OptimaHealth
A Service of Sentara

Genetic Testing for CADASIL Syndrome

Policy Number: AHS – M2069 – Genetic Testing for CADASIL Syndrome	Prior Policy Name and Number, as applicable:
Policy Revision Date: 12/08/2021	

I. Policy Description

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Tournier-Lasserve et al., 1993) is a genetic small vessel disease in which mutations in the Notch Receptor 3 (*NOTCH3*) gene located on chromosome 19 (Joutel et al., 1996) result in a clinical syndrome of adult-onset migraines (frequently with aura), progressive strokes, and cognitive decline in adults leading to severe functional impairment by the seventh decade of life (Opherk, Peters, Herzog, Luedtke, & Dichgans, 2004; Zhu & Nahas, 2016).

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx or the manual website

1. Genetic testing to confirm the diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome **MEETS COVERAGE CRITERIA** under the following conditions:

    a. Clinical signs, symptoms, and imaging results are consistent with CADASIL, indicating that the pre-test probability of CADASIL is at least in the moderate to high range (See policy guidelines for further details)

    b. Individuals in which the diagnosis of CADASIL is inconclusive following a combination of clinical presentation, magnetic resonance imaging (MRI) findings, and skin biopsy findings.

2. Genetic testing for CADASIL syndrome in asymptomatic individuals who have a first- or second-degree relative diagnosed with CADASIL syndrome **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3. Genetic testing for CADASIL syndrome in all other situations **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

CADASIL is the most common hereditary small vessel disease and is characterized by granular osmiophilic material deposits surrounding blood vessels, a prominent thickening of the vessel wall by extracellular matrix accumulation, and a progressive loss of vascular smooth muscle cells (VSMCs) (Fernandez-Susavila et al., 2018; Ferrante, Cudrici, & Boehm, 2019; Monet-Lepretre et al., 2013). Small vessel diseases such as this are an important cause of stroke and vascular cognitive decline in adults (Chabriat, Joutel, Dichgans, Tournier-Lasserve, & Bousser, 2009). VSMC dysfunction may be caused by mutations in the *NOTCH3* gene, leading to irregularities in VSMC proliferation, cell cycle affliction, senescence, and cellular apoptosis (Dziewulska, Nycz, Rajczewska-Oleszkiewicz, Bojakowski, & Sulejczak, 2018).

Individual symptoms, onset, and disease severity span a wide spectrum (Wang, 2018). Thus, descriptions of hereditary multi-infarct dementia, chronic familial vascular encephalopathy, and familial subcortical dementia, originally thought to be separate disorders, represent early reports of this condition (Dichgans, 2019). CADASIL usually presents with one or more of the following: dementia, psychiatric disturbances, migraine, and recurrent strokes (Chabriat et al., 2009; Dichgans et al., 1998; M.Wang, 2018). Rarer symptoms include lumbago, humpback, and Parkinson syndrome (Cao et al., 2019; Lim, Millar, & Zaman, 2019). Migraine with aura occurs in 55% of CADASIL cases and is often the initial manifestation of the disease (Di Donato et al., 2017). Subcortical ischemic attacks begin at a mean age of 47 years and present as lacunar syndromes (Adib-Samii, Brice, Martin, & Markus, 2010; Dichgans et al., 1998). Accumulation of lacunae, which impact executive performance and function independence, strongly correlate to clinical severity (Ling et al., 2017). Cognitive impairment associated with CADASIL is progressive; a profile of frontal lobe dysfunction, declarative memory impairment suggestive of a retrieval deficit, and relatively preserved language is often evident with this disease (Harris & Filley, 2001). A concurrent stepwise deterioration due to recurrent strokes is also common (Rutten & Lesnik Oberstein, 2016). Mood disturbances are reported in approximately 30% of individuals (Adib-Samii et al., 2010; Dichgans et al., 1998). Further, apathy, which may be independent of depression, is reported in 40% of individuals (Reyes et al., 2009).

Genetic linking of the disorder to chromosome 19 was first recognized in 1993, and the identification of the *NOTCH3* gene from the CADASIL mapped region was later discovered in 1996 (Ping & Zhao, 2018). While CADASIL was originally diagnosed via neuroimaging techniques, such as magnetic resonance imaging (MRI), the identification of the distinctive missense mutations in *NOTCH3* has allowed genetic testing to debut as the current gold standard for CADASIL diagnostics (Rutten & Lesnik Oberstein, 2016). However, MRI testing for the detection of cerebral white matter changes in the

brain is still used to assist in CADASIL diagnoses; most often, MRI imaging is used as a diagnostic measure before symptoms present (Ferrante et al., 2019).

Missense mutations in the *NOTCH3* gene typically lead to the gain or loss of a cysteine, therefore resulting in an unpaired number of cysteine residues in one of 34 highly conserved epidermal growth factor-like repeat (EGFr) domains (Joutel et al., 1996; Papakonstantinou et al., 2019; Rutten et al., 2014). This leads to an increased multimerization tendency of mutant NOTCH3 (Duering et al., 2011), toxic accumulation of the protein and extracellular matrix in disulfide cross-linked detergent-insoluble aggregates (Monet-Lepretre et al., 2013), altered neurovascular coupling (Huneau et al., 2018), and ultimately reduced cerebral blood flow, recurrent stroke, and vascular dementia (Rutten et al., 2016). However, certain *NOTCH3* mutations do not present with a cysteine change; this type of non-cysteine mutation can cause a great loss of structure in the NOTCH3 protein (Papakonstantinou et al., 2019).

