Medical Policy Manual

Genetic Testing for CADASIL Syndrome

Effective: June 1, 2017

Next Review: April 2018
Last Review: May 2017

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Mutations in the NOTCH3 gene have been causally associated with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Genetic testing is available to determine if pathogenic mutations exist in the NOTCH3 gene for patients with suspected CADASIL and their family members.

MEDICAL POLICY CRITERIA

I Genetic testing for the diagnosis of CADASIL may be considered medically necessary when all of the following criteria are met:

   A Clinical signs and symptoms are consistent with CADASIL (see Policy Guidelines); and
   B The diagnosis of CADASIL is inconclusive following alternate methods of testing, including MRI and skin biopsy.

II Genetic testing for NOTCH3 variants in adults may be considered medically necessary when there is a first- or second-degree family member with a diagnosis of CADASIL syndrome (see policy guidelines).
III Genetic testing for CADASIL syndrome for all other situations, including but not limited to testing in children, is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

The clinical presentation of CADASIL varies among and within families. The disease is characterized by five main symptoms: subcortical ischemic events, cognitive impairment, migraine with aura, mood disturbances, and apathy.

- First-degree relatives are parents, siblings, and children of an individual; and
- Second-degree relatives are grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings (siblings with one shared biological parent) of an individual.

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

BACKGROUND

CADASIL is an uncommon, autosomal dominant disease, although it is the most common cause of hereditary stroke and hereditary vascular dementia in adults. The CADASIL syndrome is an adult-onset, disabling systemic condition, characterized by migraine with aura, recurrent lacunar strokes, progressive cognitive impairment, and psychiatric disorders. The overall prevalence of the disease is unknown in the general population.

The clinical presentation of CADASIL is variable and may be confused with multiple sclerosis, Alzheimer dementia, and Binswanger disease. The specific clinical signs and symptoms, along with family history and brain magnetic resonance imaging (MRI) findings, are important in determining the diagnosis of CADASIL. The clinical features and mode of inheritance (autosomal dominant versus autosomal recessive) help to distinguish other inherited disorders in the differential diagnosis from CADASIL.

When the differential diagnosis includes CADASIL, various other tests are available for diagnosis:

- Immunohistochemistry assay of a skin biopsy sample, using a monoclonal antibody with reactivity against the extracellular domain of the NOTCH3 receptor. Positive immunostaining reveals the accumulation of NOTCH3 protein in the walls of small blood vessels.[1] Lesnick Oberstein et al. (2003) estimated sensitivity and specificity at 85-90% and 95-100%, respectively, for 2 observers of the test results in a population of patients and controls correlated with clinical, genetic and MRI parameters.[2]

- Detection of granular osmiophilic material (GOM) in the same skin biopsy sample by electron microscopy. The major component of GOM is the ectodomain of the NOTCH3 gene product.[3] GOM accumulates directly in vascular smooth muscle cells and, when present, is considered a hallmark of the disease.[4] However, GOM may not be present in all biopsy samples. Sensitivity has been reported as low as 45% and 57%, but specificity
is generally near or at 100%.[5-7]

- Genetic testing by direct sequencing of selected exons or of exons 2-24 of the NOTCH3 gene (see Scientific Evidence section below)

- Examination of brain tissue for the presence of GOM. GOM was originally described as limited to brain vessels.[8] Examination of brain biopsy or autopsy after death was an early gold standard for diagnosis. In some cases, peripheral staining for GOM has been absent even though positive results were seen in brain vessels.

**NOTCH3 VARIANTS**

Variants in NOTCH3 have been identified as the underlying cause of CADASIL. In almost all cases, the variants lead to loss or gain of a cysteine residue that could lead to increased reactivity of the NOTCH3 protein, resulting in ligand-binding and toxic effects.[9]

The NOTCH3 gene is found on chromosome 19p13.2-p13.1 and encodes the third discovered human homologue of the Drosophila melanogaster type I membrane protein NOTCH. The NOTCH3 protein consists of 2,321 amino acids primarily expressed in vascular smooth muscle cells and plays an important role in the control of vascular transduction. It has an extracellular ligand-binding domain of 34 epidermal growth factor-like repeats, traverses the membrane once, and has an intracellular domain required for signal transduction.[10]

Variants in the NOTCH3 gene have been differentiated into those that are causative of the CADASIL syndrome (pathogenic variants) and those that are of uncertain significance. Pathogenic variants affect conserved cysteine residues within 34 epidermal growth factor (EGF)-like repeat domains in the extracellular portion of the NOTCH3 protein.[10,11] More than 150 pathogenic variants have been reported in at least 500 pedigrees. NOTCH3 has 33 exons, but all CADASIL variants reported to date have been found in exons 2–24, which encode the 34 EGF-like repeats, with strong clustering in exons 3 and 4, which encode EGFR 2–5 (>40% of variants in >70% of families occur in these exons).[12]

**REGULATORY STATUS**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). NOTCH3 genetic testing is available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has not chosen to require any regulatory review of this test.

