CADASIL: a Common Form of Hereditary Arteriopathy Causing Brain Infarcts and Dementia

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary cerebrovascular disease leading to cognitive decline and dementia. CADASIL usually begins with migraine in about one third of the patients. More severe manifestations, transient ischemic attacks or recurrent strokes, appear between 30 and 50 years of age. CADASIL, however, may be diagnosed well before the first stroke on the basis of characteristic white matter hyperintensities upon magnetic resonance imaging and presence of pathognomonic granular osmiophilic material in arterial walls, including dermal arteries, since the arteriopathy is generalized. Gradual destruction of vascular smooth muscle cells (VSMC) leads to progressive wall thickening and fibrosis and luminal narrowing in small and medium-sized penetrating arteries. The reduced cerebral blood flow finally causes lacunar infarcts, mainly in the basal ganglia and fronto-temporal white matter, which lead to cognitive deficits and dementia of the subcortical vascular type. CADASIL is caused by single missense mutations or small deletions in *Notch3* gene encoding a transmembrane receptor Notch3, of which upon ligand binding a nuclear signaling protein is generated by regulated intramembrane proteolysis. Notch signaling is essential during development, regulating cellular differentiation. In adults Notch3 is expressed only in VSMCs and it may promote cell survival by inhibiting apoptosis, but its exact function is unknown. Mutations result in either a gain or loss of one (or rarely, 3) cysteine residue(s) in one of the 34 epidermal growth factor-like repeats in the extracellular amino-terminal region of Notch3. It is as yet unclear which disturbance in the Notch signaling pathway leads to the characteristic vascular pathology of CADASIL.

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Historical Aspects

In 1977 Sourander and Wålinder had described an autosomal dominantly inherited disorder characterized by multiple infarcts with onset between 30 and 40 years of age, leading to progressive dementia. They called this "hereditary multi-infarct dementia" (65). However, retrospective analysis of older reports and examination of living patients in those families have revealed that the first CADASIL family was likely described by van Bogaert et al in 1955 as a familial form of Binswanger's disease (73). The family of Sourander and Wålinder appears to present a different hereditary vascular dementia, since neither *Notch3* mutations nor CADASIL type pathognomonic granular osmiophilic material (GOM) or immunopositivity for Notch 3 ectodomains in arterial walls (unpublished observations) have been detected in material from this family.

Case reports published around that time described hereditary cerebrovascular disorders in several European families exhibiting similar clinical and pathological findings. These included hereditary multi-infarct dementia (64), chronic familial vascular encephalopathy (67), familial disorder with subcortical ischemic strokes, dementia and leukoencephalopathy (53) and familial Binswanger's syndrome (29). In the 1990s Tournier-Lasserve and coworkers embarked on genetic studies to locate the defective gene in French families. These efforts established linkage to chromosome 19 and the derivation of the disease name as CADASIL (69). Three years later the critical gene was identified to be *Notch3* at locus chromosome19p13 (32). The large number of families available further allowed a detailed description of 25 different mutations as well as the revelation of their stereotypic character (33). Rapid progress in these developments was helped by previously established knowledge that products of the *Notch* genes were cell receptors involved in a widely expressed intercellular signaling pathway (2, 46). Interestingly, Notch3 was also found to have some relationship with Alzheimer's disease (AD), since upon ligand binding Notch3 appears to be proteolytically cleaved by the same γ-secretase/presenilin-1 as β-amyloid precursor protein (APP, cf. Figure 6) (14, 15, 63), mutations of presenilin-1 being the major cause of early onset familial AD

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Table 1a. Currently known missense mutations in *Notch 3* linked to CADASIL.

No	EGF Domain	Amino acid change	Exon	Notch3 nucleotide change	% of mutations per exon*
1	1	Cys 49 Tyr	2	tGt 224 tAt	3.1
2		Arg 54 Cys		Cgt 238 Tgt	
3		Trp 71 Cys	3	tgG 291 tgT	9.2
4		Cys 76 Arg		Tgt 304 Cgt	
5	2	Arg 90 Cys		Cgt 346 Tgt	
6		Cys 93 Tyr		tGc 356 tAc	
7		Cys 93 Phe		tGc 356 tTc	
8		Arg 110 Cys		Cgt 406 Tgt	
9		Cys 117 Phe	4	tGc 428 tTc	50.8
10	3	Cys 123 Phe		tGc 446 tTc	
11		Cys 123 Tyr		tGc 446 tAc	
12		Cys 128 Tyr		tGt 461 tAt	
13		Arg 133 Cys		Cgc 475 Tgc	
14		Cys 134 Trp		tgC480tgG	
15		Arg 141 Cys		Cgc 499 Tgc	
16		Phe 142 Cys		tTc 503 tGc	
17		Cys 144 Ser		tGc 509 tCc	
18		Cys 144 Tyr		tGc 509 tAc	
19		Cys 146 Arg		Tgc 514 Cgc	
20		Tyr 150 Cys		tAc 527 tGc	
21		Arg 153 Cys		Cgc 535 Tgc	
22	4	Cys 162 Ser		Tgc 562 Agc	
23		Arg 169 Cys		Cgc 583 Tgc	
24		Gly 171 Cys		Ggt 589 Tgt	
25		Cys 174 Phe		tGc 599 tTc	
26		Cys 174 Tyr		tGc 599 tAc	
27		Ser 180 Cys		tCc 617 tGc	
28		Arg 182 Cys		Cgc 622 Tgc	
29		Cys 183 Arg		Tgc 625 Cgc	
30		Cys 183 Ser		Tgc 625 Agc	
31		Cys 185 Arg		Tgt 631 Cgt	
32		Cys 185 Gly		Tgt 631 Ggt	
33		Cys 194 Arg		Tgt 658 Cgt	
34		Cys 194 Phe		tGt 659 tTt	
35		Cys 194 Tyr		tGt 659 tAt	
36	5	Cys 206 Tyr		tGc 695 tAc	
37		Arg 207 Cys		Cgt 697 Tgt	
38		Cys 212 Ser		Tgc 712 Agc	
39		Cys 222 Gly		Tgt 742 Ggt	
40		Cys 222 Tyr		tGt 743 tAt	
41		Cys 224 Tyr		tGt 749 tAt	
42		Cys 233 Ser	5	Tgt 775 Agt	4.6
43	6	Cys 251 Arg		Tgc 829 Cgc	
44		Tyr 258 Cys		tAt 851 tGt	
45	8	Arg 332 Cys	6	Cgc 1072 Tgc	1.5
46	10	Gly 420 Cys	8	Ggt 1336 Tgt	6.2
47		Cys 428 Ser		tGt 1361 tCt	
48	11	Cys 440 Gly		Tgc 1396 Ggc	
49		Arg 449 Cys		Cgc 1423 Tgc	
50	13	Cys 542 Tyr	11	tGt 1703 tAt	7.7
51	14	Arg 544 Cys		Cgc 1708 Tgc	
52		Arg 558 Cys		Cgc 1750 Tgc	
53		Arg 578 Cys		Cgc 1810 Tgc	
54	15	Arg 607 Cys		Cgc 1897 Tgc	
55	18	Arg 728 Cys	14	Cgc 2260 Tgc	1.5
56	24	Gly 953 Cys	18	Ggc 2935 Tgc	4.6
57	25	Phe 984 Cys		tTc 3029 tGc	
58		Arg 985 Cys		Cgc 3031 Tgc	
59	26	Arg 1006 Cys	19	Cgc 3094 Tgc	6.2
60		Cys 1015 Arg		Tgc 3121 Cgc	
61		Tyr 1021 Cys		tAt 3141 tGt	
62		Arg 1031 Cys		Cgc 3169 Tgc	
63	27	Gly 1058 Cys	20	Ggt 3250 Tgt	1.5
64	31	Arg 1231 Cys	22	Cgt 3769 Tgt	1.5
65	32	Cys 1261 Arg	23	Tgc 3859 Cgc	1.5