More than 200 *NOTCH3* mutations have been reported since its original discovery in the development of CADASIL syndrome in 1996; some of these mutations result in a phenotypic change while some present as a silent mutation. A few prevalent *NOTCH3* variants include the 34 identified in EGFr. EGFr 1–6 pathogenic variants are more common in the CADASIL population than EGFr 7–34 pathogenic variants; unfortunately, patients with EGFr 1–6 variants tend to present with more severe symptoms and phenotypes (Papakonstantinou et al., 2019; Rutten et al., 2018). These severe symptoms include stroke onset an average of 12 years earlier and overall lower survival rates (Papakonstantinou et al., 2019).

The prevalence of the disease has been estimated to be at 0.8 to 5 per 100,000 individuals (Moreton, Razvi, Davidson, & Muir, 2014; Narayan, Gorman, Kalaria, Ford, & Chinnery, 2012; Razvi, Davidson, Bone, & Muir, 2005); however, many suspect that these numbers are underestimates. A more recent investigation of the frequency of the characteristic missense CADASIL mutations in a public database found a total prevalence of 3.4/1000 (Rutten et al., 2016).

Currently, no efficient treatment options to cure or prevent CADASIL syndrome are available (R. Hack, Rutten, & Lesnik Oberstein, 2019; NORD, 2019); however, recent studies have shown proof of concept for a novel application of exon skipping and are a first step towards the development of a rational therapeutic approach to treat up to 94% of CADASIL-causing mutations (Rutten et al., 2016). Further, neurofilament light chains have now been identified as a promising CADASIL biomarker and can be detected in the serum of affected patients (Ferrante et al., 2019).

Analytical Validity

There are no established diagnostic criteria for CADASIL. The phenotype is highly variable, and although imaging may be suggestive, no characteristic is pathognomonic; genetic testing remains the gold standard for diagnosis (Rutten & Lesnik Oberstein, 2016; Wang, 2018). As a heterozygous pathogenic variant in the NOTCH3 protein coding gene is well established as a main reason for CADASIL development, a CADASIL diagnosis is generally delivered based on molecular genetic testing or electron microscopy and immunohistochemistry results. Molecular genetic testing approaches may include both gene-targeted testing and in-depth genomic testing, such as exome sequencing and genome sequencing (R. Hack et al., 2019; Papakonstantinou et al., 2019).

Immunohistochemistry combined with electron microscopy of skin biopsy can be useful when molecular testing is not definitive (Rutten & Lesnik Oberstein, 2016). Immunohistochemistry assay of a skin biopsy sample for the accumulation of NOTCH3 protein in the walls of small blood vessels (Joutel

et al., 2001) has an estimated sensitivity and specificity at 85-90% and 95-100%, respectively (Lesnik Oberstein et al., 2003). Detection of granular osmiophilic material deposits (GOM) containing the ectodomain of the *NOTCH3* gene by electron microscopy (del Rio-Espinola et al., 2009; Muqtadar & Testai, 2012) had a sensitivity of 45% and a specificity of 100% (Brulin, Godfraind, Leteurtre, & Ruchoux, 2002; Malandrini et al., 2007; Markus et al., 2002).

MRI is useful to demonstrate radiologic features of CADASIL, including recent lunar infarctions and white matter hyperintensities. Computed tomography (CT) scans are less sensitive than MRI in this regard (Dichgans, 2019). MRI may also provide prognostic information. Brain lesions in CADASIL patients tend to precede symptoms by 10 to 15 years; however, a normal MRI in the fourth decade of life should not automatically rule out CADASIL syndrome even though most patients exhibit an abnormal MRI by age 35 (Samoes et al., 2016). White matter hyperintensities on MRI can be visualized in those aged 21 years and older, and lesion volume correlates with the level of disability and three-year clinical course of CADASIL (Jouvent et al., 2016). Isolated T2 hyperintensities involving the temporal poles can differentiate CADASIL from chronic microvascular ischemia due to hypertension with a sensitivity and specificity of 95% and 80%, respectively (O'Sullivan et al., 2001). Cerebral microbleeds visible on T2 weighted MRI images detected in 36% of patients with CADASIL were independently associated with an increased risk of incident ischemic stroke and may be a marker for a subgroup of patients with CADASIL who have a more severe or advanced form of the disease (Puy et al., 2017).

Guo et al. (2021) studied the role of *NOTCH3* gene mutations and variants in Alzheimer Disease (AD) and subcortical vascular dementia (SVaD). CADASIL is a common etiology of SVaD. 667 AD patients, 96 SVaD patients, and 365 healthy control participants, all recruited from the Southern Han Chinese population, were included in the study. The authors performed targeted capture sequencing on *NOTCH3* and adjacent intron regions. “Five known pathogenic variants (p.R182C, p.C201S, p.R544C, p.R607C, and p.R1006C) and two novel likely pathogenic variants (p.C201F and p.C1061F) were detected in 16 SVaD patients.” No pathogenic variants were found in AD patients. The authors concluded that the “findings broaden the mutational spectrum of NOTCH3 and validate the pathogenic role of *NOTCH3* mutations in SVaD, but do not support the notion that *NOTCH3* variation influences the risk of AD” (Guo et al., 2021).