**EVIDENCE SUMMARY**

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, which refers to how the results of the diagnostic test will be used to change management of the patient, and whether these changes in management lead to clinically important improvements in health outcomes.

ANALYTICAL VALIDITY

There is limited data on analytic validity of NOTCH3 testing were identified. The test is generally done by gene sequencing analysis, which is expected to have high analytic validity when performed under optimal conditions.

Fernandez et al described the development of a next-generation sequencing (NGS) protocol for NOTCH3 and HTRA1 genes in 70 patients referred for clinical suspicion of CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), all of whom had previously undergone Sanger sequencing of exons 3 and 4 of the NOTCH3 gene.\textsuperscript{[13]} NOTCH3 mutations were detected in 6 patients on NGS, including 2 mutations previously detected with Sanger sequencing and 4 mutations in exons 6, 11, and 19.

CLINICAL VALIDITY

Several retrospective and prospective studies have examined the association between NOTCH3 genes and cerebral autosomal dominant arteriopathy with CADASIL, as shown in Table 1. These studies have been divided into two categories:

- Part 1, diagnostic studies, in which the patients enrolled were suspected, but not confirmed to have CADASIL; and
- Part 2, clinical validity studies, in which the patients had already been diagnosed with the disease by some method other than genetic testing. The diagnostic studies are more likely to represent the target population in which the test would be used.

