Medical Policy

Blue Cross Blue Shield Blue Care Network of Michigan

Nonprofit corporations and independent licensees of the Blue Cross and Blue Shield Association

***Current Policy Effective Date: 11/1/24**
(See policy history boxes for previous effective dates)

Title: Genetic Testing of CADASIL Syndrome

Description/Background

CADASIL

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (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 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 (Table 1):

Table 1. Differential Diagnosis of CADASIL

Acquired Disorders	Inherited Disorders
• Sporadic SVD with or without hypertension as the main risk factor • Multiple sclerosis • Primary angiitis of the central nervous system	• Fabry disease • Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy • Familial SVD caused by heterozygous variants in the *HTRA1* gene • Some forms of leukodystrophy

CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; 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 features 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 selected exons or of exons 2 through 24 of the *NOTCH3* gene (see the Rationale section). Identification of a *NOTCH3* pathogenic variant definitively establishes a diagnosis of CADASIL without the need for additional diagnostic testing (eg, skin biopsy).
- Genetic testing can facilitate the differentiation of NOTCH3-associated cerebral small vessel disease (CSVD) from other CADASIL-like disorders (ed, HTRA1-associated CSVD and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy).[1]
- 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.[2] Lesnick Oberstein et al (2003) estimated the sensitivity and specificity at 85% to 90% and 95% to 100%, respectively, for 2 observers of the test results in a population of patients and controls correlated with clinical, genetic, and magnetic resonance imaging parameters.[3]
- Detection of granular osmiophilic material in the same skin biopsy sample by electron microscopy. The major component of GOM is the ectodomain of the *NOTCH3* gene product.[4] Granular osmiophilic material accumulates directly in vascular smooth muscle cells and, when present, is considered a hallmark of the disease.[5] However, granular osmiophilic material 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%.[6-8]
- Examination of brain tissue for the presence of granular osmiophilic material was originally described as limited to brain blood vessels.[9] Examination of brain biopsy or autopsy after death was an early criterion standard for diagnosis. In some cases, peripheral staining for granular osmiophilic material 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 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.[10]

The *NOTCH3* gene is found on chromosome 19p13.12 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 (EGF)-like repeats, traverses the membrane once, and has an intracellular domain required for signal transduction.[11]

Variants in the *NOTCH3* gene have been differentiated into those that are 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.[11,12] 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).[13] A study by Hack et al (2023) identified 3 clinically distinct risk categories (high, medium, and low) of *NOTCH3* EGF-like repeats using data from CADASIL and population cohorts (N=4221).[14,] 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.[15] Dupé et al (2023) investigated the phenotypic variability in individuals with CADASIL and found the mutation location in the *NOTCH3* gene (n=436) to be strongly related to clinical severity, and found male sex, arterial hypertension, and smoking to be associated with increased disease severity.[16,]

The probability that CADASIL is present is an individualized assessment depends on numerous factors such as family history, symptoms, imaging results, and other specialized testing (eg, skin biopsy). Pescini et al (2012) attempted to identify clinical factors that increase the likelihood of a pathologic mutation being present, with increasing likelihood with the presence of 1 or several factors, including 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.[17]

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* mutation 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 (FDA) has chosen not to require any regulatory review of this test.

Medical Policy Statement

Genetic testing of CADASIL syndrome is considered established in select patient populations who meet clinical criteria. This testing may be a useful diagnostic option when indicated.

Inclusionary and Exclusionary Guidelines

Inclusions

- Genetic testing of *NOTCH3* to confirm a diagnosis of CADASIL syndrome is considered established when clinical signs, symptoms, and imaging results (MRI) are suggestive of CADASIL syndrome.

- Genetic testing of (*NOTCH3*) of an asymptomatic individual who has a first- or second-degree relative with CADASIL syndrome is established when:

    - There is a family member (first- or second-degree) with a known variant, targeted genetic testing of the known *NOTCH3* familial variant is considered established.

- The family member's genetic status is unknown, genetic testing of *NOTCH3* is considered established.
  Note: First-degree relative: parent, full-sibling, child
  Second-degree relative: grandparent, grandchild, aunt, uncle, nephew, niece, half-sibling

Exclusions:

- All other situations not addressed in the inclusions above are excluded.

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81406*

*Code 81406 includes: *NOTCH3* (notch 3) (eg, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]), targeted sequence analysis (eg, exons 1-23).