Cho et al. (2021) performed an analysis on whole-exome sequencing data from 200,632 participants in the UK Biobank. The authors note that CADASIL is considered rare, but there is a higher frequency of cysteine-altering *NOTCH3* variants which could increase risk of apparently sporadic lacunar stroke. The authors compared frequency of stroke, vascular dementia, clinical features of CADASIL, and MRI white matter hyperintensity volume between carriers and non-carriers of 67 cysteine-altering *NOTCH3* variants. “*NOTCH3* variant carriers had increased risk of stroke (OR: 2.33, p=0.0004) and vascular dementia (OR: 5.00, p=0.007), and increased white matter hyperintensity volume (standardised difference: 0.52, p<0.001) and white matter ultrastructural damage on diffusion MRI (standardised difference: 0.72, p<0.001).” The authors concluded that “cysteine-changing *NOTCH3* variants are more common in the general population than expected from CADASIL prevalence and are risk factors for apparently 'sporadic' stroke and vascular dementia” (Cho et al., 2021).

Gravesteijn et al. (2021) studied the effect of *NOTCH3* variant position on NOTCH3 protein aggregation load. Vascular NOTCH3 aggregation was measured in skin biopsies and brain tissue from CADASIL patients. “CADASIL patients with an EGFr 7-34 variant have significantly less vascular NOTCH3 aggregation than patients with an EGFr 1-6 variant.” The authors concluded that *NOTCH3* variant

position may be a factor that underlies differences in CADASIL disease severity (Gravesteijn et al., 2021).

Clinical Validity and Utility

One study has reported that the sequence analysis of *NOTCH3* is 95-100% sensitive and 100% specific to establish the diagnosis of CADASIL (Dotti et al., 2005; Peters et al., 2005; Tikka et al., 2009; Yin et al., 2015). A preliminary scale was proposed to screen for patients who should undergo *NOTCH3* gene analysis with a sensitivity of 96.7% and a specificity of 74.2% (Pescini et al., 2012). Another study of Russian patients with clinically suspected CADASIL concluded that careful assessment of genealogical, clinical, and neuroimaging data in patients with lacunar stroke can help select patients with a high probability of finding mutations on genetic screening (Abramycheva et al., 2015). In the absence of clinical features suggestive of CADASIL, screening of patients with lacunar stroke, leukoarosis, and migraine have low yield (de Vries, Frants, Ferrari, & van den Maagdenberg, 2009; Dong et al., 2003).

As individual symptoms and disease severity span a wide spectrum, it must be noted that symptom onset alone cannot warrant a CADASIL syndrome diagnosis. Researchers previously screened 123 patients who exhibited two common CADASIL symptoms: lacunar stroke and transient ischemic attack. These participants were genetically tested for CADASIL; it was determined that only 12.5% had a *NOTCH3* mutation, showing that common CADASIL symptoms are shared with many other disorders (Bersano et al., 2018). This highlights the importance of genetic testing as a diagnostic measure. Further, three features were found to be significantly associated with a CADASIL diagnosis: "A family history of stroke, the presence of dementia and external capsule lesions on MRI" (Bersano et al., 2018).

CADASIL was first diagnosed by visualizing granular osmiophilic material (GOM) in the tunica media of small arteries through light microscopy. Although GOM deposit is the pathological hallmark of CADASIL, *NOTCH3* genetic sequencing is the confirmative diagnostic tool. While most genetic tests use Sanger sequencing methods to target specific *NOTCH3* exons, next-generation sequencing (NGS) and whole exome sequencing (WES) have proven to deliver greater efficacy. One study has reported that NGS and WES have increased sensitivity to detect low frequency variants of *NOTCH3* mutations compared to Sanger sequencing. Through Sanger sequencing, 10.8% of tests were able to identify *NOTCH3* mutations compared to 15.8% of tests identifying mutations through next-generation sequencing. With NGS, the results were in concordance with Sanger sequencing, but it extended the capacity to detect mutations and previously unreported variants. As diagnostic sequencing techniques continue to advance, NGS and WES may play an important role in identifying other genes involved with CADASIL (Dunn et al., 2020).

Rutten et al. (2018) analyzed the effect of *NOTCH3* pathogenic variant (PV) location on CADASIL disease variability. The authors correlated PV position with brain MRI lesion load, age of first stoke, and survival on 664 European CADASIL patients. "CADASIL patients with an EGFr 1–6 pathogenic variant have a 12-year earlier onset of stroke than those with an EGFr 7–34 pathogenic variant, lower survival, and higher white matter hyperintensity volumes." The authors concluded that *NOTCH3* PV location is "the most important determinant of CADASIL disease severity"(Rutten et al., 2018).

Mukai et al. (2020) correlated genotypes and phenotypes of 179 Japanese CADASIL probands. The authors identified 68 mutations, "p.Cys388Arg, p.Cys435Phe, p.Gly481Cys, p.Cys743Tyr, and p.Cys1009Phe were novel ones." The authors then analyzed genotype-phenotype correlations on the three most common mutations. "p.Arg141Cys showed typical CADASIL phenotypes, whereas

p.Arg75Pro showed mild and atypical phenotypes, a low frequency of stroke/TIA [transient ischemic attack], high frequency of hypertension, and low frequency of temporal pole lesions. p.Arg182Cys showed various initial symptoms other than stroke/TIA". The authors also studied mutation location and the age of stroke/TIA onset, and found that mutations of EGFr 1-6 (excluding p.Arg75Pro) were significantly correlated with a younger age of stroke/TIA onset than mutations in EGFr 7-43. The authors concluded that the data clarified genotype-phenotype correlations and the effect of mutation location on the age of stroke/TIA onset in Japanese CADASIL probands (Mukai et al., 2020).

