Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL) is the most common hereditary subcortical vascular dementia. It is caused by the defective NOTCH3 gene, which encodes a transmembrane receptor; over 170 different mutations are known. The main clinical features are migraine with aura (often atypical or isolated), strokes, cognitive decline/dementia and psychiatric symptoms. Executive and organizing cognitive functions are impaired first, memory is affected late. Typical MRI findings are T2 weighted hyperintensities in temporopolar white matter and the capsula externa. Smooth muscle cells in small arteries throughout the body degenerate and vessel walls become fibrotic. In the brain, this results in circulatory disturbances and lacunar infarcts, mainly in cerebral white matter and deep gray matter. The exact pathogenesis is still open: a dominant-negative toxic effect is suggested, possibly related to Notch3 misfolding. Diagnosis is reached either by identifying a pathogenic NOTCH3 mutation or by electron microscopic demonstration of granular osmiophilic material in a (skin) biopsy. Only symptomatic treatment is available at present.
NOTCH3 gene encoding a 280 kDa transmembrane receptor [1, Suppl. table in 2]. The mutations lead to progressive vascular smooth muscle cell (VSMC) degeneration, thickening and fibrosis of the vessel walls and accumulation of the Notch3 extracellular domain (N3ECD) on the VSMCs. The four most common characteristic clinical symptoms of CADASIL are migraine with aura, recurrent ischemic attacks, cognitive decline and psychiatric symptoms. Only symptomatic therapy is available and thus, as a progressive disease, CADASIL leads to severe dementia and finally to death, commonly within 20-25 years after symptoms have occurred.

- **Historical Annotation**

The first CADASIL family was most likely reported by van Bogaert et al. in 1955 as a familial form of Binswanger's disease.[3] This honor was long ascribed to Sourander and Wålinder, who in 1977 described an autosomal dominant 'hereditary multi-infarct dementia' with onset around 30-40 years of age.[4] However, this family was proven to have another hereditary vascular dementia since no NOTCH3 mutation, or deposition of CADASIL pathognomonic granular osmiophilic material (GOM) or N3ECD in arterial walls was detected.[5]

The linkage of the defective gene to chromosome 19 was established in 1993 by Tournier-Lasserve et al. who, at the same time, gave the disease its present lengthy but descriptive name, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[6] In 1996, the defective gene was reported to be NOTCH3 at chr19p13 [1] and the next year a detailed description of the stereotypic mutations (see below) in CADASIL was published.[7]

- **Epidemiology**

  - **Incidence & Prevalence**

    CADASIL has been reported worldwide in all ethnic groups. With better knowledge of its clinical picture and improved diagnostic examinations, the number of CADASIL families is steadily increasing. The greatest number of families have so far been identified among European Caucasians, while in the USA and Canada, the number of reported cases has been surprisingly low considering the size of their populations, the high standard of neurology and the high publishing activity in those countries. Exact incidence and prevalence figures are not available. In the UK, prevalence has been estimated to be at least one per 100,000.[8] In the west of Scotland, the prevalence of CADASIL with definite diagnosis is 1.98, and if patients with predicted diagnosis are included it is 4.14 per 100,000.[9] These figures are identical to those in Finland where the prevalence of molecular genetically verified patients is approximately two, and when based on family history it is four per 100,000 [Own Unpublished Observation]. One of nine (11%) patients aged 50 years or below with lacunar infarcts and leukoaraiosis suffered from CADASIL, whereas among patients aged 65 years and below the frequency was one of 48 patients (2%; with estimated 90% sensitivity of the genetic analysis, based on screening for mutations in exons 3, 4, 5 and 6 by PCR followed by analysis for single-stranded conformational polymorphism).[10,11] However, definite prevalence figures must await analyses on large enough populations.

- **Clinical Features**

  Females and males are equally affected. The four cardinal manifestations of CADASIL are:

  1. Migraine with aura (aura being often atypical or isolated)
  2. Ischemic attacks (transient or strokes)
  3. Cognitive decline and dementia
  4. Psychiatric symptoms

The age of onset of CADASIL varies greatly, which also depends on the criterion used for the onset of the disease. Since migraine (see below) is as common as an independent disease, the age of onset is usually given on the basis of the first ischemic attack. However, it is difficult to distinguish between a transient ischemic attack and isolated migrainous aura (without headache), which may be very severe and long lasting in CADASIL. The age at the first ever stroke varies from approximately 25 to 70 years with a reported peak around 40-50 years of age and with great variation even within the same family,[12-14] and even between monozygotic twins.[15] Thus, in general, the site of NOTCH3 mutation does not seem to influence the onset and course of the disease,[16] but a lower age of onset has been reported
to associate with mutations p.Cys174>Tyr [17] and p.Cys455>Arg.[18] CADASIL is usually slowly progressive and it leads to death within a mean of 23 years (range: 3 to 43 years).[13] A few late-onset patients survived to old age, the eldest known CADASIL patient being a Finnish female, who died at the age of 96 years after a 28-year-long course [S Tuisku, Pers. Comm].

- **Migraine**

Recurrent migraineous headache is a common symptom, it occurs in up to 60% of CADASIL patients and in 22-40% of the patients associated with aura. Migraine attacks may begin even before the age of 10 years, but more commonly during the third or fourth decade.[12,13,19] The aura is commonly a visual or sensory disturbance, often atypical and long lasting and exceptionally severe, causing even hemiplegia, in which case hemiplegic migraine is a differential diagnostic possibility.[20] Because migraine is so common it may be overlooked as a manifestation of CADASIL. The severity of migraine may become milder, less frequent or even cease after the first ischemic attack.[13] On the other hand, in females, the first migraneous attack often occurs or the attacks are aggravated in late pregnancy or during puerperium, often associated with other neurological symptoms.[21]

- **Ischemic Attacks**

The most common manifestation (85%) in symptomatic CADASIL patients is recurrent ischemic insults of variable severity.[12-14] The first insults are often transient ischemic attacks with rapid recovery, which are difficult to distinguish from severe migraineous aura or episodes of hemiplegic migraine unless ischemia can be verified by MRI. Most commonly the strokes are typical lacunar syndromes, such as pure motor or pure sensory strokes, dysarthria clumsy hand syndrome, expressive dysphasia or visual field defects, whereas major strokes due to large infarcts in the territories of the main cerebral arteries are very rare.[22,23] With progression, difficulties in locomotion and movement disorders (including vascular Parkinsonism due to infarcts in basal ganglia), pseudobulbar paresis and urinary incontinence may appear. Finally, patients lose their ambulation. Strokes may also be caused by infarcts in the brain stem and rarely in the spinal cord. In 5-20% of patients, subcortical dementia develops without diagnosed clinical strokes[24, 25, Own Unpublished Data], although silent infarcts may be detected in MRI in the white matter (WM) or basal ganglia, where the symptomatic infarcts are also located (Figure 1).