Other codes (investigational, not medically necessary, etc.):

N/A

Rationale

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

TESTING INDIVIDUALS WITH SUSPECTED CADASIL SYNDROME

Clinical Context and Test Purpose

The purposes of genetic testing of symptomatic individuals with suspected cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome are to establish the diagnosis of CADASIL without skin biopsy or other invasive testing and to aid in reproductive planning when the diagnosis cannot be made clinically.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with suspected CADASIL.

Interventions

The test being considered is genetic testing for *NOTCH3* variants.
Genetic testing is used to confirm a diagnosis of CADASIL. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Comparators
The following practice is currently being used: standard clinical management without genetic testing.

Outcomes
The potential beneficial outcome of primary interest would be changes in management associated with improved outcomes initiated based on confirming a genetic diagnosis of CADASIL. Reductions in skin biopsies or other invasive tests to confirm diagnosis of CADASIL are also potential beneficial outcomes.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to inappropriate initiation of treatments or psychological harm after receiving positive test results. False-negative test results can lead to lack of medical or neurologic treatments or surveillance.

The time frame for outcomes measures varies from short-term development of symptoms to long-term changes in disease status and outcomes.

Study Selection Criteria
For the evaluation of clinical validity of the tests, studies that met the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described
- Included a validation cohort separate from development cohort

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Several retrospective and prospective studies have examined the association between *NOTCH3* variants and CADASIL, as shown in Table 2. Studies have been divided into 2 categories: Part 1: Diagnostic studies, in which 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.

The results of the clinical validity studies 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 ranging from 90% to 100%. Limited data on specificity derive from testing small numbers of healthy controls, and no false-positive *NOTCH3* variants have been reported in these populations. The diagnostic yield studies have reported a variable yield

(range, 10%-54%). These lower numbers likely reflect testing in heterogeneous populations that include Individuals with other disorders.

Testing Strategy

Identification of a *NOTCH3* pathogenic variant establishes a diagnosis of CADASIL. For individuals suspected of CADASIL:

- Perform targeted sequencing and analysis of specific *NOTCH3* exons (eg, exon 4 only, exons 2–6) OR
- Perform general testing of *NOTCH3* exons (eg, exons 2-24 or all 33 exons)
- If no *NOTCH3* pathogenic variant is identified, skin biopsy is warranted for immunohistochemical staining for *NOTCH3* protein and/or electron microscopy for granular osmiophilic material.

Table 2. Association Between *NOTCH3* and CADASIL Diagnosis: Results From Studies Supporting *NOTCH3* Genotyping Test Claims

Study	Patients Evaluated	*NOTCH3* Exons Sequenced	Results	
Part 1: Diagnostic Studies			***Diagnostic Yield***	***Specificity***
Mosca et al (2011)[10]	Patients: 140 with clinical suspicion of CADASIL (Italian, Chinese) Selection: History of premature strokes; migraine with aura; vascular dementia; suggestive MRI findings; consistent family history; or combination of previous criteria	Direct sequencing of exons 2-8, 10, 14, 19-20, 22	Patients: 14 with pathogenic variants located in 10 exons. 126 patients free of pathogenic variants Family members: Analysis of 15 additional family members identified 11 of the same pathogenic variants	NR
Lee et al (2009)[18]	Patients: 39 with suspected CADASIL (Chinese); 100 healthy elderly controls ≥80 y Selection: Suggestive MRI findings and at least 1 of the following: young age at onset, cognitive decline, psychiatric disorders, or consistent family history	Direct sequencing of exons 2-23	Patients: 9 different SNVs identified in 21/39 patients Family members: No data	100% No variants in 100 healthy elderly controls
Markus et al (2002)[8]	Patients: 83 with suspected CADASIL (U.K.) Selection: Patients were <60 y with recurrent lacunar stroke with leukoaraiosis on neuroimaging. Migraine, psychiatric disorders, or dementia could occur but were not essential.	Direct sequencing of exons 3-4; SSCP of exons 2, 5-23	Patients: 15 SNVs identified in 48 families with 116 symptomatic patients, 73% in exon 4, 8% in exon 3, 6% in exons 5 and 6 Family members: No data	NR
Choi et al (2013)[9]	Patients: 151 consecutive patients (Korean) Selection: History of acute ischemic stroke, neurologic exam, cranial computed tomography, or MRI	Bidirectional sequencing of exons 3, 4, 6, 11,18	Patients: 6 (4%) found with identical *NOTCH3* variant (R544C; exon11). Of these, all had preexisting lacunar infarction, 5 (83.3%) had grade 2-3 white-matter hyperintensity lesions, and a history of hypertension; history of stroke and dementia higher in patients with variants Family members: No data	NR
Yin et al (2015)[19]	Patients: 47 subjects from 34 families (Chinese) diagnosed with suspected CADASIL Diagnosis/selection: MRI abnormalities and presence of >1 typical symptom (eg, migraine, stroke, cognitive deficits, psychiatric symptoms) or presence of atypical symptoms with positive family history	Testing method per Joutel et al(1997)[17]: exons 3 and 4 screened first; if no variants detected, remaining exons analyzed	Patients: 6 known familial variants identified in 8 families and 2 novel pathogenic variants identified in 2 families (exons 3 and 4),and 1 VUS identified in 1 family (exon 2).Overall *NOTCH3* pathogenic variant prevalence: 29.4%	NR

