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The pericyte: A critical cell in the pathogenesis of CADASIL

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ABSTRACT

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small vessel disease presenting with migraine, mood and cognitive disorders, focal neurological deficits, recurrent ischemic attacks, lacunar infarcts and brain white matter changes. As they age, CADASIL patients invariably develop cognitive impairment and subcortical dementia. CADASIL is caused by missense mutations in the *NOTCH3* gene resulting in a profound cerebral vasculopathy affecting primarily arterial vascular smooth muscle cells, which target the microcirculation and perfusion. Based on a thorough review of morphological lesions in arteries, veins, and capillaries in CADASIL, we surmise that arteriolar and capillary pericyte damage or deficiency appears a key feature in the pathogenesis of the disease. This may affect critical pericyte-endothelial interactions causing stroke injury and vasomotor disturbances. Changes in microvascular permeability due to perhaps localized blood-brain barrier alterations and pericyte secretory dysfunction likely contribute to delayed neuronal as well as glial cell death. Moreover, pericyte-mediated cerebral venous insufficiency may explain white matter lesions and the dilatation of Virchow-Robin perivascular spaces typical of CADASIL. The postulated central role of the pericyte offers some novel approaches to the study and treatment of CADASIL and enable elucidation of other forms of cerebral small vessel diseases and subcortical vascular dementia.

Abbreviations

aVSMC	artery-type smooth-muscle cell
BBB	blood-brain barrier
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
Cys	cysteine
EGFr	epidermal growth factor receptor
GOM	granular osmiophilic material
MRI	magnetic resonance imaging
SVD	small vessel disease
vSMC	vascular smooth muscle cells
WMLs	white matter lesions

1. Introduction

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common genetic small vessel disease (SVD). Since its first molecular identification in France in 1995, CADASIL has become widely recognized and numerous cases have been found not only in Europe but also around the world, including large families in Colombia [3,80,136]. Given its highly variable phenotype, CADASIL probably affects hundreds of thousands of individuals. The prevalence rate is estimated to be 2–5 per 100,000 persons in the general population. However, precise prevalence rates globally are not known but it is certainly more common than other hereditary dementia causing disorders such as familial Alzheimer's disease [13]. To this date, it remains an untreatable disease leading inexorably to progressive disability, subcortical vascular dementia, and premature death of every diagnosed patient.

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In this review, we focus on the pathology of the pericyte in CADASIL. Pericytes are unique contractile cells interspersed on capillaries and venules that reportedly control local blood flow [8]. Pericyte cell densities may vary along capillaries depending on type of vascularity in brain regions as well as other tissues [2,30,34,35]. Based on a comprehensive analysis of systemic and cerebral lesions in CADASIL, we postulate that alterations of the pericyte are likely to be critical to the pathogenesis of CADASIL in the CNS. Our reappraisal of prevailing concepts on CADASIL is based on review of morphological material dating back as early as 1994–95, complemented by genetic animal research and neuroimaging observations.

2. CADASIL as cerebral SVD

CADASIL is caused by more than 280 predominantly missense mutations of the *NOTCH3* gene, encoding a transmembrane receptor, NOTCH3 [62]. Mutations lie within the 34 epidermal growth factor receptor (EGFr) domains of *NOTCH3*. In recent years, upon screening of large exome databases incorporating different peoples worldwide, it appears that the frequency of archetypal cysteine altering *NOTCH3* (*NOTCH3*cys) mutations in the EGFr domains is estimated to be more than 100-fold higher than expected from estimates of the typical CADASIL genotype [66,111]. Until recently, it was maintained that mutations in *NOTCH3* gene were fully penetrant to produce the disease phenotype. However, clinical manifestations of the typical CADASIL phenotype begin in the third decade and include migraine with aura, recurrent ischemic strokes, apathy, mood and cognitive impairment, subcortical dementia and premature death [22]. Magnetic resonance imaging (MRI) of the brain shows T_2-weighted hyperintense periventricular and deep white matter lesions (WMLs) [22] (Fig. 1). With progression of the disease, multiple lacunar infarcts in the hemispheric white matter and basal ganglia are seen on MRI T_1-weighted sequences [23]. The neuropathology of CADASIL is characterized by lacunar infarcts, white matter attenuation, microinfarcts, microhemorrhages, perivascular dilated spaces and severe arteriolosclerosis (Fig. 1). However, large extracranial and intracranial arteries are mostly spared. Of vital importance is the observation that clinical signs and symptoms are attributable to CADASIL pathology in the CNS. Even the kidney, a very sensitive organ to arterial-type vascular disease, is largely free of functional impairment in CADASIL patients.

