2

INTERPRETATION OF NOTCH3 MUTATIONS IN THE DIAGNOSIS OF CADASIL

Julie Rutten[1], Joost Haan[2,3], Gisela Terwindt[3], Sjoerd van Duinen[4], Elles Boon[5], Saskia Lesnik Oberstein[5]

[1] Department of Human Genetics, Leiden University Medical Center, [2] Department of Neurology, Rijnland Hospital, [3] Department of Neurology, Leiden University Medical Center, [4] Department of Pathology, Leiden University Medical Center,[5] Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Expert Review of Molecular Diagnostics. 2014; 14(5):593-603.

ABSTRACT

CADASIL is an autosomal dominantly inherited disease, characterized by mid-adult onset of cerebrovascular disease and dementia. CADASIL is caused by mutations in the *NOTCH3* gene which encodes the NOTCH3 protein. Pathogenic mutations in CADASIL are highly distinctive in the sense that they typically lead to the loss or gain of a cysteine residue in one of the 34 epidermal growth factor-like repeat (EGFr) domains of the NOTCH3 protein. The majority are missense mutations, but small deletions, insertions and splice-site mutations have been reported, which typically also lead to a numerical cysteine alteration. Whether numerical cysteine altering mutations are a rule in CADASIL remains subject of debate, as there are reports suggesting pathogenicity of other types of mutations. However, for most of these the association with CADASIL was later revoked or is questionable. Here, we discuss and provide recommendations for the interpretation of *NOTCH3* mutations in the diagnosis of CADASIL.

INTRODUCTION

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is a hereditary small vessel disease, caused by mutations in the *NOTCH3* gene. CADASIL patients can present with a history of migraine with aura, young to mid adult onset of cerebrovascular disease, mood disturbance, apathy, cognitive decline progressing to dementia, and diffuse white matter lesions and subcortical infarcts on neuroimaging.

Compared to other inherited brain disorders with a comparable impact, such as Huntington's chorea or inherited early-onset Alzheimer's dementia, CADASIL is still relatively unknown in the medical community. This is not so much due to the fact that it is a rare disease, but more to the fact that there is only a short history of recognition of the disease. CADASIL was first described as a clearly defined and separate disease entity in the early 1990's,[1-3] followed by the identification of mutations in the *NOTCH3* gene in 1996.[4] Since then, *NOTCH3* mutation screening has allowed for the identification of CADASIL families all over the globe, with an ever increasing prevalence due to increased awareness of the disease, diagnosis in clinically less severe or atypical cases and improved diagnostic tools. The minimum prevalence of CADASIL has been estimated to be between 2 and 4 per 100.000,[5-7] but the actual prevalence will probably prove to be higher. In the Netherlands, for example, the number of diagnosed CADASIL families started out with seven in 1998 and has increased to more than 150 Dutch families in 2013, with new families still being identified at a steady rate. (Lesnik Oberstein and Boon, unpublished results)

The clinical diagnosis CADASIL is made based on a combination of the following: otherwise unexplained cerebral ischemic events and/or cognitive decline at a relatively young age, distinctive brain MRI abnormalities and a family history with a dominant pattern of inheritance of stroke or dementia. The clinical diagnosis can be confirmed by the detection of a characteristic cysteine-altering mutation in *NOTCH3*. If the results of molecular testing are unclear or comprehensive *NOTCH3* screening is not available, the diagnosis can be confirmed by taking a skin biopsy. This shows pathognomonic vessel wall abnormalities, including positive NOTCH3 immunostaining and the presence of electron dense deposits called granular osmiophilic material (GOM).[8,9] The correct diagnostic interpretation of *NOTCH3* variants other than the typical cysteine-altering missense mutations requires expertise in both the clinical features and the distinguishing molecular aspects of CADASIL. Incorrect interpretation of mutations can lead to an erroneous CADASIL diagnosis, with far-reaching implications for the patient and his or her family members. Here, we provide recommendations for the clinical diagnosis and interpretation of *NOTCH3* mutations in CADASIL.

NOTCH3 GENE, NOTCH3 PROTEIN AND CADASIL PATHOGENESIS

The *NOTCH3* gene is one of the four mammalian NOTCH homologues. *NOTCH3* contains 33 exons encoding the NOTCH3 protein, a single pass transmembrane protein of 2321 amino acids predominantly expressed in vascular smooth muscle cells (VSMC).[10] Here, it plays an important role in VSMC maturation and differentiation.[11] The NOTCH3 protein is composed of an extracellular domain (NOTCH3^{ECD}), non-covalently bound to an intracellular domain. (Figure 1) After binding of a ligand (Delta-like or Jagged) to the NOTCH3^{ECD} , the protein undergoes two proteolytic cleavage steps, leading to translocation of the intracellular domain to the nucleus where it functions as a nuclear transcription factor.[12] The NOTCH3^{ECD} consists of 34 epidermal growth factor- like repeat (EGFr) domains. These are modular protein subunits of approximately 40 amino acids which, by definition, each contain a fixed

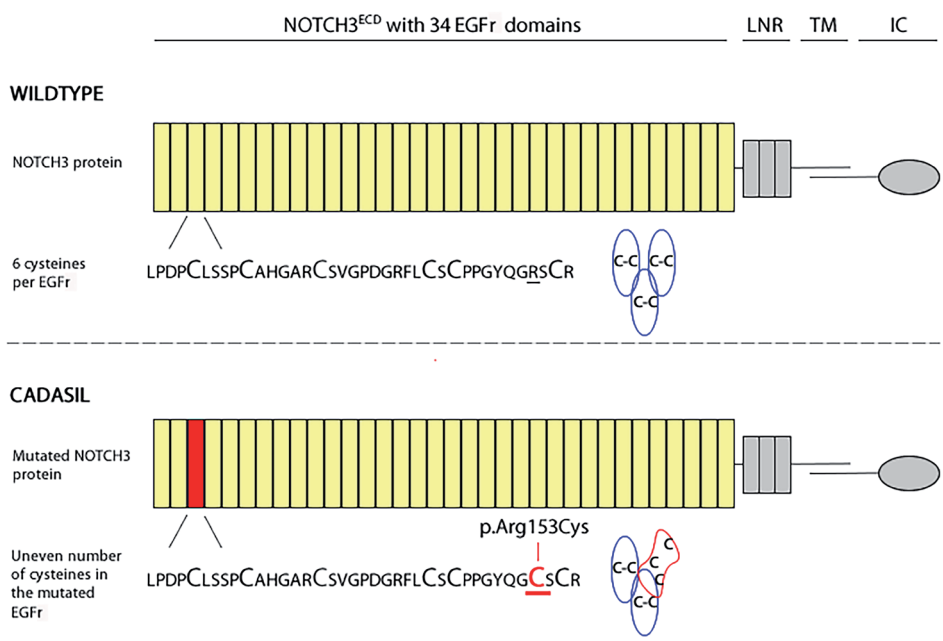

Figure 1. Schematic representation of the NOTCH3 protein. The NOTCH3 protein is a transmembrane protein, composed of an extracellular domain (NOTCH3^{ECD}), a transmembrane (TM) and an intracellular domain (IC). The NOTCH3^{ECD} contains 34 EGFr and three NOTCH/Lin repeats(LNR). The EGFr, each composed of approximately 40 amino acids, all contain 6 cysteine amino acids which form 3 disulphide bridges. In this example of CADASIL mutated NOTCH3 (with the representative p.Arg153Cys mutation), the number of cysteine residues in EGFr 3 is changed from 6 to 7, leaving one cysteine unpaired and disrupting normal disulphide bridge formation.

number of 6 cysteine residues. In pairs, these cysteines form three disulphide bridges which are important for EGFr secondary structure. *NOTCH3* mutations in CADASIL invariably lead to an uneven number of cysteines (typically 5 or 7) in the mutated EGFr.[13] This results in an unpaired cysteine, which is predicted to disrupt normal disulphide bridge formation, causing misfolding of EGFr and increased NOTCH3 multimerization.[14,15]

In patients, $NOTCH3^{ECD}$ is seen to accumulate in the vessel wall, in close proximity to VSMC.[10] This $NOTCH3^{ECD}$ accumulation has a direct or indirect toxic effect on VSMC, leading to VSMC degeneration.[9,16,17] The arteriopathy in CADASIL is systemic,[9] as is also illustrated by the presence of vascular pathology in for example skin arterioles,[8,17] but the small penetrating cerebral and leptomeningeal arteries are most severely affected.[18] Next to $NOTCH3^{ECD}$ accumulation and the presence of GOM, affected arteries show a thickened vessel wall with lumen stenosis, abundance of extracellular matrix proteins and destruction of vascular smooth muscle cells.[19,20] The vessel wall changes result in an impaired cerebrovascular reactivity and decreased cerebral blood flow, believed to cause both chronic cerebral ischemia and acute ischemic events.[21-23]

CLINICAL DIAGNOSIS BASED ON SYMPTOMS, BRAIN MRI AND FAMILY HISTORY

The main symptoms in CADASIL, affecting the majority of patients, are recurrent ischemic events (transient ischemic attacks and strokes) and cognitive decline leading to vascular dementia. Migraine with aura, psychiatric disturbances and apathy are other frequent symptoms. Most patients experience their first stroke around 45-50 years of age,[24-27] but the range of age at onset is broad, varying from as early as the third decade to as late as the eighth decade. In the majority of patients, ischemic events are recurrent,[24,26,27] eventually resulting in severe disability.[25] Cognitive decline initially manifests as a decreased executive function,[28-30] followed by a slowly progressive and/or stepwise deterioration in cognitive function which becomes apparent in daily activities around the age of 50, leading to vascular dementia in about 80% of patients.[25] Migraine is diagnosed in approximately 35% of CADASIL patients. The migraine attacks are often the presenting clinical symptom with a mean age at onset of around 26 years, and are remarkable by the high prevalence of auras (90% of patients).[24,27] Often, the migraine attacks are atypical, with prolonged, brainstem or hemiplegic auras and confusion, fever, meningeal signs or even coma. Also, during gestation and shortly after childbirth there appears to be an increased risk of transient neurological symptoms,[31] mostly resembling migraine auras. Approximately one third of CADASIL patients have psychiatric problems, usually depressions.[24,27] Apathy is a common symptom in

later stages.[32] Less frequent symptoms include epileptic seizures (5-10%), usually secondary to stroke,[3,24,26,33] and acute encephalopathy.[3,27,34-36]

The suspicion of CADASIL often arises when brain MRI imaging reveals symmetrically distributed white matter hyperintensities (WMH), which are much too extensive for the patient's age. (Figure 2) MRI abnormalities in CADASIL precede clinical symptoms of ischemic events and cognitive decline by as much as 10-15 years, increase with age and have a recognizable, but not pathognomonic, distribution. In the early stages the WMH, best seen on T2- weighted images or fluid –attenuated inversion recovery (FLAIR) sequences, are small and punctuate and are often seen to initiate in the anterior temporal lobes, periventricular frontal white matter, and external capsules,[37-39] eventually affecting all of the white matter. Lacunar infarcts, most readily detected on T1- weighted images, can be seen from the age of 30 onwards, and are found in the basal ganglia, thalamus, brainstem and subcortical white matter.[37] Microbleeds, predominantly seen in the subcortical white matter and thalamus, and dilated perivascular spaces are frequently

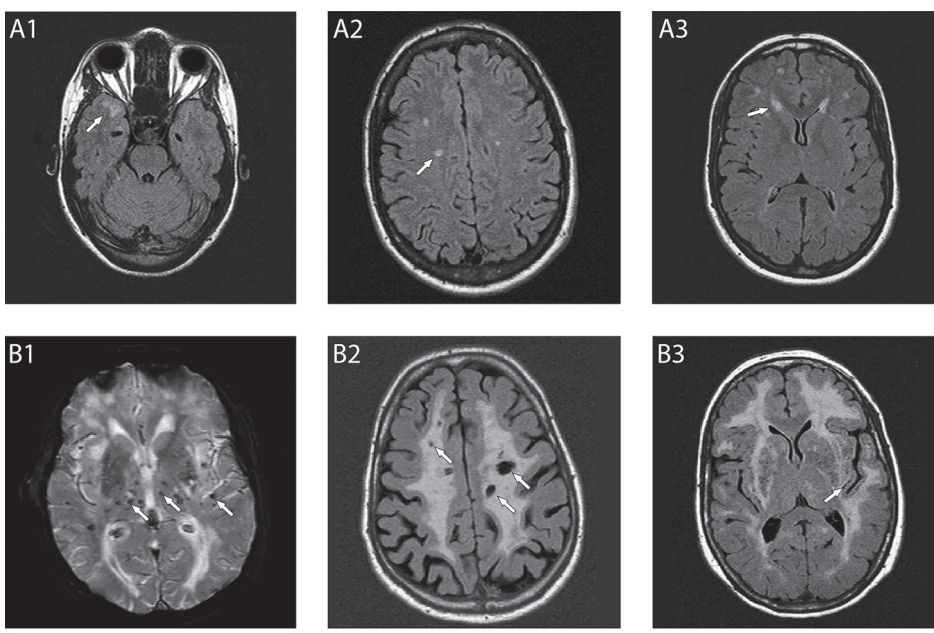

Figure 2. Brain MRI abnormalities in CADASIL patients. In the early stages of CADASIL, brain MRI abnormalities consist of small circumscript white matter hyperintensities (WMH) (indicated by arrows), as shown here in the anterior temporal lobes (A1), in the semioval center (A2) and periventricularly (A3). (FLAIR images) Brain MRI images of patients with advanced stages of CADASIL showing confluent WMH (B1-B3), microbleeds (three marked with an arrow) (B1), lacunar infarcts (three marked with an arrow) (B2) and dilated perivascular spaces (B3). (B1: T2*- weighted gradient echo images, B2, B3: FLAIR images)

encountered.[40-42] The MRI lesion load and pattern can vary quite significantly, such that some patients hardly show any lacunar infarcts even at later stages, or lack WMH in the temporal lobes.[43] A consistent factor is the presence of symmetrical WMH, often visible from the early twenties onward, but present in most if not all patients from 35 years of age. MRI abnormalities in CADASIL can resemble those seen in other diseases, such as other types of small vessel disease or multiple sclerosis. In fact, a significant number of CADASIL patients are first erroneously diagnosed as having multiple sclerosis.[44]

The family history of a CADASIL patient typically shows an autosomal dominant inheritance pattern of stroke and dementia, and family members often have migraine with aura or mood disturbances. However, the severity of the disease is variable, also within families. It has been noted, that CADASIL patients frequently report a negative family history,[45] which in many cases can be attributed to the fact that affected family members, especially in older generations, have received other diagnoses such as Alzheimer's dementia or multiple sclerosis. Taking this into account, a thorough family history will usually reveal affected family members. However, a small number of CADASIL patients with *de novo NOTCH3* mutations have been reported, so a negative family history does not rule out CADASIL.[46,47]

CADASIL can be diagnosed in a plethora of clinical presentations or settings, where the early onset of stroke, white matter hyperintensities on brain MRI and a family history of stroke, dementia or migraine with aura, may each contribute more or less strongly in leading the clinician to consider CADASIL. However, CADASIL should always, be considered in an individual presenting with otherwise unexplained ischemic events or cognitive decline and symmetrical white matter hyperintensities on brain MRI.