![Figure 1. The lacunar infarcts (arrow) are located either in the cerebral white matter or in the basal ganglia, whereas the cortex is well preserved. Reproduced with permission from [14].](image)

Major parenchymal brain hemorrhages (PBHs) are relatively uncommon in CADASIL (although in a small Korean cohort of 20 patients as many as 25% were struck by a PBH[26]), and antithrombotic therapy and hypertension appear to be contributory factors.[26-28] Small microbleeds (see below section, Imaging) are considered to indicate an increased risk of PBH, although these themselves are most often asymptomatic.

- **Cognitive Decline & Dementia**
When the tissue damage is of sufficient severity, cognitive decline ensues affecting predominantly frontal lobe functions in particular causing executive dysfunctions. The decline is detectable in neuropsychological tests as early as the prestroke phase of the disease.[29,30] Patients have impaired executive and organizing functions, general mental and psychomotor slowing, poor concentration and narrowing of the field of interest, whereas they perform relatively well in the routine Mini-Mental State Examination. Later on, memory and other cognitive functions are affected, leading to a subcortical type of vascular dementia.[29,31,32] Cognitive decline becomes clinically manifest between 40 and 70 years of age and approximately 80% of the CADASIL patients have become demented by 65 years of age.[13] The MRI load of lacunar infarcts is independently associated with the severity of cognitive dysfunction, whereas WM hyperintensity lesion load and microbleeds do not associate after correcting for age in cross-sectional studies.[33,34]

- **Psychiatric Manifestations**

Mood disturbances are CADASIL patients' most common psychiatric disorder (present in ~20-30%).[12,13,28] As expected, depression is the most common disturbance, especially in those patients who have confronted the disease in their family and have a preserved insight of their disease. Manic episodes are rare. Psychotic disorders were common in a large French family, wherefore they were considered to represent a variant phenotype of CADASIL.[35] A single CADASIL patient with schizophrenia has been reported.[36] In addition, paranoia, agitation and aggression, dysthymia and emotional lability have been described.[37,38] However, their relationship to CADASIL has not been established.

- **Additional Clinical Findings**

Routine clinical laboratory examinations are nearly always noncontributory.

Epileptic seizures occur in approximately 7-10% of the patients. They occur most often at the later stages of the disease, but only rarely does CADASIL present with epileptic seizures.[13,16,25,39] Nonconvulsive status epilepticus with focal neurological deficit has also been reported, which may be misinterpreted as an ischemic stroke.[40] An acute reversible encephalopathy ('CADASIL coma': confusion, fever, headache and fits) that poses a risk of misdiagnosis of encephalitis has been reported to be an underdiagnosed presentation of CADASIL.[41] Sensory motor neuropathy has been detected both electrophysiologically and by nerve biopsy.[42] Bothersome urge incontinence has been observed in many CADASIL patients [Kalimo H, Unpublished Results].

CADASIL is a generalized arteriopathy even though the symptoms are almost exclusively neurological and the ischemic lesions are in the CNS. Thus, myocardial ischemia could be expected to be common in CADASIL patients. The reports are contradictory. Lesnik Oberstein et al. found that almost 25% (10 out of 41) of NOTCH3 mutation carriers had evidence of myocardial infarction.[43] On the contrary, Cumurciuc et al. found no ECG evidence for myocardial infarction or ischemia, conduction disturbances or arrhythmias in CADASIL patients compared with healthy controls.[44] In the latest study, Rufa et al. presented data consistent with autonomic nervous derangement. They suggested that CADASIL patients may be at risk for life-threatening arrhythmias, which might be one explanation for their higher risk of sudden unexpected death.[45]

In a recent meta-analysis, CADASIL patients were reported to also suffer from neuromuscular symptoms, weakness, wasting, reduced/exaggerated tendon reflexes, abnormal nerve conduction and electromyography. Their muscle biopsies had revealed alterations indicating mitochondrial pathology: ragged red muscle fibers, reduced COX staining, decreased complex I respiratory chain activity, abnormally structured mitochondria or mitochondrial DNA (mtDNA) mutations.[46]

In conclusion, most of these additional clinical findings are not specific and, thus, not of diagnostic significance in CADASIL, but should be considered in the care of CADASIL patients.[47]

- **Risk Factors**

The occurrence of cardiovascular risk factors is similar to that of the general population. Hypertension is uncommon, which differs from other cerebrovascular diseases.[12,16,25] Smoking may increase the risk for stroke in CADASIL patients. In some, but not all, patient cohorts hypercholesterolemia has been common. CADASIL patients may be more susceptible to their harmful effects because of lesser cardiovascular reserve.[48-50]

- **Imaging**
Hyperintensities on T2-weighted (T2w) MRI in temporopolar WM, periventricular cerebral WM (Figures 2A & 2B) and capsula externa are characteristic early findings in CADASIL,[51] and at advanced stages virtually all cerebral WM is involved (Figure 2C). These WM findings are comparable with the MRI diagnosis of leukoaraiosis and may be deceptively reminiscent of multiple sclerosis. Diffusion tensor MRI reveals a marked increase in water diffusivity, and the diffusion can occur more freely in any direction indicating loss of anisotropy.[52] This reflects microstructural change with enlargement of the extracellular space due to vasogenic edema, possibly associated with myelin and axonal damage, an interpretation well compatible with the microscopic picture in CADASIL WM. These alterations are also detectable in the normal appearing WM outside the T2w hyperintensities indicating an incipient microstructural abnormality.

Figure 2. MRI findings in cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy.

(A) Hyperintensities in anterior temporal lobes in T2-weighted (T2w) MRI are characteristic early alterations in CADASIL. A 29-year-old female at the time of her first transient ischemic attack. (B) Another typical T2w MRI alteration is punctiform periventricular hyperintensities. A subjectively healthy 40-year-old male. (C) Advanced stage of dementia with extensive white matter hyperintensities. A 54-year-old male. (D and E) In fluid-attenuated inversion recovery MRI, lacunar infarcts are visible both in the white matter and in basal ganglia (arrows). (D) A 57-year-old female. (E) A 54-year-old male. Asterisk in D denotes frontal horn of the lateral ventricle.