Notes: Above mutation results compiled from following references and unpublished data: Ceroni et al (7); De Lange et al (13); Dichgans et al (17-19); Escary et al (24); Joutel et al (32, 33); Kalaria et al (unpublished data); Kotorii et al (44); Lesnik Oberstein et al (47); Low and Kalaria (unpublished data); Markus et al (unpublished data); Oliveri et al (56); Tuominen et al (70); Wang et al (75).

* Percentages of total of 65 different mutations in each exon. In addition to above at least 20 nucleotide substitutions not involving amino acid change (neutral mutation) and at least 8 substitutions causing an amino acid change but not associated with disease have been reported. These occur in several different exons.

Abbreviations: A or a, adenine; Arg, arginine; Asp, asparagine; C or c, cytosine; Cys, cysteine; EGF, epidermal growth factor; G or g, guanine; Gly, glycine; Phe, phenylalanine; Ser, serine; T or t, thymine; Tyr, tyrosine.

No	EGF Domain	Amino acid change	Exon	Notch3 nucleotide change
1	2	Deletion of 5 amino acids from Asp80 to Ser84, including Cys82	3	Deletion from A317 to G331
2		Deletion of 7 amino acids from Gly114 to Pro120 including Cys117	4	Deletion from G419 to G439 due to A to G transition in the 3' acceptor splice of exon 4
3	3	Deletion of 3 amino acids from Arg153 to Cys155		Deletion from C537 to G545
4	6	Deletion of 15 amino acids from Asp239 to Asp253 including Cys240, Cys245, Cys251	5	Deletion fromC792 to A836

Table 1b. Currently known deletions in *Notch 3* linked to CADASIL.

(30). The progress in CADASIL research after the gene discovery has been mainly directed to detailed clinical and imaging studies, the basic pathogenetic mechanisms of the disease remaining largely unknown (21, 34, 66, 77). The identification and diagnosis of CADASIL patients increased substantially when the molecular genetic confirmation of the defective gene combined with ultrastructural findings in diseased arteries became available. However, CADASIL still appears to be underdiagnosed and may be missed even in stroke clinics.

Clinical Picture

Epidemiology. CADASIL occurs worldwide and in many different ethnic groups. The greatest number of families have so far been identified among European Caucasians, while in the United States and Canada the number of reported cases has been surprisingly low considering the size of the populations. Thus far, 2 large reports on the clinical picture of CADASIL have been published: 45 members from 7 French families (8) and 102 patients from 28 German and one Austrian family (17). Exact prevalence numbers have not been reported, but the estimates are that in Finland, with a relatively high frequency and fairly good general awareness of this entity among clinicians, the prevalence is about 4 per 100 000. This estimate is realistic in many European countries given that well over 100 genetically confirmed families exist in France, Germany and the United Kingdom (MMR, RNK and M Dichgans, personal communication). However, there are estimated to be at least 500 CADASIL familes worldwide, suggesting that this disorder is much more common than familial AD.

Onset and duration. The 4 principal symptoms of CADASIL are migraine with aura, ischemic attacks (transient or strokes), psychiatric symptoms and cognitive decline or dementia. The age of onset varies greatly, also depending on the criterion used for the onset of the disease. Migraine attacks may begin even before the age of 10 years, but more commonly during the third decade. However, because of its commonness as an independent disease, the age of onset is usually not given on the basis of migraine but the first ever stroke.

The timing of the first ischemic attack is also widely variable even within the same family (17, 39), thus, the variation in onset does not depend on the type of gene defect. In Finland among about 100 CADASIL patients with the same R133C mutation the age of onset varies from 28 to almost 60 years and in a Swedish pair of identical twins, twin B had his first symptoms at the age of 39, 30 years earlier than his mother, while twin A is still asymptomatic at the age of 50 years (42). Furthermore, it may be difficult to distinguish between a transient ischemic attack (TIA) and migraneous aura, which may be very severe and long lasting in CADASIL. The reported peak in ischemic episodes is around 40 to 50 years of age and the latest age of first stroke is in the seventh decade. The mean age of onset in the German/Austrian kindreds was 37 years (17), in the large Finnish family about 40 years (64) and in the French families 45 years (8). CADASIL is slowly progressive with exacerbations associated with recurrent strokes. The disease leads to death within 10 to 20 years. The oldest known patient is a 94-year-old female in Finland (S. Tuisku, personal communication).

Migraine. Recurrent headache, most frequently in the form of migraine with aura, is a common symptom in CADASIL patients, being reported in 22 to 38% of the patients with mean age of onset at 26 to 38 years (8, 17). The aura is commonly a visual or sensory disturbance, but it is often atypical, long lasting or exceptionally severe. The aura may appear even as hemiplegia (8), which has been explained by the close localization of the gene for hemiplegic migraine, which encodes for the α1A-subunit of P/Q type calcium channel (57). Because the headache or migraine is so common, patients do not necessarily mention it spontaneously. The character of the migraine may become milder after the first ischemic episode, as reported to occur in the majority of German/Austrian patients, whose headaches either ceased or migraine attacks became markedly less frequent (17). On the other hand, pregnancy may be associated with aggravation of migraine (S. Tuominen et al., unpublished observations).