WHEN TO PERFORM A SKIN BIOPSY IN CADASIL

If *NOTCH3* screening is unavailable or does not reveal a typical cysteine altering missense mutation in a patient otherwise presenting with a convincing CADASIL phenotype, then taking a skin biopsy for NOTCH3 immunostaining and electron microscopy (EM) is recommended. (figure 3) The sensitivity of NOTCH3 immunostaining of a skin biopsy has been reported to be between 85-100%, and the specificity between 90-100%.[8,48] The detection of GOM in the vessel wall using electron microscopy is considered pathognomonic for CADASIL. Most studies report the presence of GOM deposits in all CADASIL patients,[49-52] with only two studies reporting low GOM detection rate.[53,54] Skin biopsy analysis using both NOTCH3 immunostaining and EM should therefore allow for a conclusive confirmation or rejection of the diagnosis when performed by an experienced (neuro)pathologist.

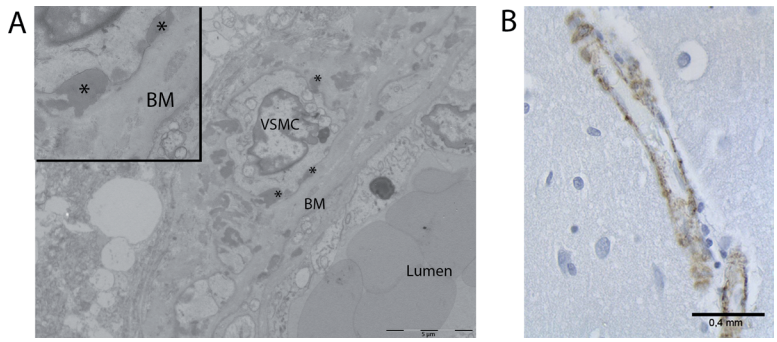

Figure 3. Vessel wall abnormalities in CADASIL. (A) Electron microscopy on brain tissue from a deceased CADASIL patient. Pathognomic deposits of granular osmiophillic material (GOM) are seen, surrounding the vascular smooth muscle cell (VSMC). (B) NOTCH3 immunohistochemistry of a skin biopsy showing the typical granular positive NOTCH3 staining of the vessel wall. BM= basement membrane, *=GOM, VSMC=vascular smooth muscle cell.

NOTCH3 MUTATIONS IN CADASIL

A typical CADASIL causing mutation is a heterozygous *NOTCH3* missense mutation that leads to a numerical cysteine amino acid change in one of the 34 EGFr of the NOTCH3 protein.(see supplementary table 1 for a list of known CADASIL- causing missense mutations) Pathogenic mutations have been found throughout exons 2-24, which are the exons that encode the EGFr, but the majority of mutations are found in exon 4. The prevalence of mutations in other exons varies between countries; in the French, German and English CADASIL population exon 3 is the second most frequently mutated exon,[13,53,55] whereas in Dutch CADASIL patients, exon 11 is the second most frequently mutated exon.[56] These geographic differences probably are due to the presence of founder mutations.

When a clinical diagnosis of CADASIL is suspected, sequencing of exons 2-24 of the *NOTCH3* gene, including flanking intronic sequences, is the most reliable method to confirm the diagnosis. In patients with a typical clinical and radiological presentation and family history, the mutation detection rate exceeds 95%. (Lesnik Oberstein, unpublished observations) Detection of the disease- causing mutation then allows for (predictive) DNA-testing of family members. However, sequencing exons 2-24 is costly and can be time-consuming, and is not available in all countries. Targeted sequencing strategies are used in centres that do not offer complete *NOTCH3* sequencing analysis, usually sequencing analysis of only the most frequently mutated exons. In cases with a confirmed clinical diagnosis by skin biopsy but no mutation detected by sequencing analysis, additional molecular techniques can be performed to detect larger deletions or duplications, or to assess the potential effect of novel variants on splicing.

Although it has been suggested that some mutations may be associated with a milder or more severe phenotype,[25,57] so far no clear genotype- phenotype correlations have been found.[24,25,58,59] As disease severity can vary not only between, but also within families, and even between monozygotic twins,[60] it is likely that modifying factors other than the *NOTCH3* mutation play a role in determining the course of the disease.

Homozygous and compound heterozygous NOTCH3 mutations

A small number of CADASIL patients with homozygous *NOTCH3* mutations have been reported.[36,61-64] (Table 1) In some of these patients, the phenotype was found to be more severe, whereas others reported clinical severity similar to heterozygous mutation carriers. Overall, patients with homozygous mutations described so far have a phenotype within the normal CADASIL spectrum, and variation in severity may as readily be attributed to the natural variability seen in CADASIL.

Reference	Homozygous mutation	Exon	Amino acid change	Age patient	Age at first stroke
Tuominen et al.[62]	c.397C>T	4	p.Arg133Cys	52	28
Vinciguerra et al.[64]	c.547T>A	4	p.Cys183Ser	44	40
Ragno et al.[36]	c.1582G>T	10	p.Gly528Cys	54	No stroke at age 54
Liem et al.[61]	c.1732C>T	11	p.Arg578Cys	65	64
Soong et al.[63]	c.1630C>T c.1630C>T	11 11	p.Arg544Cys p.Arg544Cys	63 68	63 58

Table 1. Homozygous mutations in CADASIL patients. A small number of CADASIL-causing mutations in a homozygous state have been reported. The severity of symptoms in these patients seems to be within the CADASIL spectrum. Mutations are described according to HGVS nomenclature,[93] and may therefore differ from the mutation description in the original research article.

One CADASIL patient has been reported who has a typical cysteine-altering missense mutation on one of his *NOTCH3* alleles, but also a large intragenic *NOTCH3* deletion, leading to a premature stop codon, on his other allele.[65] In effect, this means he is functioning on only one *NOTCH3* allele which, moreover, harbors a CADASIL-causing mutation. The phenotype of this patient was within the normal CADASIL spectrum. In patients with a *NOTCH3* mutation on one allele and a large *NOTCH3* deletion on the other allele, the mutation may be interpreted to be a homozygous mutation if only sequencing analysis is performed. Therefore, in mutations appearing to be homozygous, the presence of a *NOTCH3* deletion on

one of the alleles should be excluded, for example by performing MLPA analysis. Although this has no clinical consequences for the patient, because the disease severity in these patients is within the normal CADASIL spectrum, it is of importance for the counseling of family members.

Small NOTCH3 deletions, duplications, splice site mutations and a deletion/insertion

Although the vast majority of CADASIL causing mutations are missense mutations, a few other rare types of *NOTCH3* mutations have been reported, which also lead to the typical CADASIL-associated uneven number of cysteine residues in EGFr. These include some small intragenic deletions, duplications, splice site mutations and a deletion/insertion.[14,25,43,51,66-71] (Table 2) As these mutations lead to a typical numerical cysteine alteration in NOTCH3 EGFr, they are likely to be pathogenic, although the clinical diagnosis was not confirmed by skin biopsy in all cases and molecular analysis was not always comprehensive.

Deletions leading to altered spacing of cysteine residues

In one study, a 12 base pair deletion not involving a cysteine residue was reported to be associated with CADASIL.[72] The diagnosis was confirmed by skin biopsy, segregated with the clinical phenotype and all EGFr encoding exons had been sequenced. Although not resulting in a numerical cysteine alteration, this deletion does result in altered spacing between two cysteines in an EGFr, thereby possibly disrupting normal disulphide bridge formation. As no RNA analysis was performed, it cannot be excluded that the deletion results in abnormal splicing and thereby potentially alters the number of cysteine residues.

A second study describes a small intronic deletion leading to retention of intron 3.[73] This results in an insertion of 25 amino acids between the 5th and 6th cysteine of EGFr 2, thereby likely disturbing disulphide bridge formation between these two cysteines.

NOTCH3 VARIANTS NOT ALTERING CYSTEINE RESIDUES IN EGFR

NOTCH3 mutations not leading to a numerical cysteine amino acid change in NOTCH3 EGFr have also been described to be associated with CADASIL. Upon closer scrutiny, however, these associations often remain uncertain, or pathogenicity was later disproven.

Ref.	Mutation	Exon / intron	RNA analysis	Amino acid change	Numerical cysteine alteration	Sequencing analysis all EGFr encoding exons	Diagnosis confirmed by skin biopsy
Wang et al.[43]	c.226_234del	exon 3	-	p.Cys76_ Leu78del	deletion 1 cysteine EGFr 1	yes	yes
Opherk et al.[25]	c.231_248del	exon 3	-	p.Gln77_ Cys82del	deletion 1 cysteine EGFr 2	not reported	not reported
Mazzei et al.[66]	c.277_279dup	exon 3	-	p.Cys93dup	insertion 1 cysteine EGFr 2	not reported	yes
Dichgans et al.[14]	c.239_253del c.459_467del	exon 3 exon 4	- -	p.Asp80_ Ser84del p.Arg153_ Cys155del	deletion 1 cysteine EGFr 2 deletion 1 cysteine EGFr 3	no† no†	yes yes
Joutel et al.[67]	c.341-2A>G	intron 3	r.341_361del	p.Gly114_ Pro120del	deletion 1 cysteine EGFr 2	yes	yes
Dichgans et al.[68]	c.714_758del	exon 5	-	p.Asp239_ Asp253del	deletion 3 cysteines EGFr 6	no‡	no
Lackovic et al.[71]	c.955_956delGCinsTG	exon 6	-	p.Ala319Cys	Replacement alanine with cysteine	no±	yes
Lee et al.[69]	c.1057_1071dup	exon 7	-	p.Asp353_ Ser357dup	insertion 1 cysteine EGFr 9	yes	yes
Tikka et al.[51]	c.1300_1308dup	exon 8	-	p.Glu434_ Leu436dup	insertion 1 cysteine EGFr 11	not reported	yes
Saiki et al.[70]	c.2411-1G>T	intron 15	r.2411_2566del	p.Gly804_ Asn856delinsAsp	deletion 1 cysteine EGFr 20 deletion complete EGFr 21 deletion 1 cysteine EGFr 22	not reported	yes

Table 2. Small *NOTCH3* deletions, duplications, splice site mutations and a deletion/insertion leading to a numerical cysteine alteration. Pathogenic mutations other than missense mutations are rare in CADASIL. The reported small deletions, duplications, splice site mutations and deletion/insertion lead to a numerical cysteine amino acid change in one of the EGFr of NOTCH3. Mutations are described according to HGVS nomenclature,[93] and may therefore differ from the mutation description in the original research article.