Hack et al. (2020) performed a cross-sectional study using 118 participants with a *NOTCH3* cysteine altering variant and 184 age- and sex-matched control participants. Clinical, neuroimaging, and whole-exome data was compared. There was no difference in dementia, mild cognitive impairment, migraine with aura, or depression prevalence. Participants with a *NOTCH3* cysteine altering variant had a higher had a higher risk of stroke, white matter hyperintensity, and lacunas after age 65. The authors note that the classic mid-adult onset CADASIL phenotype was not reported, suggesting "*NOTCH3* variants do not only cause the rate and more severe hereditary CADASIL but are much more commonly associated with a milder [cerebral small vessel disease] SVD phenotype, specifically when these variants are located in EGFr 7 to 35" (R. J. Hack et al., 2020).

Liu et al. (2021) tracked clinical and MRI data of three patients from a family in China over seven years. Genetic tests confirmed CADASIL diagnosis on all three participants, including a novel mutation of p.C533S on exon 10 of *NOTCH3*. The same heterozygous mutations were detected across family members. The authors conclude that there is "distinct heterogeneity of CADASIL patients in the same family with the same mutation" (Liu et al., 2021).

Chen et al. (2021) assessed the diagnostic utility of using NGS and MRI data for the diagnosis of adult onset leukodystrophy. The authors used a panel of 200 neurodegeneration-related genes and an MRI brain-based diagnostic algorithm from 45 patients with young-onset cognitive impairment with leukodystrophy. All of the patients with an established genetic diagnosis had MRI brain patterns consistent with their diagnosis. 51.4% of patients with MRI changes consistent with vascular cognitive impairment secondary to small vessel disease (VCI-SVD) had pathogenic variants: 89.5% of which were pathogenic *NOTCH3* and 11.5% of which were *HTRA1* variants. The authors concluded that the results "demonstrated a high diagnostic utility incorporating a targeted neurodegeneration gene panel and MRI-based diagnostic algorithms in young-onset cognitive impairment patients with leukodystrophy" (Chen et al., 2021).

Predictive Testing of At-Risk Family Members

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 a factor that delays onset of disease, but this is a general recommendation that is not altered by genetic testing. Goldman (2015) has suggested that asymptomatic family members follow the guidelines for presymptomatic testing for Huntington disease (HDSA, 2016).

CADASIL genetic testing may assist decision making in areas such as employment choices and reproductive decision making. However, the impact of these decisions on health outcomes is uncertain. Further, the testing of asymptomatic at-risk individuals with nonspecific or equivocal symptoms is not useful in predicting age of onset, severity, type of symptoms, or rate of progression

in asymptomatic individuals (Rutten & Lesnik Oberstein, 2016). Initial data from Reyes, Kurtz, Herve, Tournier-Lasserve, and Chabriat (2012) show that predictive testing is rarely requested and has a high dropout rate.

Di Donato et al. (2017) state that the MRI of an unaffected family member could have a similar impact to a genetic test because MRIs are able to accurately predict CADASIL disease development before symptoms present. Therefore, the potential implications of MRI testing should be shared before this type of testing is completed.

V. Guidelines and Recommendations

American Heart Association and American Stroke Association (Kleindorfer et al., 2021; Powers et al., 2019; Smith et al., 2017)

The American Heart Association and American Stroke Association do not provide any recommendations on rare genetic causes of cerebral small vessel disease, such as CADASIL, but they do provide suggestions on when rare genetic causes could be suspected. They suggest that the diagnosis could be made on the basis of testing for mutations in the *NOTCH3* gene (Kleindorfer et al., 2021; Powers et al., 2019; Smith et al., 2017).

European Federation of Neurological Societies (Burgunder et al., 2010)

The European Federation of Neurological Societies guideline on the molecular diagnosis of channelopathies, epilepsies, migraine, stroke, and dementias notes that most *NOTCH3* mutations occur within exons 3 and 4 and suggests direct sequencing of these 2 exons if clinical suspicion is high (Burgunder et al., 2010).

U.S. Preventive Services Task Force (USPSTF, 2021)

As of 10/05/2021, the USPSTF has not published guidelines for the genetic testing of CADASIL patients.

European Academy of Neurology (EAN) (Mancuso et al., 2020)

The European Academy of Neurology (EAN) released guidelines for monogenic cerebral small-vessel disease (cSVD), including diagnosis and management of CADASIL. EAN suggests that the first line diagnosis for CADASIL should be genetic testing, but diagnosis can also be established by skin biopsy with electron microscopy revealing granular osmiophilic material (GOM). Most *NOTCH3* variants causing CADASIL are due to a loss or gain of a cysteine in the EGFR repeats. Some non-cysteine changing variants have been reported, but most of these non-cysteine changing variants do not lead to a diseased state. If genetic testing reveals a non-cysteine changing variant, electron microscopy to visualize GOM is a useful tool to confirm CADASIL diagnosis. If the *NOTCH3* variant is of unknown significance, CADASIL diagnosis can be established with skin biopsy via electron microscopy or immunohistochemistry of the NOTCH3 extracellular domain. The guideline recommends "considering" a CADASIL diagnosis in any patient with "unexplained symmetrical periventricular WMHs [white matter hyperintensities] and a positive family history of migraine with aura, stroke, mood disorders or dementia". The guideline also notes that CADASIL cannot be ruled out in the presence of "common cerebrovascular risk factors and extensive WMHs" or in "the absence of a medical or family history of migraine with aura". The guideline remarks that "although most patients

have a family history, if the clinical and imaging phenotype is consistent with CADASIL the diagnosis should be considered" (Mancuso et al., 2020).

