MAYO CLINIC LABORATORIES

Test Definition: NTC3Z

NOTCH3 Gene, Full Gene Analysis, Varies

Overview

Useful For

Establishing a molecular diagnosis in individuals with features of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and *NOTCH3*-related disorders

Identifying disease-causing variants within the *NOTCH3* gene known to be associated with CADASIL and *NOTCH3*-related disorders, allowing for predictive testing of at-risk family members

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *NOTCH3* gene associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and other *NOTCH3*-related disorders. See Method Description for additional details.

Identification of a pathogenic variant may assist with diagnosis, prognosis, clinical management, familial screening, recurrence risk assessment, and genetic counseling for *NOTCH3*-related disorders.

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture testing will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Informed Consent for Genetic Testing
- Molecular Genetics: Neurology Patient Information
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

Method Name

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

NY State Available

Yes

Specimen

Specimen Type
Varies

Ordering Guidance
Targeted testing (also called site-specific or known variant testing) is available for variants identified in this gene. See FMTT / Familial Variant, 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.

Submit only 1 of the following specimens:

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 whole blood specimen in original tube. **Do not aliquot**.
Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblast
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred)/Refrigerated (<24 hours)
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular

Testing. An additional 3 to -4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Blood spot
Container/Tube:
Preferred: Collection card (Whatman Protein Saver 903 Paper)
Acceptable: PerkinElmer 226 (formerly Ahlstrom 226) filter paper or blood spot collection card
Specimen Volume: 5 Blood spots
Collection Instructions:
1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect Dried Blood Spot Samples.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry
Specimen Stability Information: Ambient (preferred)/Refrigerated
Additional Information:
1. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
2. For collection instructions, see Blood Spot Collection Instructions
3. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
4. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

Specimen Type: Saliva
Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.
Supplies: Saliva Swab Collection Kit (T786)
Specimen Volume: 1 Swab
Collection Instructions: Collect and send specimen per kit instructions.
Additional Information: Due to lower concentration of DNA yielded from saliva, it is possible that additional specimen may be required to complete testing.
Specimen Stability Information: Ambient 30 days

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:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Molecular Genetics: Neurology Patient Information
3. If not ordering electronically, complete, print, and send a Neurology Specialty Testing Client Test Request (T732) with the specimen.

Specimen Minimum Volume

See Specimen Required

Reject Due To

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

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small vessel disorder and common genetic cause of stroke and dementia in adults. Onset of clinical symptoms typically occurs in mid-adulthood and may include recurrent ischemic stroke and transient ischemic attacks, cognitive decline that progresses to dementia, migraine with aura, and psychiatric disturbances. Symmetric and progressive white matter hyperintensities, lacunes of presumed vascular origin, and subcortical infarcts are characteristic neuroimaging findings. Granular osmophilic material (GOM) detected by electron microscopy on skin fibroblasts is considered a pathognomonic finding for CADASIL.

Disease-causing variants in the *NOTCH3* gene cause CADASIL. Most individuals with CADASIL inherit the condition from a parent, but rare *de novo* variants have been reported. The family history may appear negative due to variable expressivity of the condition and failure to recognize symptoms in other affected family members. Further, *NOTCH3* is comprised of repetitive epidermal growth-factor like repeat (EGFr) domains. Reported pathogenic variants typically result in either loss of or gain of cysteine residues within EGFr domains; those impacting EGFr domains 1-6 are fully penetrant, while those impacting EGRr domains 7-34 may be associated with mild disease or incomplete penetrance.

Heterozygous pathogenic variants in *NOTCH3* also cause autosomal dominant lateral meningocele syndrome (LMS). LMS is a rare condition associated with multiple lateral meningoceles, hearing loss, developmental delay, hypotonia, joint hyperlaxity, and variable additional congenital malformations. LMS typically occurs due to a *de novo* disease-causing variant, but rare instances of inheritance from an affected parent have been reported.

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.(1) 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 additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors 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. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

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.

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. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur the laboratory may issue an amended report.

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.(1) 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. 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
2. Ferrante E, Cudrici C, Boehm M: CADASIL: new advances in basic science and clinical perspectives. Curr Opin Hematol. 2019 May; 26(3):193-198
3. Rutten J, Van Eijsden B, Duering M et al: The effect of NOTCH3 pathogenic variant position on CADASIL disease severity: NOTCH3 EGFr 1-6 pathogenic variant are associated with a more severe phenotype and lower survival compared with EGFr 7-34 pathogenic variant. Genet Med. 2019 Mar; 21(3):676-682
4. Canalis E: The skeleton of lateral meningocele syndrome. Front Genet. 2021 Jan 14;11620334

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 gene analyzed, as well as some other regions that have known disease-causing 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 deletion/insertions (delins) 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 -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 the *NOTCH3* gene is NM_000435.3. Reference transcript numbers may be updated due to transcript re-versioning. 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.

Gene analyzed: *NOTCH3*

PDF Report

Supplemental

Day(s) Performed
Varies

Report Available
28 to 42 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Blood spots, saliva, cultured fibroblasts, skin biopsy, cord blood: 1 month

Performing Laboratory Location
Rochester

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
81479
88233-Tissue culture, skin, solid tissue biopsy (if appropriate)
88240-Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NTC3Z	NOTCH3 Gene, Full Gene Analysis	103950-2

Result ID	Test Result Name	Result LOINC® Value
616564	Test Description	62364-5
616565	Specimen	31208-2
616566	Source	31208-2
616567	Result Summary	50397-9
616568	Result	82939-0
616569	Interpretation	69047-9
616570	Resources	In Process
616571	Additional Information	48767-8

616572	Method	85069-3
616573	Genes Analyzed	82939-0
616574	Disclaimer	62364-5
616575	Released By	18771-6