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***Preauthorization is required.***

*The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.*

Populations	Interventions	Comparators	Outcomes
Individuals: <ul style="list-style-type: none"> <li>With suspected CADASIL syndrome</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>NOTCH3 genetic testing</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>Standard clinical management without genetic testing</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>Overall survival</li> <li>Test accuracy</li> <li>Test validity</li> <li>Changes in reproductive decision making</li> <li>Change in disease status</li> <li>Morbid events</li> </ul>
Individuals: <ul style="list-style-type: none"> <li>Who are asymptomatic with family members who have CADASIL syndrome</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>Targeted genetic testing for a known NOTCH3 familial variant</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>Standard clinical management without genetic testing</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>Overall survival</li> <li>Test accuracy</li> <li>Test validity</li> <li>Other test performance measures</li> <li>Changes in reproductive decision making</li> <li>Change in disease status</li> <li>Morbid events</li> </ul>
Individuals: <ul style="list-style-type: none"> <li>Who are asymptomatic with family members who have CADASIL syndrome whose genetic status is unknown</li> </ul>	Interventions of interest are: <ul style="list-style-type: none"> <li>NOTCH3 genetic testing</li> </ul>	Comparators of interest are: <ul style="list-style-type: none"> <li>Standard clinical management without genetic testing</li> </ul>	Relevant outcomes include: <ul style="list-style-type: none"> <li>Overall survival</li> <li>Test accuracy</li> <li>Test validity</li> <li>Other test performance measures</li> <li>Changes in reproductive decision making</li> <li>Change in disease status</li> <li>Morbid events</li> </ul>

### DESCRIPTION

Variants 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 variants exist in the NOTCH3 gene for patients with suspected CADASIL and their family members.

## SUMMARY OF EVIDENCE

For individuals with suspected CADASIL syndrome who receive NOTCH3 genetic testing, the evidence includes case reports, case series, and genotype-phenotype correlation studies evaluating the clinical validity and genetic testing yield for NOTCH3. Relevant outcomes are overall survival, test accuracy and validity, changes in reproductive decision making, change in disease status, and morbid events. The clinical validity studies have demonstrated that a NOTCH3 pathogenic variant is found in a high percentage of patients with a clinical diagnosis of CADASIL, with studies reporting a clinical sensitivity of 90% to 100%. Limited data on specificity derives from testing small numbers of healthy controls, and no false-positive NOTCH3 pathogenic variants have been reported in these populations. The diagnostic yield studies have reported a variable yield, ranging from 10% to 54%. These lower numbers likely reflect testing in heterogeneous populations that include patients with other disorders. No direct evidence was identified demonstrating outcome improvements associated with genetic testing for CADASIL. However, a chain of evidence can be constructed to demonstrate that identification of a NOTCH3 pathogenic variant establishes the diagnosis of CADASIL without the need for a skin biopsy and reduces the need for other diagnostic tests used to exclude other conditions in a differential diagnosis. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic with family members who have CADASIL syndrome who receive targeted genetic testing for a known NOTCH3 familial variant, 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 asymptomatic 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 (e.g., production, 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 disease onset. A chain of evidence can be constructed to demonstrate that identification of a NOTCH3 familial variant predicts future development of CADASIL in an asymptomatic individual, 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. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic with family members who have CADASIL syndrome whose genetic status is unknown who receive NOTCH3 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 asymptomatic family members of an individual with known CADASIL whose genetic status is unknown, knowledge of the presence of a NOTCH3 pathogenic variant may lead to changes in lifestyle decisions for the affected individual (e.g., 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 disease onset. A chain of evidence can be constructed to demonstrate that identification of a NOTCH3 pathogenic variant predicts future development of CADASIL in an asymptomatic individual, 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. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

## POLICY

Genetic testing for a NOTCH3 variant to confirm the diagnosis of CADASIL syndrome in a patient may be considered **medically necessary** under the following conditions:

- Clinical signs, symptoms, skin biopsy and imaging results are consistent with CADASIL, indicating that the pretest probability of CADASIL is at least in the moderate-to-high range (see Policy Guidelines); and

- The diagnosis of CADASIL is inconclusive following alternative methods of testing, including skin biopsy and magnetic resonance imaging.

For individuals who are asymptomatic with a family member with a diagnosis of CADASIL syndrome:

- If there is a family member (first- and second-degree relative) with a known variant, targeted genetic testing of the known NOTCH3 familial variant may be considered **medically necessary**.
- If the family member's genetic status is unknown, genetic testing of NOTCH3 (see Policy Guidelines) may be considered **medically necessary**.

Genetic testing for a NOTCH3 variant to confirm the diagnosis of CADASIL syndrome in all other situations, is considered **investigational**.

## POLICY GUIDELINES

Genetic testing for NOTCH3 comprises targeted sequencing of specific exons (e.g., exon 4 only, exons 2-6), general sequencing of NOTCH3 exons (e.g., exons 2-24 or all 33 exons), or targeted testing for known NOTCH3 pathogenic variants.

The probability that CADASIL is present in an individualized assessment depends on numerous factors such as family history, symptoms, imaging results, and other specialized testing such as skin biopsy.