Overall, the EAN remarks that "CADASIL can only be definitively confirmed by genetic testing, revealing a *NOTCH3* mutation altering the number of cysteines in one of the 34 EGFr domains of the NOTCH3 protein" (Mancuso et al., 2020).

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration (FDA)

No U.S. Food and Drug Administration-cleared tests were found with the keyword "NOTCH3" as of 10/05/2021; a total of 25 U.S. Food and Drug Administration-cleared tests were found with the keyword "genotyping." Additionally, many labs have developed specific tests that they must validate and perform in house. *NOTCH3* sequencing is therefore a laboratory developed test (LDT). These LDTs are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

B. Centers for Medicare & Medicaid Services (CMS)

N/A

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81406	Molecular pathology procedure, level 7 Gene: ***NOTCH3*** (notch 3) (eg, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]), targeted sequence analysis (eg, exons 1-23).

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

Abramycheva, N., Stepanova, M., Kalashnikova, L., Zakharova, M., Maximova, M., Tanashyan, M., . . . Illarioshkin, S. (2015). New mutations in the Notch3 gene in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *J Neurol Sci, 349*(1-2), 196-201. doi:10.1016/j.jns.2015.01.018

Adib-Samii, P., Brice, G., Martin, R. J., & Markus, H. S. (2010). Clinical spectrum of CADASIL and the effect of cardiovascular risk factors on phenotype: study in 200 consecutively recruited individuals. *Stroke, 41*(4), 630-634. doi:10.1161/STROKEAHA.109.568402

Bersano, A., Bedini, G., Markus, H. S., Vitali, P., Colli-Tibaldi, E., Taroni, F., . . . Candelise, L. (2018). The role of clinical and neuroimaging features in the diagnosis of CADASIL. *J Neurol, 265*(12), 2934-2943. doi:10.1007/s00415-018-9072-8

Brulin, P., Godfraind, C., Leteurtre, E., & Ruchoux, M. M. (2002). Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. *Acta Neuropathol, 104*(3), 241-248. doi:10.1007/s00401-002-0530-z

Burgunder, J. M., Finsterer, J., Szolnoki, Z., Fontaine, B., Baets, J., Van Broeckhoven, C., . . . Gasser, T. (2010). EFNS guidelines on the molecular diagnosis of channelopathies, epilepsies, migraine, stroke, and dementias. *Eur J Neurol, 17*(5), 641-648. doi:10.1111/j.1468-1331.2010.02985.x

Cao, L., Zhang, Q., Yuan, Y., Liu, L., He, L., Zhang, C., . . . You, Y. (2019). [CADASIL with clinical manifestations of lumbago, hunchback and Parkinson's syndrome]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 36*(9), 922-925. doi:10.3760/cma.j.issn.1003-9406.2019.09.017

Chabriat, H., Joutel, A., Dichgans, M., Tournier-Lasserve, E., & Bousser, M. G. (2009). Cadasil. *Lancet Neurol, 8*(7), 643-653. doi:10.1016/S1474-4422(09)70127-9

Chen, Z., Tan, Y. J., Lian, M. M., Tandiono, M., Foo, J. N., Lim, W. K., . . . Ng, A. S. L. (2021). High Diagnostic Utility Incorporating a Targeted Neurodegeneration Gene Panel With MRI Brain Diagnostic Algorithms in Patients With Young-Onset Cognitive Impairment With Leukodystrophy. *Front Neurol, 12*, 631407. doi:10.3389/fneur.2021.631407

Cho, B. P. H., Nannoni, S., Harshfield, E. L., Tozer, D., Gräf, S., Bell, S., & Markus, H. S. (2021). NOTCH3 variants are more common than expected in the general population and associated with stroke and vascular dementia: an analysis of 200 000 participants. *J Neurol Neurosurg Psychiatry, 92*(7), 694-701. doi:10.1136/jnnp-2020-325838

de Vries, B., Frants, R. R., Ferrari, M. D., & van den Maagdenberg, A. M. (2009). Molecular genetics of migraine. *Hum Genet, 126*(1), 115-132. doi:10.1007/s00439-009-0684-z

del Rio-Espinola, A., Mendioroz, M., Domingues-Montanari, S., Pozo-Rosich, P., Sole, E., Fernandez-Morales, J., . . . Montaner, J. (2009). CADASIL management or what to do when there is little one can do. *Expert Rev Neurother, 9*(2), 197-210. doi:10.1586/14737175.9.2.197

Di Donato, I., Bianchi, S., De Stefano, N., Dichgans, M., Dotti, M. T., Duering, M., . . . Federico, A. (2017). Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) as a model of small vessel disease: update on clinical, diagnostic, and management aspects. *BMC Med, 15*. doi:10.1186/s12916-017-0778-8