Ischemic strokes. The key feature of CADASIL is recurrent ischemic insults of variable severity. In 71% of the German/Austrian patients the presenting manifestation of the disease was a transient ischemic attack (TIA) or stroke (17). TIA can be difficult to distinguish from a severe migranous aura. Most often the strokes are focal (lacunar), clinically manifest as pure motor or pure sensory strokes, dysarthria-clumsy hand syndrome, expressive dysphasia or visual field defects. As the disease progresses pseudobulbar paresis and difficulties in locomotion may appear and finally patients lose their ambulation. In 10 to 15% of patients dementia develops without identified episodes of clinical stroke (55). The infarcts causing these symptoms are almost

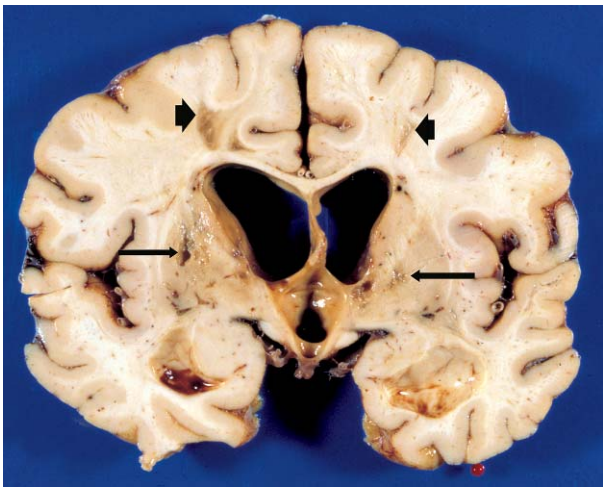

Figure 1. Numerous, mainly lacunar infarcts are seen in the basal ganglia (thin arrows) and white matter (thick arrows) in the coronal slice of brain from a 62-year-old woman with CADASIL.

invariably subcortical, located in the white matter or basal ganglia (Figure 1), but they may also occur in the brain stem and rarely in the spinal cord.

Psychiatric symptoms. Mood disturbances are exhibited by more than 20% of CADASIL patients. Depression is expectedly the most common disturbance, especially in those patients who have preserved insight of their disease. Manic episodes are rare. Psychotic disorders were common in one French family, considered to represent a variant phenotype of CADASIL (74), but these occur also in families with a common phenotype. One CADASIL patient with schizophrenia has been reported (45).

Cognitive decline and dementia. The disturbances in cerebral blood flow (CBF) lead to cognitive decline, predominantly in frontal lobe functions, detectable already in the prestroke phase of the disease. The patients have impaired executive and organizing functions, general mental slowing, poor concentration and slowing of motor functions, whereas they perform relatively well in the routine Mini Mental Status Examination (MMSE) test. Later on, obviously parallel to the cumulative tissue destruction, memory and other cognitive functions are affected leading to a subcortical type of dementia (68; Amberla et al, submitted), which fulfill the suggested criteria for a subcortical vascular dementia (23). Cognitive decline becomes clinically manifest between 40 and 70 years of age, with about 80% of the CADASIL patients aged over 65 years being demented (17)

Additional findings and risk factors. Epileptic seizures, most commonly partial or generalized, occur in about 6 to 10 % of the patients, usually at a late stage of the disease (17). Episodes of disturbed consciousness with raised intracranial pressure may rarely occur even as a presenting sign (26). CADASIL patients are usually normotensive, but they may have other vascular risk factors, such as smoking, high serum cholesterol, obesity or use of contraceptive pills, the role of which in modifying the clinical picture has not been assessed in detail (11). Interestingly, the more severely affected monozygous twin (see above) did smoke. Routine laboratory examinations are usually noncontributory.

Imaging

T2 weighted (T2w) and FLAIR sequence magnetic resonance imaging (MRI) with high resolution and sensitivity are the most important diagnostic imaging methods. With T2w MRI it is possible to detect changes highly suggestive of CADASIL diagnosis already in asymptomatic carriers of the gene defect (Figure 2A). Even in an 18-year-old CADASIL subject re-evaluation of the “routinely negative” T2w MRI images disclosed minimal abnormalities (T. Kurki, unpublished observation). Hyperintensities on T2w and FLAIR MRI in cerebral white matter, especially in anterior temporal lobes (Figure 2B), periventricular regions and external capsule, are the characteristic findings in CADASIL. (Figures 2A-D). These are compatible with the imaging diagnosis of leukoaraiosis (4) and deceptively reminiscent of multiple sclerosis. Periventricular hyperintensities are so common in CADASIL (present in 96% of patients) that their absence in practice excludes this diagnosis (11).

Diffusion tensor MRI of the white matter has revealed marked increase in water diffusivity, and the diffusion can occur more freely in any direction, meaning loss of anisotropy (10). This is considered to reflect a microstructural change with enlargement of the extracellular space due to vasogenic edema, possibly associated with myelin and axonal damage, ie, this imaging finding is well compatible with the microscopic picture in CADASIL. These alterations were also detectable outside the T2w lesions in the normal appearing white matter indicating an early microstructural abnormality.

In symptomatic patients, who have sustained strokes (in 10-15% of cases not noted by the patient), variable number of small infarcts can be identified in T1 weight-