† exons 2 and 3 screened; ‡ exons 2-5 screened; ± exons 2-6 screened

NOTCH3 missense mutations not involving a cysteine residue

The first group of mutations with an uncertain association with CADASIL are missense mutations in *NOTCH3* which do not involve a cysteine residue. Various studies have reported such variants to be causative of CADASIL.[43,74-81] (Table 3) In the majority of these reports however, the association is not proven, because either *NOTCH3* was incompletely screened and thus a typical cysteine altering mutation in another exon was not excluded, or the clinical diagnosis was not confirmed by skin biopsy.[75,76,78-80] In four studies, describing the p.Ala1020Pro, p.Arg213Lys, p.Tyr1098Ser, and p.Arg75Pro variants respectively, sequencing of exons 2-24 was comprehensive and the diagnosis was confirmed by skin biopsy.[43,74,77,81] However, the p.Ala1020Pro, and also the p.His170Arg mutation have been reported to be polymorphisms by others.[82] (Supplementary table 2) That polymorphisms are mistakenly interpreted as causative mutations is further suggested by the fact that these variants have been found to co-segregate with a typical cysteine altering missense mutation.[83] (Lesnik Oberstein and Boon, unpublished findings)

The p.Arg213Lys and p.Tyr1098Ser mutations were found in a Japanese and a Chinese patient, respectively, but segregation analysis was not performed. Also for these mutations it remains possible they are polymorphisms, as a coinciding cysteine altering mutation, such as a large deletion or intronic mutation, might have been missed due to technical reasons. This may also apply to the p.Arg61Trp mutation, of which the pathogenicity was described as uncertain.[84] A possible exception may be the p.Arg75Pro mutation, which has been frequently reported in Japanese, Chinese and Korean patients.[43,74,85,86] In two of these families, comprehensive sequencing of *NOTCH3* was performed, the mutation segregated with affected family members and GOM were present in skin biopsy.[74] However, MRI abnormalities were not typical for CADASIL and NOTCH3 immunostaining was not performed. Therefore, drawing definite conclusions regarding the relation between this mutation and CADASIL remains precarious, but it cannot be excluded that this mutation does cause CADASIL or a CADASIL-like phenotype. In summary, with one possible exception, there is no compelling evidence that missense mutations that do not involve the loss or gain of a cysteine residue are associated with CADASIL. Therefore, if such a non-cysteine altering mutation is detected, the clinical diagnosis should be critically re-evaluated and, if the clinical symptoms are persuasive, be confirmed by skin biopsy. If the skin biopsy shows the typical vessel wall abnormalities seen in CADASIL, molecular testing should be elaborated by analysis of larger deletions or duplications using for example MLPA, and the potential effect on splicing of variants with unknown pathogenicity should be ascertained. Finally, if possible, segregation of the mutation with affected family members should be determined.

Reference	Mutation	Exon	Amino acid change	Sequencing analysis all EGFr encoding exons	Diagnosis confirmed by skin biopsy or autopsy
Brass et al.[84]	c.259C>T	2	p.Arg61Trp	yes	yes
Mizuno et al.[74]	c.224G>C	3	p.Arg75Pro	yes	yes
Ampuero et al.[75]	c.451C>G** c.509A>G*	4 4	p.Gln151Glu p.His170Arg	no†	not reported
Roy et al.[76]	c.605C>T	4	p.Ala202Val	no‡	not reported
Kotorii et al.[77]	c.638G>A	4	p.Arg213Lys	yes	yes
Uchino et al.[78]	c.709G>A*	5	p.Val237Met	not reported	not reported
Ferreira et al.[79]	c.1729A>G	11	p.Thr577Ala	not reported	not reported
Ferreira et al.[80]	c.2932A>C	18	p.Ser978Arg	not reported	not reported
Scheid et al.[81]	c.3058G>C*	19	p.Ala1020Pro	yes	yes
Wang et al.[43]	c.3292A>T	20	p.Tyr1098Ser	yes	yes

Table 3. *NOTCH3* missense variants not involving a cysteine residue which have been suggested to be causative of CADASIL. Missense variants not involving cysteine residues have been reported in association with CADASIL in several studies. Most of these were later described to be polymorphisms, or their pathogenicity is questionable due to incomplete molecular analysis or uncertain clinical diagnosis. One possible exception is the p.Arg75Pro mutation, which was found to segregate with the clinical phenotype in two thoroughly studies families.[74] Mutations are described according to HGVS nomenclature,[93] and may therefore differ from the mutation description in the original research article. †exons 2-6,8,11,14,18-19, 22-23 screened; ‡ exons 2-4, 11, 18-19 screened. * Also described as a polymorphism [94,95] (Supplementary table 2) ** Also described as a variant with unknown pathogenicity [82]

NOTCH3 mutations leading to loss-of-function

A second group of mutations about which there is debate as to their pathogenicity in CADASIL, are those leading to loss of NOTCH3 function (out-of-frame deletions or stop mutations). These mutations have been only rarely reported and there is no convincing evidence that such loss-of-function mutations cause CADASIL. As in the case of the non- cysteine altering missense mutations, the reports mentioning an association between CADASIL phenotype and loss-of-function mutations either lack comprehensive *NOTCH3* molecular analysis, or the clinical diagnosis has not been confirmed.[87,88] The hypothesis that loss-of-function mutations may also cause CADASIL is further discredited by the fact that *NOTCH3* loss-of-function mutations have been found in thoroughly studied individuals in whom CADASIL was clinically excluded,[65] as well as by the fact that Notch3 knock-out mice do not develop a CADASIL phenotype.[89] In summary, loss of *NOTCH3* function mutations appear not to cause CADASIL, and should be considered a coincidental finding until proven otherwise.

NOTCH3 MUTATIONS OUTSIDE OF THE EGFR ENCODING EXONS

In the initial report on the identification of *NOTCH3* mutations in CADASIL, one mutation in exon 25 was described.[4] Later reports have shown that mutations located outside EGFr encoding exons 2-24 do not lead to a CADASIL phenotype.[90-92]

EXPERT COMMENTARY

Upon the discovery of *NOTCH3* as the causative gene for CADASIL in 1996, it was clear that mutations were highly distinctive, namely all were missense mutations leading to a cysteine alteration in one of the 34 EGFr of NOTCH3. In the past two decades, this has remained a consistent finding in CADASIL patients reported from all over the world. However, there have also been a substantial number of reports suggesting that other types of mutations, mostly missense mutations that do not involve a cysteine, can also cause CADASIL. To date there is no convincing evidence that these mutations indeed cause CADASIL and they should be considered to be coincidental findings, until proven otherwise. Incorrect interpretation of mutations leads to erroneous diagnosis, with far reaching implications for both the patient and his or her family members. In order to be certain of the diagnosis and the pathogenicity of the mutation, the analysis of patients with mutations that do not alter cysteines should always include comprehensive molecular *NOTCH3* screening to exclude a coinciding cysteine-altering mutation, as well as a thorough clinical (re-)evaluation, including skin biopsy. This should preferably be performed by an experienced team, including a clinical laboratory geneticist, clinical geneticist, neurologist, neuroradiologist and neuropathologist.

FIVE-YEAR VIEW

Nearly two decades after the identification of *NOTCH3* as the causative gene for CADASIL, it has become clear that the disease is much more prevalent than initially assumed and it is now considered the most prevalent type of hereditary vascular dementia. However, there is likely still a substantial number of undiagnosed patients, due to lack of awareness of the disease and the non-specificity of many of the signs and symptoms. However, once the diagnosis has been considered, it is readily confirmed by the presence of a cysteine altering missense mutation in *NOTCH3*. As molecular testing is becoming more widely available, we expect that in the coming 5 years patients will be even more frequently diagnosed and a clearer picture will emerge of both the prevalence and the clinical spectrum of the disease. Thus far, there is no therapy to delay the onset of the disease. Future rational therapeutic strategies may be those modifying, down- regulating

or capturing the toxic mutated protein. In order to determine the effect of such therapeutic strategies in the future, a thorough and complete knowledge of both the mutational spectrum and the natural history of CADASIL is of vital importance.

KEY ISSUES

- CADASIL is caused by gain-of-function mutations in *NOTCH3*, leading to toxic NOTCH3 accumulation in the vessel wall.
- Only *NOTCH3* mutations that alter the number of cysteines in one of the 34 epidermal growth factor repeat (EGFr) domains of the NOTCH3 protein have been proven to cause CADASIL:
  - The great majority are missense mutations
  - Mutations are found only in EGFr-encoding exons (2-24)
  - Most mutations are located in exon 4
- Some CADASIL patients with homozygous and compound heterozygous mutations have been described. The symptoms of these patients seem to be within the normal CADASIL spectrum.
- Small *NOTCH3* in-frame deletions, insertions or splice-site mutations can also cause CADASIL. These typically also alter the number of cysteines in one of the 34 EGFr of NOTCH3.
- Mutations in *NOTCH3* leading to loss of NOTCH3 function do not cause CADASIL. These rare mutations include mutations leading to a frame-shift and stop mutations.
- Missense mutations in *NOTCH3* not altering a cysteine residue are unlikely to be pathogenic, and should be considered coincidental findings until proven otherwise. If such a mutation is detected, the following should be considered:
  - A coinciding cysteine altering mutation may have been missed due to technical reasons or incomplete sequencing analysis.
  - The clinical diagnosis of CADASIL may be incorrect and should be confirmed by electron microscopy and NOTCH3 immunohistochemistry on a skin biopsy.

REFERENCES

1. Tournier-Lasserve,E, Iba-Zizen,MT, Romero,N, and Bousser,MG Autosomal dominant syndrome with strokelike episodes and leukoencephalopathy. *Stroke* 22, 1297-1302 (1991).
2. Baudrimont,M, Dubas,F, Joutel,A, Tournier-Lasserve,E, and Bousser,MG Autosomal dominant leukoencephalopathy and subcortical ischemic stroke. A clinicopathological study. *Stroke* 24, 122-125 (1993).
3. Chabriat,H, Vahedi,K, Iba-Zizen,MT, Joutel,A, Nibbio,A, Nagy,TG, Krebs,MO, Julien,J, Dubois,B, Ducrocq,X, et al. Clinical spectrum of CADASIL: a study of 7 families. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Lancet* 346, 934-939 (1995).
4. Joutel,A, Corpechot,C, Ducros,A, Vahedi,K, Chabriat,H, Mouton,P, Alamowitch,S, Domenga,V, Cecillion,M, Marechal,E, Maciazek,J, Vayssiere,C, Cruaud,C, Cabanis,EA, Ruchoux,MM, Weissenbach,J, Bach,JF, Bousser,MG, and Tournier-Lasserve,E Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* 383, 707-710 (1996).
5. Narayan,SK, Gorman,G, Kalaria,RN, Ford,GA, and Chinnery,PF The minimum prevalence of CADASIL in northeast England. *Neurology* 78, 1025-1027 (2012).
6. Kalimo,H, Ruchoux,MM, Viitanen,M, and Kalaria,RN CADASIL: a common form of hereditary arteriopathy causing brain infarcts and dementia. *Brain Pathol.* 12, 371-384 (2002).
7. Razvi,SS, Davidson,R, Bone,I, and Muir,KW The prevalence of cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) in the west of Scotland. *J.Neurol.Neurosurg.Psychiatry* 76, 739-741 (2005).
8. Joutel,A, Favrole,P, Labauge,P, Chabriat,H, Lescoat,C, Andreux,F, Domenga,V, Cecillon,M, Vahedi,K, Ducros,A, Cave-Riant,F, Bousser,MG, and Tournier-Lasserve,E Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. *Lancet* 358, 2049-2051 (2001).
9. Ruchoux,MM, Guerouaou,D, Vandenhaute,B, Pruvo,JP, Vermersch,P, and Leys,D Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Acta Neuropathol.* 89, 500-512 (1995).
10. Joutel,A, Andreux,F, Gaulis,S, Domenga,V, Cecillon,M, Battail,N, Piga,N, Chapon,F, Godfrain,C, and Tournier-Lasserve,E The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J.Clin.Invest* 105, 597-605 (2000).
11. Domenga,V, Fardoux,P, Lacombe,P, Monet,M, Maciazek,J, Krebs,LT, Klonjkowski,B, Berrou,E, Mericskay,M, Li,Z, Tournier-Lasserve,E, Gridley,T, and Joutel,A Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. *Genes Dev.* 18, 2730-2735 (2004).
12. Wang,T, Baron,M, and Trump,D An overview of Notch3 function in vascular smooth muscle cells. *Prog.Biophys.Mol.Biol.* 96, 499-509 (2008).
13. Joutel,A, Vahedi,K, Corpechot,C, Troesch,A, Chabriat,H, Vayssiere,C, Cruaud,C, Maciazek,J, Weissenbach,J, Bousser,MG, Bach,JF, and Tournier-Lasserve,E Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet* 350, 1511-1515 (1997).
14. Dichgans,M, Ludwig,H, Muller-Hocker,J, Messerschmidt,A, and Gasser,T Small in-frame deletions and missense mutations in CADASIL: 3D models predict misfolding of Notch3 EGF-like repeat domains. *Eur.J.Hum.Genet.* 8, 280-285 (2000).
15. Duering,M, Karpinska,A, Rosner,S, Hopfner,F, Zechmeister,M, Peters,N, Kremmer,E, Haffner,C, Giese,A, Dichgans,M, and Opherk,C Co-aggregate formation of CADASIL-mutant NOTCH3: a single-particle analysis. *Hum.Mol.Genet.* (2011).
16. Monet-Lepretre,M, Haddad,I, Baron-Menguy,C, Fouillot-Panchal,M, Riani,M,

Domenga-Denier,V, Dussaule,C, Cognat,E, Vinh,J, and Joutel,A Abnormal recruitment of extracellular matrix proteins by excess Notch3ECD: a new pathomechanism in CADASIL. *Brain* 136, 1830-1845 (2013).