Pescini et al (2012) attempted to identify clinical factors that increase the likelihood of a pathogenic variant being present. Table PG1 summarizes the pooled frequency of clinical and radiologic features, and the points assigned for each finding. 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%).

Table PG1. Pooled Frequency of Clinical and Radiologic Features

Features	No. With NOTCH3 Variant	Percent With NOTCH3 Variant	Points
<b>Clinical</b>			
Migraine	239/463	52%	1
Migraine with aura	65/85	76%	3
Transient ischemic attack/stroke	380/526	72%	1 (2 if <50 y)
Psychiatric disturbance	106/380	28%	1
Cognitive decline	188/434	43%	3
<b>Radiologic</b>			
LE	277/277	100%	3
LE extended to temporal pole	174/235	74%	1
LE extended to external capsule	228/303	75%	5
Subcortical infarcts	210/254	83%	2

Adapted from Pescini et al (2012).

LE: leukoencephalopathy.

### Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence protocol updates starting in 2017 (see Table PG2). The Society's nomenclature is recommended by the Human Variome Project, the HUMAN Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG3 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely

benign,” and “benign”—to describe variants identified that cause Mendelian disorders.

Table PG2. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG3. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

## GENETIC COUNSELING

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

## BACKGROUND

### CADASIL

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

### Diagnosis

The differential diagnosis of CADASIL includes the following conditions (see Table 1).

Table 1. Differential Diagnosis of CADASIL

Acquired Disorders	Inherited Disorders
<ul style="list-style-type: none"> <li>Sporadic SVD with or without hypertension as the main risk factor</li> <li>Multiple sclerosis</li> <li>Primary angiitis of the central nervous system</li> </ul>	<ul style="list-style-type: none"> <li>Fabry disease</li> <li>Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy</li> <li>Familial SVD caused by heterozygous variants in the HTRA1 gene</li> <li>Some forms of leukodystrophy</li> </ul>

SVD: small vessel disease.

Since the clinical presentation of CADASIL varies, the condition 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 findings, are extremely important in diagnosing CADASIL. The clinical fea-

tures and mode of inheritance (autosomal dominant versus autosomal recessive) help to distinguish CADASIL from other inherited disorders in a differential diagnosis.

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

- Genetic testing, by direct sequencing of select exons or of exons 2 through 24 of the NOTCH3 gene. Identification of a NOTCH3 pathogenic variant definitively establishes a diagnosis of CADASIL without the need for additional diagnostic testing (e.g., skin biopsy).
- 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 the NOTCH3 protein in the walls of small blood vessels.<sup>1</sup> Lesnick Oberstein et al (2003) estimated the sensitivity and specificity at 85% to 90% and 95% to 100%, respectively, for two observers of the test results in a population of patients and controls correlated with clinical, genetic, and magnetic resonance imaging parameters.<sup>2</sup>
- 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.<sup>3</sup> GOM accumulates directly in vascular smooth muscle cells and, when present, is considered a hallmark of the disease.<sup>4</sup> 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%.<sup>5-7</sup>
- Examination of brain tissue for the presence of GOM was originally described as limited to brain blood vessels.<sup>8</sup> Examination of brain biopsy or autopsy after death was an early criterion standard for diagnosis. In some cases, peripheral staining for GOM has been absent even though positive results were seen in brain blood vessels.

#### *NOTCH3 Variants*

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

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 2321 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 (EGF)-like repeats, traverses the membrane once, and has an intracellular domain required for signal transduction.<sup>10</sup>

Variants in the NOTCH3 gene have been differentiated into those causative of the CADASIL syndrome (pathogenic variants) and those of uncertain significance. Pathogenic variants affect conserved cysteine residues within 34 EGF-like repeat domains in the extracellular portion of the NOTCH3 protein.<sup>10,11</sup> 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 occurred in exons 2 to 24, which encode the 34 EGF-like repeats, with strong clustering in exons 3 and 4, which encode EGF receptors 2 to 5 (>40% of variants in >70% of families occur in these exons).<sup>12</sup> Some studies have indicated that the clinical variability in CADASIL presentation, particularly about the development of white-matter hyperintensities on magnetic resonance imaging, may be related to genetic modifiers outside the NOTCH3 locus, but the specific role of these modifiers is not well-delineated.<sup>13</sup> The probability that CADASIL is present in an individualized assessment depends on numerous factors such as family history, symptoms, imaging results, and other specialized testing (e.g., skin biopsy). Pescini et al (2012) attempted to identify clinical factors that increase the likelihood of a pathogenic variant being present, with increasing likelihood with the presence of one or several factors, including a migraine, migraine with aura, transient ischemic attack/stroke,

psychiatric disturbance, cognitive decline, leukoencephalopathy (with greater risk for leukoencephalopathy extending to the temporal pole or external capsule), and subcortical infarcts.<sup>14</sup>

## 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 Amendments (CLIA). Genetic testing of NOTCH3 is available under the auspices of the CLIA. Laboratories that offer LDTs must be licensed by the CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

## RELATED PROTOCOL

Preimplantation Genetic Testing

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Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

## REFERENCES

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.

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