Abramycheva et al (2015)[21]	Patients: 30 unrelated patients with suspected CADASIL	Direct sequencing of exons 2-23 via PCR	Patients: 16 SNVs identified in 18 unrelated patients, 12 of which had been previously described and 4 were novel (C194G, V252M, C338F, C484G)	NR
Maksemous et al (2016)[22]	Patients: 44 with suspected clinical diagnosis of CADASIL previously screened for standard Sanger sequencing exons (3, 4) and/or (2, 11, 18, 19) and classified as negative for known pathogenic variants	Custom NGS panel	Patients: 6 typical CADASIL pathogenic variants identified in 7/44 patients	NR
Gorukmez et al (2023)[23]	**Patients:** 368 individuals with suspected CADASIL based on radiological and clinical findings	NGS	**Patients:** 30 variants (17 novel) were detected in 44 individuals from 40 families in exons 2 to 24, 25, 31, and 33	NR
Part 2: Clinical Validity Studies			***Sensitivity***	***Specificity***
Peters et al (2005)[24]	Patients: 125 unrelated patients diagnosed with CADASIL Diagnosis/selection: Skin biopsy-proven CADASIL patients	Bidirectional sequencing of all exons	Sensitivity: 96% Patients: 54 distinct variants in 120 (96.0%) of 125 patients. In 5(4.0%) patients, no variants identified. Family members: No data	NR
Tikka et al (2009)[25]	Patients: 131 patients from 28 families diagnosed with CADASIL (Finnish, Swedish, French) Diagnosis/selection: EM examination of skin biopsy was performed; 26 asymptomatic controls from CADASIL families	Direct sequencing of exons 2-24	Sensitivity: 100% Patients: 131 CADASIL patients were pathogenic variant-positive Family members: No data No pathogenic variant reported per family or per unrelated individual	100% No pathogenic variants in 26 negative controls
Dotti et al (2005)[26]	Patients: 28 unrelated, consecutively diagnosed patients with CADASIL (Italian) Diagnosis/selection: Patients diagnosed via clinical and MRI criteria	DHPLC, followed by confirmatory sequencing of identified pathogenic variants	Sensitivity: 100% Patients: All 28 had pathogenic variants	NR
Joutel et al (1997)[20]	Patients: 50 unrelated patients with a clinical suspicion of CADASIL and 100 healthy controls Diagnosis/selection: History of recurrent strokes, migraine with aura, vascular dementia, or a combination; brain MRI with suggestive findings; and consistent familial history	SSCP or heteroduplex analysis of all exons, followed by confirmatory sequencing of identified variants	Sensitivity: 90% Patients: 45/50CADASIL patients had variants	100% No variants in 100 healthy controls

CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; DHPLC: denaturing high-performance liquid chromatography; EM: electron microscope; MRI: magnetic resonance imaging; NGS: next-generation sequencing; NR: not reported; PCR: polymerase chain reaction; SNV: single nucleotide variant; SSCP: single-stranded conformational polymorphism; VUS: variant of uncertain significance.

Section Summary: Clinically Valid

The clinical sensitivity of genetic testing is high given that *NOTCH3* is the only gene for which pathogenic variants are known to cause CADASIL. In clinical situations where diagnosis of CADASIL cannot be confirmed by other methods (clinical presentation, magnetic resonance imaging [MRI] findings), identification of a pathogenic variant in *NOTCH3* establishes a diagnosis of CADASIL.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if individuals receive correct therapy, more effective therapy, or avoid unnecessary therapy, or testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for individuals managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RCTs).