Dichgans, M. (2019). Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) - UpToDate. In J. Dashe (Ed.), *UpToDate*. Retrieved from https://www.uptodate.com/contents/cerebral-autosomal-dominant-arteriopathy-with-subcortical-infarcts-and-leukoencephalopathy-cadasil?search=cadasil&source=search_result&selectedTitle=1~16&usage_type=default&display_rank=1

Dichgans, M., Mayer, M., Uttner, I., Bruning, R., Muller-Hocker, J., Rungger, G., . . . Gasser, T. (1998). The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol, 44*(5), 731-739. doi:10.1002/ana.410440506

Dong, Y., Hassan, A., Zhang, Z., Huber, D., Dalageorgou, C., & Markus, H. S. (2003). Yield of screening for CADASIL mutations in lacunar stroke and leukoaraiosis. *Stroke, 34*(1), 203-205. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12511775

Dotti, M. T., Federico, A., Mazzei, R., Bianchi, S., Scali, O., Conforti, F. L., . . . Quattrone, A. (2005). The spectrum of Notch3 mutations in 28 Italian CADASIL families. *J Neurol Neurosurg Psychiatry, 76*(5), 736-738. doi:10.1136/jnnp.2004.048207

Duering, M., Karpinska, A., Rosner, S., Hopfner, F., Zechmeister, M., Peters, N., . . . Opherk, C. (2011). Co-aggregate formation of CADASIL-mutant NOTCH3: a single-particle analysis. *Hum Mol Genet, 20*(16), 3256-3265. doi:10.1093/hmg/ddr237

Dunn, P. J., Maksemous, N., Smith, R. A., Sutherland, H. G., Haupt, L. M., & Griffiths, L. R. (2020). Investigating diagnostic sequencing techniques for CADASIL diagnosis. *Hum Genomics, 14*(1), 2. doi:10.1186/s40246-019-0255-x

Dziewulska, D., Nycz, E., Rajczewska-Oleszkiewicz, C., Bojakowski, J., & Sulejczak, D. (2018). Nuclear abnormalities in vascular myocytes in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Neuropathology, 38*(6), 601-608. doi:10.1111/neup.12519

Fernandez-Susavila, H., Mora, C., Aramburu-Nunez, M., Quintas-Rey, R., Arias, S., Collado, M., . . . Campos, F. (2018). Generation and characterization of the human iPSC line IDISi001-A isolated from blood cells of a CADASIL patient carrying a NOTCH3 mutation. *Stem Cell Res, 28*, 16-20. doi:10.1016/j.scr.2018.01.023

Ferrante, E. A., Cudrici, C. D., & Boehm, M. (2019). CADASIL: new advances in basic science and clinical perspectives. *Curr Opin Hematol, 26*(3), 193-198. doi:10.1097/moh.0000000000000497

Goldman, J. S. (2015). Genetic testing and counseling in the diagnosis and management of young-onset dementias. *Psychiatr Clin North Am, 38*(2), 295-308. doi:10.1016/j.psc.2015.01.008

Gravesteijn, G., Hack, R. J., Mulder, A. A., Cerfontaine, M. N., van Doorn, R., Hegeman, I. M., . . . Lesnik Oberstein, S. A. J. (2021). NOTCH3 variant position is associated with NOTCH3 aggregation load in CADASIL vasculature. *Neuropathol Appl Neurobiol*. doi:10.1111/nan.12751

Guo, L., Jiao, B., Liao, X., Xiao, X., Zhang, W., Yuan, Z., . . . Shen, L. (2021). The role of NOTCH3 variants in Alzheimer's disease and subcortical vascular dementia in the Chinese population. *CNS Neurosci Ther, 27*(8), 930-940. doi:10.1111/cns.13647

Hack, R., Rutten, J., & Lesnik Oberstein, S. A. J. (2019). CADASIL. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. Seattle (WA): University of Washington, Seattle

Hack, R. J., Rutten, J. W., Person, T. N., Li, J., Khan, A., Griessenauer, C. J., . . . Zand, R. (2020). Cysteine-Altering NOTCH3 Variants Are a Risk Factor for Stroke in the Elderly Population. *Stroke, 51*(12), 3562-3569. doi:10.1161/strokeaha.120.030343

Harris, J. G., & Filley, C. M. (2001). CADASIL: neuropsychological findings in three generations of an affected family. *J Int Neuropsychol Soc, 7*(6), 768-774. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11575598

HDSA. (2016). HDSA Genetic Testing Protocol for HD. Retrieved from http://hdsa.org/wp-content/uploads/2015/02/HDSA-Gen-Testing-Protocol-for-HD.pdf. from Huntington's Disease Society of America http://hdsa.org/wp-content/uploads/2015/02/HDSA-Gen-Testing-Protocol-for-HD.pdf

Huneau, C., Houot, M., Joutel, A., Beranger, B., Giroux, C., Benali, H., & Chabriat, H. (2018). Altered dynamics of neurovascular coupling in CADASIL. *Ann Clin Transl Neurol, 5*(7), 788-802. doi:10.1002/acn3.574

Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., . . . Tournier-Lasserve, E. (1996). Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature, 383*(6602), 707-710. doi:10.1038/383707a0

Joutel, A., Favrole, P., Labauge, P., Chabriat, H., Lescoat, C., Andreux, F., . . . Tournier-Lasserve, E. (2001). Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. *Lancet, 358*(9298), 2049-2051. doi:10.1016/S0140-6736(01)07142-2