In symptomatic CADASIL patients who have experienced strokes (in ~10-15% of cases infarcts are silent), a variable number of small (lacunar) infarcts can be identified in T1w and fluid attenuation inversion recovery (FLAIR) MRI (Figures 2D & 2E) and computerized tomography (CT). The infarcts are most commonly located in cerebral WM and
Small hypointensities in T2w gradient echo MR corresponding to microbleeds are detectable in 31-69% of the CADASIL patients. Microbleeds show a preference for cortical-subcortical regions, subcortical WM, thalamus and brainstem. Their frequency increases with patients' age and volume of T2w lesions.[53,54] They are more common in patients with antiaggregant therapy.[53] The hypointensities are due to perivascular accumulations of hemosiderin-containing macrophages, indicating focal extravasation of red blood cells. Microbleeds also occur in other small-vessel diseases of the CNS, such as arteriolosclerosis and amyloid angiopathy. The significance of microbleeds for the pathogenesis of CADASIL is still uncertain, since they are clinically silent and most common in the relatively spared cerebral cortex, 82% being outside the T2w hyperintensities or ischemic lesions. Thus, microbleeds were considered to be largely an independent manifestation of the underlying angiopathy.[54] Neither does the number of microbleeds associate with the severity of cognitive dysfunction.[33] Nevertheless, they are considered to predict an increased risk of PBH.[53,54]

Conventional cerebral angiography is non-contributory, and it appears to carry a considerably increased risk of complications, most often hemorrhages.[55]

- **Circulatory Disturbances in CADASIL**

Reduced cerebral blood flow (CBF) has been demonstrated in CADASIL with several functional imaging methods, such as single photon emission CT (SPECT),[24] PET,[56,57] MRI bolus tracking method[58,59] and Doppler sonography.[58] PET examination has shown that the reduction of CBF in the WM already appears at presymptomatic stage and becomes more clear beyond 30 years of age, at which age first strokes may also appear (Figures 3A & 3B). In the cerebral cortex, CBF is not reduced until later symptomatic stages, and even then only to a lesser degree in concordance with the paucity of cortical infarctions. In a presymptomatic subject, the oxygen extraction fraction (OEF) was increased, indicating that the brain tissue could compensate for the decreased CBF by increasing the OEF. On the other hand, not until later stages parallel to the development of dementia were cerebral metabolic rate of oxygen (CMRO2)[56] and glucose consumption (CMRgluc)[57] reduced, indicating that the decrease in CBF is due to the arteriopathy not secondary to tissue loss. With the MRI bolus tracking method, decreased CBF and cerebral blood volume (CBV) were recorded within areas of T2w hyperintensities in the WM, where reductions were more severe in demented than in nondemented patients. A trend of reduced CBV was already observed within the normal-appearing WM.[58] Furthermore, the hemodynamic reserve is reduced in CADASIL patients, since the acetazolamide-induced increase in CBF and CBV was lower in T2w hyperintense areas.[58] Prolonged arteriovenous cerebral transit time in both disabled and nondisabled CADASIL patients has been demonstrated by Doppler sonography.[60]

Since CADASIL is a generalized arteriopathy, derangement of vasoregulation in the systemic vasculature is not surprising. Stenborg et al. showed impaired endothelium-dependent vasodilation in resistance arteries in the forearm of CADASIL patients, but not in a large conduit artery, a. brachialis, which resulted in reductions in both basal and stimulated blood flow.[61] Similarly, transgenic mice expressing mutant NOTCH3 showed early impairment in flow-mediated vasodilatation in resistance vessels, although extrapolation of mouse results to humans requires caution (see below section, Animal models).[62]
Pathology

Biopsy Findings

Although the symptoms of CADASIL are almost exclusively neurological, blood vessels (mainly medium sized and small arteries) throughout the body are affected (Figures 4-6). A pathognomonic feature of CADASIL is the presence of GOM (Figures 4, 5A & 5B) detectable with electron microscope (EM) on the VSMCs in the walls of the affected vessels. GOM was originally demonstrated by Baudrimont et al. and Ruchoux et al. [63,64] and it has not been detected in any other disease. GOM is located either in indentations on the degenerating VSMCs or free between these cells, the basal lamina of which is usually irregularly thickened (Figures 4, 5A & 5B). Both accumulation of GOM and degeneration of VSMCs are detectable already before 20 years of age (Figure 5B).[65,66] The exact composition of GOM is not yet clear, but on the basis of immunoelectron microscopy N3ECD has been suggested to be a major component of GOM.[67] By light microscopic immunohistochemistry with antibodies against N3ECD, introduced by Joutel et al. , the vessel walls stain diffusely positively (Figure 6A).[68] With confocal microscopy N3ECD immunoreactivity appears as dot-like accumulations on the surface of VSMCs, in concordance with the appearance of GOM deposits in EM (Figure 6B).

Figure 4. Electron micrograph of a dermal artery from a patient at an early stage of the disease. A 28-year old male with p.Arg133Cys mutation. Note the widened subendothelial spaces (asterisks) and irregular smooth muscle cells (M) on which there are three deposits of granular osmiophilic material (arrows). E: Endothelial cell.
Figure 5. Electron micrograph showing presence of granular osmiophilic material. (A) In a 48-year-old patient who had already suffered from a couple of strokes relatively abundant granular osmiophilic material (GOM; asterisks) is present in the indentations of two vascular smooth muscle cells. (B) A small amount of GOM is already present in a 19-year-old patient carrying the p.Arg133Cys mutation. GOM is located in a deep invagination, which might be difficult to detect by immunohistochemistry.

Figure 6. Since CADASIL is a generalized arteriopathy, its characteristic pathological alterations are also visible in skin biopsy. (A) A 57-year-old female with p.Arg133Cys mutation. N3ECD is present in the wall of the small dermal arteries (asterisks). (B) A 62-year-old male with p.Tyr1069Cys mutation. Confocal microscopy demonstrates N3ECD immunoreactivity as small dots along the vessel wall, a finding compatible with the deposits of GOM (see Figures 4 & 5).

- **Autopsy Findings**

Macroscopy. In accordance with the imaging findings, there are multiple small (lacunar) infarcts in the WM and/or deep GM, whereas the cerebral cortex is markedly well preserved (Figure 1). Lacunar infarcts are also relatively common in the brain stem. Even though the microbleeds are fairly common (see above section, Imaging), parenchymal brain hemorrhages are relatively uncommon. These have most often occurred in patients treated with anticoagulants or antiaggregants, or subjected to arteriography.