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 an individual 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 individuals 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) attempted to identify clinical factors that increase the likelihood of a pathologic mutation being present and therefore might be helpful in selecting individuals for testing.[17] 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 individuals with CADASIL. Table 3 summarizes the pooled frequency of clinical and radiologic features.

Table 3. Clinical and Radiologic Features in Patients with NOTCH3 Variants

Features	No. With *NOTCH3* Variant	Percent With NOTCH3 Variant	Points
Clinical			
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
Radiologic			
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)[17]
LE: leukoencephalopathy.

Using these frequencies, a preliminary scoring system was developed and tested in 61 individuals with *NOTCH3* pathogenic variants, and in 54 individuals with phenotypic features of

CADASIL who were *NOTCH3*-negative. With the addition of family history and age at onset of transient ischemic attack or stroke, a scoring system was developed, as provided in Table 3. The authors recommended that a total score of 14 be used to select individuals for testing because this score resulted in a high sensitivity (96.7%) and 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.[4,11] Four studies were found that addressed the efficacy of potential treatments for CADASIL.

Two randomized, double-blind, placebo-controlled studies evaluated the efficacy and safety of potential treatments for CADASIL. Dichgans et al (2008) showed there was no significant difference between donepezil (n=84) and placebo (n=77) in the primary cognitive endpoint, the cognitive subscale of the Vascular AD Assessment Scale score.[27,] De Maria et al (2014) found no significant difference between sapropterin (n=32) and placebo (n=29) in change in reactive hyperemia peripheral arterial tonometry response (mean difference, 0.19: 95% confidence interval, -0.18 to 0.56).[28,]

Two single-arm studies also evaluated the efficacy of potential treatments for CADASIL. Huang et al (2010) found treatment with acetazolamide (N=16) resulted in a significant increase of blood mean flow velocity in the middle cerebral artery (57.68 cm/s) compared with mean flow velocity in the middle cerebral artery at rest before treatment (67.12 cm/s; p=.001).[29,] During the treatment period, none of the subjects developed new neurologic symptoms, and the original symptoms in these individuals (eg, headaches, dizziness) were relieved. Peters et al (2007), evaluated the use of 3-hydroxy-3-methylglutaryl-coenzyme A-reductase inhibitors (statins) in 24 CADASIL subjects treated with atorvastatin for 8 weeks.[30,] There was no significant treatment effect on mean flow velocity (p=.5) or cerebral vasoreactivity, as assessed by hypercapnia (p=.5) or intravenous l-arginine (p=.4) in the overall cohort. However, an inverse correlation was found between vasoreactivity at baseline and changes of both CO_2- and l-arginine-induced vasomotor response (both p<.05). Short-term treatment with atorvastatin resulted in no significant improvement of hemodynamic parameters in the overall cohort of CADASIL subjects.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Genetic testing of individuals with suspected CADASIL may have clinical utility by:
- Establishing a diagnosis of CADASIL in an individual with signs and symptoms of the disease, particularly when other disorders are being considered, without the need for a skin biopsy.
- 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. Preimplantation testing is addressed elsewhere (see medical policy, *Genetic Testing - Preimplantation*).

Section Summary: Clinically Useful
Direct evidence for the clinical utility of genetic testing of individuals with suspected CADASIL is lacking. No specific clinical treatment for CADASIL has established efficacy. However, a

chain of evidence for the clinical validity of *NOTCH3* pathogenic variants in establishing a diagnosis of CADASIL leading to initiation of supportive care in the form of practical help, emotional support, and counseling may provide a chain of evidence for potential clinical utility.

TARGETED FAMILIAL VARIANT TESTING IN ASYMPTOMATIC INDIVIDUALS WITH RELATIVES WHO HAVE CADASIL SYNDROME

Clinical Context and Test Purpose

The purposes of targeted familial variant testing of asymptomatic individuals with family members who have CADASIL are to screen at-risk individuals and predict the development of disease, to determine the need for surveillance, and to aid in reproductive planning.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is asymptomatic individuals with relatives who have CADASIL syndrome.

Interventions

The following test is currently being used: targeted familial variant testing of *NOTCH3*.