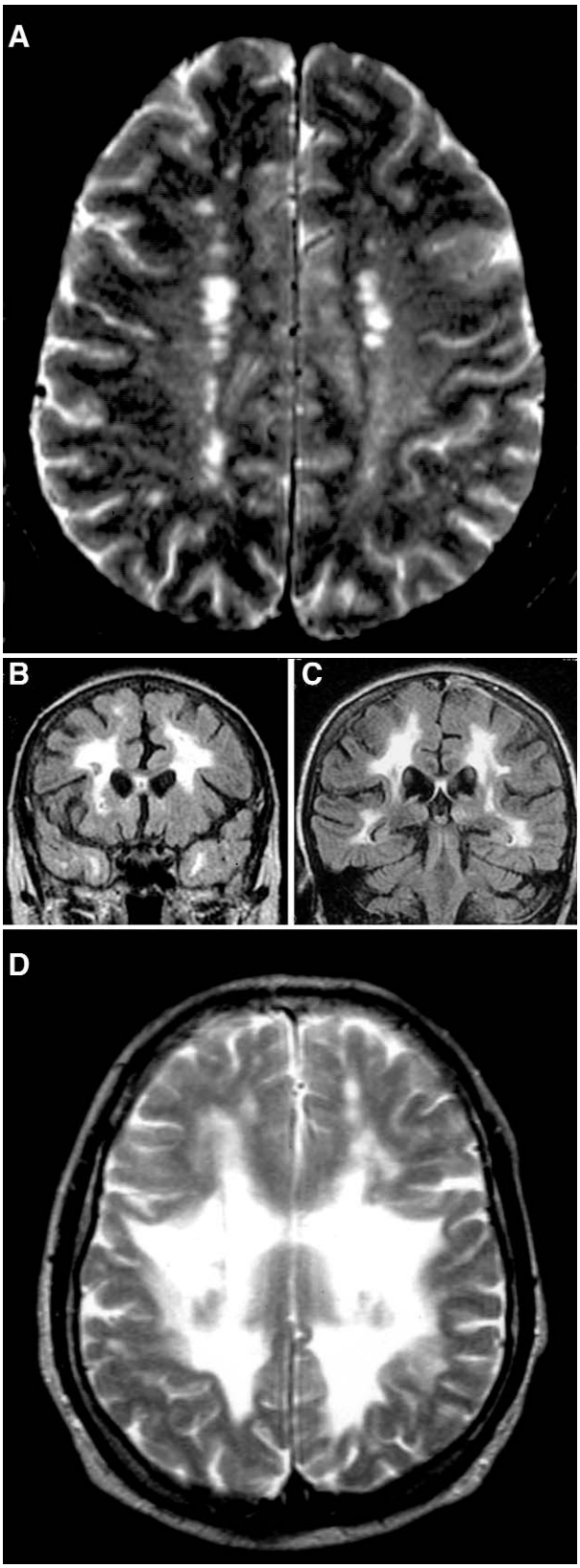

Figure 2. A. T2-weighted MRI image of a subjectively healthy 40-year-old CADASIL-patient with R133 C mutation of *Notch* 3. Small nodular hyperintensities are present in the white matter around the ventricles as an early sign of the disease. **B** and **C**. FLAIR sequences on MRI in a 56-year-old CADASIL patient with R153C mutation. Note in (**B**) clear hyperintense signals in temporal poles in addition to involvement of centrum semiovale. **D**. In a 55-year old moderately demented patient homozygous for R133C mutation the T2-weighted hyperintensities are confluent reflecting severe involvement. Scans **A** and **D** courtesy of Dr Timo Kurki, Turku University Hospital, Finland, **B** and **C** courtesy of Dr P.G. Cleland, Sunderland Hospital, U.K.

ed MRI and computed-assisted tomography (CT). The infarcts are most commonly located in the white matter and deep grey matter (basal ganglia), whereas the cerebral cortex remains relatively intact (cf. Figure 1).

In T2 weighted gradient echo MRI small hyperintensities corresponding to microbleeds have been discovered mainly in cerebral cortex (20) or in thalamus (48). Their frequency increased with age and volume of T2w lesions. These represent perivascular accumulations of hemosiderin containing macrophages indicating focal extravasation of red blood cells. Microbleeds are not specific for CADASIL, since they occur also in other small vessel diseases of CNS, such as hypertension and amyloid angiopathy. The significance of microbleeds for the pathogenesis of CADASIL is still uncertain, since they are found only in about a third to half of patients. These are clinically silent and also occur in the cerebral cortex. Their localization, however, does not correspond to the T2w MRI changes (82% of the microbleeds were outside the T2w hyperintensities) or to ischemic lesions, and, thus, microbleeds were considered to be independent manifestations of the underlying angiopathy (20). The relationship between the microbleeds and significant intracerebral hemorrhages (which are uncommon in CADASIL) has not been established. Since microbleeds are assumed to increase risk of intracerebral hemorrhages they should be taken into account when undertaking diagnosis and management (48). Conventional cerebral angiography appears to carry a considerably increased risk of complication, most often hemorrhage (16) and therefore its use is contraindicated in CADASIL.

Reduced CBF has been demonstrated in CADASIL with several functional imaging methods. Single photon emission computed tomography (SPECT) (55) and positron emission tomography (PET) (9) have been used to evaluate few CADASIL patients. In the PET study CBF was reduced both in the presynptomatic subject and in the demented patient, but oxygen consumption ($CMRO_2$) was lowered only in the demented

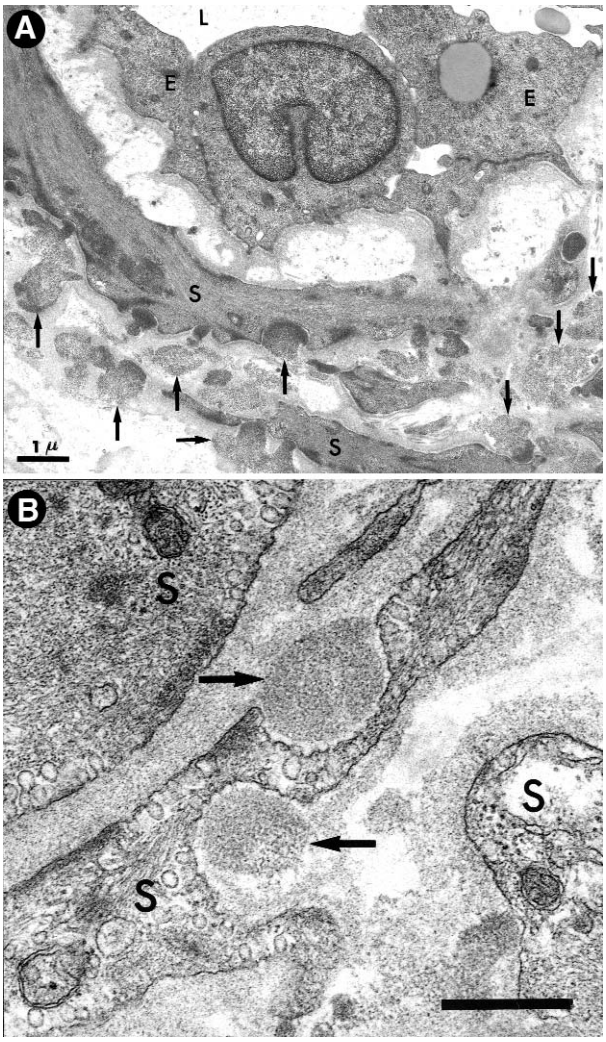

Figure 3. Electron microscopic findings in a skin biopsy from a CADASIL patient. **A**. Smooth muscle cells (S) in the wall of a small dermal artery are irregular in shape and between them there are deposits of characteristic granular osmiophilic material (GOM, arrows). Note the widening of the subendothelial space. **B**. At a higher magnification GOM (arrows) is seen in indentations of a smooth muscle cell (S) within thickened basal lamina. Note that caveolae are present along the entire plasma membrane and not only underneath GOM. L = lumen. E = endothelium, S = smooth muscle cell. Bar: **A**. 1 μm, **B**. 0.5 μm.