17. Ruchoux,MM, Chabriat,H, Bousser,MG, Baudrimont,M, and Tournier-Lasserve,E Presence of ultrastructural arterial lesions in muscle and skin vessels of patients with CADASIL. *Stroke* 25, 2291-2292 (1994).
18. Chabriat,H, Joutel,A, Dichgans,M, Tournier-Lasserve,E, and Bousser,MG Cadasil. *Lancet Neurol.* 8, 643-653 (2009).
19. Kalaria,RN and Kalimo,H Introduction: Non-atherosclerotic cerebrovascular disorders. *Brain Pathol.* 12, 337-342 (2002).
20. Miao,Q, Paloneva,T, Tuominen,S, Poyhonen,M, Tuisku,S, Viitanen,M, and Kalimo,H Fibrosis and stenosis of the long penetrating cerebral arteries: the cause of the white matter pathology in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Brain Pathol.* 14, 358-364 (2004).
21. Chabriat,H, Pappata,S, Ostergaard,L, Clark,CA, Pachot-Clouard,M, Vahedi,K, Jobert,A, Le,BD, and Bousser,MG Cerebral hemodynamics in CADASIL before and after acetazolamide challenge assessed with MRI bolus tracking. *Stroke* 31, 1904-1912 (2000).
22. Pfefferkorn,T, von Stuckrad-Barre,S, Herzog,J, Gasser,T, Hamann,GF, and Dichgans,M Reduced cerebrovascular CO(2) reactivity in CADASIL: A transcranial Doppler sonography study. *Stroke* 32, 17-21 (2001).
23. Tuominen,S, Miao,Q, Kurki,T, Tuisku,S, Poyhonen,M, Kalimo,H, Viitanen,M, Sipila,HT, Bergman,J, and Rinne,JO Positron emission tomography examination of cerebral blood flow and glucose metabolism in young CADASIL patients. *Stroke* 35, 1063-1067 (2004).
24. Dichgans,M, Mayer,M, Uttner,I, Bruning,R, Muller-Hocker,J, Rungger,G, Ebke,M, Klockgether,T, and Gasser,T The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann.Neurol.* 44, 731-739 (1998).
25. Opherk,C, Peters,N, Herzog,J, Luedtke,R, and Dichgans,M Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. *Brain* 127, 2533-2539 (2004).
26. Desmond,DW, Moroney,JT, Lynch,T, Chan,S, Chin,SS, and Mohr,JP The natural history of CADASIL: a pooled analysis of previously published cases. *Stroke* 30, 1230-1233 (1999).
27. Adib-Samii,P, Brice,G, Martin,RJ, and Markus,HS Clinical spectrum of CADASIL and the effect of cardiovascular risk factors on phenotype: study in 200 consecutively recruited individuals. *Stroke* 41, 630-634 (2010).
28. Peters,N, Opherk,C, Danek,A, Ballard,C, Herzog,J, and Dichgans,M The pattern of cognitive performance in CADASIL: a monogenic condition leading to subcortical ischemic vascular dementia. *Am.J.Psychiatry* 162, 2078-2085 (2005).
29. Buffon,F, Porcher,R, Hernandez,K, Kurtz,A, Pointeau,S, Vahedi,K, Bousser,MG, and Chabriat,H Cognitive profile in CADASIL. *J.Neurol.Neurosurg.Psychiatry* 77, 175-180 (2006).
30. Amberla,K, Waljas,M, Tuominen,S, Almkvist,O, Poyhonen,M, Tuisku,S, Kalimo,H, and Viitanen,M Insidious cognitive decline in CADASIL. *Stroke* 35, 1598-1602 (2004).
31. Roine,S, Poyhonen,M, Timonen,S, Tuisku,S, Marttila,R, Sulkava,R, Kalimo,H, and Viitanen,M Neurologic symptoms are common during gestation and puerperium in CADASIL. *Neurology* 64, 1441-1443 (2005).
32. Reyes,S, Viswanathan,A, Godin,O, Dufouil,C, Benisty,S, Hernandez,K, Kurtz,A, Jouvent,E, O'Sullivan,M, Czernecki,V, Bousser,MG, Dichgans,M, and Chabriat,H Apathy: a major symptom in CADASIL. *Neurology* 72, 905-910 (2009).
33. Haan,J, Lesnik Oberstein,SA, and Ferrari,MD Epilepsy in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Cerebrovasc.Dis.* 24, 316-317 (2007).
34. Feuerhake,F, Volk,B, Ostertag,CB, Jungling,FD, Kassubek,J, Orszagh,M, and Dichgans,M Reversible coma with raised intracranial pressure: an unusual

clinical manifestation of CADASIL. *Acta Neuropathol.* 103, 188-192 (2002).

35. Schon,F, Martin,RJ, Prevett,M, Clough,C, Enevoldson,TP, and Markus,HS "CADASIL coma": an underdiagnosed acute encephalopathy. *J.Neurol.Neurosurg. Psychiatry* 74, 249-252 (2003).
36. Ragno,M, Pianese,L, Morroni,M, Cacchio,G, Manca,A, Di,MF, Silvestri,S, Miceli,C, Scarcella,M, Onofrj,M, and Trojano,L "CADASIL coma" in an Italian homozygous CADASIL patient: comparison with clinical and MRI findings in age-matched heterozygous patients with the same G528C NOTCH3 mutation. *Neurol.Sci.* (2013).
37. Chabriat,H, Levy,C, Taillia,H, Iba-Zizen,MT, Vahedi,K, Joutel,A, Tournier-Lasserve,E, and Bousser,MG Patterns of MRI lesions in CADASIL. *Neurology* 51, 452-457 (1998).
38. Skehan,SJ, Hutchinson,M, and MacErlaine,DP Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR findings. *AJNR Am.J.Neuroradiol.* 16, 2115-2119 (1995).
39. Auer,DP, Putz,B, Gossl,C, Elbel,G, Gasser,T, and Dichgans,M Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. *Radiology* 218, 443-451 (2001).
40. Lesnik Oberstein,SA, van den Boom,R, van Buchem,MA, van Houwelingen,HC, Bakker,E, Vollebregt,E, Ferrari,MD, Breuning,MH, and Haan,J Cerebral microbleeds in CADASIL. *Neurology* 57, 1066-1070 (2001).
41. Dichgans,M, Holtmannspotter,M, Herzog,J, Peters,N, Bergmann,M, and Yousry,TA Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. *Stroke* 33, 67-71 (2002).
42. van den Boom,R, Lesnik Oberstein,SA, van Duinen,SG, Bornebroek,M, Ferrari,MD, Haan,J, and van Buchem,MA Subcortical lacunar lesions: an MR imaging finding in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Radiology* 224, 791-796 (2002).
43. Wang,Z, Yuan,Y, Zhang,W, Lv,H, Hong,D, Chen,B, Liu,Y, Luan,X, Xie,S, and Wu,S NOTCH3 mutations and clinical features in 33 mainland Chinese families with CADASIL. *J.Neurol.Neurosurg.Psychiatry* 82, 534-539 (2011).
44. Phillips,CD and Zuckerman,SJ CADASIL can mimic multiple sclerosis. *J.La State Med.Soc.* 162, 174 (2010).
45. Razvi,SS, Davidson,R, Bone,I, and Muir,KW Is inadequate family history a barrier to diagnosis in CADASIL? *Acta Neurol.Scand.* 112, 323-326 (2005).
46. Coto,E, Menendez,M, Navarro,R, Garcia-Castro,M, and Alvarez,V A new de novo Notch3 mutation causing CADASIL. *Eur.J.Neurol.* 13, 628-631 (2006).
47. Joutel,A, Dodick,DD, Parisi,JE, Cecillon,M, Tournier-Lasserve,E, and Bousser,MG De novo mutation in the Notch3 gene causing CADASIL. *Ann.Neurol.* 47, 388-391 (2000).
48. Lesnik Oberstein,SA, van Duinen,SG, van den Boom,R, Maat-Schieman,ML, van Buchem,MA, van Houwelingen,HC, Hegeman-Kleinn,IM, Ferrari,MD, Breuning,MH, and Haan,J Evaluation of diagnostic NOTCH3 immunostaining in CADASIL. *Acta Neuropathol.* 106, 107-111 (2003).
49. Ebke,M, Dichgans,M, Bergmann,M, Voelter,HU, Rieger,P, Gasser,T, and Schwendemann,G CADASIL: skin biopsy allows diagnosis in early stages. *Acta Neurol.Scand.* 95, 351-357 (1997).
50. Mayer,M, Straube,A, Bruening,R, Uttner,I, Pongratz,D, Gasser,T, Dichgans,M, and Muller-Hocker,J Muscle and skin biopsies are a sensitive diagnostic tool in the diagnosis of CADASIL. *J.Neurol.* 246, 526-532 (1999).
51. Tikka,S, Mykkanen,K, Ruchoux,MM, Bergholm,R, Junna,M, Poyhonen,M, Yki-Jarvinen,H, Joutel,A, Viitanen,M, Baumann,M, and Kalimo,H Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. *Brain* 132, 933-939 (2009).
52. Morroni,M, Marzioni,D, Ragno,M, Di,BP, Cartechini,E, Pianese,L, Lorenzi,T, Castellucci,M, and Scarpelli,M Role of electron microscopy in the diagnosis of cadasil syndrome: a study of 32 patients. *PLoS.One.* 8, e65482 (2013).

53. Markus,HS, Martin,RJ, Simpson,MA, Dong,YB, Ali,N, Crosby,AH, and Powell,JF Diagnostic strategies in CADASIL. *Neurology* 59, 1134-1138 (2002).

54. Malandrini,A, Gaudiano,C, Gambelli,S, Berti,G, Serni,G, Bianchi,S, Federico,A, and Dotti,MT Diagnostic value of ultrastructural skin biopsy studies in CADASIL. *Neurology* 68, 1430-1432 (2007).

55. Peters,N, Opherk,C, Bergmann,T, Castro,M, Herzog,J, and Dichgans,M Spectrum of mutations in biopsy-proven CADASIL: implications for diagnostic strategies. *Arch.Neurol.* 62, 1091-1094 (2005).

56. Oberstein,SA Diagnostic strategies in CADASIL. *Neurology* 60, 2020 (2003).

57. Arboleda-Velasquez,JF, Lopera,F, Lopez,E, Frosch,MP, Sepulveda-Falla,D, Gutierrez,JE, Vargas,S, Medina,M, Martinez De,AC, Lebo,RV, Slaugenhaupt,SA, Betensky,RA, Villegas,A, Arcos-Burgos,M, Rivera,D, Restrepo,JC, and Kosik,KS C455R notch3 mutation in a Colombian CADASIL kindred with early onset of stroke. *Neurology* 59, 277-279 (2002).

58. Singhal,S, Bevan,S, Barrick,T, Rich,P, and Markus,HS The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. *Brain* 127, 2031-2038 (2004).

59. Dichgans,M, Filippi,M, Bruning,R, Iannucci,G, Berchtenbreiter,C, Minicucci,L, Uttner,I, Crispin,A, Ludwig,H, Gasser,T, and Yousry,TA Quantitative MRI in CADASIL: correlation with disability and cognitive performance. *Neurology* 52, 1361-1367 (1999).

60. Mykkanen,K, Junna,M, Amberla,K, Bronge,L, Kaariainen,H, Poyhonen,M, Kalimo,H, and Viitanen,M Different clinical phenotypes in monozygotic CADASIL twins with a novel NOTCH3 mutation. *Stroke* 40, 2215-2218 (2009).

61. Liem,MK, Lesnik Oberstein,SA, Vollebregt,MJ, Middelkoop,HA, van der Grond,J, and Helderman-van den Enden AT Homozygosity for a NOTCH3 mutation in a 65-year-old CADASIL patient with mild symptoms: a family report. *J.Neurol.* 255, 1978-1980 (2008).

62. Tuominen,S, Juvonen,V, Amberla,K, Jolma,T, Rinne,JO, Tuisku,S, Kurki,T, Marttila,R, Poyhonen,M, Savontaus,ML, Viitanen,M, and Kalimo,H Phenotype of a homozygous CADASIL patient in comparison to 9 age-matched heterozygous patients with the same R133C Notch3 mutation. *Stroke* 32, 1767-1774 (2001).

63. Soong,BW, Liao,YC, Tu,PH, Tsai,PC, Lee,IH, Chung,CP, and Lee,YC A homozygous NOTCH3 mutation p.R544C and a heterozygous TREX1 variant p.C99MfsX3 in a family with hereditary small vessel disease of the brain. *J.Chin Med.Assoc.* 76, 319-324 (2013).

64. Vinciguerra,C, Rufa,A, Bianchi,S, Sperduto,A, De,SM, Malandrini,A, Dotti,MT, and Federico,A Homozygosity and severity of phenotypic presentation in a CADASIL family. *Neurol.Sci.* (2013).

65. Rutten,JW, Boon,EM, Liem,MK, Dauwerse,JG, Pont,MJ, Vollebregt,E, Maat-Kievit,AJ, Ginjaar,HB, Lakeman,P, van Duinen,SG, Terwindt,GM, and Lesnik Oberstein,SA Hypomorphic NOTCH3 Alleles Do Not Cause CADASIL in Humans. *Hum.Mutat.* 34, 1486-1489 (2013).

66. Mazzei,R, Guidetti,D, Ungaro,C, Conforti,FL, Muglia,M, Cenacchi,G, Lanza,PL, Patitucci,A, Sprovieri,T, Riguzzi,P, Magariello,A, Gabriele,AL, Citrigno,L, Preda,P, and Quattrone,A First evidence of a pathogenic insertion in the NOTCH3 gene causing CADASIL. *J.Neurol.Neurosurg.Psychiatry* 79, 108-110 (2008).

67. Joutel,A, Chabriat,H, Vahedi,K, Domenga,V, Vayssiere,C, Ruchoux,MM, Lucas,C, Leys,D, Bousser,MG, and Tournier-Lasserve,E Splice site mutation causing a seven amino acid Notch3 in-frame deletion in CADASIL. *Neurology* 54, 1874-1875 (2000).

68. Dichgans,M, Herzog,J, and Gasser,T NOTCH3 mutation involving three cysteine residues in a family with typical CADASIL. *Neurology* 57, 1714-1717 (2001).