Asymptomatic individuals with family members with CADASIL may be referred to a medical geneticist for investigation of genetic status for carrying a known familial variant. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Comparators

The following practice is currently being used: standard clinical management without genetic testing.

Outcomes

The potential beneficial outcomes of primary interest would be confirming or excluding the need for surveillance or changes in reproductive decision making. A negative genetic test result would eliminate the need for surveillance to detect the development of symptoms and disease. A positive genetic test result would confirm a need for active surveillance and inform the reproductive decision process.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary medical or neurologic surveillance of asymptomatic individuals. False-negative test results can lead to lack of medical or neurologic surveillance.

The time frame for outcome measures varies from short-term surveillance of asymptomatic individuals for the development of signs or symptoms of CADASIL to long-term development of the disease.

Study Selection Criteria

For the evaluation of clinical validity of the tests, studies that met the eligibility criteria described in the first indication were considered.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

See the clinical validity discussion in the *Testing Individuals With Suspected CADASIL Syndrome* section.

Testing Strategy
Identification of a *NOTCH3* pathogenic variant establishes a diagnosis of CADASIL in both symptomatic and asymptomatic individuals. For testing in asymptomatic individuals with family members who have CADASIL:

- When the proband's *NOTCH3* pathogenic variant is known, conduct targeted familial variant testing to determine genetic status.

The testing strategy described here is a general approach for targeted genetic testing for a known pathogenic variant previously identified in a family member (familial variant) with CADASIL.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if individuals receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No randomized trials were identified addressing outcomes managed with CADASIL testing.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Genetic testing of asymptomatic individuals with family members who have CADASIL may have clinical utility by:

- Confirming or excluding the need for surveillance based on the presence or absences of a known familial variant.
- Informing the reproductive decision making process in preimplantation testing, prenatal (in utero) testing or altering reproductive planning decisions when a known *NOTCH3* familial variant is present in a parent. Preimplantation testing is addressed elsewhere (see medical policy, *Genetic Testing-Preimplantation*).

Genetic counseling is recommended to discuss the impact of positive or negative test results, followed by molecular testing if desired.[5] At present, for an asymptomatic individual, knowledge of familial variant status will generally not lead to any management changes that can prevent or delay the onset of the disorder. Avoiding tobacco use can be a factor that delays the onset of disease, but this is a general recommendation that is not altered by genetic testing. However, a negative test may preclude the need for surveillance for complications. Genetic testing may also assist reproductive decision making.

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.

Section Summary: Clinically Useful
Direct evidence for the clinical utility of genetic testing of asymptomatic relatives of individuals with CADASIL is lacking. No specific clinical treatment for CADASIL has established efficacy. However, a chain of evidence can be developed to for potential clinical utility, particularly for reproductive decision-making process for preimplantation and/or prenatal testing.

GENETIC TESTING OF *NOTCH3* IN ASYMPTOMATIC INDIVIDUALS WITH RELATIVES WHO HAVE CADASIL AND UNKNOWN GENETIC STATUS

Clinical Context and Test Purpose
The purposes of genetic testing of *NOTCH3* in asymptomatic individuals with family members with CADASIL whose genetic status is unknown are to screen at-risk individuals, to predict development of disease, to determine the need for surveillance and to aid in reproductive planning.

The following PICO was used to select literature to inform this review

Populations
The relevant population of interest is asymptomatic individuals with relatives who have CADASIL and whose genetic status is unknown.

Interventions
The test being considered is genetic testing of *NOTCH3* variants.

Asymptomatic individuals with family members who have CADASIL may be referred to a medical geneticist for investigation of genetic status for carrying a known familial variant. Referral for genetic counseling is important for the explanation of genetic disease, heritability, genetic risk, test performance, and possible outcomes.

Comparators
The following practice is currently being used: standard clinical management without genetic testing.

Outcomes
The potentially beneficial outcomes of primary interest would be confirming or excluding the need for surveillance or changes in reproductive decision making. A negative genetic test result would eliminate the need for surveillance to detect development of symptoms and disease. A positive genetic test result would confirm a need for active surveillance and also inform the reproductive decision-making process.

Potential harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary medical or neurological

surveillance of asymptomatic individuals. False-negative test results can lead to lack of medical or neurological surveillance.

The time frame for outcomes measures varies from short-term surveillance of asymptomatic individuals for the development of signs or symptoms of CADASIL to long-term development of disease.