Histopathology. Histological stainings reveal markedly thickened small arteries in WM with accumulation of granular material in the degenerating tunica media. This material is basophilic in hematoxylin and eosin and red in periodic acid-Schiff stainings (Figures 7A-7C). Decreased immunopositivity for α-smooth muscle actin reveals degeneration of
the VSMCs (Figure 7D). The accumulation of N3ECD may be verified by immunohistochemical staining (Figure 7E). It is the accumulation of extracellular matrix proteins, including various types of collagens and laminin outside the degenerating VSMCs, that causes the thickening of the vessel walls (Figure 7F). On the basis of the stainings described above, CADASIL can be distinguished from the two other arteriopathies with thickened walls: in arteriolosclerosis (Binswanger disease) and cerebral amyloid angiopathy, the walls are homogeneously stained, either collagen or similar to amyloid.

![Image of histopathological findings in CADASIL patients' brain](image)

**Figure 7.** Histopathological findings in CADASIL patients' brain. A) A small arteriole from the cerebral white matter of a control person. (B) The wall of a corresponding arteriole from a CADASIL patient is markedly thickened and the lumen stenosed. Note the basophilic granular material in the thick media and adventitia. (C) The granular material in B is PAS-positive. (D) The degeneration of vascular smooth muscle cells (VSMCs) appears either as almost complete loss of a-SMA-stainable VSMCs (left arteriole) or their irregular pattern (right arteriole). (E) N3ECD accumulates in the tunica media of affected arterioles. (F) The degenerated VSMCs have been replaced by abundant collagen I.

SMA: a-smooth muscle actin; N3ECD: Notch3 extracellular domain; PAS: Periodic acid-Schiff.

The marked thickening of the walls of small penetrating arteries in cerebral WM is reflected numerically in the highly significant increase of sclerotic index (SI = 1-internal diameter/external diameter; 0.75 vs 0.35-0.40 in controls).[69] Within WM, the small arterioles (<130 µm external diameter) are most severely affected, and at the same time they become stenosed with significantly smaller mean internal (luminal) diameters (Figures 8 & 9).[69] Finally, these arterioles must become severely enough obliterated or thrombosed to give rise to lacunar infarcts in cerebral WM (Figures 1 & 8). In the cerebral cortex, the small arteries and arterioles are also thickened (SI: 0.56 vs 0.48 in controls) and their lumina somewhat narrowed (Figure 9), but to a considerably lesser extent than in WM, explaining the absence of cortical infarcts.[69] Corresponding to the presence of the microbleeds in imaging, perivascular deposits of hemosiderin may be found.[54] These are located predominantly in GM, which may simply depend on the fact that the
walls of arterioles in GM are less thickened than in WM and therefore more fragile[69] and prone to extravasation.

Figure 8. Hogressive fibrosis of the affected arterioles in cerebral white matter may lead to complete obliteration of the affected arteriole. Note the loose texture of the surrounding infarcted tissue.

CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy; GM: Grey matter; LN: Lentiform nucleus; n: Numbers of CADASIL patients (elderly with pArg133Cys mutation, mean age: 63.4 ± 2.9 years, range 60-68 years, young with pCys174Arg mutation, age 32 years); n1: Numbers of controls for GM and WM ; n2: Numbers of LN controls; WM: White matter.

Interestingly, the small arteries in the deep gray matter of basal ganglia (nucleus lentiformis) - the other region in the brain, where lacunar infarcts occur in CADASIL - behave differently. These vessels are not stenosed (Figure 9) as in WM even though their walls are thickened, suggesting a different, most probably hemodynamic, pathogenesis for the lacunar infarcts in the basal ganglia.[70]

These findings suggest that there is a fundamental difference between the cortical and WM arteries. The response of VSMCs to the gene defect appear to be markedly different, which is also reflected in the magnitude of the fibrotic reaction. Similarly, it was recently shown in NOTCH3 -knockout mice that the contractile activity of VSMCs in the aorta is different from that of cerebral arteries.[71]

Since NOTCH3 is mainly or exclusively expressed in VSMCs, the main interest has been targeted at these cells and their degeneration. Ruchoux and Maurage have performed the most detailed structural analysis of endothelium in skin and skeletal muscle biopsies.[72] They found attenuation of endothelial cells and increased density of their
cytoplasm with accumulation of microfilament bundles. Widening of subendothelial space has also been observed (Figure 4). Interaction between endothelial and vascular smooth muscle cells has been identified as a central process in the regulation of vascular formation, stabilization, remodeling and function.[73] During development, expression of Notch3 signaling appears to be necessary for correct specification of arterial and venous characteristics of the VSMCs in the blood vessel walls.[74] Thus, the pathological findings in CADASIL patients' endothelium may reflect functional disturbances: we have shown impaired endothelium-dependent vasodilation in CADASIL patients' forearm resistance arteries,[61] and in addition, other studies have suggested endothelial dysfunction.[75]

- Genetics

Linkage of the CADASIL gene to chromosome 19 was discovered in 1993;[6] 3 years later the defective gene was identified as NOTCH3 in 19p13.1-13.2.[1] A great majority (well over 95%) of CADASIL cases are due to missense point mutations in N3ECD in exons 2-23 (Figure 10). Over 170 different point mutations have been identified.[2] Virtually all point mutations result in an amino acid substitution involving cysteine, either a replacement of a wild-type cysteine with another amino acid or vice versa, resulting in an uneven number of cysteines (normal number six) in the affected EGF-like repeat in N3ECD. In addition to the point mutations, eight different deletions have been described. These result in a loss of either one or three cysteine residues and, thus, similarly in an uneven number of cysteines, which is also the consequence of one mutation with insertion of an extra cysteine [Suppl. table in 2].

![Notch signaling diagram](image)

**Figure 10.** Notch signaling. Notch3 is constitutively cleaved by furin (S1) and the bipartite molecule is inserted to the plasma membrane. The Notch3 extracellular domain (N3ECD) consists of 34 EGF-like repeats, followed by three notch/lin-12 repeats, a transmembrane domain and an NICD, which contains five ankyrin repeats. The binding site of the ligand (in human delta or jagged) is at EGF repeats 10-11. Upon binding, N3ECD is cleaved at 12 amino acids external to the intramembranous domain (S2). Thereafter occurs the intramembranous S3 cleavage and NICD enters the nucleus to release the repressor molecules CoR and HDAC, which bind to a transcription regulator of the CSL family, RBP-Jk and activate transcription. CADASIL mutations are in EGF repeats 1-32 (exons 2-23) between the asterisks. The non-CADASIL Notch3 mutation is located in exon 25 (triangle). TACE: TNF-a-converting enzyme; CoRHAc and MAML1: Components of an activation complex; NICD: Notch3 intracellular domain.