69. Lee,SJ, Meng,H, Elmadhoun,O, Blaivas,M, and Wang,MM Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy affecting an African American man:

identification of a novel 15-base pair NOTCH3 duplication. *Arch.Neurol.* 68, 1584-1586 (2011).

70. Saiki,S, Sakai,K, Saiki,M, Kitagawa,Y, Umemori,T, Murata,K, Matsui,M, and Hirose,G Varicose veins associated with CADASIL result from a novel mutation in the Notch3 gene. *Neurology* 67, 337-339 (2006).
71. Lackovic,V, Bajcetic,M, Lackovic,M, Novakovic,I, Labudovic,BM, Pavlovic,A, Zidverc-Trajkovic,J, Dzolic,E, Rovcanin,B, Sternic,N, and Kostic,V Skin and sural nerve biopsies: ultrastructural findings in the first genetically confirmed cases of CADASIL in Serbia. *Ultrastruct.Pathol.* 36, 325-335 (2012).
72. Mazzei,R, Conforti,FL, Lanza,PL, Sprovieri,T, Lupo,MR, Gallo,O, Patitucci,A, Magariello,A, Caracciolo,M, Gabriele,AL, Fera,F, Valentino,P, Bono,F, Cenacchi,G, Santoro,G, Muglia,M, and Quattrone,A A novel Notch3 gene mutation not involving a cysteine residue in an Italian family with CADASIL. *Neurology* 63, 561-564 (2004).
73. Bianchi,S, Dotti,MT, Gallus,GN, D'Eramo,C, Di,D, I, Bernardi,L, Maletta,R, Puccio,G, Bruni,AC, and Federico,A First deep intronic mutation in the NOTCH3 gene in a family with late-onset CADASIL. *Neurobiol.Aging* 34, 2234-12 (2013).
74. Mizuno,T, Muranishi,M, Torugun,T, Tango,H, Nagakane,Y, Kudeken,T, Kawase,Y, Kawabe,K, Oshima,F, Yaoi,T, Itoh,K, Fushiki,S, and Nakagawa,M Two Japanese CADASIL families exhibiting Notch3 mutation R75P not involving cysteine residue. *Intern.Med.* 47, 2067-2072 (2008).
75. Ampuero,I, Alegre-Abarrategui,J, Rodal,I, Espana,A, Ros,R, Sendon,JL, Galloway,EG, Cervello,A, Caminero,AB, Zabala,A, Erro,E, Jarauta,F, Morlan,L, Lopez-Valdes,E, Aladro,Y, Seijo,M, Rivas,GG, Munoz,DG, and de Yebenes,JG On the diagnosis of CADASIL. *J.Alzheimers.Dis.* 17, 787-794 (2009).
76. Roy,B, Maksemous,N, Smith,RA, Menon,S, Davies,G, and Griffiths,LR Two novel mutations and a previously unreported intronic polymorphism in the NOTCH3 gene. *Mutat.Res.* 732, 3-8 (2012).
77. Kotorii,S, Takahashi,K, Kamimura,K, Nishio,T, Arima,K, Yamada,H, Uyama,E, Uchino,M, Suenaga,A, Matsumoto,M, Kuchel,G, Rouleau,GA, and Tabira,T Mutations of the notch3 gene in non-caucasian patients with suspected CADASIL syndrome. *Dement.Geriatr.Cogn Disord.* 12, 185-193 (2001).
78. Uchino,M, Hirano,T, Uyama,E, and Hashimoto,Y Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and CADASIL-like disorders in Japan. *Ann.N.Y.Acad.Sci.* 977, 273-278 (2002).
79. Ferreira,S, Malheiro,F, and Oliveira,JP Novel human pathological mutations. Gene symbol: NOTCH3. Disease: cerebral autosomal dominant aretriopathy with subcortical infarcts and leukoencephalopathy. *Hum.Genet.* 121, 651-652 (2007).
80. Ferreira,S, Fontoura,P, Guerreiro,R, and Oliveira,JP Novel human pathological mutations. Gene symbol: NOTCH3. Disease: cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *Hum.Genet.* 121, 649-650 (2007).
81. Scheid,R, Heinritz,W, Leyhe,T, Thal,DR, Schober,R, Strenge,S, von Cramon,DY, and Froster,UG Cysteine-sparing notch3 mutations: cadasil or cadasil variants? *Neurology* 71, 774-776 (2008).
82. Ungaro,C, Mazzei,R, Conforti,FL, Sprovieri,T, Servillo,P, Liguori,M, Citrigno,L, Gabriele,AL, Magariello,A, Patitucci,A, Muglia,M, and Quattrone,A CADASIL: extended polymorphisms and mutational analysis of the NOTCH3 gene. *J.Neurosci.Res.* 87, 1162-1167 (2009).
83. Quattrone,A and Mazzei,R Cysteine-sparing notch3 mutations: cadasil or cadasil variants? *Neurology* 72, 2135-2136 (2009).
84. Brass,SD, Smith,EE, Arboleda-Velasquez,JF, Copen,WA, and Frosch,MP Case records of the Massachusetts General Hospital. Case 12-2009. A 46-year-old man with migraine, aphasia, and hemiparesis and similarly affected family members. *N.Engl.J.Med.* 360, 1656-1665 (2009).

85. Kim,Y, Choi,EJ, Choi,CG, Kim,G, Choi,JH, Yoo,HW, and Kim,JS Characteristics of CADASIL in Korea: a novel cysteine-sparing Notch3 mutation. *Neurology* 66, 1511-1516 (2006).
86. Kim,YE, Yoon,CW, Seo,SW, Ki,CS, Kim,YB, Kim,JW, Bang,OY, Lee,KH, Kim,GM, Chung,CS, and Na,DL Spectrum of NOTCH3 mutations in Korean patients with clinically suspicious cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Neurobiol.Aging* 35, 726 (2014).
87. Dotti,MT, De,SN, Bianchi,S, Malandrini,A, Battisti,C, Cardaioli,E, and Federico,A A novel NOTCH3 frameshift deletion and mitochondrial abnormalities in a patient with CADASIL. *Arch.Neurol.* 61, 942-945 (2004).
88. Weiming,F, Yuliang,W, Youjie,L, Xinsheng,L, Shuyang,X, and Zhaoxia,L A novel Notch3 deletion mutation in a Chinese patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *J.Clin.Neurosci.* 20, 322-323 (2013).
89. Cognat,E, Baron-Menguy,C, Domenga-Denier,V, Cleophax,S, Fouillade,C, Monet-Lepretre,M, Dewerchin,M, and Joutel,A Archetypal Arg169Cys Mutation in NOTCH3 Does Not Drive the Pathogenesis in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leucoencephalopathy via a Loss-of-Function Mechanism. *Stroke* (2014).
90. Fouillade,C, Chabriat,H, Riant,F, Mine,M, Arnoud,M, Magy,L, Bousser,MG, Tournier-Lasserve,E, and Joutel,A Activating NOTCH3 mutation in a patient with small-vessel-disease of the brain. *Hum.Mutat.* 29, 452 (2008).
91. Martignetti,JA, Tian,L, Li,D, Ramirez,MC, Camacho-Vanegas,O, Camacho,SC, Guo,Y, Zand,DJ, Bernstein,AM, Masur,SK, Kim,CE, Otieno,FG, Hou,C, Abdel-Magid,N, Tweddale,B, Metry,D, Fournet,JC, Papp,E, McPherson,EW, Zabel,C, Vaksmann,G, Morisot,C, Keating,B, Sleiman,PM, Cleveland,JA, Everman,DB, Zackai,E, and Hakonarson,H Mutations in PDGFRB Cause Autosomal-Dominant Infantile Myofibromatosis. *Am.J.Hum.Genet.* 92, 1001-1007 (2013).
92. Bersano,A, Ranieri,M, Ciammola,A, Cinnante,C, Lanfranconi,S, Dotti,MT, Candelise,L, Baschirotto,C, Ghione,I, Ballabio,E, Bresolin,N, and Bassi,MT Considerations on a mutation in the NOTCH3 gene sparing a cysteine residue: a rare polymorphism rather than a CADASIL variant. *Funct.Neurol.* 27, 247-252 (2012).
93. den Dunnen,JT and Antonarakis,SE Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum.Mutat.* 15, 7-12 (2000).
94. Ross,OA, Soto-Ortolaza,AI, Heckman,MG, Verbeeck,C, Serie,DJ, Rayaprolu,S, Rich,SS, Nalls,MA, Singleton,A, Guerreiro,R, Kinsella,E, Wszolek,ZK, Brott,TG, Brown,RD, Jr., Worrall,BB, and Meschia,JF NOTCH3 Variants and Risk of Ischemic Stroke. *PLoS.One.* 8, e75035 (2013).
95. Schmidt,H, Zeginigg,M, Wiltgen,M, Freudenberger,P, Petrovic,K, Cavalieri,M, Gider,P, Enzinger,C, Fornage,M, Debette,S, Rotter,JI, Ikram,MA, Launer,LJ, and Schmidt,R Genetic variants of the NOTCH3 gene in the elderly and magnetic resonance imaging correlates of age-related cerebral small vessel disease. *Brain* 134, 3384-3397 (2011).

No	Nucleotide change	Amino acid change	Exon	EGFR	Reference
1	c.127T>G	p.Cys43Gly	2	1	Peters et al.[3], Opherk et al.[4]
2	c.128G>T	p.Cys43Phe	2	1	Lesnik Oberstein [5]
3	c.145T>G	p.Cys49Gly	2	1	Oki et al.[6]
4	c.145T>C	p.Cys49Arg	2	1	Wang et al.[7]
5	c.146G>A	p.Cys49Tyr	2	1	Joutel et al.[8]
6	c.146G>T	p.Cys49Phe	2	1	Peters et al.[3], Opherk et al.[4]
7	c.157G>T	p.Gly53Cys	2	1	Wang et al.[7]
8	c.160C>T	p.Arg54Cys	2	1	Escary et al.[9]
9	c.179C>G	p.Ser60Cys	2	1	Peters et al.[3], Opherk et al.[4]
10	c.193T>G	p.Cys65Gly	2	1	Cleves et al.[10], HGMD
11	c.194G>C	p.Cys65Ser	2	1	Peters et al.[3], Opherk et al.[4]
12	c.194G>A	p.Cys65Tyr	2	1	Bianchi et al.[11]
13	c.199T>A	p.Cys67Ser	3	1	Tikka et al.[1]
14	c.200G>A	p.Cys67Tyr	3	1	Moon et al.[12]
15	c.213G>T	p.Trp71Cys	3	1	Joutel et al.[13]
16	c.226T>C	p.Cys76Arg	3	1	Markus et al.[14]
17	c.228T>G	p.Cys76Trp	3	1	Peters et al.[3], Opherk et al.[4]
18	c.259T>C	p.Cys87Arg	3	2	Peters et al.[3], Opherk et al.[4]
19	c.260G>A	p.Cys87Tyr	3	2	Opherk et al.[4]
20	c.265G>T	p.Gly89Cys	3	2	Pavlovic et al.[15], Lackovic et al.[16]
21	c.268C>T	p.Arg90Cys	3	2	Joutel et al.[8]
22	c.278G>A	p.Cys93Tyr	3	2	Kalimo et al.[17]
23	c.278G>T	p.Cys93Phe	3	2	Dichgans et al.[18]
24	c.316T>C	p.Cys106Arg	3	2	Yamada et al.[19]
25	c.318C>G	p.Cys106Trp	3	2	Opherk et al.[4]
26	c.322T>C	p.Cys108Arg	3	2	Wang et al.[20]
27	c.323G>C	p.Cys108Ser	3	2	Testi et al.[21]
28	c.323G>A	p.Cys108Tyr	3	2	Peters et al.[3], Opherk et al.[4]
29	c.324C>G	p.Cys108Trp	3	2	Rojas-Marcos et al.[22]
30	c.328C>T	p.Arg110Cys	3	2	Joutel et al.[8]
31	c.349T>C	p.Cys117Arg	4	2	Wang et al.[7]
32	c.350G>T	p.Cys117Phe	4	2	Dichgans et al.[23]
33	c.350G>C	p.Cys117Ser	4	2	Spinicci et al.[24]
34	c.350G>A	p.Cys117Tyr	4	2	Ampuero et al.[25]
35	c.353C>G	p.Ser118Cys	4	3	Lee et al.[26]
36	c.368G>A	p.Cys123Tyr	4	3	Escary et al.[9]
37	c.368G>T	p.Cys123Phe	4	3	Dichgans et al.[18]