Study Selection Criteria
For the evaluation of clinical validity of the tests, studies that met the eligibility criteria described in the first indication were considered.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

See the clinical validity discussion in the *Testing Individuals with Suspected CADASIL Syndrome* section.

Testing Strategy
For testing in asymptomatic individuals with family members who have CADASIL whose genetic status is unknown:

- Perform targeted sequencing and analysis of specific *NOTCH3* exons (eg, exon 4 only, exons 2–6) OR
- Perform general testing of *NOTCH3* exons (eg, exons 2-24 or all 33 exons)

This testing strategy to perform sequence analysis of multiple *NOTCH3* exons to identify pathogenic variants is a general approach for genetic testing for *NOTCH3*.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if individuals receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for individuals managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No randomized trials were identified addressing outcomes managed with CADASIL testing.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Genetic testing of asymptomatic individuals with family members who have CADASIL may have clinical utility by:

- Confirming or excluding the need for surveillance based on the presence or absence of a *NOTCH3* pathogenic variant.

- Informing the reproductive decision making process in preimplantation testing, prenatal (in utero) testing or altering reproductive planning decisions when a known *NOTCH3* pathogenic variant is present in a parent. Preimplantation testing is addressed elsewhere (see Policy, *Genetic Testing-Preimplantation*).

Section Summary: Clinical Utility
Similar to the case where there is a known family variant associated with CADASIL, direct evidence for the clinical utility of genetic testing of asymptomatic relatives of individuals with CADASIL is lacking. However, a chain of evidence can be developed to support the clinical utility of testing, as outlined above.

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 in the exclude other conditions in a differential diagnosis. The evidence is sufficient to determine that the technology results in 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 (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 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 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 (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 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 improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Clinical Input Received through Physician Specialty Societies and Academic Medical Centers

2013

In response to requests made by the Blue Cross Blue Shield Association, input was received from 1 physician specialty society and 3 academic medical centers while their policy was under review in 2013. Most reviewers disagreed with statement that genetic testing to confirm the diagnosis of CADASIL was investigational. All reviewers expressed support for testing to confirm the diagnosis in select patients, particularly when the diagnosis of CADASIL is inconclusive, and when the pretest likelihood of CADASIL is moderate to high. In addition to consensus among reviewers, contextual factors in support of medical necessity are present for this indication, ie, there is a highly suggestive chain of evidence; high-quality trials are unlikely to be performed, and there is a potential for reducing harms by avoiding additional testing and avoiding anticoagulants and antiplatelet agents when the disease is present.

Reviewers also agreed with the recommendation that testing is medically necessary for a first- or second-degree relative, when there is a known pathologic variant (familial variant) in the family. For this indication, contextual factors in support of medical necessity were not present. High-quality trials are unlikely to be performed.

2020

Clinical consultation was obtained in 2020 indicating that skin biopsy prior to NOTCH3 testing is not necessary; skin biopsy should be reserved for patients where NOTCH3 genetic testing is inconclusive (eg, variants of uncertain significance).

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in ‘Supplemental Information’ if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

No guidelines with US representation or that were informed by a systematic review were identified. One position statement was identified.

American Heart Association

In a 2023 scientific statement, the American Heart Association reviewed the current clinical, genetic, and imaging aspects of CADASIL to provide prevention, management, and therapeutic considerations to support future treatment recommendations.[31] In consideration of when to test for *NOTCH3* mutations, they state to "[consider gene testing in patients with small vessel stroke before 55 years] of age with a paucity of vascular risk factors (eg, normotensive, nondiabetic, nonsmoker) or positive family history of CADASIL."

U.S. Preventive Services Task Force Recommendations

Not applicable.

Ongoing and Unpublished Clinical Trials

Several ongoing studies that might influence this review are listed in Table 4.

Table 4. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT04310098	Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy Registry Study	1000	Mar 2049
NCT06148051	AusCADASIL: An Australian Cohort of CADASIL	300	March 2027
NCT05677880	Unraveling the Early Phases of Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL)	500	Jan 2026
NCT05072483	Natural History Study of CADASIL	140	June 2041

Government Regulations

National:

There is no national coverage determination addressing genetic testing for CADASIL syndrome.

Local:

There is no active local coverage determination or article that addresses genetic testing of CADASIL syndrome, or genetic testing of *NOTCH3*.

Palmetto GBA MolDX has no LCD regarding *NOTCH3* or CADASIL.