There is a marked clustering of mutations at the N-terminus of the NOTCH gene: approximately 62% of the reported mutations are located within exons 3, 4, 5 and 8 encoding for the first five EGF repeats [Suppl. table in 2, 6]. In two families, de novo mutations causing p.Arg182>Cys[76] or p.Cys128>Gly[77] substitutions have been verified. One CADASIL patient has been diagnosed to be homozygous for the c.397C>T mutation, which causes a relatively common p.Arg133>Cys substitution.[65] The homozygous patient's phenotype was still within the normal spectrum although relatively severe. Since this patient's heterozygous son appears to have a similar phenotype CADASIL appears to follow the classic definition of dominant pattern of inheritance, in other words, the phenotypes of the heterozygous and homozygous patients are indistinguishable. Finally, seven CADASIL patients/families with a noncysteine mutation have been reported.[2,78,79] Interestingly, a noncysteine mutation in exon 25 (p.Leu1515>Pro; Figure 10) of NOTCH3 was recently shown to cause a non-CADASIL small-vessel disease without accumulation of
N3ECD or GOM on the VSMCs. This mutation causes constitutively active Notch3 signaling exceeding the wild-type activity approximately tenfold.[80]

- **Notch3 Biochemistry**

Notch3 is a member of the Notch family, which in mammals has four members (Notch1-4). Notch signaling is very important during development and, accordingly, Notch molecules are highly conserved during evolution. Orthologous genes with a high degree of homology exist from nematodes to man.[81] The Notch pathway is important, for example in stem cell renewal, proliferation, determination of cell fate and differentiation and apoptosis during organogenesis, including vasculogenesis.[81-84]

- **Structure**

The human NOTCH3 gene has 33 exons and encodes for a 2321 amino acid Notch3 receptor with a single transmembrane domain (Figure 10).[1,85] The N-terminal extracellular part of the molecule contains 34 EGF-like repeats followed by three notch/lin-12 repeats. The recognition site of the ligand is at EGF repeats 10 and 11. Each EGF repeat contains six cysteines, which form sulphur bridges when the protein domain is folded. The intracellular domain on the Notch3 contains RBP-J?-associated molecule region in the juxtamembrane part, five ankyrin repeats and a C-terminal PEST-motif.

- **Ligands**

Delta-like family (DLL1, 3 and 4) and Serrate family Jagged (JAG1 and JAG2) are the ligands for Notch receptors. [86-88] The molecular structure of Delta and Jagged is very similar to that of Notch. They are transmembrane proteins with large extracellular domains containing several EGF-repeats. VSMCs express both the ligand and the receptor on the cell surface but the signal transduction seems to occur only between neighboring cells.[89,90]

- **Signaling**

The molecular mechanism of receptor maturation, ligand binding and transcription activation are presumed to be similar for all Notch receptors. Notch receptors are cleaved three times during maturation and signaling (Figure 10). First, Notch is translated as a single polypeptide chain and cleaved by a furin-like convertase in the Golgi (S1 cleavage). The two cleavage products are held together by metal ions (Ca2+) generating a bipartite molecule that is inserted into the plasma membrane.[91] The ligand binding induces the second proteolytic cleavage by TNF-a-converting enzyme (TACE, ADAM-17) at the site S2 located 12 amino acids external to the intramembranous domain. [92] N3ECD is released and transcendocytosed with the ligand to the ligand-expressing cell (Figure 11, item D). Finally, the Notch transmembrane-intracellular domain is cleaved (S3) within the plasma membrane by ?-secretase, [93] which is a complex of presenilin1, nicastrin, Aph-1 and Pen-2.[94] This same enzyme cleaves ?-amyloid precursor protein, participating in the production of the pathogenic ?-amyloid peptide in Alzheimer's disease. S3-cleavage releases the Notch3 intracellular domain (NICD), which then enters the nucleus and binds a DNA-binding CSL family of transcriptional regulators (CBF1, Suppressor of Hairless and Lag-1, and CBF1/RBP-J? in mammals).[81,82] The NICD replaces co-repressors from the CSL and forms a transcription-activating complex in which it further recruits co-activator proteins (Figure 10). Exactly how Notch3 regulates the development and differentiation of VSMCs is unknown. Neither has the exact function of Notch3 been clarified in adult animals, in which Notch3 appears to be exclusively expressed in VSMCs,[95,96] which may have different molecular phenotypic and physiological characteristics in different vascular beds.[69,71] Interestingly, in a recent article the ischemic lesions induced by occlusion of the proximal middle cerebral artery by a filament were reported to be approximately two-times larger in homozygous NOTCH3 -knockout mice than in wild-type or heterozygous NOTCH3 +/- mice. This indicates a striking susceptibility to ischemic stroke, which was normalized if wild-type human NOTCH3 was conditionally expressed in the NOTCH3 +/- mice. Thus, it was suggested that Notch3 defines a key determinant of stroke burden through regulation of VSMC function.[71]
Figure 11. Possible (proven and hypothetical) pathogenic effects in association with mutated (misfolded?) Notch3. NICD: Notch3 intracellular domain. Notch Signaling is Regulated by Endocytosis

In experiments using Drosophila or mouse skeletal muscle myoblasts, it has been shown that the ligand-N3ECD complex is internalized to the signal-donor cell.[89,90] After the first interaction between Notch and ligand, both the signal-donor and signal-receiving cell will continue the signal activation cascade by clustering new receptors on the signal-receiving cell and new/recycled ligands on the signal-donor cell surface.[97,98] It is not definitely clarified whether the ligand is first internalized to enter a special endocytic pathway for conversion from inactive into active form to be then recycled onto the signal-donor cell in the activated form, or whether initial interaction between the ligand and N3ECD is needed for the ligand recycling cascade to begin.[97,98] As for the role of endocytosis in the pathogenesis of CADASIL, see the below section: Internalization of the N3ECD.