No	Nucleotide change	Amino acid change	Exon	EGFR	Reference
38	c.382T>G	p.Cys128Gly	4	3	Coto et al.[27]
39	c.383G>A	p.Cys128Tyr	4	3	Kalimo et al.[17]
40	c.383G>T	p.Cys128Phe	4	3	LOVD
41	c.391G>T	p.Gly131Cys	4	3	Ungaro et al.[28]
42	c.397C>T	p.Arg133Cys	4	3	Joutel et al.[8]
43	c.402C>G	p.Cys134Trp	4	3	Joutel et al.[29]
44	c.421C>T	p.Arg141Cys	4	3	Joutel et al.[8]
45	c.425T>G	p.Phe142Cys	4	3	Kalimo et al.[17]
46	c.431G>A	p.Cys144Tyr	4	3	Dichgans et al.[18]
47	c.431G>C	p.Cys144Ser	4	3	Dichgans et al.[18]
48	c.431G>T	p.Cys144Phe	4	3	Grigg et al.[30]
49	c.434C>G	p.Ser145Cys	4	3	Opherk et al.[4]
50	c.436T>C	p.Cys146Arg	4	3	Joutel et al.[8]
51	c.437G>A	p.Cys146Tyr	4	3	Malandrini et al.[31]
52	c.445G>T	p.Gly149Cys	4	3	Peters et al.[3], Opherk et al.[4]
53	c.449A>G	p.Tyr150Cys	4	3	Dichgans et al.[18]
54	c.457C>T	p.Arg153Cys	4	3	Joutel et al.[8]
55	c.463T>A	p.Cys155Ser	4	3	Ampuero et al.[25]
56	c.464G>A	p.Cys155Tyr	4	3	LOVD
57	c.464G>C	p.Cys155Ser	4	3	Opherk et al.[4]
58	c.484T>A	p.Cys162Ser	4	4	Escary et al.[9]
59	c.484T>C	p.Cys162Arg	4	4	Andreadou et al.[32]
60	c.486C>G	p.Cys162Trp	4	4	Lesnik Oberstein et al.[33]
61	c.493G>T	p.Gly165Cys	4	4	Ampuero et al.[25]
62	c.505C>T	p.Arg169Cys	4	4	Joutel et al.[34]
63	c.511G>T	p.Gly171Cys	4	4	Joutel et al.[8]
64	c.520T>C	p.Cys174Arg	4	4	Santa el al.[35]
65	c.520T>A	p.Cys174Ser	4	4	Tikka et al.[1]
66	c.521G>A	p.Cys174Tyr	4	4	Dichgans et al.[23]
67	c.521G>T	p.Cys174Phe	4	4	Kotorii et al.[36]
68	c.539C>G	p.Ser180Cys	4	4	Escary et al.[9]
69	c.542T>G	p.Phe181Cys	4	4	Granild-Jensen et al.[37]
70	c.544C>T	p.Arg182Cys	4	4	Joutel et al.[34]
71	c.547T>A	p.Cys183Ser	4	4	Dichgans et al.[18]
72	c.547T>C	p.Cys183Arg	4	4	Dichgans et al.[23]
73	c.548G>T	p.Cys183Phe	4	4	Peters et al.[3], Opherk et al.[4]
74	c.553T>C	p.Cys185Arg	4	4	Joutel et al.[8]

No	Nucleotide change	Amino acid change	Exon	EGFR	Reference
75	c.553T>G	p.Cys185Gly	4	4	Joutel et al.[29]
76	c.553T>A	p.Cys185Ser	4	4	Adib-Samii et al.[38], HGMD
77	c.566A>G	p.Tyr189Cys	4	4	Lesnik Oberstein et al.[5]
78	c.580T>A	p.Cys194Ser	4	4	Markus et al.[14]
79	c.580T>C	p.Cys194Arg	4	4	Kalimo et al.[17]
80	c.581G>A	p.Cys194Tyr	4	4	Escary et al.[9]
81	c.581G>T	p.Cys194Phe	4	4	Dichgans et al.[18]
82	c.581G>C	p.Cys194Ser	4	4	Adib-Samii et al.[38], HGMD
83	c.601T>C	p.Cys201Arg	4	5	Uyguner et al.[39]
84	c.602G>A	p.Cys201Tyr	4	5	Opherk et al.[4]
85	c.616T>C	p.Cys206Arg	4	5	Matsumoto et al.[40]
86	c.617G>A	p.Cys206Tyr	4	5	Escary et al.[9]
87	c.619C>T	p.Arg207Cys	4	5	Lesnik Oberstein et al.[41]
88	c.634T>A	p.Cys212Ser	4	5	Joutel et al.[8]
89	c.635G>A	p.Cys212Tyr	4	5	Bentley et al.[42]
90	c.636C>G	p.Cys212Trp	4	5	Spinnici et al.[24]
91	c.659A>G	p.Tyr220Cys	4	5	Rojas-Marcos et al.[43]
92	c.664T>G	p.Cys222Gly	4	5	Joutel et al.[8]
93	c.665G>A	p.Cys222Tyr	4	5	Kalimo et al.[17]
94	c.665G>C	p.Cys222Ser	4	5	Wang et al.[7]
95	c.671G>A	p.Cys224Tyr	4	5	Joutel et al.[8]
96	c.697T>A	p.Cys233Ser	5	5	Joutel et al.[29]
97	c.697T>C	p.Cys233Arg	5	5	Adib-Samii et al.[38], HGMD
98	c.698G>A	p.Cys233Tyr	5	5	Peters et al.[3], Opherk et al.[4]
99	c.699T>G	p.Cys233Trp	5	5	Lesnik Oberstein [5]
100	c.719G>C	p.Cys240Ser	5	6	Opherk et al.[4]
101	c.733T>C	p.Cys245Arg	5	6	Peters et al.[3], Opherk et al.[4]
102	c.733T>A	p.Cys245Ser	5	6	Razvi et al.[44]
103	c.751T>C	p.Cys251Arg	5	6	Markus et al.[14]
104	c.751T>A	p.Cys251Ser	5	6	Lesnik Oberstein [5]
105	c.751T>G	p.Cys251Gly	5	6	Viskelis et al.[45]
106	c.752G>A	p.Cys251Tyr	5	6	Tikka et al.[1]
107	c.773A>G	p.Tyr258Cys	5	6	Joutel et al.[8]
108	c.778T>G	p.Cys260Gly	5	6	De Silva et al.[46]
109	c.779G>A	p.Cys260Tyr	5	6	Peters et al.[3], Opherk et al.[4]
110	c.812G>T	p.Cys271Phe	6	6	Kam-Ming Au et al.[47]
111	c.886G>T	p.Gly296Cys	6	7	Garcia-Estevez et al.[48]

No	Nucleotide change	Amino acid change	Exon	EGFR	Reference
112	c.895A>T	p.Ser299Cys	6	7	Golomb et al.[49]
113	c.994C>T	p.Arg332Cys	6	8	Oliveri et al.[50]
114	c.1004C>G	p.Ser335Cys	6	8	Peters et al.[3], Opherk et al.[4]
115	c.1010A>G	p.Tyr337Cys	6	8	Lesnik Oberstein [5]
116	c.1012T>C	p.Cys338Arg	6	8	Dotti et al.[51]
117	c.1078T>C	p.Cys360Arg	7	9	Kim et al.[52]
118	c.1096T>C	p.Cys366Arg	7	9	HGMD
119	c.1098T>G	p.Cys366Trp	7	9	Pradotto et al.[53]
120	c.1135T>C	p.Cys379Arg	7	9	Del Rio-Espínola et al.[54]
121	c.1136G>C	p.Cys379Ser	7	9	Peters et al.[3], Opherk et al.[4]
122	c.1144G>T	p.Gly382Cys	7	9	Lesnik Oberstein [5]
123	c.1163G>A	p.Cys388Tyr	7	9	Ishida et al.[55]
124	c.1183T>C	p.Cys395Arg	7	10	Peters et al.[3], Opherk et al.[4]
125	c.1187C>G	p.Ser396Cys	7	10	Testi et al.[21]
126	c.1241C>G	p.Ser414Cys	8	10	Kim et al.[52]
127	c.1258G>T	p.Gly420Cys	8	10	Joutel et al.[29]
128	c.1261C>T	p.Arg421Cys	8	10	Peters et al.[3], Opherk et al.[4]
129	c.1279C>T	p.Arg427Cys	8	10	HGMD
130	c.1282T>C	p.Cys428Arg	8	10	Dotti et al.[51]
131	c.1283G>A	p.Cys428Tyr	8	10	Peters et al.[3], Opherk et al.[4]
132	c.1283G>C	p.Cys428Ser	8	10	Joutel et al.[29]
133	c.1303T>C	p.Cys435Arg	8	11	Lesnik Oberstein [5]
134	c.1318T>A	p.Cys440Ser	8	11	Federico et al.[56]
135	c.1318T>G	p.Cys440Gly	8	11	Markus et al.[14]
136	c.1318T>C	p.Cys440Arg	8	11	Peters et al.[3], Opherk et al.[4]
137	c.1337G>C	p.Cys446Ser	8	11	Opherk et al.[4]
138	c.1337G>T	p.Cys446Phe	8	11	Lesnik Oberstein et al.[33]
139	c.1345C>T	p.Arg449Cys	8	11	Thomas et al.[57]
140	c.1363T>C	p.Cys455Arg	8	11	Arboleda-Velasquez et al.[58]
141	c.1364G<A	p.Cys455Tyr	8	11	Kim et al.[52]
142	c.1370G>C	p.Cys457Ser	8	11	Adib-Samii et al.[38], HGMD
143	c.1394A>G	p.Tyr465Cys	9	11	Lesnik Oberstein [5]
144	c.1450T>G	p.Cys484Gly	9	12	LOVD
145	c.1451G>A	p.Cys484Tyr	9	12	Opherk et al.[4]
146	c.1451G>T	p.Cys484Phe	9	12	Peters et al.[3]
147	c.1484G>A	p.Cys495Tyr	9	12	Peters et al.[3], Opherk et al.[4]
148	c.1510T>C	p.Cys504Arg	10	12	Lee et al.[59]

No	Nucleotide change	Amino acid change	Exon	EGFR	Reference
149	c.1531T>C	p.Cys511Arg	10	13	Peters et al.[3], Opherk et al.[4]
150	c.1532G>T	p.Cys511Phe	10	13	Mosca et al.[60]
151	c.1532G>A	p.Cys511Tyr	10	13	Bianchi et al.[61]
152	c.1582G>T	p.Gly528Cys	10	13	Dotti et al.[51]
153	c.1592G>C	p.Cys531Ser	10	13	Mazzei et al.[62]
154	c.1594C>T	p.Arg532Cys	10	13	Bianchi et al.[63]
155	c.1624T>C	p.Cys542Arg	11	13	Kim et al.[52]
156	c.1625G>A	p.Cys542Tyr	11	13	Joutel et al.[8]
157	c.1630C>T	p.Arg544Cys	11	14	Lesnik Oberstein et al.[41]
158	c.1645T>C	p.Cys549Arg	11	14	Lesnik Oberstein [5]
159	c.1646G>A	p.Cys549Tyr	11	14	Peters et al.[3], Opherk et al.[4]
160	c.1672C>T	p.Arg558Cys	11	14	Joutel et al.[8]
161	c.1703G>A	p.Cys568Tyr	11	14	Ferreira et al.[64]
162	c.1721A>G	p.Tyr574Cys	11	14	Mazzei et al.[65]
163	c.1732C>T	p.Arg578Cys	11	14	Joutel et al.[34]
164	c.1735T>C	p.Cys579Arg	11	14	Roy et al.[66]
165	c.1759C>T	p.Arg587Cys	11	15	Kim et al.[67]
166	c.1771T>C	p.Cys591Arg	11	15	LOVD
167	c.1774C>T	p.Arg592Cys	11	15	Praline et al.[68], HGMD
168	c.1790G>C	p.Cys597Ser	11	15	Bohlega et al.[69]
169	c.1816T>C	p.Cys606Arg	11	15	Testi et al.[21]
170	c.1819C>T	p.Arg607Cys	11	15	Escary et al.[9]
171	c.1918C>T	p.Arg640Cys	12	16	LOVD
172	c.1999G>T	p.Gly667Cys	13	17	LOVD
173	c.2038C>T	p.Arg680Cys	13	17	Pradotto et al.[70]
174	c.2129A>G	p.Tyr710Cys	13	18	Rutten et al.[71]
175	c.2149C>T	p.Arg717Cys	14	18	HGMD
176	c.2182C>T	p.Arg728Cys	14	18	Joutel et al.[8]
177	c.2324G>C	p.Cys775Ser	15	20	Peters et al.[3]
178	c.2815T>C	p.Cys939Arg	18	24	Testi et al.[21]
179	c.2857G>T	p.Gly953Cys	18	24	Markus et al.[14]
180	c.2923G>T	p.Gly975Cys	18	25	Kotorii et al.[72]
181	c.2929T>A	p.Cys977Ser	18	25	Lee et al.[26]
182	c.2951T>G	p.Phe984Cys	18	25	Escary et al.[9]
183	c.2953C>T	p.Arg985Cys	18	25	Joutel et al.[8]
184	c.2963G>A	p.Cys988Tyr	18	25	Kim et al.[67]
185	c.2989T>G	p.Cys997Gly	18	25	Ungaro et al.[28]

No	Nucleotide change	Amino acid change	Exon	EGFR	Reference
186	c.3011G>A	p.Cys1004Tyr	19	26	Guerrot et al.[73]
187	c.3016C>T	p.Arg1006Cys	19	26	Joutel et al.[8]
188	c.3037G>T	p.Gly1013Cys	19	26	Testi et al.[21]
189	c.3043T>C	p.Cys1015Arg	19	26	Lesnik Oberstein et al.[41]
190	c.3062A>G	p.Tyr1021Cys	19	26	Kalimo et al.[17]
191	c.3065G>T	p.Cys1022Phe	19	26	Vedeler et al.[74]
192	c.3084G>T	p.Trp1028Cys	19	26	Viana-Babtista et al.[75]
193	c.3091C>T	p.Arg1031Cys	19	26	Joutel et al.[8]
194	c.3172G>T	p.Gly1058Cys	20	27	Kalimo et al.[17]
195	c.3182G>A	p.Cys1061Tyr	20	27	LOVD
196	c.3206A>G	p.Tyr1069Cys	20	27	Tikka et al.[1]
197	c.3226C>T	p.Arg1076Cys	20	27	Lesnik Oberstein et al.[33]
198	c.3296G>A	p.Cys1099Tyr	20	28	Ferreira et al.[76]
199	c.3393C>G	p.Cys1131Trp	21	29	Pescini et al.[77]
200	c.3427C>T	p.Arg1143Cys	21	29	HGMD
201	c.3691C>T	p.Arg1231Cys	22	31	Joutel et al.[8]
202	c.3750C>G	p.Cys1250Trp	23	32	Del Rio-Espínola et al.[78]
203	c.3781T>C	p.Cys1261Arg	23	32	Joutel et al.[13]
204	c.3782G>A	p.Cys1261Tyr	23	32	Peters et al.[3], Opherk et al.[4]
205	c.3893G>T	p.Cys1298Phe	24	33	Rinnoci et al.[79]
206	c.3944G>A	p.Cys1315Tyr	24	33	Valenti et al.[80]