There is a retired Wisconsin Physicians Service Insurance Corporation Article, MolDX: Excluded Test List (A55247), which lists 81406 for NOTCH3, tsa.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

Genetic Testing – Preimplantation
Genetic Testing and Counseling

References

1. Yuan L, Chen X, Jankovic J, et al. CADASIL: A NOTCH3-associated cerebral small vessel disease. J Adv Res. Jan 02 2024. PMID 38176524
2. Joutel A, Favrole P, Labauge P et al. Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. Lancet. Dec 15 2001; 358(9298):2049-51. PMID 11755616
3. Lesnik Oberstein SA, van Duinen SG, van den Boom R et al. Evaluation of diagnostic NOTCH3 immunostaining in CADASIL. Acta Neuropathol. Aug 2003; 106(2):107-111. PMID 12756589
4. Muqtadar H, Testai FD. Single gene disorders associated with stroke: a review and update on treatment options. Curr Treat Options Cardiovasc Med. Jun 2012; 14(3):288-97. PMID 22528196
5. del Rio-Espinola A, Mendioroz M, Domingues-Montanari S et al. CADASIL management or what to do when there is little one can do. Expert Rev Neurother. Feb 2009; 9(2):197-210. PMID 19210195
6. Malandrini A, Gaudiano C, Gambelli S et al. Diagnostic value of ultrastructural skin biopsy studies in CADASIL. Neurology. Apr 24 2007; 68(17):1430-1432. PMID 17452591
7. Brulin P, Godfraind C, Leteurtre E et al. Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. Acta Neuropathol. Sep 2002; 104(3):241-248. PMID 12172909
8. Markus HS, Martin RJ, Simpson MA et al. Diagnostic strategies in CADASIL. Neurology. Oct 22 2002; 59(8):1134-1138. PMID 12395806
9. Choi JC, Lee KH, Song SK et al. Screening for NOTCH3 Gene Variants Among 151 Consecutive Korean Patients With Acute Ischemic Stroke. J Stroke Cerebrovasc Dis. Nov 29 2013; 22(5):608-614. PMID 22133740
10. Mosca L, Marazzi R, Ciccone A et al. NOTCH3 gene variants in subjects clinically suspected of CADASIL. J Neurol Sci. Aug 15 2011; 307(1-2):144-148. PMID 21616505
11. Hack RJ, Rutten J, Lesnik Oberstein SAJ. In: Adam MP, Feldman J, Mirzaa GM, et al., eds. GeneReviews, Seattle, WA: University of Washington; 2019.
12. Donahue CP, Kosik KS. Distribution pattern of Notch3 variants suggests a gain-of-function mechanism for CADASIL. Genomics. Jan 2004; 83(1):59-65. PMID 14667809
13. Chabriat H, Joutel A, Dichgans M et al. Cadasil. Lancet Neurol. Jul 2009; 8(7):643-653. PMID 19539236
14. Hack RJ, Gravesteijn G, Cerfontaine MN, et al. Three-tiered EGFr domain risk stratification for individualized NOTCH3-small vessel disease prediction. Brain. Jul 03 2023; 146(7): 2913-2927. PMID 36535904
15. Opherk C, Gonik M, Duering M, et al. Genome-wide genotyping demonstrates a polygenic risk score associated with white matter hyperintensity volume in CADASIL. Stroke. Apr 2014;45(4):968-972. PMID 24578207
16. Dupé C, Guey S, Biard L, et al. Phenotypic variability in 446 CADASIL patients: Impact of NOTCH3 gene mutation location in addition to the effects of age, sex and vascular risk factors. J Cereb Blood Flow Metab. Jan 2023; 43(1): 153-166. PMID 36254369