Table 1. Studies of the association of NOTCH3 with CADASIL

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients Evaluated</th>
<th>NOTCH3 Exons Evaluated</th>
<th>Results</th>
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<tbody>
<tr>
<td>Part 1 Diagnostic Studies</td>
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<td>Diagnostic Yield</td>
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<tr>
<td>Choi et al. 2011\textsuperscript{[8]}</td>
<td>Patients: 151 consecutive Korean patients with acute ischemic stroke. Patient Selection: History of acute ischemic stroke, neurologic exam, cranial computed tomography or MRI.</td>
<td>Bidirectional sequencing of exons 3, 4, 6, 11 and 18.</td>
<td>Patients: 6 patients (4%) were found with the identical NOTCH3 mutation (R544C; exon 11). Of these, all had pre-existing lacunar infarction, 5 (83.3%) had grade 2-3 white-matter hyperintensity lesions, and a history of hypertension; a history of stroke and dementia was higher in patients with mutations. Family Members: No data for additional family members.</td>
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<td>NOTCH3 Exons Evaluated</td>
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<td>Lee et al. 2009[14]</td>
<td>Patients: 39 patients with suspected CADASIL (China). 100 healthy elderly controls</td>
<td>Direct sequencing of exons 2-23.</td>
<td>Patients: 9 different point mutations identified in 21/39 patients. Family members: No data for additional family members 100% No mutations found in 100 healthy elderly controls.</td>
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<td>Markus et al. 2002[7]</td>
<td>Patients: 83 patients with suspected CADASIL (UK). Patients were younger than 60 years of age with recurrent lacunar stroke with leukoaraiosis on neuroimaging. Migraine, psychiatric disorders, or dementia could occur but were not essential.</td>
<td>Direct sequencing of exons 3-4; SSCP of exons 2, 5-23.</td>
<td>Patients: 15 different point mutations identified in 48 families with a total of 116 symptomatic patients, 73% in exon 4, 8% in exon 3, and 6% in exons 5 and 6. Family Members: No data for additional family members</td>
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<td>Yin et al. 2015[15]</td>
<td>Patients: 47 subjects from 34 families (Chinese) diagnosed with suspected CADASIL Patient diagnosis/selection: MRI abnormalities and the presence of more than 1 typical symptom (eg, migraine, stroke, cognitive deficits, psychiatric symptoms) or the presence of atypical symptoms with a positive family history</td>
<td>Testing method: exons 3 and 4 screened first; if no mutations detected, remaining exons analyzed</td>
<td>Patients: 6 known mutations were identified in 8 families and 2 novel mutations were identified in 2 families (exons 3 and 4), and 1 VUS was identified in 1 family (exon 2). Overall NOTCH3 mutation prevalence: 29.4%</td>
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<td>NOTCH3 Exons Evaluated</td>
<td>Results</td>
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<td>Maksemous et al. 2016[16]</td>
<td>Patients: 44 patients with suspected CADASIL previously screened for standard sequencing exons (3 and 4, and/or 2, 11, 18, 19) by Sanger sequencing and classified as negative for known pathogenic variants</td>
<td>Custom NGS panel</td>
<td>Patients: 6 typical CADASIL variants were identified in 7/44 patients.</td>
</tr>
<tr>
<td><strong>Part 2 Clinical Validity Studies</strong></td>
<td><strong>Sensitivity</strong></td>
<td><strong>Specificity</strong></td>
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<tr>
<td>Choi et al. 2013[17]</td>
<td>Patients: 73 unrelated patients diagnosed with CADASIL between 2004-2009. <strong>Patient Diagnosis/Selection:</strong> Patients were diagnosed via clinical and MRI, and stroke history.</td>
<td>Bidirectional sequencing of R544C (exon 11).</td>
<td>Patients: 65 of 73 Patients (90.3%) had the same R544C genotype.</td>
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<tr>
<td>Tikka et al. 2009[18]</td>
<td>Patients: 131 patients from 28 families diagnosed with CADASIL (Finnish, Swedish, and French). <strong>Patient Diagnosis/Selection:</strong> EM examination of skin biopsy was performed; 26 asymptomatic controls from CADASIL families.</td>
<td>Direct sequencing of exons 2-24.</td>
<td>Sensitivity: 100% <strong>Patients:</strong> 131 CADASIL patients were mutation positive. <strong>Family Members:</strong> No data for additional family patients. No mutation reporting per family or per unrelated individual.</td>
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<tr>
<td>Dotti et al. 2005[19]</td>
<td>Patients: 28 unrelated, consecutively diagnosed patients with CADASIL (Italian). <strong>Patient Diagnosis/Selection:</strong> Patients were diagnosed via clinical and MRI.</td>
<td>DHPLC, followed by confirmatory sequencing of identified mutations.</td>
<td>Sensitivity: 100%. <strong>Patients:</strong> All 28 patients had mutations.</td>
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<tr>
<td>Peters et al. 2005[20]</td>
<td>Patients: 125 unrelated patients diagnosed with CADASIL. <strong>Patient Diagnosis/Selection:</strong> Skin biopsy-proven CADASIL pts referred between 1994 and 2003 (German).</td>
<td>Bidirectional sequencing of all exons.</td>
<td>Sensitivity: 96% <strong>Patients:</strong> 54 distinct mutations in 120 (96.0%) of the 125 patients. In 5 patients (4.0%), no mutation was identified.</td>
</tr>
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</table>
The results of the clinical validity studies demonstrate that a NOTCH3 mutation is found in a high percentage of patients with a clinical diagnosis of CADASIL, with studies reporting a clinical sensitivity of 90-100%. Limited data on specificity is from testing small numbers of healthy controls, and no false positive NOTCH3 mutations have been reported in these populations. The diagnostic yield studies report a variable diagnostic yield, ranging from 10-54%. These lower numbers likely reflect testing in heterogeneous populations that include patients with other disorders.

**CLINICAL UTILITY**

Genetic testing may have clinical utility in several situations. The clinical situations addressed in herein are:

- confirmation of a clinical diagnosis of CADASIL in an individual with signs and symptoms of the disease; and
- Informing the reproductive decision making process in preimplantation testing, prenatal (in utero) testing or altering reproductive planning decisions when a NOTCH3 pathogenic variant is present in a parent.

**Confirmation of a CADASIL Diagnosis**

The clinical specificity of genetic testing for CADASIL is high, and false-positive results have not been reported in studies of clinical validity. Therefore, a positive genetic test in a patient with clinical signs and symptoms of CADASIL is sufficient to confirm the diagnosis with a high degree of certainty. The clinical sensitivity is also relatively high, in the range of 90% to 100% for patients with a clinical diagnosis of CADASIL. This indicates that a negative test reduces the likelihood that CADASIL is present. However, because false-negative tests do occur, a negative test is less definitive in ruling out CADASIL. Whether a negative test is sufficient to rule out CADASIL depends on the pretest likelihood that CADASIL is present.
Pescini et al. (2012) published a study that attempted to identify clinical factors that increase the likelihood of a pathologic mutation being present and therefore might be helpful in selecting patients for testing. The authors first performed a systematic review to determine the frequency with which clinical and radiologic factors are associated with a positive genetic test. Evidence was identified from 15 clinical series of patients with CADASIL. Table 2 summarizes the pooled frequency of clinical and radiologic features.