Supplementary table 1. Currently known cysteine altering missense mutations in *NOTCH3* causing CADASIL. This list is compiled data from a list of CADASIL causing mutations described by Tikka et al.[1] in 2009, supplemented with mutations described thereafter. All mutations are described according to HGVS nomenclature, and may therefore differ from the mutation description in the original research article. LOVD= leiden open variation database (www.lovd.nl) [2], HGMD= human gene mutation database

No	Amino acid change	Exon	EGFR	Reference
1	p.Arg103Gln	3	2	Schmidt et al.[81]
2	p.His170Arg	4	4	Joutel et al.[8]
3	p.Pro496Leu	9	12	Joutel et al.[8]
4	p.Ser497Leu	9	12	Schmidt et al.[81]
5	p.Ser502Phe	9	12	Schmidt et al.[81]
6	p.Val764Ala	14	19	Schmidt et al.[81]
7	p.His981Tyr	18	25	Ross et al.[82]
8	p.Ala1020Pro	19	26	Schmidt et al.[81]
9	p.His1133Gln	21	29	Joutel et al.[8]
10	p.Val1183Met	22	30	Joutel et al.[8]
11	p.His1235Leu	22	31	Schmidt et al.[81]
12	p.Arg1262Leu	23	32	Schmidt et al.[81]

Supplementary table 2. *NOTCH3* missense variants not involving a cysteine residue described as non- pathogenic. In healthy individuals, variants have been detected which lead to an amino acid change in the EGFR encoding exons of NOTCH3, but which do not involve a cysteine residue. Some of these polymorphisms, namely pHis170Arg and p.Ala1020Pro have also erroneously been reported to be pathogenic. Note: this list results from polymorphisms described in larger studies in healthy individuals, and is not a complete list of all *NOTCH3* polymorphisms described.

REFERENCES

1. Tikka,S, Mykkanen,K, Ruchoux,MM, Bergholm,R, Junna,M, Poyhonen,M, Yki-Jarvinen,H, Joutel,A, Viitanen,M, Baumann,M, and Kalimo,H Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. *Brain* 132, 933-939 (2009).
2. Fokkema,IF, Taschner,PE, Schaafsma,GC, Celli,J, Laros,JF, and den Dunnen,JT LOVD v.2.0: the next generation in gene variant databases. *Hum.Mutat.* 32, 557-563 (2011).
3. Peters,N, Opherk,C, Bergmann,T, Castro,M, Herzog,J, and Dichgans,M Spectrum of mutations in biopsy-proven CADASIL: implications for diagnostic strategies. *Arch.Neurol.* 62, 1091-1094 (2005).
4. Opherk,C, Peters,N, Herzog,J, Luedtke,R, and Dichgans,M Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. *Brain* 127, 2533-2539 (2004).
5. Oberstein,SA Diagnostic strategies in CADASIL. *Neurology* 60, 2020 (2003).
6. Oki,K, Nagata,E, Ishiko,A, Shimizu,A, Tanaka,K, Takahashi,K, Tabira,T, Katayama,T, and Suzuki,N Novel mutation of the Notch3 gene in a Japanese patient with CADASIL. *Eur.J.Neurol.* 14, 464-466 (2007).
7. Wang,Z, Yuan,Y, Zhang,W, Lv,H, Hong,D, Chen,B, Liu,Y, Luan,X, Xie,S, and Wu,S NOTCH3 mutations and clinical features in 33 mainland Chinese families with CADASIL. *J.Neurol.Neurosurg.Psychiatry* 82, 534-539 (2011).
8. Joutel,A, Vahedi,K, Corpechot,C, Troesch,A, Chabriat,H, Vayssiere,C, Cruaud,C, Maciazek,J, Weissenbach,J, Bousser,MG, Bach,JF, and Tournier-Lasserve,E Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet* 350, 1511-1515 (1997).
9. Escary,JL, Cecillon,M, Maciazek,J, Lathrop,M, Tournier-Lasserve,E, and Joutel,A Evaluation of DHPLC analysis in mutational scanning of Notch3, a gene with a high G-C content. *Hum.Mutat.* 16, 518-526 (2000).
10. Cleves,C, Friedman,NR, Rothner,AD, and Hussain,MS Genetically confirmed CADASIL in a pediatric patient. *Pediatrics* 126, e1603-e1607 (2010).
11. Bianchi,S, Dotti,MT, De,SN, Stromillo,ML, and Federico,A Novel human pathological mutations. Gene symbol: NOTCH3. Disease: CADASIL, exon 2 mutation. *Hum. Genet.* 122, 558-559 (2007).
12. Moon,SY, Kim,HY, Seok,JI, Kwon,JC, Ki,CS, Kim,JW, Suh,YL, and Na,DL A novel mutation (C67Y)in the NOTCH3 gene in a Korean CADASIL patient. *J.Korean Med. Sci.* 18, 141-144 (2003).
13. Joutel,A, Corpechot,C, Ducros,A, Vahedi,K, Chabriat,H, Mouton,P, Alamowitch,S, Domenga,V, Cecillion,M, Marechal,E, Maciazek,J, Vayssiere,C, Cruaud,C, Cabanis,EA, Ruchoux,MM, Weissenbach,J, Bach,JF, Bousser,MG, and Tournier-Lasserve,E Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* 383, 707-710 (1996).
14. Markus,HS, Martin,RJ, Simpson,MA, Dong,YB, Ali,N, Crosby,AH, and Powell,JF Diagnostic strategies in CADASIL. *Neurology* 59, 1134-1138 (2002).
15. Pavlovic,AM, Dobricic,V, Semnic,R, Lackovic,V, Novakovic,I, Bajcetic,M, and Sternic,N A novel Notch3 Gly89Cys mutation in a Serbian CADASIL family. *Acta Neurol.Belg.* 113, 299-302 (2013).
16. Lackovic,V, Bajcetic,M, Lackovic,M, Novakovic,I, Labudovic,BM, Pavlovic,A, Zidverc-Trajkovic,J, Dzolic,E, Rovcanin,B, Sternic,N, and Kostic,V Skin and sural nerve biopsies: ultrastructural findings in the first genetically confirmed cases of CADASIL in Serbia. *Ultrastruct.Pathol.* 36, 325-335 (2012).
17. Kalimo,H, Ruchoux,MM, Viitanen,M, and Kalaria,RN CADASIL: a common form of hereditary arteriopathy causing brain infarcts and dementia. *Brain Pathol.* 12, 371-384 (2002).
18. Dichgans,M, Ludwig,H, Muller-Hocker,J, Messerschmidt,A, and Gasser,T Small in-frame deletions and missense mutations in CADASIL: 3D models predict misfolding

of Notch3 EGF-like repeat domains. *Eur.J.Hum.Genet.* 8, 280-285 (2000).

19. Yamada,K, Sakai,K, Akazawa,K, Sugimoto,N, Nakagawa,M, and Mizuno,T Detection of early neuronal damage in CADASIL patients by q-space MR imaging. *Neuroradiology* 55, 283-290 (2013).
20. Wang,ZX, Lu,H, Zhang,Y, Bu,DF, Niu,XY, Zhang,Z, Huang,YN, and Yuan,Y [NOTCH3 gene mutations in four Chinese families with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy]. *Zhonghua Yi.Xue. Za Zhi.* 84, 1175-1180 (2004).
21. Testi,S, Malerba,G, Ferrarini,M, Ragno,M, Pradotto,L, Mauro,A, and Fabrizi,GM Mutational and haplotype map of NOTCH3 in a cohort of Italian patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *J.Neurol.Sci.* 319, 37-41 (2012).
22. Rojas-Marcos,I, Encarnacion,M, Martinez-Yelamos,S, Ferrer,I, Arbizu,T, Gil-Peralta,A, and Garcia-Lozano,JR Gene symbol: NOTCH3. Disease: CADASIL. *Hum.Genet.* 115, 175 (2004).
23. Dichgans,M, Filippi,M, Bruning,R, Iannucci,G, Berchtenbreiter,C, Minicucci,L, Uttner,I, Crispin,A, Ludwig,H, Gasser,T, and Yousry,TA Quantitative MRI in CADASIL: correlation with disability and cognitive performance. *Neurology* 52, 1361-1367 (1999).
24. Spinicci,G, Conti,M, Cherchi,MV, Mancosu,C, Murru,R, and Carboni,N Unusual clinical presentations in subjects carrying novel NOTCH3 gene mutations. *J.Stroke Cerebrovasc.Dis.* 22, 539-544 (2013).
25. Ampuero,I, Alegre-Abarrategui,J, Rodal,I, Espana,A, Ros,R, Sendon,JL, Galloway,EG, Cervello,A, Caminero,AB, Zabala,A, Erro,E, Jarauta,F, Morlan,L, Lopez-Valdes,E, Aladro,Y, Seijo,M, Rivas,GG, Munoz,DG, and de Yebenes,JG On the diagnosis of CADASIL. *J.Alzheimers.Dis.* 17, 787-794 (2009).
26. Lee,YC, Yang,AH, Liu,HC, Wong,WJ, Lu,YC, Chang,MH, and Soong,BW Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: two novel mutations in the NOTCH3 gene in Chinese. *J.Neurol.Sci.* 246, 111-115 (2006).
27. Coto,E, Menendez,M, Navarro,R, Garcia-Castro,M, and Alvarez,V A new de novo Notch3 mutation causing CADASIL. *Eur.J.Neurol.* 13, 628-631 (2006).
28. Ungaro,C, Sprovieri,T, Conforti,FL, Consoli,D, Citrigno,L, Liguori,M, Quattrone,A, and Mazzei,R Gene symbol: NOTCH3. Disease: CADASIL. *Hum.Genet.* 123, 554 (2008).
29. Joutel,A, Favrole,P, Labauge,P, Chabriat,H, Lescoat,C, Andreux,F, Domenga,V, Cecillon,M, Vahedi,K, Ducros,A, Cave-Riant,F, Bousser,MG, and Tournier-Lasserve,E Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. *Lancet* 358, 2049-2051 (2001).
30. Grigg,R, Lea,R, Sullivan,AA, Curtain,R, MacMillian,J, and Griffiths,L Identification of a novel mutation C144F in the Notch3 gene in an Australian CADASIL pedigree. *Hum.Mutat.* 16, 449-450 (2000).
31. Malandrini,A, Albani,F, Palmeri,S, Fattapposta,F, Gambelli,S, Berti,G, Bracco,A, Tammaro,A, Calzavara,S, Villanova,M, Ferrari,M, Rossi,A, and Carrera,P Asymptomatic cores and paracrystalline mitochondrial inclusions in CADASIL. *Neurology* 59, 617-620 (2002).
32. Andreadou,E, Papadimas,G, and Sfagos,C A novel heterozygous mutation in the NOTCH3 gene causing CADASIL. *Swiss. Med.Wkly.* 138, 614-617 (2008).
33. Lesnik Oberstein,SA, van den Boom,R, van Buchem,MA, van Houwelingen,HC, Bakker,E, Vollebregt,E, Ferrari,MD, Breuning,MH, and Haan,J Cerebral microbleeds in CADASIL. *Neurology* 57, 1066-1070 (2001).
34. Joutel,A, Corpechot,C, Ducros,A, Vahedi,K, Chabriat,H, Mouton,P, Alamowitch,S, Domenga,V, Cecillion,M, Marechal,E, Maciazek,J, Vayssiere,C, Cruaud,C, Cabanis,EA, Ruchoux,MM, Weissenbach,J, Bach,JF, Bousser,MG, and Tournier-Lasserve,E Notch3 mutations in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a mendelian condition causing stroke and vascular dementia. *Ann.N.Y.Acad.Sci.* 826, 213-217 (1997).