17. Pescini F, Nannucci S, Bertaccini B et al. The Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL) Scale: a screening tool to select patients for NOTCH3 gene analysis. Stroke. Nov 2012; 43(11):2871-2876. PMID 22996955
18. Lee YC, Liu CS, Chang MH et al. Population-specific spectrum of NOTCH3 mutations, MRI features and founder effect of CADASIL in Chinese. J Neurol. Feb 2009; 256(2):249-255. PMID 19242647
19. Yin X, Wu D, Wan J, et al. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: Phenotypic and mutational spectrum in patients from mainland China. Int J Neurosci. Sep 1 2015;125(8):585-592. PMID 25105908
20. Joutel A, Vahedi K, Corpechot C et al. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. Lancet. Nov 22 1997; 350(9090):1511-1515. PMID 9388399
21. Abramycheva N, Stepanova M, Kalashnikova L, et al. New variants in the Notch3 gene in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). J Neurol Sci. Feb 15 2015;349(1-2):196-201. PMID 25623805
22. Maksemous N, Smith RA, Haupt LM, et al. Targeted next generation sequencing identifies novel NOTCH3 gene mutations in CADASIL diagnostics patients. Hum Genomics. Nov 24 2016;10(1):38. PMID 27881154
23. Gorukmez O, Gorukmez O, Topak A, et al. NOTCH3 Variants in Patients with Suspected CADASIL. Ann Indian Acad Neurol. 2023; 26(4): 484-490. PMID 37970308
24. Peters N, Opherk C, Bergmann T, et al. Spectrum of variants in biopsy-proven CADASIL: implications for diagnostic strategies. Arch Neurol. Jul 2005;62(7):1091-1094. PMID 16009764
25. Tikka S, Mykkanen K, Ruchoux MM, et al. Congruence between NOTCH3 variants and GOM in 131 CADASIL patients. Brain. Apr 2009;132(Pt 4):933-939. PMID 19174371
26. Dotti MT, Federico A, Mazzei R, et al. The spectrum of Notch3 variants in 28 Italian CADASIL families. J Neurol Neurosurg Psychiatry. May 2005;76(5):736-738. PMID 15834039
27. Dichgans M, Markus HS, Salloway S et al. Donepezil in patients with subcortical vascular cognitive impairment: a randomised double-blind trial in CADASIL. Lancet Neurol. Apr 2008; 7(4):310-318. PMID 18296124
28. De Maria R, Campolo J, Frontali M, et al. Effects of sapropterin on endothelium-dependent vasodilation in patients with CADASIL: a randomized controlled trial. Stroke. Oct 2014;45(10):2959-2966. PMID 25184356
29. Huang L, Yang Q, Zhang L et al. Acetazolamide improves cerebral hemodynamics in CADASIL. J Neurol Sci. May 15 2010; 292(1-2):77-80. PMID 20227091
30. Peters N, Freilinger T, Opherk C et al. Effects of short term atorvastatin treatment on cerebral hemodynamics in CADASIL. J Neurol Sci. Sep 15 2007; 260(1-2):100-105. PMID 17531269
31. Meschia JF, Worrall BB, Elahi FM, et al. Management of Inherited CNS Small Vessel Diseases: The CADASIL Example: A Scientific Statement From the American Heart Association. Stroke. Oct 2023; 54(10): e452-e464. PMID 37602377

The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through 5/20/24, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
5/1/12	2/21/12	2/21/12	Joint policy established
7/1/13	4/16/13	4/22/13	Routine maintenance - Replaced unlisted procedure code with new CPT code 81406 (effective 1/1/13). Updated references.
11/1/14	8/19/14	8/19/14	Policy status changed from experimental/investigational to established. Title changed from “Genetic Testing - NOTCH3 Genotyping for Diagnosis of CADASIL” to “Genetic Testing of CADASIL Syndrome.”
7/1/16	4/19/16	4/19/16	Routine maintenance

7/1/17	6/7/17	6/6/17	Routine maintenance. Updated inclusions regarding testing of family members.
5/1/18	2/20/18	2/20/18	Routine maintenance. Policy status to EST. Updated inclusions: incorporation of CADASIL scale; genetic testing allowed if highly suggestive based on scale and to avoid skin biopsy; exclusions updated. References updated. Updated Medicare information.
5/1/19	2/19/19		Routine maintenance
5/1/20	2/18/20		Routine maintenance
5/1/21	4/1/21		Routine maintenance. Inclusions/exclusions: reference to skin biopsy deleted. Ref 28 added. Inclusions edited.
11/1/21	8/17/21		Routine maintenance
11/1/22	8/16/22		Routine maintenance (ls)
11/1/23	8/15/23		Routine maintenance (jf) Vendor Managed: NA
11/1/24	8/20/24		Routine maintenance (jf) Vendor Managed: NA Added Ref 1,11,14,16,23,31

Next Review Date: 3rd Qtr, 2025

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING OF CADASIL SYNDROME

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered; criteria apply
BCNA (Medicare Advantage)	See Government Regulations section.
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.