Jouvent, E., Duchesnay, E., Hadj-Selem, F., De Guio, F., Mangin, J. F., Herve, D., . . . Chabriat, H. (2016). Prediction of 3-year clinical course in CADASIL. *Neurology, 87*(17), 1787-1795. doi:10.1212/WNL.0000000000003252

Kleindorfer, D. O., Towfighi, A., Chaturvedi, S., Cockroft, K. M., Gutierrez, J., Lombardi-Hill, D., . . . Williams, L. S. (2021). 2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association. *Stroke, 52*(7), e364-e467. doi:10.1161/str.0000000000000375

Lesnik Oberstein, S. A., van Duinen, S. G., van den Boom, R., Maat-Schieman, M. L., van Buchem, M. A., van Houwelingen, H. C., . . . Haan, J. (2003). Evaluation of diagnostic NOTCH3 immunostaining in CADASIL. *Acta Neuropathol, 106*(2), 107-111. doi:10.1007/s00401-003-0701-6

Lim, H. K., Millar, Z. A., & Zaman, R. (2019). CADASIL and Bipolar Affective Disorder. *Psychiatr Danub, 31*(Suppl 3), 591-594.

Ling, Y., De Guio, F., Duering, M., Jouvent, E., Herve, D., Godin, O., . . . Chabriat, H. (2017). Predictors and Clinical Impact of Incident Lacunes in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy. *Stroke, 48*(2), 283-289. doi:10.1161/strokeaha.116.015750

Liu, Y., Huang, S., Yu, L., Li, T., Diao, S., Chen, Z., . . . Fang, Q. (2021). A Chinese CADASIL Family with a Novel Mutation on Exon 10 of Notch3 Gene. *J Stroke Cerebrovasc Dis, 30*(8), 105674. doi:10.1016/j.jstrokecerebrovasdis.2021.105674

M.Wang, A. l. o. o. p. (2018). *Handbook of Clinical Neurology* (Vol. 148).

Malandrini, A., Gaudiano, C., Gambelli, S., Berti, G., Serni, G., Bianchi, S., . . . Dotti, M. T. (2007). Diagnostic value of ultrastructural skin biopsy studies in CADASIL. *Neurology, 68*(17), 1430-1432. doi:10.1212/01.wnl.0000264018.46335.c8

Mancuso, M., Arnold, M., Bersano, A., Burlina, A., Chabriat, H., Debette, S., . . . Markus, H. S. (2020). Monogenic cerebral small-vessel diseases: diagnosis and therapy. Consensus recommendations of the European Academy of Neurology. *Eur J Neurol, 27*(6), 909-927. doi:10.1111/ene.14183

Markus, H. S., Martin, R. J., Simpson, M. A., Dong, Y. B., Ali, N., Crosby, A. H., & Powell, J. F. (2002). Diagnostic strategies in CADASIL. *Neurology, 59*(8), 1134-1138. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12395806

Monet-Lepretre, M., Haddad, I., Baron-Menguy, C., Fouillot-Panchal, M., Riani, M., Domenga-Denier, V., . . . Joutel, A. (2013). Abnormal recruitment of extracellular matrix proteins by excess Notch3 ECD: a new pathomechanism in CADASIL. *Brain, 136*(Pt 6), 1830-1845. doi:10.1093/brain/awt092

Moreton, F. C., Razvi, S. S., Davidson, R., & Muir, K. W. (2014). Changing clinical patterns and increasing prevalence in CADASIL. *Acta Neurol Scand, 130*(3), 197-203. doi:10.1111/ane.12266

Mukai, M., Mizuta, I., Watanabe-Hosomi, A., Koizumi, T., Matsuura, J., Hamano, A., . . . Mizuno, T. (2020). Genotype-phenotype correlations and effect of mutation location in Japanese CADASIL patients. *J Hum Genet, 65*(8), 637-646. doi:10.1038/s10038-020-0751-9

Muqtadar, H., & Testai, F. D. (2012). Single gene disorders associated with stroke: a review and update on treatment options. *Curr Treat Options Cardiovasc Med, 14*(3), 288-297. doi:10.1007/s11936-012-0179-4

Narayan, S. K., Gorman, G., Kalaria, R. N., Ford, G. A., & Chinnery, P. F. (2012). The minimum prevalence of CADASIL in northeast England. *Neurology, 78*(13), 1025-1027. doi:10.1212/WNL.0b013e31824d586c

NORD. (2019). CADASIL. Retrieved from https://rarediseases.org/rare-diseases/cadasil/

O'Sullivan, M., Jarosz, J. M., Martin, R. J., Deasy, N., Powell, J. F., & Markus, H. S. (2001). MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. *Neurology, 56*(5), 628-634. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11245715

Opherk, C., Peters, N., Herzog, J., Luedtke, R., & Dichgans, M. (2004). Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. *Brain, 127*(Pt 11), 2533-2539. doi:10.1093/brain/awh282

Papakonstantinou, E., Bacopoulou, F., Brouzas, D., Megalooikonomou, V., D'Elia, D., Bongcam-Rudloff, E., & Vlachakis, D. (2019). NOTCH3 and CADASIL syndrome: a genetic and structural overview. *EMBnet J, 24*. doi:10.14806/ej.24.0.921

Pescini, F., Nannucci, S., Bertaccini, B., Salvadori, E., Bianchi, S., Ragno, M., . . . Pantoni, L. (2012). The Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL) Scale: a screening tool to select patients for NOTCH3 gene analysis. *Stroke, 43*(11), 2871-2876. doi:10.1161/STROKEAHA.112.665927

Peters, N., Opherk, C., Bergmann, T., Castro, M., Herzog, J., & Dichgans, M. (2005). Spectrum of mutations in biopsy-proven CADASIL: implications for diagnostic strategies. *Arch Neurol, 62*(7), 1091-1094. doi:10.1001/archneur.62.7.1091

Ping, S., & Zhao, L.-R. (2018). *Current Understanding of Pathology and Therapeutic Status for CADASIL*.