### Table 2. Clinical and Radiological Features in Patients with NOTCH3 Mutations

<table>
<thead>
<tr>
<th>Features</th>
<th>No. With NOTCH3 Mutation</th>
<th>Percent With NOTCH3 Mutation, %</th>
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<tbody>
<tr>
<td><strong>Clinical features</strong></td>
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<tr>
<td>Migraine</td>
<td>239/463</td>
<td>52%</td>
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<tr>
<td>Migraine with aura</td>
<td>65/85</td>
<td>76%</td>
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<tr>
<td>Transient ischemic attack/stroke</td>
<td>380/526</td>
<td>72%</td>
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<tr>
<td>Psychiatric disturbance</td>
<td>106/380</td>
<td>28%</td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>188/434</td>
<td>43%</td>
</tr>
<tr>
<td><strong>Radiologic features</strong></td>
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</tr>
<tr>
<td>LE (leukoencephalopathy)</td>
<td>277/277</td>
<td>100%</td>
</tr>
<tr>
<td>LE extended to temporal pole</td>
<td>174/235</td>
<td>74%</td>
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<tr>
<td>LE extended to external capsule</td>
<td>228/303</td>
<td>75%</td>
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<tr>
<td>Subcortical infarcts</td>
<td>210/254</td>
<td>83%</td>
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</table>

Using these frequencies, a preliminary scoring system was developed and tested in 61 patients with NOTCH3 mutations, and in 54 patients with phenotypic features of CADASIL who were NOTCH3-negative. With the addition of family history and age at onset of transient ischemic attack (TIA)/stroke, a scoring system was developed with the following point values: migraine (1); migraine with aura (3); TIA/stroke (1); TIA/stroke 50 years old or younger (2); psychiatric disturbance (1); cognitive decline (3); leukoencephalopathy (3); leukoencephalopathy extended to temporal pole (1); leukoencephalopathy extended to external capsule (5); subcortical infarcts (2); family history, 1 generation (1); and family history, 2 generations or more (2). The authors recommended that a total score of 14 be used to select patients for testing, because this score resulted in a high sensitivity (96.7%) and a moderately high specificity (74.2%).

Currently, no specific clinical treatment for CADASIL has established efficacy. Supportive care in the form of practical help, emotional support, and counseling are appropriate for affected individuals and their families. Four studies were found that addressed the efficacy of potential treatments for CADASIL.

A double-blind, placebo-controlled trial evaluating the efficacy and safety of donepezil hydrochloride (HCl) in individuals with CADASIL was conducted. The study showed donepezil HCl had no effect on the primary cognitive endpoint, the Cognitive subscale of the Vascular AD Assessment Scale score in patients with CADASIL and cognitive impairment.

Another study evaluated the efficacy and tolerance of a 24-week treatment with acetazolamide 250 mg/d to improve cerebral hemodynamics in CADASIL patients (n=16). Treatment with acetazolamide resulted in a significant increase of mean blood flow velocity (MFV) in the middle cerebral artery (MCA) compared with MFV in the MCA at rest before treatment (57.68±12.7 cm/s vs 67.12±9.4 cm/s; p=0.001). During the treatment period, none of the subjects developed new neurologic symptoms, and the original symptoms in these patients (eg, headaches, dizziness) were relieved.
A third study evaluated the use of 3-hydroxy-3-methylglutaryl-coenzyme A-reductase inhibitors (statins) in 24 CADASIL subjects treated with atorvastatin for 8 weeks. Treatment was started at 40 mg, followed by a dosage increase to 80 mg after 4 weeks. Transcranial Doppler sonography measuring MFV in the MCA was performed at baseline and at the end of treatment. There was no significant treatment effect on MFV (p=0.5) or cerebral vasoreactivity, as assessed by hypercapnia (p=0.5) or intravenous L-arginine (p=0.4) in the overall cohort. However, an inverse correlation was found between vasoreactivity at baseline and changes of both CO2- and L-arginine--induced vasomotor response (both p<0.05). Short-term treatment with atorvastatin resulted in no significant improvement of hemodynamic parameters in the overall cohort of CADASIL subjects.[25]

