MAYO CLINIC LABORATORIES

Test Definition: RETZZ

RET Full Gene Analysis

Overview

Useful For

Evaluation for patients with a personal or family history suggestive of a multiple endocrine neoplasia type 2 (MEN2) or Hirschsprung disease (HSCR)
Establishing a diagnosis of MEN2 or HSCR allowing for targeted cancer surveillance based on associated risks
Identifying variants within genes known to be associated with MEN2 or HSCR allowing for predictive testing of at-risk family members

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in one gene associated with multiple endocrine neoplasia type 2 and Hirschsprung disease: *RET*. See Method Description for additional details.
Identification of a pathogenic variant may assist with diagnosis, prognosis, clinical management, familial screening, and genetic counseling for multiple endocrine neoplasia type 2 and Hirschsprung disease.

Special Instructions

- Molecular Genetics: Inherited Cancer Syndromes Patient Information
- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing.

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

For a comprehensive hereditary cancer panel that includes the *RET* gene, consider 1 of the following
-ENDCP / Hereditary Endocrine Cancer Panel, Varies
-HPGLP / Hereditary Paraganglioma/Pheochromocytoma Panel, Varies
-THYRP / Hereditary Thyroid Cancer Panel, Varies
Testing for *RET* gene as part of a customized panel is available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.
Targeted testing for familial variants (also called site-specific or known mutations testing) is available for this gene. For more information see FMTT / Familial Mutation, Targeted Testing, Varies.

Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.
Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: Any anticoagulant
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube. **Do not** aliquot.
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated

Forms

1. **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)
2. Molecular Genetics: Inherited Cancer Syndromes Patient Information(T519) in Special Instructions
3. If not ordering electronically, complete, print, and send a Oncology Test Request (T729) with the specimen.

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Minimum Volume

See Specimen Required

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)		

Clinical & Interpretive

Clinical Information

Variants in the *RET* proto-oncogene are associated with two distinct, and in rare cases, overlapping clinical syndromes: multiple endocrine neoplasia type 2 (MEN2) and Hirschsprung disease (HSCR).(1)
Multiple endocrine neoplasia type 2:
MEN2 is an autosomal dominant cancer syndrome, which has classically been divided into 3 subtypes: MEN 2A, MEN 2B, and familial medullary thyroid carcinoma (FMTC). The characteristic features of MEN 2A include medullary thyroid carcinoma (MTC), pheochromocytoma, and primary hyperparathyroidism.(1-3) MEN 2B is characterized by early-onset MTC, pheochromocytoma, mucosal neuromas, and distinctive facies with enlarged lips. Other features of MEN 2B include enlarged nerves of the gastrointestinal tract (ganglioneuromatosis), marfanoid habitus, hypotonia, and corneal nerve thickening.(1-3) FMTC has traditionally been diagnosed in families of MTC in the absence of pheochromocytoma or parathyroid involvement. All MEN2 subtypes are inherited in an autosomal dominant inheritance pattern. The

National Comprehensive Cancer Network and the American Thyroid Association provide recommendations regarding the medical management of individuals with MEN2 syndrome.(1,4)

Hirschsprung disease:

Hirschsprung disease (HSCR) is a congenital disorder of impaired intestinal motility, also known as aganglionic megacolon.(1,5,6) Variable lengths of the colon may be affected, resulting in either total aganglionosis, long-segment HSCR, or short-segment HSCR. HSCR affects approximately 1 in 5000 live births and is resolved via surgical intervention.(1,5,6) HSCR can result from chromosome abnormalities, single gene disorders (both syndromic and non-syndromic), a combination of variants in multiple genes, and unknown causes.(1,5,6) Pathogenic *RET* variants are considered the most common cause of HSCR cases though, particularly in families with multiple cases of HSCR and long segment disease.(1,5,6) It has been reported that up to 50% of familial cases of HSCR and up to 33% of sporadic HSCR cases are due to *RET* germline variants.(1,5,6)

While gain of function variants in *RET* are typically associated with MEN2, loss of function variants have been reported in patients with HSCR including full or partial *RET* gene deletions.(1) In addition to clearly pathogenic *RET* variants that cause HSCR, additional benign variants in *RET* (which may not be causative in themselves) confer increased susceptibility to HSCR.(1)

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics (ACMG) recommendations.(7) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of further testing options or for assistance in the interpretation of these results, Mayo Clinic Laboratory genetic counselors can be contacted at 800-533-1710.

Technical Limitations:

Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Insertions/deletions (indels) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller indels.

Deletion/Duplication Analysis:

This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.
This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.
For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor at 800-533-1710.
If the patient has had an allogeneic hematopoietic stem cell transplant or a recent heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.
Reclassification of Variants:
At this time, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages health care providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time.
Variant Evaluation:
Evaluation and categorization of variants is performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline. Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.
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 periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgement.

Clinical Reference

1. Eng C: Multiple endocrine neoplasia type 2. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. GeneReviews [Internet]. University of Washington, Seattle; 1999. Updated August 15, 2019. Accessed July 7, 2021. Available at www.ncbi.nlm.nih.gov/books/NBK1257/
2. American Thyroid Association Guidelines Task Force, Wells SA, Asa SL, Dralle H, et al: Revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma. Thyroid. 2015 Jun 1; 25(6):567-610
3. Moline J, Eng C: Multiple endocrine neoplasia type 2: an overview. Genet Med. 2011;13(9):755-764
4. Haddad RI, Nasr C, Bischoff L, et al: NCCN Guidelines Insights: Thyroid Carcinoma, Version 2.2018. J Natl Compr Canc Netw. 2018 Dec;16(12):1429-1440
5. Ruiz-Ferrer M, Fernandez RM, Antinolo G, et al: A complex additive model of inheritance for Hirschsprung disease is supported by both RET mutations and predisposing RET haplotypes. Genet Med. 2006;8(11):704-710
6. de Pontual L, Pelet A, Trochet D, et al: Mutations of the RET gene in isolated and syndromic Hirschsprung's disease in human disclose major and modifier alleles at a single locus. J Med Genet. 2006 May;43(5):419-423
7. 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

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of variants in coding regions and intron/exon boundaries of the *RET* gene analyzed, as well as some other regions that have known pathogenic variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for insertions/deletions (indels) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction (PCR)-based quantitative method is performed to test for the presence of deletions and duplications in the gene analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.

The reference transcript for *RET* gene is NM_020975.6. Reference transcript numbers may be updated due to transcript reversioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.(Unpublished Mayo method)

PDF Report

Supplemental

Specimen Retention Time

Whole Blood: 2 weeks (if available); Extracted DNA: 3 months

Performing Laboratory Location

Rochester

Fees & Codes

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

81406