cation and red blood cell dehydration. Pseudohyperkalemia (loss of red cell potassium when cold or at room temperature) can be a feature. Symptoms include compensated to mild hemolytic anemia, moderate splenomegaly, elevated reticulocyte count, increased mean corpuscular volume (MCV), variably increased mean corpuscular hemoglobin concentration (MCHC), perinatal edema, and a tendency for iron overload. Red cells exhibit various shape abnormalities on blood smear, including elliptocytes, schizocytes, and rare stomatocytes.

The majority of symptomatic DHSt cases reported to date have been associated with gain-of-function alterations in the mechanosensitive cation channel gene, *PIEZO1*. However, recent studies have identified families with DHSt associated with variants in the *KCNN4* gene.(1-4) *KCNN4* encodes for the Gardos channel, the erythroid voltage-independent potassium channel that is activated by intracellular calcium.(4) Pathogenic alterations in *KCNN4* result in decreased intracellular total cation and potassium levels. Cases reported have shown abnormal ektacytometry curves typical of hereditary xerocytosis. Clinical features of *KCNN4* patients include hemolytic anemia of variable severity that can be more severe in the perinatal period. Splenectomy may have no efficacy in symptom improvement in the few cases with *KCNN4* variants.(2, 5)

This test is best interpreted in the context of protein studies and peripheral blood findings. This can be provided by also ordering RBCME / Red Blood Cell Membrane Evaluation, Blood. Please fill out the information sheet and indicate that *KCNN4* testing was ordered. Providing CBC data and clinical notes will also allow more precise interpretation of results.

Reference Values

An interpretive report will be provided.

Interpretation

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics and Genomics (ACMG) recommendations as a guideline.(3) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Cautions

Clinical:

Some individuals may have a sequence variant that is not identified by the methods performed. The absence of a variation, therefore, does not eliminate the possibility of hereditary hemolytic anemia or a related disorder. 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 hereditary hemolytic anemia or a related disorder, it is often useful to test first-degree family members to help establish the clinical significance of variants of unknown significance.

At this time, it is not standard practice for the laboratory to systematically review likely pathogenic variants or variants of uncertain significance that have been previously 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.

Technical:

Some genetic or genomic alterations, such as large insertion/deletion (indel) events, copy number alterations, and gene translocation events are not detected by this assay. The depth of coverage may be variable for some target regions, but assay performance below the minimum acceptable criteria or for failed regions will be noted. Additionally, rare polymorphisms may be present that could lead to false-negative or false-positive results. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion and duplication analysis. If the patient has had an allogenic blood transfusion, these results may be inaccurate due to the presence of donor DNA.

Clinical Reference

1. Glogowska E, Lezon-Geyda K, Maksimova Y, et al: Mutations in the Gardos channel (KCNN4) are associated with hereditary xerocytosis. Blood. 2015 Sep 10;126(11):1281-1284. doi: 10.1182/blood-2015-07-657957

2. Andolfo I, Russo R, Manna F, et al: Novel Gardos channel mutations linked to dehydrated hereditary stomatocytosis (xerocytosis). Am J Hematol. 2015 Oct;90(10):921-926. doi: 10.1002/ajh.24117

3. 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 May;17(5):405-424. doi: 10.1038/gim.2015.30

4. Rapetti-Mauss R, Lacoste C, Picard V, et al: A mutation in the Gardos channel is associated with hereditary xerocytosis. Blood 2015 Sep;126(11):1273-1280. doi: 10.1182/blood-2015-04-642496

5. Iolascon A, Andolfo I, Barcellini W, et al: Working Study Group on Red Cells and Iron of the EHA. Recommendations regarding splenectomy in hereditary hemolytic anemias. Haematologica. 2017 Aug;102(8):1304-1313. doi: 10.3324/haematol.2016.161166

6. Andolfo I, Russo R, Rosato BE, et al: Genotype-phenotype correlation and risk stratification in a cohort of 123 hereditary stomatocytosis patients. Am J Hematol. 2018 Dec;93(12):1509-1517. doi: 10.1002/ajh.25276

7. Gallagher PG: Disorders of erythrocyte hydration. Blood. 2017 Dec 21;130(25):2699-2708. doi: 10.1182/blood-2017-04-590810

8. Fermo E, Bogdanova A, Petkova-Kirova P, et al: 'Gardos Channelopathy': a variant of hereditary Stomatocytosis with complex molecular regulation. Sci Rep. 2017 May 11;7(1):1744. doi: 10.1038/s41598-017-01591-w

Performance

Method Description

Total genomic DNA is extracted from the sample and the full *KCNN4* gene is amplified by PCR followed by Sanger sequencing of the 5â€™ and 3â€™UTRs and exons 1-9, including 10 base pairs on each intron/exon boundary.Â Review of the sequence data is performed using a combination of automated calls and manual inspection.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

8 to 10 weeks

Specimen Retention Time

DNA: 3 months

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 US Food and Drug Administration.

CPT Code Information

81479

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
KCNN4	KCNN4 Full Gene Sequencing, V	In Process

Result ID	Test Result Name	Result LOINC Value
607810	Interpretation	82939-0
607811	Signing Pathologist	19139-5