patient. In presymptomatic subjects the oxygen extraction fraction (OEF) was increased indicating that the brain tissue could still compensate for the decreased CBF by increasing OEF (9). In a Finnish study on young presymptomatic and mildly affected CADASIL patients CBF in cerebral cortex was not reduced until later symptomatic stages, but in the white matter significant reductions were observed already at the preclinical or early clinical stage of the disease (Tuominen et al, submitted). These results indicate that the decrease in CBF is due to the arteriopathy, not secondary to tissue loss. At the later stage, when the white matter and basal ganglia infarcts have appeared, the $CMRO_2$ and glucose consumption (CMR_{gluc}) decrease in parallel to tissue loss and development of dementia (9, Tuominen et al, submitted). With MRI bolus tracking method decreased CBF (12) and cerebral blood volume (CBV) (5, 12) were recorded within areas of T2w hyperintensities in the white matter, where reductions were more severe in demented than in nondemented patients. However, a trend towards reduced (12) or normal (5) CBV was recorded within normal-appearing white matter. Furthermore, the hemodynamic reserve is reduced in CADASIL patients, since the acetazolamide induced increase in CBF and CBV was lower in T2w hyperintense areas of white matter (12). The arteriopathy of CADASIL was also evinced by Doppler sonography, which revealed diminished arteriovenous cerebral transit time in both disabled and nondisabled CADASIL patients (49)

Pathology

Biopsy findings. Even though the symptoms of CADASIL are almost exclusively neurological, vascular changes are not limited to the cerebral arteries, but are seen in the medium-sized and small arteries and in some veins of almost all organs. This ubiquity provides an easy tissue source for identification of the pathognomonic structural changes as well as for *intra vitam* histopathological verification of CADASIL. The most common source is skin biopsy, and for CADASIL specific granular omiophilic material (GOM; Figures 3A, B) can virtually always be detected between degenerating VSMCs in the walls of dermal arterioles (41, 54, 59). Recently it was demonstrated that in CADASIL the extracellular domain cleaved from Notch3 in response to ligand binding accumulates outside the degenerating VSMCs and can be visualised immunohistochemically (Figure 4) (34, 36). Common neuropathological biopsies from muscle or peripheral nerve can also be used for detection of GOM (61, 62). Both accumulation of GOM and degeneration of VSMCs appear to begin early, being detectable in skin biopsies of CADASIL subjects already before 20 years of age (70). In spite of deposition of connective tissue, stenosis of dermal arterioles does not occur as suggested by the lack of difference in the sclerotic index (1-lumen diameter/external diameter) of arteries in skin biopsies from CADASIL patients compared to controls (6).

The pathognomonic GOM is located either in indentations of degenerating arterial VSMCs or free between these cells, often within the thickened basal lamina (Figures 3A, B). GOM deposits vary in size from 0.2 to 0.8 μm, and are composed of 10 to 15 nm granules, which in turn sometimes appear to be conglomerates of tiny 2 to 4 nm granules (Figure 3B) (41, 59-61). There is no filamentous component in GOM. The composition of GOM has not yet been definitely identified, even though Notch3 protein was suspected to be one component. Beneath GOM in the VSMC indentations there are often small caveolae, which have been suggested to be related to the accumulation of GOM. However, caveolae are common structures in VSMCs in general (37) and also present outside GOM indentations, wherefore their pathogenetic significance remains unclear.

Post-mortem brain findings. In accordance with imaging, multiple small (lacunar) infarcts in the white matter or deep grey matter are seen at post-mortem examination of CADASIL patients' brains (Figure 1). Lacunar infarcts also occur frequently in the brain stem. On the other hand, as the CBF observations imply, the cerebral cortex is relatively preserved (Figure 5A). Unlike in β–amyloid angiopathy (38, 40), intracerebral hemorrhages are uncommon, having occurred most often in patients treated with anticoagulants or antiaggregants, or subjected to arteriography.

Histological sections show that the walls of small and medium-sized leptomeningeal and penetrating arteries are markedly thickened. Characteristic granular material, which is on H&E staining basophilic, PAS-positive (Figures 5B, C) and immunopositive for Notch3 ectodomains (Figure 4B), accumulates in the degenerating tunica media. Destruction of the smooth muscle cells can be demonstrated by immunostaining for smooth muscle α-actin (Figure 5D). The thickening of the arterial wall appears to be mainly due to accumulation of extracellular matrix proteins (7, 37), including various types of collagen and laminin (Figures 5E, F). The presence of the above-mentioned granular material (Figure 5B, C) serves as a distinguishing feature from the fibrotic vasculopathy occurring for example in arterial hypertension and Binswanger's disease (38, 40, 61). The affected arteries of CADASIL are completely negative in alkaline Congo red staining, which excludes deposition of amyloid as the cause of the vascular pathology. Electron microscopy confirms the destruction of smooth muscle cells in the arterial walls and also discloses in greater detail the accumulation the pathognomonic GOM (cf. Figure 3, see above).

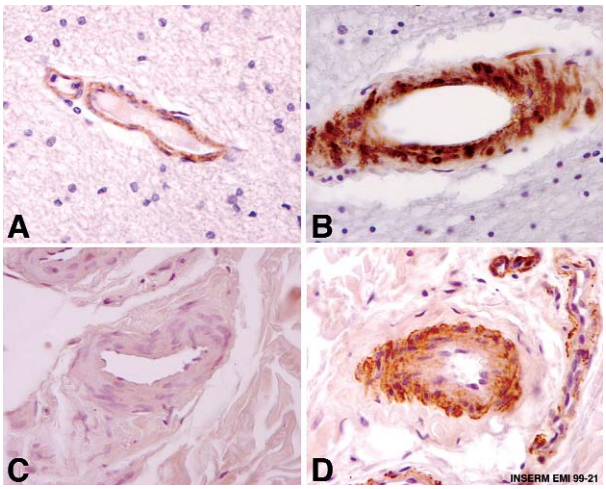

Figure 4. Immunohistochemical localization of Notch3 extodomain shows minor non-specific staining in a control cerebral arteriole (**A**) while a control skin arteriole (**C**) is entirely negative. In CADASIL patients there is strong granular staining both in a brain (**B**) and in a skin arteriole (**D**). Immunostaining with antibody 1E4 and hematoxylin counterstain. (Courtesy of Dr Anne Joutel)

In the only autopsy study some VSMC degeneration and GOM were found electron microscopically in the arteries of most internal organs and even in aorta (60). Yet, non-neurological symptomatic diseases attributable to these changes are virtually nonexistent in CADASIL. For example, the disease process in coronary arteries very rarely results in myocardial infarction (8, 17, 61), and accordingly the coronary arteries may disclose minimal light microscopic changes even in patients with advanced cerebrovascular pathology.