35. Santa,Y, Uyama,E, Chui,DH, Arima,M, Kotorii,S, Takahashi,K, and Tabira,T Genetic, clinical and pathological studies of CADASIL in Japan: a partial contribution of Notch3 mutations and implications of smooth muscle cell degeneration for the pathogenesis. *J.Neurol.Sci.* 212, 79-84 (2003).
36. Kotorii,S, Takahashi,K, Kamimura,K, Nishio,T, Arima,K, Yamada,H, Uyama,E, Uchino,M, Suenaga,A, Matsumoto,M, Kuchel,G, Rouleau,GA, and Tabira,T Mutations of the notch3 gene in non-caucasian patients with suspected CADASIL syndrome. *Dement.Geriatr. Cogn Disord.* 12, 185-193 (2001).
37. Granild-Jensen,J, Jensen,UB, Schwartz,M, and Hansen,US Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy resulting in stroke in an 11-year-old male. *Dev.Med.Child Neurol.* 51, 754-757 (2009).
38. Adib-Samii,P, Brice,G, Martin,RJ, and Markus,HS Clinical spectrum of CADASIL and the effect of cardiovascular risk factors on phenotype: study in 200 consecutively recruited individuals. *Stroke* 41, 630-634 (2010).
39. Uyguner,ZO, Siva,A, Kayserili,H, Saip,S, Altintas,A, Apak,MY, Albayram,S, Isik,N, Akman-Demir,G, Tasyurekli,M, Oz,B, and Wollnik,B The R110C mutation in Notch3 causes variable clinical features in two Turkish families with CADASIL syndrome. *J.Neurol.Sci.* 246, 123-130 (2006).
40. Matsumoto,H, Tsumoto,M, Yamamoto,T, Takahashi,K, Tahira,T, Ugawa,Y, and Tsuji,S [A case of early stage CADASIL showing only dizziness and vertigo with a novel mutation of Notch 3 gene]. *Rinsho Shinkeigaku* 45, 27-31 (2005).
41. Oberstein,SA, Ferrari,MD, Bakker,E, van,GJ, Kneppers,AL, Frants,RR, Breuning,MH, and Haan,J Diagnostic Notch3 sequence analysis in CADASIL: three new mutations in Dutch patients. Dutch CADASIL Research Group. *Neurology* 52, 1913-1915 (1999).
42. Bentley,P, Wang,T, Malik,O, Nicholas,R, Ban,M, Sawcer,S, and Sharma,P CADASIL with cord involvement associated with a novel and atypical NOTCH3 mutation. *J.Neurol.Neurosurg.Psychiatry* (2011).
43. Rojas-Marcos,I, Garcfa-Lozano,R, Lozano,P, Gil-NTciga,E, Gil-Peralta,A, and Bautista,J Gene symbol: NOTCH3. *Hum. Genet.* 120, 917 (2007).
44. Razvi,SS, Davidson,R, Bone,I, and Muir,KW The prevalence of cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) in the west of Scotland. *J.Neurol.Neurosurg.Psychiatry* 76, 739-741 (2005).
45. Vikelis,M, Papatriantafyllou,J, and Karageorgiou,CE A novel CADASIL-causing mutation in a stroke patient. *Swiss. Med.Wkly.* 137, 323-325 (2007).
46. De Silva,KR, Gamage,R, Dunuwille,J, Gunarathna,D, Sirisena,D, Weerasinghe,A, Amarasinghe,PH, Hosomi,A, and Mizuno,T Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL): a patient from Sri Lanka. *J.Clin.Neurosci.* 16, 1492-1493 (2009).
47. Au,KM, Li,HL, Sheng,B, Chow,TC, Chen,ML, Lee,KC, and Chan,AY A novel mutation (C271F) in the Notch3 gene in a Chinese man with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Clin. Chim.Acta* 376, 229-232 (2007).
48. Garcia-Estevez,DA, Barros-Angueira,F, and Navarro,C [CADASIL: brief report on a family with a new p.G296C mutation in exon 6 of the Notch-3 gene]. *Rev.Neurol.* 51, 729-732 (2010).
49. Golomb,MR, Sokol,DK, Walsh,LE, Christensen,CK, and Garg,BP Recurrent hemiplegia, normal MRI, and NOTCH3 mutation in a 14-year-old: is this early CADASIL? *Neurology* 62, 2331-2332 (2004).
50. Oliveri,RL, Muglia,M, De,SN, Mazzei,R, Labate,A, Conforti,FL, Patitucci,A, Gabriele,AL, Tagarelli,G, Magariello,A, Zappia,M, Gambardella,A, Federico,A, and Quattrone,A A novel mutation in the Notch3 gene in an Italian family with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: genetic and magnetic resonance spectroscopic findings. *Arch.Neurol.* 58, 1418-1422 (2001).

51. Dotti,MT, Federico,A, Mazzei,R, Bianchi,S, Scali,O, Conforti,FL, Sprovieri,T, Guidetti,D, Aguglia,U, Consoli,D, Pantoni,L, Sarti,C, Inzitari,D, and Quattrone,A The spectrum of Notch3 mutations in 28 Italian CADASIL families. *J.Neurol.Neurosurg.Psychiatry* 76, 736-738 (2005).
52. Kim,YE, Yoon,CW, Seo,SW, Ki,CS, Kim,YB, Kim,JW, Bang,OY, Lee,KH, Kim,GM, Chung,CS, and Na,DL Spectrum of NOTCH3 mutations in Korean patients with clinically suspicious cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Neurobiol.Aging* 35, 726 (2014).
53. Pradotto,L, Azan,G, Doriguzzi,C, Valentini,C, and Mauro,A Sporadic vascular dementia as clinical presentation of a new missense mutation within exon 7 of NOTCH3 gene. *J.Neurol.Sci.* 271, 207-210 (2008).
54. del Rio-Espinola,A, Fernandez-Cadenas,I, Mendioroz,M, Gutierrez-Agullo,M, Fernandez,MT, Fernandez-Morales,J, Delgado,P, Domingues-Montanari,S, Sole,E, and Montaner,J Novel human pathological mutations. Gene symbol: NOTCH3. Disease: CADASIL. *Hum.Genet.* 127, 473-474 (2010).
55. Ishida,C, Sakajiri,K, Yoshita,M, Joutel,A, Cave-Riant,F, and Yamada,M CADASIL with a novel mutation in exon 7 of NOTCH3 (C388Y). *Intern.Med.* 45, 981-985 (2006).
56. Federico,A, Bianchi,S, and Dotti,MT The spectrum of mutations for CADASIL diagnosis. *Neurol.Sci.* 26, 117-124 (2005).
57. Thomas,NJ, Morris,CM, Scaravilli,F, Johansson,J, Rossor,M, De,LR, St,CD, Nicoll,J, Blank,C, Coulthard,A, Bushby,K, Ince,PG, Burn,D, and Kalaria,RN Hereditary vascular dementia linked to notch 3 mutations. CADASIL in British families. *Ann.N.Y.Acad.Sci.* 903, 293-298 (2000).
58. Arboleda-Velasquez,JF, Lopera,F, Lopez,E, Frosch,MP, Sepulveda-Falla,D, Gutierrez,JE, Vargas,S, Medina,M, Martinez De,AC, Lebo,RV, Slaugenhaupt,SA, Betensky,RA, Villegas,A, Arcos-Burgos,M, Rivera,D, Restrepo,JC, and Kosik,KS C455R notch3 mutation in a Colombian CADASIL kindred with early onset of stroke. *Neurology* 59, 277-279 (2002).
59. Lee,YC, Liu,CS, Chang,MH, Lin,KP, Fuh,JL, Lu,YC, Liu,YF, and Soong,BW Population-specific spectrum of NOTCH3 mutations, MRI features and founder effect of CADASIL in Chinese. *J.Neurol.* 256, 249-255 (2009).
60. Mosca,L, Marazzi,R, Ciccone,A, Santilli,I, Bersano,A, Sansone,V, Grosso,E, Mandrile,G, Giachino,DF, Adobbati,L, Corengia,E, Agostoni,E, Fiumani,A, Gallone,S, Scarpini,E, Guidotti,M, Sterzi,R, Ajmone,C, Marocchi,A, and Penco,S NOTCH3 gene mutations in subjects clinically suspected of CADASIL. *J.Neurol.Sci.* (2011).
61. Bianchi,S, Scali,O, Dotti,MT, Pantoni,L, Parnetti,L, Inzitari,D, and Federico,A Gene symbol: NOTCH3. Disease: CADASIL. *Hum.Genet.* 118, 535 (2005).
62. Mazzei,R, Conforti,FL, Ungaro,C, Liguori,M, Sprovieri,T, Patitucci,A, Magariello,A, Gabriele,AL, Muglia,M, and Quattrone,A Gene symbol: NOTCH3. *Hum.Genet.* 121, 295 (2007).
63. Bianchi,S, Dotti,MT, Perretti,A, De,RA, Manganelli,F, and Federico,A Novel human pathological mutations. Gene symbol: NOTCH3. Disease: CADASIL. *Hum.Genet.* 122, 558 (2007).
64. Ferreira,S, Silva,RS, and Oliveira,JP Novel human pathological mutations. Gene symbol: NOTCH3. Disease: cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *Hum.Genet.* 121, 649 (2007).
65. Mazzei,R, Conforti,FL, Ungaro,C, Liguori,M, Magariello,A, Gabriele,AL, Patitucci,A, Sprovieri,T, Muglia,M, and Quattrone,A Gene symbol: NOTCH3. *Hum.Genet.* 121, 296 (2007).
66. Roy,B, Maksemous,N, Smith,RA, Menon,S, Davies,G, and Griffiths,LR Two novel mutations and a previously unreported intronic polymorphism in the NOTCH3 gene. *Mutat.Res.* 732, 3-8 (2012).
67. Kim,Y, Kim,JS, Kim,G, No,YJ, and Yoo,HW Two novel mutations of the NOTCH3 gene in Korean patients with CADASIL. *Mutat.Res.* 593, 116-120 (2006).

68. Praline,J, Limousin,N, Vourc'h,P, Pallix,M, Debiais,S, Guennoc,AM, Andres,CR, and Corcia,P CADASIL and ALS: a link? *Amyotroph.Lateral.Scler.* 11, 399-401 (2010).
69. Bohlega,S Novel mutation of the notch3 gene in arabic family with CADASIL. *Neurol.Int.* 3, e6 (2011).
70. Pradotto,L, Orsi,L, Daniele,D, Caroppo,P, Lauro,D, Milesi,A, Sellitti,L, and Mauro,A A new NOTCH3 mutation presenting as primary intracerebral haemorrhage. *J.Neurol.Sci.* 315, 143-145 (2012).
71. Rutten,JW, Boon,EM, Liem,MK, Dauwerse,JG, Pont,MJ, Vollebregt,E, Maat-Kievit,AJ, Ginjaar,HB, Lakeman,P, van Duinen,SG, Terwindt,GM, and Lesnik Oberstein,SA Hypomorphic NOTCH3 Alleles Do Not Cause CADASIL in Humans. *Hum.Mutat.* 34, 1486-1489 (2013).
72. Kotorii,S, Goto,H, Kondo,T, Matsuo,H, Takahashi,K, and Shibuya,N [Case of CADASIL showing spontaneous subcortical hemorrhage with a novel mutation of Notch3 gene]. *Rinsho Shinkeigaku* 46, 644-648 (2006).
73. Guerrot,D, Francois,A, Boffa,JJ, Boulos,N, Hanoy,M, Legallicier,B, Triquenot-Bagan,A, Guyant-Marechal,L, Laquerriere,A, Freguin-Bouilland,C, Ronco,P, and Godin,M Nephroangiosclerosis in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: is NOTCH3 mutation the common culprit? *Am.J.Kidney Dis.* 52, 340-345 (2008).
74. Vedeler,C and Bindoff,L A family with atypical CADASIL. *J.Neurol.* (2011).
75. Viana Babtista, M. The spectrum of NOTCH3 mutations in portugese patients with CADASIL: implications for diagnostic strategies. 23 (suppl 2), 75. 2007.
76. Ferreira,S, Costa,C, and Oliveira,JP Novel human pathological mutations. Gene symbol: NOTCH3. Disease: cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *Hum. Genet.* 121, 649 (2007).
77. Pescini,F, Bianchi,S, Salvadori,E, Poggesi,A, Dotti,MT, Federico,A, Inzitari,D, and Pantoni,L A pathogenic mutation on exon 21 of the NOTCH3 gene causing CADASIL in an octogenarian paucisymptomatic patient. *J.Neurol.Sci.* 267, 170-173 (2008).
78. del Rio-Espinola,A, Fernandez-Cadenas,I, Mendioroz,M, Gutierrez-Agullo,M, Fernandez,MT, Fernandez-Morales,J, Maisterra,O, Domingues-Montanari,S, Garcia-Patos,V, Sole,E, and Montaner,J Novel human pathological mutations. Gene symbol: NOTCH3. Disease: CADASIL. *Hum.Genet.* 127, 474 (2010).
79. Rinnoci,V, Nannucci,S, Valenti,R, Donnini,I, Bianchi,S, Pescini,F, Dotti,MT, Federico,A, Inzitari,D, and Pantoni,L Cerebral hemorrhages in CADASIL: report of four cases and a brief review. *J.Neurol.Sci.* 330, 45-51 (2013).
80. Valenti,R, Bianchi,S, Pescini,F, D'Eramo,C, Inzitari,D, Dotti,MT, and Pantoni,L First report of a pathogenic mutation on exon 24 of the NOTCH3 gene in a CADASIL family. *J.Neurol.* (2011).
81. Schmidt,H, Zeginigg,M, Wiltgen,M, Freudenberger,P, Petrovic,K, Cavalieri,M, Gider,P, Enzinger,C, Fornage,M, Debette,S, Rotter,JI, Ikram,MA, Launer,LJ, and Schmidt,R Genetic variants of the NOTCH3 gene in the elderly and magnetic resonance imaging correlates of age-related cerebral small vessel disease. *Brain* 134, 3384-3397 (2011).
82. Ross,OA, Soto-Ortolaza,AI, Heckman,MG, Verbeeck,C, Serie,DJ, Rayaprolu,S, Rich,SS, Nalls,MA, Singleton,A, Guerreiro,R, Kinsella,E, Wszolek,ZK, Brott,TG, Brown,RD, Jr., Worrall,BB, and Meschia,JF NOTCH3 Variants and Risk of Ischemic Stroke. *PLoS.One.* 8, e75035 (2013).