- Pathogenesis
  - Molecular Pathogenesis

The exact molecular pathogenetic mechanism causing CADASIL is unknown. It has been suggested that proteolytic processing and targeting of Notch3 receptor, signaling or ligand binding and internalization of the cleaved N3ECD could be affected.[28,95,99]

Notch3 Processing & Targeting. Although alternative proteolytic processing has been suggested to be a possible cause of CADASIL, no definite proof has been presented.[100,101] It seems clear that in CADASIL the mutated Notch3...
Signaling & Ligand Binding. Some CADASIL cases are caused by mutations that lead to the loss of ligand binding on Notch3 (Figure 11). The clinical outcome is, however, identical to those with the mutation outside of the ligand-binding site. Thus, it would be difficult to explain that loss of function would be the cause of CADASIL. Several pieces of evidence oppose the loss of function of Notch3, for example, Joutel et al. [100] and Peters et al. [101] demonstrated that only specific mutations located in the ligand-binding site in EGF repeats 10-11 (p.Cys428>Ser and p.Cys455>Arg) exhibited a significant reduction of transcriptional activity via the RBP/Jk pathway (Figures 10 & 11) while all other mutations tested were shown to have close to normal signaling activity. Furthermore, Notch3-deficient mice do not develop CADASIL pathognomonic GOM deposits.[102] Physiological levels of wild-type human Notch3 and the mutant pArg90>Cys human Notch3 rescued the arterial defects of NOTCH3 -/- mice to similar degrees and exhibit normal levels of Notch3/RBP-Jk activity in brain arteries.[103] And finally, the similar phenotype in the homozygous and heterozygous CADASIL patients also favors a mechanism other than loss of receptor function[65] as does the lack of truncating mutations in CADASIL.[101]

Evidence against gain of Notch3 receptor function is based on the same experiments as for loss of function, in particular, cells expressing mutated Notch3 show normal signaling activity.[100,101] However, it was pointed out that the experiments were limited to the major Notch signaling pathway, and CADASIL mutations may affect other Notch signaling pathways that have been shown to exist.[101,104-106] Gain of Notch3 function has been reported in a patient with cerebral small-vessel disease lacking GOM deposits and Notch3 accumulation. This mutation is located in the juxtamembranous region of Notch3, which constitutes the heterodimerization domain (Figure 10). The mutation destabilizes the metal ion bridge leading to a ligand-independent, constitutively-active receptor.[80] Thus, it seems that gain of function leads to a cerebral small-vessel disease different from CADASIL.

Internalization of the N3ECD. It has been shown that in CADASIL, the accumulated Notch3 contains only its ECD. [95] This accumulation of N3ECD on the surface of VSMCs has been considered pivotal in CADASIL, although the reason for the accumulation has remained unclear. Joutel et al. found almost identical amounts of N3ECD in CADASIL patients regardless of whether their mutations were located at or outside the binding site, indicating that Jagged1 binding is not a prerequisite for N3ECD accumulation.[100] Normally, N3ECD would be transendocytosed with the ligand to the signal-donor cell (Figure 11).[89] The ligand ECD bound to the Notch ECD as a trans-complex is assumed to place mechanical strain on the Notch3 protein, which exposes the cleavage site to be targeted by TACE for S2 cleavage.[98] Inefficient ligand binding could lead to weaker mechanical strain, and inhibition of S2 cleavage and subsequent internalization of N3ECD.[97]

Misfolding. Since practically all the CADASIL mutations lead to an uneven number of cysteine residues in the mutated EGF repeat, normal formation of sulphur bridges does not occur and the Notch3 receptor 3D structure is altered. This may cause secondary modifications in Notch3 processing, such as impaired proteolytic S1 cleavage by furin or O-linked glycosylation by Fringe and aberrant dimerization of Notch3, which could all contribute to the accumulation of N3ECD on the VSMC surface.[107] Usually a cell can determine the ability of a protein to form disulphide bridges at the endoplasmic reticulum (ER). If for any reason the protein cannot form the predefined number of disulphur bridges, the protein is directed to ubiquitylation and subsequent proteosomal degradation.[108] The mutated Notch3 seems to escape this degradation and is transported to the cell surface as a misfolded protein. [95,101,107-109] CADASIL cells are oxidatively stressed and have upregulated proteasomal degradation, which are typical signs for impaired sulphur bridge formation, misfolding of proteins and ER quality control failure.[110] These problems in the protein folding and degradation can lead to degeneration of VSMCs. Misfolding of the receptor does not seem to influence the ligand binding or the CSL/RBP-Jk-signaling if the mutation is not on the ligand-binding site.[100,101] Thus, inactivation of the canonical Notch3 signaling is not necessary for CADASIL. The only feature common to all CADASIL mutations is the accumulation of N3ECD on the VSMC surface. In the transgenic mice expressing mutated (p.Arg90>Cys) human Notch3, Monet et al. reported normal Notch3 function in vivo (mice) with no dominant-negative interfering activity despite accumulation of mutated N3ECD on the VSMCs as in CADASIL.[103] Thus, they suggested that the mutated Notch3 does not have such a dominant negative 'sop up' effect as suggested by Spinner (Figure 11).[111] Whether the accumulation in mice is comparable to that in the decades-long human CADASIL is an open question. In humans, N3ECD accumulation may become excessive and pathogenic with age and gradually lead to disturbances in the cell-cell and cell-extracellular matrix interactions, including ligand binding.[111] But all in all, we do not yet know whether the accumulated N3ECD is really pathogenic or is just a marker for ongoing cellular damage.
Animal Models

Functional Pathogenesis

CADASIL leads to arteriopathy causing blood flow disturbances, especially in the resistant arteries.[61,62] The key proteins in VSMCs contraction are myosin and actin. SMC-specific smooth muscle cell α-actin is a direct target of the canonical Notch3 pathway.[115] The expression of constitutively active Notch3ΔE in cultured VSMCs resulted in increased actin stress fibers and steady-state levels of polymerized actin.[74] In authentic human CADASIL cells, several proteins interacting with the actin cytoskeleton were differentially expressed and the ability of these cells to contract was impaired.[110] These studies suggest that Notch3 is involved in the regulation of the actin cytoskeleton and may act as a sensor or a signal transmitter that enables VSMC to respond to mechanical stretching of the vessel wall.