The cerebrospinal fluid (CSF) from CADASIL patients reveals oligoclonal bands but these seem indistinguishable from those in other disorders. However, there may be CSF proteins of interest (39). Unlu et al (72) reported increased presence of factor B of the alternative complement pathway, but it is likely that other modifying factors—either genetic or epigenetic—contribute to the phenotypic variations in age at onset, number of cerebrovascular incidents, degree of dementia and general morbidity.

Genetics and Notch Signaling

The human *Notch3* gene has 33 exons and it encodes a Notch3 receptor protein of 2321 amino acids with a single transmembrane domain (Figure 6) (32). The extracellular N-terminal part of the molecule contains 34 epidermal growth factor (EGF) type repeats followed by

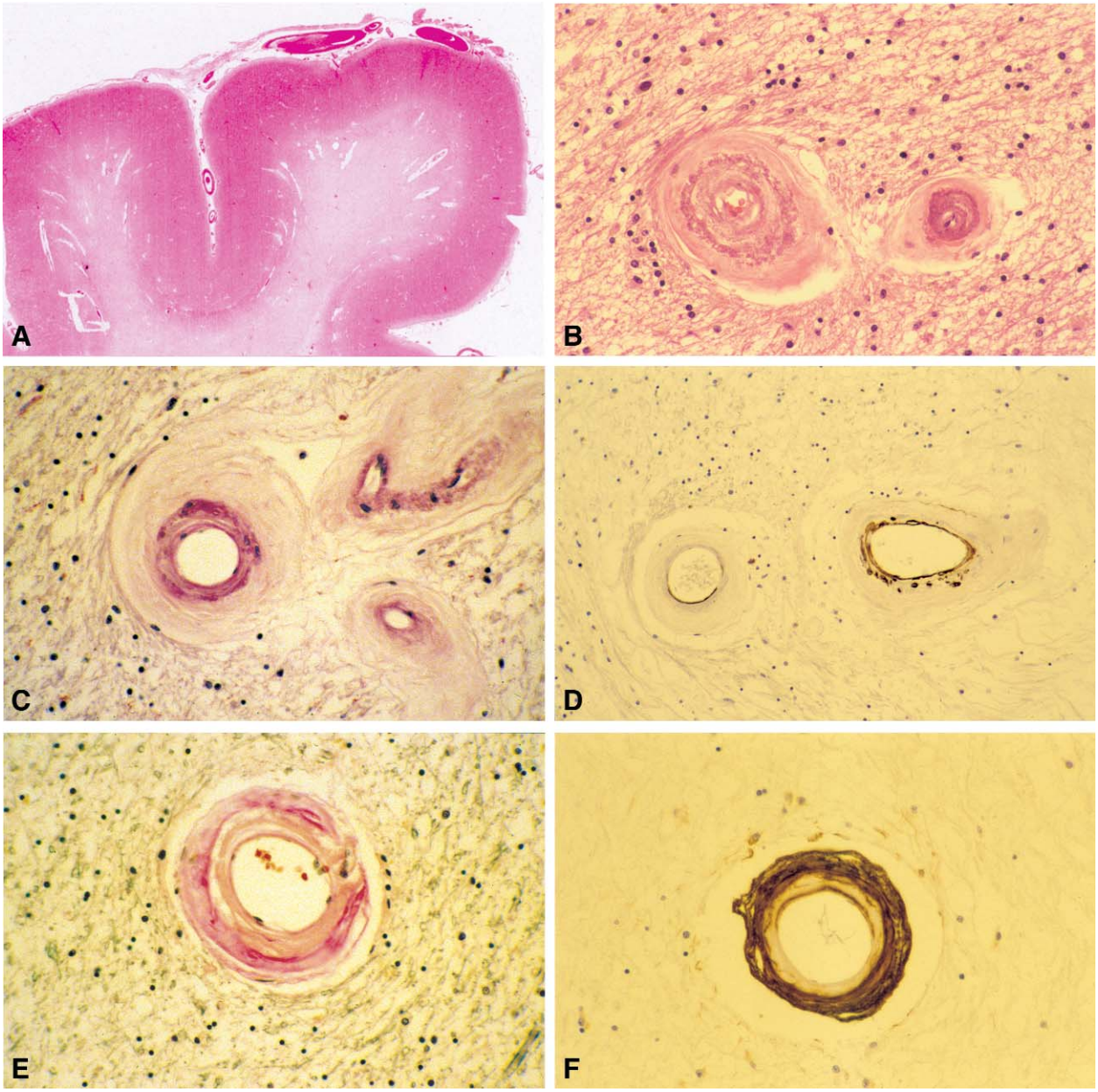

Figure 5. Microscopic changes in the brain parenchyme. **A**. Cortex is relatively well preserved, whereas the underlying white matter is damaged. **B**. The lumina of two small white matter arteries are narrowed and their walls are markedly thickened. Basophilic granular material replaces smooth muscle cells of the tunica media. **C**. In PAS staining the granular material accumulated in the arterial wall is intensely red. **D**. The number of smooth muscle cells (brown) in the arterial wall is markedly reduced due to their severe degeneration. **E**. The thickened wall around the inner part with the granular material is fibrotic, staining red in van Gieson staining and (**F**) strongly immunopositively for type I collagen. **A** and **B**: H&E, **C**: PAS, **D**: anti-smooth muscle α-actin and hematoxylin counterstain, **E**: van Gieson and **F**: anti-type I collagen and hematoxylin counterstain. Magnifications: **A**: 3×; **B-F**: 170×

three notch/lin-12 repeats. On the cytoplasmic side there are 6 ankyrin repeats (33). Notch3 gene/protein is a member of the Notch family, which in mammals has 4 members and which has a very important role during development. Notch receptor molecules are the name-giving elements of the Notch signaling pathway, which may be the most widely used signaling pathway in animal development. This is also evident in the fact that Notch molecules are highly homologous in organisms ranging from nematodes to man (2, 3, 50).