Powers, W. J., Rabinstein, A. A., Ackerson, T., Adeoye, O. M., Bambakidis, N. C., Becker, K., . . . Tirschwell, D. L. (2019). Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke, 50*(12), e344-e418. doi:doi:10.1161/STR.0000000000000211

Puy, L., De Guio, F., Godin, O., Duering, M., Dichgans, M., Chabriat, H., & Jouvent, E. (2017). Cerebral Microbleeds and the Risk of Incident Ischemic Stroke in CADASIL (Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy). *Stroke, 48*(10), 2699-2703. doi:10.1161/strokeaha.117.017839

Razvi, S. S., Davidson, R., Bone, I., & Muir, K. W. (2005). The prevalence of cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) in the west of Scotland. *J Neurol Neurosurg Psychiatry, 76*(5), 739-741. doi:10.1136/jnnp.2004.051847

Reyes, S., Kurtz, A., Herve, D., Tournier-Lasserve, E., & Chabriat, H. (2012). Presymptomatic genetic testing in CADASIL. *J Neurol, 259*(10), 2131-2136. doi:10.1007/s00415-012-6468-8

Reyes, S., Viswanathan, A., Godin, O., Dufouil, C., Benisty, S., Hernandez, K., . . . Chabriat, H. (2009). Apathy: a major symptom in CADASIL. *Neurology, 72*(10), 905-910. doi:10.1212/01.wnl.0000344166.03470.f8

Rutten, J. W., Dauwerse, H. G., Gravesteijn, G., van Belzen, M. J., van der Grond, J., Polke, J. M., . . . Lesnik Oberstein, S. A. (2016). Archetypal NOTCH3 mutations frequent in public exome: implications for CADASIL. *Ann Clin Transl Neurol, 3*(11), 844-853. doi:10.1002/acn3.344

Rutten, J. W., Haan, J., Terwindt, G. M., van Duinen, S. G., Boon, E. M., & Lesnik Oberstein, S. A. (2014). Interpretation of NOTCH3 mutations in the diagnosis of CADASIL. *Expert Rev Mol Diagn, 14*(5), 593-603. doi:10.1586/14737159.2014.922880

Rutten, J. W., & Lesnik Oberstein, S. A. J. (2016). Cadasil. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20301673. https://www.ncbi.nlm.nih.gov/pubmed/20301673

Rutten, J. W., Van Eijsden, B. J., Duering, M., Jouvent, E., Opherk, C., Pantoni, L., . . . Lesnik Oberstein, S. A. J. (2018). The effect of NOTCH3 pathogenic variant position on CADASIL disease severity: NOTCH3 EGFr 1-6 pathogenic variant are associated with a more severe phenotype and lower survival compared with EGFr 7-34 pathogenic variant. *Genet Med*. doi:10.1038/s41436-018-0088-3

Samoes, R., Alves, J. E., Taipa, R., Silva, J., Melo Pires, M., & Pereira-Monteiro, J. M. (2016). CADASIL: MRI may be normal in the fourth decade of life - a case report. *Cephalalgia, 36*(11), 1082-1085. doi:10.1177/0333102415618613

Smith, E. E., Saposnik, G., Biessels, G. J., Doubal, F. N., Fornage, M., Gorelick, P. B., . . . Council on, H. (2017). Prevention of Stroke in Patients With Silent Cerebrovascular Disease: A Scientific Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke, 48*(2), e44-e71. doi:10.1161/STR.0000000000000116

Tikka, S., Mykkanen, K., Ruchoux, M. M., Bergholm, R., Junna, M., Poyhonen, M., . . . Kalimo, H. (2009). Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. *Brain, 132*(Pt 4), 933-939. doi:10.1093/brain/awn364

Tournier-Lasserve, E., Joutel, A., Melki, J., Weissenbach, J., Lathrop, G. M., Chabriat, H., . . . et al. (1993). Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. *Nat Genet, 3*(3), 256-259. doi:10.1038/ng0393-256

USPSTF. (2021). Published Recommendations. Retrieved from https://www.uspreventiveservicestaskforce.org/BrowseRec/Index

Wang, M. (2018). Cadasil. *Handb Clin Neurol, 148*, 733-743. doi:10.1016/B978-0-444-64076-5.00047-8

Yin, X., Wu, D., Wan, J., Yan, S., Lou, M., Zhao, G., & Zhang, B. (2015). Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: Phenotypic and mutational spectrum in patients from mainland China. *Int J Neurosci, 125*(8), 585-592. doi:10.3109/00207454.2014.951929

Zhu, S., & Nahas, S. J. (2016). CADASIL: Imaging Characteristics and Clinical Correlation. *Curr Pain Headache Rep, 20*(10), 57. doi:10.1007/s11916-016-0584-6