De Maria et al. reported the results of a randomized, double-blinded trial comparing sapropterin with placebo for adults with CADASIL.[26] Sapropterin is a synthetic analog of tetrahydrobiopterin, which is an essential cofactor in nitric oxide synthesis in endothelial cells. Given nitric oxide’s role in cerebrovascular function, the authors hypothesized that sapropterin supplementation would improve cerebral endothelium-dependent vasodilation in CADASIL patients. Endothelial dysfunction was assessed using the reactive hyperemia peripheral arterial tonometry (RH-PAT) response, which has been shown to be impaired in patients with CADASIL syndrome. Peripheral arterial tonometry (PAT) is a noninvasive, quantitative test that measures changes in digital pulse volume during reactive hyperemia (RH) and evaluates the endothelial function of resistance arteries and nitric oxide–mediated changes in microvascular response. The study randomized 61 subjects from 38 families, 32 to sapropterin and 29 to placebo. In intention-to-treat analysis, there was no significant difference in change in RH-PAT response (mean difference in RH-PAT change, 0.19: 95% confidence interval, -0.18 to 0.56). Both groups demonstrated improvements in RH-PAT values over the course of the study, but, after results were adjusted for age, sex, and clinical characteristics, the improvement was not associated with treatment.

**Genetic Testing of NOTCH3 in Relatives of Patients with CADASIL**

For individuals that have family members with CADASIL syndrome who receive genetic testing, the evidence is limited. Relevant outcomes are overall survival, test accuracy and validity, changes in reproductive decision making, change in disease status, and morbid events. For family members of an individual with known CADASIL, knowledge of the presence of a familial variant may lead to changes in lifestyle decisions for the affected individual (eg, reproduction, employment). However, the impact of these lifestyle decisions on health outcomes is uncertain, and there are no interventions for asymptomatic individuals that are known to delay or prevent the onset of disease. A chain of evidence can be constructed to demonstrate that identification of a NOTCH3 familial variant predicts future development of CADASIL in asymptomatic individuals, eliminates the need for additional diagnostic testing, allows for earlier monitoring for development of systems, aids in reproductive planning and helps determine the likelihood of an affected offspring.

It has been suggested that asymptomatic family members follow the guidelines for presymptomatic testing for Huntington disease. Genetic counseling is recommended to discuss the impact of positive or negative test results, followed by molecular testing if desired.[4] For an asymptomatic individual, knowledge of mutation status will generally not lead to any management changes that can prevent or delay the onset of the disorder. Avoiding tobacco use may be one factor that delays onset of disease, but this is a general recommendation that is not altered by genetic testing.
EUROPEAN FEDERATION OF NEUROLOGICAL SOCIETIES (EFNS)

The ENFS guidelines[27] state that among patients with suspected genetic disorders, “direct sequencing of exons 3 and 4 in the Notch3 gene is suggested as a first step if clinical suspicion for CADASIL is high (Level B).” According to guideline authors, a Level B rating is defined as “probably useful/predictive or not useful/predictive” and requires “at least one convincing class II study or overwhelming class III evidence.” The methods used to formulate these recommendations are based upon expert consensus.

 SUMMARY

There is enough research to show that testing for NOTCH3 variants can help diagnose CADASIL in patients whose diagnosis cannot be confirmed by other methods. Therefore, genetic testing to confirm the diagnosis of CADASIL syndrome may be considered medically necessary when the policy criteria are met.

There is enough evidence to show that testing for mutations associated with CADASIL in individuals who have a family member with the disease can help patients make reproductive planning decisions and avoid unnecessary diagnostic testing. Therefore, genetic testing for NOTCH3 variants in adults that have a first- or second-degree family member with a diagnosis of CADASIL syndrome may be considered medically necessary.

There is not enough research to show that genetic testing for CADASIL improves health outcomes or decision-making in patients that do not meet the policy criteria. Therefore, genetic testing for CADASIL syndrome in all other situations, including but not limited to testing in children, is considered investigational.

 REFERENCES


<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
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<tbody>
<tr>
<td>CPT</td>
<td>81406</td>
<td>Molecular pathology procedure, Level 7</td>
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<tr>
<td>HCPCS</td>
<td>None</td>
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*Date of Origin: April 2013*