Notch signaling pathway shows some unique characteristics, which most likely are relevant also for the development of CADASIL (4). Firstly, it appears to transduce signals between neighboring cells in immediate contact, since the ligands (Delta and Serrate) binding to Notch receptors are considered to also be strictly cell bound. On this basis, the exchange of information between the endothelial and smooth muscle cells may be pivotal for the development and maintenance of the arterial tunica muscularis. Secondly, Notch receptor molecules undergo 3 regulated proteolytic cleavages (Figure 6, S1-S3). The primary protein product is first constitutively cleaved by a furin-like convertase (S1), the cleavage products bind to each other to generate a heterodimer, which is inserted in the plasma membrane. The next proteolytic event (S2) occurs upon ligand binding in the extracellular part of Notch3 by TNFα-converting enzyme (TACE), also called metalloprotease ADAM-17, precisely 12 amino acids outside of the plasma membrane —similar to the process by which α-secretase acts upon APP (43). This cleavage releases the extracellular domain to the extracellular space (this may be of central importance in the pathogenesis of CADASIL, see below). The third cleavage (S3) within the plasma membrane by γ-secretase-like activity, which appears to be the same as in the cleavage of β-amyloid in AD and which at present is considered to be a complex of presenilin1 and nicastrin (25), releases the Notch3 intracellular domain (NICD), which then translocates to the nucleus and binds a DNA binding protein called CSL (also called RBP-Jk) to regulate transcription of genes (2, 4). This represents a novel signaling paradigm called regulated intramembrane proteolysis (RIP), which appears to be an important phenomenon generating proteins for nuclear signaling (22). In AD the amyloidogenic β–peptide is also generated by RIP and at present the C-terminal intracellular fragment of APP has been suggested to also have nuclear signaling function similar to NICD (Figure 6) (22).

During development the Notch pathway regulates tissue differentiation (2, 3, 47). In general, its function is associated with prevention of differentiation of primitive cells during organogeneses, including myogenesis. During so-called lateral inhibition the signaling cells expressing ligand molecules (Delta or Serrate) differentiate, whereas in the Notch expressing receiving cells the Notch intracellular domain is cleaved and translocated to the nucleus, where it inhibits transcription—during myogenesis of muscle specific proteins. However, the exact role of Notch3 in VSMC during development remains to be defined. The exact function of Notch3 in

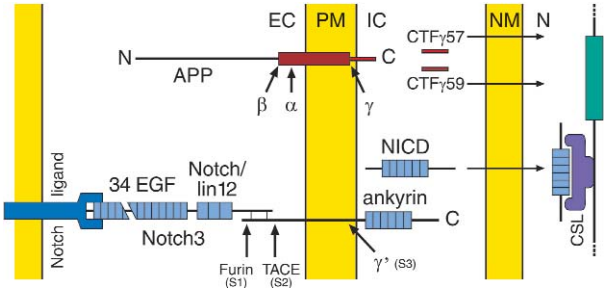

Figure 6. Presumed process and function of the Notch3 protein. Subsequent to translation the primary protein product is constitutively cleaved by furin like convertase (S1) and the subunits form a homodimer, which is inserted in the plasma membrane (PM). Upon ligand (Serrate or Delta) binding the extracellular part (ectodomain) is released at S2 by TNFα-converting enzyme (TACE) and the Notch3 intracellular domain (NICD) is cleaved by γ-secretase, which is possibly the same as that cleaving β-amyloid precursor protein (APP) in Alzheimer's disease. NICD (and possibly also C-terminal fractions of APP = CTFγ57 or CTFγ59) is then translocated to nucleus (N), where it regulates transcription associated with a DNA binding protein CSL (RBP-Jk). The fact that vascular smooth muscle cells also express APP mRNA (Revesz et al, in this issue) further adds interest in this analogy. EGF = epidermal growth factor, EC = extracellular and IC = intracellular compartments, NM = nuclear membrane.

adult animals, in which it is exclusively expressed in VSMCs, is unknown (34, 58). Recent data have provided evidence that Notch3 may be essential for preventing apoptosis of VSMCs (76).

More than 95% of CADASIL cases to date are due to missense point mutations in the extracellular domain of Notch3 (Figure 6, Table 1). There is a marked clustering of mutations at the 5′ end of the Notch3 gene (33, Table 1). At least 65 different pathogenic point mutations have been identified from codon 49 in exon 2 to codon 1261 in exon 23 (Table 1a). However, in about 70% of patients the mutation is located within exons 3 and 4, which encode the first 5 EGF repeats. The clustering of mutations within these exons is useful to quickly identify new suspected cases. All the point mutations result in amino acid substitutions, either a replacement of a cysteine with another amino acid or vice versa. Thus, instead of the normal even number of 6 cysteines the mutated EGF repeat contains an uneven number, either 5 or 7 cysteines. In addition to the point mutations, 4 different small deletions have been described (Table 1b), which result in a loss of either one or 3 cysteine residues and thereby they also cause a change from an even to uneven number of cysteines in the deleted EGF repeat. However, Tabira et al (44) have recently found a family with a *Notch3* mutation not involving a cysteine

residue. They described the presence of a R213K mutation in a Japanese family with clinical features characteristic of typical CADASIL.

Several families with typical CADASIL features have failed to reveal any abnormalities in *Notch3* suggesting that other genetic forms of CADASIL-like disorders may exist. While sporadic cases of CADASIL may occur these are likely to be rare. Joutel et al (35), however, reported a de novo mutation causing R182C substitution. Recently, a CADASIL patient homozygous for mutation C475T causing a relatively common R133C substitution was described (70). The homozygous male patient had early onset and severe clinical and imaging findings, but not to such an extent that homozygosity could be considered to definitely aggravate symptoms, ie, CADASIL appears to follow the classical definition of a dominant disease, according to which the heterozygous and homozygous patients are clinically indistinguishable.