In a recent article, Notch signaling was linked to PDGF signaling, another key determinant of VSMC biology.[104] It was demonstrated that PDGF-receptor β is a novel immediate Notch target gene. PDGF signaling is important in vascular development and for homeostasis of blood vessels. Four different ligands (A-D) bind to PDGF-receptor tyrosine kinases (PDGFR-a and -β), which activate several intracellular signaling cascades. In particular, PDGFR-β/PDGFR-β signaling has been shown to be important, since defects in this pathway cause vascular abnormalities such as hemorrhage, microaneurysms and VSMC hypoplasia.[116] PDGF released in vascular injury leads to downregulation of VSMC marker genes, increased VSMC proliferation and vascular remodeling.[117] PDGF stimulation inhibits Notch3 expression and signaling and increases Jagged1 expression.[116-119] PDGFR-β was recently demonstrated to be an immediate downstream target gene of Notch3 activation.[104] Notch3-deficient adult mice exhibit vascular abnormalities and have reduced PDGFR-β expression in arteries of the tail lateral bundle. Furthermore, Notch-ligand stimulation of VSMCs resulted in significantly reduced induction of PDGFR-β expression in authentic human CADASIL cells (with mutation p.Arg133Cys) than in control cells.[104] The relevance of PDGFR-β expression by Notch3 in CADASIL is not self evident, but it could explain why mutations in ligand binding and nonligand-binding sites cause a similar disease.

- Functional Pathogenesis

Whatever the pathogenetic mechanism causing the degeneration of VSMCs, is the key functional consequence of impaired blood flow in small cerebral arteries to such an extent that lacunar infarcts ensue (Figure 1). The structural alterations described above must be causally related to such CBF impairment. The destruction of VSMCs leads to secondary fibrosis with marked thickening of the walls of small arteries as well as reduced compliance, and in the WM arterioles also to stenosis of the lumen (Figures 7-9).[69] Consequently, CBF is decreased, as discussed in the above section on Circulatory disturbances. The small penetrating WM arteries may become completely obliterated by the fibrotic process, but most likely the final cause of the lacunar infarcts is thrombosis in the affected small arteries, this view is supported by detection of fibrin degradation products in the plasma of CADASIL patients with recent strokes [Ilveskero, Unpublished Observation]. The fibrosis and stenosis in the penetrating WM arterioles is significantly more severe than in the cortical ones[69] and the lack of collaterals makes the WM more vulnerable. All these factors explain the predominant localization of infarcts in WM and sparing of cortex.

On the other hand, the pathogenesis of common deep GM infarcts in basal ganglia (Figure 1) may have another, possibly hemodynamic mechanism, since despite thickening of the walls such stenosis as in WM arterioles does not occur in nucleus lentiformis arterioles (Figure 9).[70] In fact, hemodynamic perturbations due to rigidity of fibrosed arterioles could also contribute to WM infarcts. Thus, not only could the severe and most probably late-occurring stenosis cause WM infarcts (Figure 8), but also impaired vasodilation of the thick walled WM arterioles result in focal ischemia. Increased blood flow into, and dilatation of, larger arteries necessitates flow-mediated vasodilatation of 'downstream' vessels to avoid 'steal' into high-flow vessels. A partial failure of vasodilatation in WM resistance arterioles with a consequent decrease of blood flow might occur early in CADASIL.[61] The dilatation of the fibrosed vessels is understandably decreased, which has also been verified as the lesser vasodilatory effect of acetazolamide.[58] Furthermore, this phenomenon of flow-mediated vasodilatation is normally greater in the cerebral vasculature than in other vascular beds, increasing the risk of 'steal' of blood flow from one vascular territory to another.[120] The increased cortical and reduced WM blood flow in PET studies of young CADASIL patients could reflect such a 'steal'.[57] And the lack of collaterals makes the subcortical area vulnerable to the 'steal' effect, similar to stenosis/occlusion.

- Animal Models
Two approaches to develop transgenic mouse models of CADASIL have been reported. The French CADASIL team produced transgenic mice, in which SM22a promoter drives humanNOTCH3 carrying p.Arg90>Cys substitution due to c.268C>T mutation. The findings in these experiments were, however, not compared with transgenic mice overexpressing wild-type human Notch3. By the age of 17-20 months the mice overexpressing the mutant Notch3 developed in both cerebral and peripheral arteries similar vascular changes similar to CADASIL patients. Accumulation of N3ECD in arterial walls was verified by immunohistochemistry. Electron microscopy disclosed degeneration of VSMCs and deposition of small GOMs. VSMCs appeared to lose their normal anchorage to adjacent extracellular matrix before the above mentioned deposits appeared. Despite these alterations, no parenchymal damage was seen in the brains of the transgenic mice. In functional testing, the cerebrovascular reactivity was found to be compromised in these animals: CBF autoregulation was shifted towards higher blood pressures. Furthermore, the flow-induced dilatation was reduced and the pressure-induced myogenic tone was increased, which had occurred at the age of 10 months prior to the abovementioned structural changes, indicating early vascular dysfunction. These results are in concordance with vascular dysfunction in CADASIL patients, in whom vasoactivity to acetazolamide and carbon dioxide is impaired as is flow-mediated vasodilatation in upper extremity resistance arteries. Furthermore, retinal arteries in young CADASIL patients are constricted as in hypertension. This mouse model was also used to show that the arterial defects of NOTCH3/- knockout mice can be similarly rescued by expression at comparable and physiological levels of both the wild-type human Notch3 and the mutant p.Arg90>Cys human Notch3 (see above section, Molecular pathogenesis).

In another study, a mouse knock-in model for one of the most prevalent human CADASIL mutations, p.Arg141>Cys, was generated by making the corresponding alteration p.Arg142>Cys in mouse Notch3. These mice, however, did not develop a CADASIL-like phenotype. The mutated mouse Notch3 receptor was normally processed, N3ECD was not accumulated, brain MRI was normal and no abnormal behavior or brain pathology was detected. This failure was suggested to be at least in part due to species difference or the propensity of different mutations to produce a phenotype in mouse, which might not be an ideal experimental animal in which to study late-onset diseases such as CADASIL.

- Diagnosis & Differential Diagnosis in CADASIL

  o Diagnosis

A patient suffering from a cerebrovascular accident at a young age is usually examined by MRI. In CADASIL, the MRI findings (Figures 1A-1D) are characteristic and almost specifically diagnostic (see previous page, Imaging). The yield of positive gene analysis for CADASIL was 11% among patients below 50 years of age who had lacunar infarcts and leukoaraiosis. Thus, it is worthwhile to perform genetic testing in such a population of patients. Migraine with aura alone often does not usually motivate to perform MRI, since migraine/headache is so common. Despite the low probability, CADASIL should be considered, especially in late-onset migraine, if the aura is exceptionally severe or if it begins or is aggravated during late pregnancy or puerperium. Similar symptoms in relatives favor the possibility of CADASIL, even though migraine as such may also be familial.