Pathogenesis

It is possible that the alteration from an even to uneven number of cysteine residues affects the formation of sulphur bridges and thereby the 3-dimensional structure of the extracellular part of the Notch3 receptor molecule. Consequently, dimerization of Notch3 molecules, binding ligands, disposal of the cleaved extracellular domain and other molecular interactions may be affected (33, 38). These alterations presumably lead to defective signaling in the VSMCs and cause their degeneration. Since CADASIL is an autosomally dominantly inherited disease, both alternatives, haploinsufficiency (ie, one wild type allele of *Notch3* not being sufficient to maintain normal cellular function) and toxic gain of function, are plausible. In favor of insufficient Notch3 signaling it was recently reported both in vitro and in injured rat carotid artery that activation of Notch3 signaling was associated with upregulation of c-FLIP, which is an inhibitor of Fas ligand induced apoptosis. Thus, Notch3 may protect VSMCs from apoptosis and be essential for their survival (76). It has been demonstrated in vitro that selected mutations of Notch3 did not impair cell-surface expression or ligand binding (31). Therefore, the impairment in Notch3 signalling may be secondary to the pathological accumulation of mutated Notch3 ectodomains on the surface of VSMCs (34). These molecules could "sop up ligand without transmitting a signal" (66), which could consequently lead to gradual destruction of VSMCs, eg, by apoptosis (76).

The question of how damage to arteries leads to cerebrovascular disturbance of such severity that infarctions ensue remains open. Retinal arteries appear constricted at a fairly early stage, possibly already before the loss of VSMCs and the secondary fibrosis has caused the narrowing (Tuominen et al, in preparation). This and the subsequent progressive thickening of the arterial walls obviously reduce the arterial lumina and their compliance and consequently CBF as discussed above in the imaging section. Small cerebral arteries may become severely obliterated by the fibrotic process (Miao et al, unpublished observations), but opposite views have also been presented, with a hypothesis that there is no true stenosis but rather hypotonicity of the vascular wall (more easily collapsible) weakened by the loss of VSMCs (6). However, a likely final cause of the infarct is thrombosis in affected arteries, since fibrin degradation products can be detected in the plasma of CADASIL patients with recent infarcts (S Ilveskero et al, unpublished observations). The tissue destruction then translates to cognitive decline, which finally develops into dementia of subcortical vascular type.

The predominant localization of infarcts to the white matter and deep gray matter can be explained by the distribution of the cerebral arteries and circulation (40). Those areas of the brain are supplied by relatively long penetrating arteries of the end artery type without efficient collateral connections to the neighboring arteries. Furthermore, penetrating arteries become tortuous with age. In the cerebral cortex the density of arteries is greater and they are shorter. The walls of the cortical arteries are also significantly less thickened than in the deeper parenchyma (Miao et al, unpublished observations) and the same applies to cortical and white matter capillaries (6).

Diagnosis and Differential Diagnosis in CADASIL

Diagnosis. In a family without previous knowledge of a similar disease in relatives the first affected member is often examined either for a minor cerebrovascular accident (transient ischemic attack or stroke) at an exceptionally young age or for severe migraine. The former patients are most likely examined by MRI, and the characteristic, though not specifically diagnostic white matter changes should alert the radiologist. Migraine with aura is a very common early symptom, reported in 22 to 38% of patients (8, 17), but migraine alone often does not motivate to perform MRI, since migraine/headache is so common an ailment. Despite the low probability, physicians treating patients with migraneous headache should take CADASIL into consideration, especially if the aura is exceptionally severe.

The appearance of similar symptoms in relatives supports the possibility of CADASIL, even though migraine as such may also be familial (77).

Positive MRI findings necessitate electron microscopic or immunohistochemical examination of a skin biopsy, which are relatively easy to perform. Presence of GOM in the arterial walls can be considered definitely diagnostic of CADASIL, because GOM has not been found in any other disease (40, 59, 61). In many biopsies of suspected but not genetically verified patients various types of granular debris can be found between degenerative SMCs, but such debris is different and can be distinguished from true GOM. Although we have detected GOM in all genetically verified CADASIL patients, we make a reservation that a negative result does not absolutely exclude CADASIL, since GOM deposits may be too focal or the biopsy has not been deep enough, in which cases repeating the biopsy may solve the problem.

The identification of GOM and morphological diagnosis of CADASIL have been made by electron microscopy. Recently, the development of a monoclonal antibody to the extracellular domain of Notch3, which accumulates in the arterial wall, has provided a technically easier immunohistochemical method (Figure 4) for morphological diagnostics with high sensitivity (96%) and specificity (100%) (36) . Genetic screening for *Notch3* mutations still provides the most definitive diagnosis. The large number of different mutations (Table 1) makes the search tedious and expensive if the mutations are not found in exons 3 and 4, where 60% of the mutations appear and can be readily screened for.

Differential diagnosis. Since all CADASIL cases diagnosed have so far been familial the presence of CADASIL in affected relatives of appropriate age would be implicated. However, a similar familial disease in which migraine is associated with stroke-like symptoms is familial hemiplegic migraine (FHM), but the FHM patients usually recover from attacks. The defective gene *CACNL1A4* in one type of FHM is located close to *Notch3* in Chr 19p13. Stroke may also associate with "independent" migraine, and migraine has been traditionally included among risk factors for stroke. However, a detailed analysis could verify an increased risk of stroke only for young women (71a).

Recurrent strokes of other etiologies as well as other vascular dementias must also be taken into consideration. Recurrent embolizations may cause multiple lacunar infarcts and they should be excluded by adequate examinations. White matter infarcts are a feature of Binswanger's disease and a hereditary form of Binswanger's disease has been described, although this disorder could be a CADASIL like disease of unknown etiology (29). Hypertension is commonly associated with Binswanger's disease, whereas CADASIL patients are usually normotensive (11, 39). Yet, risk factors of cerebrovascular disease are not uncommon in CADASIL. Chabriat et al (11) identified at least one risk factor of stroke in 44% of their patients. Recently, a recessively inherited CADASIL-like disease was described and named CARASIL, but its arteriopathy is arteriosclerotic and the patients also have skeletal degenerative changes (78). Finally, strokes in young persons may be caused by a mitochondrial encephalopathy, especially MELAS, but in these diseases infarcts are usually cortical and located in occipital lobes (1, 51). Interestingly, a novel pathogenic mutation 5650G>A in the *tRNAAla* gene in mtDNA was reported in an R133C CADASIL patient (27) and in patients with migraine, mitochondrial haplotype U is a risk factor for occipital stroke (52).

Therapeutic Possibilities

At present, only symptomatic therapy is available. Because the vascular pathology has been thought to give rise to local thromboses, anticoagulant (8) or antiaggregant has been tried without positive results and in most patients also without negative effects. However, a few patients on these drugs have died of intracerebral haemorrhage, although the causal relationship to the medication has not been established with certainty. On the other hand, the slow and unpredictable progression of CADASIL makes evaluation of therapeutic effects very difficult. Acutely, acetazolamide may be of help to CADASIL patients with excruciating migraine (28).

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