Positive clinical and MRI findings necessitate either gene testing or electron microscopic/immunohistochemical examination of a skin biopsy. Molecular genetic identification of a pathogenic NOTCH3 mutation gives the definite diagnosis. The great number (>170) of different mutations makes the search for less common mutations relatively cumbersome, even though analyses for the entire pathogenic regions of NOTCH3 gene begin to be available. The most efficient strategy to search for the NOTCH3 mutations depends on the patient's family history and the mutational background in the population of which the suspected patient is a member of. In families with a known mutation, one should of course test directly for that mutation. In populations with known founder or major mutations, the analyses should be directed to those mutations, restriction analysis being usually the most practical method. In populations with no known founder or other prevalent mutations, the mutational hot-spot regions - exons 3, 4, 5 and 8, of the NOTCH3 gene - should be analyzed first and approximately 80% coverage exons 2, 6, 11 and 18 should be included.

Owing to the problems mentioned above in the determination of the NOTCH3 mutation, a rapid and reliable screening test has been called for. The EM analysis for GOM (Figures 3 & 4) seems to provide such a test; it is easy to perform and the presence of GOM in the arterial walls appears to be 100% specific. Besides, we have detected GOM in representative skin biopsies from all CADASIL patients with an identified NOTCH3 mutation, in other words, the sensitivity also appears to be 100%. Thus, the detection of GOM seems to allow definite diagnosis of CADASIL. Since the arteriopathy is generalized, GOM can be detected in many different intra vitam tissue...
biopsies. Most commonly, the biopsy is taken from skin but other neuropathological biopsies, for example, from muscle or peripheral nerve, can also be used.[73,129] Some caution is, however, needed since the biopsy might not have been deep enough (small arteries located in deep dermis or upper subcutis are optimal), in which cases the biopsy needs to be repeated. Besides, true GOM needs to be distinguished from the nonspecific granular debris present between degenerative VSMCs in some other diseases.[2]

Immunohistochemical demonstration of accumulated N3ECD provides another morphological diagnostic method,[68] but an inherent caveat of this technique - false-positive immunoreactivity in other diseases - erodes its specificity.[43] In addition, detection of small amounts of N3ECD at the early stages of the disease may be problematic (Figure 4B). Thus, we highly recommend the electron microscopic analysis of GOM as the morphological diagnostic method.[2]

- **Differential Diagnosis**

All CADASIL cases diagnosed so far have been familial and, thus, the presence of affected relatives lends support for CADASIL. In familial hemiplegic migraine (FHM), migraine is associated with stroke-like symptoms, but the FHM patients usually recover better from the attacks. Genetic tests for the defective genes (e.g., for CACNL1A4 in one type of FHM) or EM analysis should allow correct diagnosis. Stroke may also associate with 'independent' migraine, and migraine has been traditionally included among risk factors for stroke, although this may be true only for young women.[130]

Recurrent embolizations, such as in cardiac arrhythmias or coagulation disturbances, may cause multiple lacunar infarcts and they should be excluded by adequate examinations. WM infarcts are also a feature of subcortical arteriosclerotic encephalopathy (SAE;Binswanger's disease). Hypertension is commonly associated with SAE, whereas CADASIL patients are most often normotensive, but risk factors of stroke, such as hypertension and hypercholesterolemia, also occur in CADASIL patients.[25,43,51] Finally, strokes in young persons may be caused by a mitochondrial encephalopathy, especially mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome, but in these diseases infarcts are usually cortical and located in posterior parts of the brain.[131] Interestingly, the frequency of mutations in mitochondrial DNA appears to be higher in CADASIL patients than in the general population.[132]

- **Therapeutic Possibilities**

The slow and unpredictable progression of CADASIL makes evaluation of therapeutic effects very difficult. At present, only symptomatic therapy is available. Acutely, acetazolamide may be of help to CADASIL patients with excruciating migraine,[133] whereas ergotamines and triptans should not be used in the treatment of the migraneous headache, since they may cause vasospasms that may further impair the already compromised CBF. Since the vascular pathology has been thought to give rise to local thromboses, anticoagulants or antiaggregants have been tried without definite positive effects. In a few patients, these medications may have caused fatal parenchymal brain hemorrhages. Since blood flow is impaired in the diseased arterioles due to progressive stenosis and rigidity, increased blood viscosity, for example, due to polycythemia or dehydration, appears to be harmful and should be avoided [Own Unpublished Observations]. For the same reason, medications causing cerebral hypoperfusion, such as tricyclic antidepressants, neuroleptics and hypotensive drugs, should be administered with caution. Refraining from smoking is recommended since smoking can increase the risk for stroke.[15]

Statins reduce the risk of stroke, especially in patients with coronary artery disease.[134] The role of statins is still unknown in CADASIL,[134] but one half of the patients had hypercholesterolemia and therefore treatment with statins is indicated.

Cognitive decline begins before ischemic attacks. Cholinergic deficits have been found in CADASIL patients in late middle age (>45-50 years)[136-138] and, therefore, cholinesterase inhibitors may alleviate cognitive decline. In a placebo-controlled study with donepezil, executive functions were moderately improved, but the clinical relevance of this treatment is still unknown.[139]

- **Future Perspective**

The pathogenesis of CADASIL is still unsettled. Most CADASIL mutations do not halt canonical Notch3 signaling. Thus, the definite cause(s) of the degeneration of the VSMCs and the subsequent fibrosis of the arterioles appears to be independent from the classical Notch3 signaling cascade and is perhaps caused by a still uncharacterized secondary
signaling pathway of the same receptor. Furthermore, the mechanism by which the blood flow in different parts of the brain is impaired has not been definitively clarified, including the exact role of the endothelial malfunction. Finally, none of the presently tested medications appears to offer effective therapeutic intervention.

Thus, new approaches should focus on the characterization of the still vaguely described alternative receptor targets and on the possible prevention of a dominant negative/toxic effect of Notch3 (e.g., the accumulation of mutated N3ECD on the VSMCs) or compensation for somehow defective signaling. Even though VSMCs of the cerebral arterioles are beyond the blood-brain barrier, their close proximity to the blood flow should nonetheless allow the future drug(s) to reach their target with relative ease.

References


5. Low WC, Junna M, Börjesson-Hanson A et al.: Hereditary multi-infarct dementia of the Swedish type is a novel disorder different from NOTCH 3 causing CADASIL. Brain 130, 357-367 (2007).


110. Ihalainen S, Soliymani R, Ivanainen E et al.: Proteome analysis of cultivated vascular smooth muscle cells from


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**Addendum**

- A new version of topic of the month publication is uploaded in my web site every month (it remains for a month and is changed with the monthly update of the neurology bulletin at: [http://neurology.yassermetwally.com](http://neurology.yassermetwally.com))
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