Upon electron microscopy, the vascular changes consist of deposition of 0.2- to 2-μm granular osmiophilic material (GOM) surrounding indentations of vascular smooth muscle cells (vSMCs) and damaged capillary pericytes and moderate to severe fibrosis and thickening of arterial walls [10,27,94,105,137] (Fig. 1). GOM deposits are associated with meningeal vessels as well as perforating arteries, arterioles and juxtaposed to capillaries but not major intracranial arteries. GOM is specifically labeled with antibodies against the extracellular portion of NOTCH3 (N3ECD) but not by antibodies to the intracellular domain of NOTCH3 [61]. The N3ECD positive GOM is exclusively found in CADASIL but not in any other familial SVDs [58,137]. The extensive distribution of N3ECD-GOM complexes within meninges, arteries, arterioles, and particularly capillaries even in the white matter (WM) [27, 137] suggests that aggregated NOTCH3 fragments are major components of GOM deposits, which may be eliminated via perivascular routes [20]. Consistent with profound reductions in vSMC, Viitanen and colleagues [130] demonstrated mitochondrial alterations including reduced antioxidant potential in vSMC of CADASIL patients with the *Arg133Cys NOTCH3* mutation. This finding corroborates with prolonged retention of mutant *NOTCH3* aggregates in the endoplasmic reticulum and increased sensitivity to cellular stresses [120].

Other key pathological sequelae likely triggered by the mutant *NOTCH3* gene have also been demonstrated in different studies. An important component of the pathological process is delayed cell death; neuronal apoptotic cell death in the cerebral cortex as well as vascular cells particularly in areas with status cribrosus, both in basal ganglia and subcortical WM [49]. Neuronal apoptosis appears to predominate in cortical layers 3 and 5 [49] and its severity correlates with the degree of dementia and the extent of ischemic lesions and axonal WM damage. In addition, astrocytes particularly in the white matter undergo massive autophagic cell death via transformation into clasmatodendrocytes [55]. It is now also apparent that there are distinct classes of WM changes in CADASIL. As previously thought pericyte coverage loss and blood-brain barrier (BBB) leakage may not be the primary drivers of WMLs but there could be direct effects of mutant *NOTCH3* on neurovascular or gliovascular elements and myelin pathophysiology to explain all the WM hyperintensities [101].

3. Arteriopathy and the vSMC in CADASIL

In lieu of the generic term vSMC, we use here "artery-type smooth-muscle cell" (aSMC) to describe the vascular mural cells containing contractile smooth muscle filaments observed in arteries and arterioles [15]. In the capillary, the equivalent mural cell is the pericyte. Patients diagnosed with CADASIL bear widespread damage of arterial vSMCs which impacts on vasomotor functions of the cerebral arterial circulation with unique specificity manifested as cerebral SVD [64]. Arterial SMC damage is found throughout the entire arterial tree including arteries and arterioles perforating into the white matter [105]. By contrast, this is different with hypertension, diabetes or hypercholesterolemia, entities including their risk factors are not exclusively neurological and produce numerous systemic manifestations [9,89,93]. However, neuromuscular [36,81,115,116] and ostensible cardiac impairments [28] have been described in CADASIL.

The NOTCH3 receptor is primarily expressed in membranes of vSMC and pericytes in adult mammalian arteries [61], capillaries and veins, in both the cerebral and the systemic vasculature, correlating with the pathological findings [123]. Since endothelial cells can induce the differentiation of mural cells through activation and induction of NOTCH3 it is plausible that mutant NOTCH3 alters differentiation of mural cells via loss of local autoregulation and NOTCH3 ligands such as JAGGED1 [79]. However, Arboleda-Velasquez et al. [4] have shown that two phenotypically distinct CADASIL mutations, *Cys455Arg* and *Arg1031-Cys*, define different hypomorphic activity states of NOTCH3. Furthermore, they were able to simulate these states to ischemic stroke susceptibility in mouse models. Proteomic analysis of brain vessels carrying the same CADASIL mutations indicated that extracellular associated clusterin and collagen 18 α1 or endostatin were also GOM components [4]. It is plausible aggregation and accumulation of N3ECD promote abnormal recruitment of functionally important extracellular matrix proteins that ultimately cause toxicity. Dysregulation of tissue inhibitor of metalloproteinases 3 or TIMP3 activity could lead to mutant N3ECD toxicity by impairing extracellular matrix homeostasis and influence early blood flow deficits [124] plus contribute to WMLs [19,87, 124]. These findings collectively also link aberrant NOTCH signaling with ischemic cerebral SVD [4].

In general, the phenotype of CADASIL is largely driven by fully penetrant effects of mutant *NOTCH3*. However, several reports have suggested that coexisting vascular risk factors modify the disease course. For example, smoking, hypertension and hyperhomocysteinemia (>12 ug mol/l) increase risk for stroke or migraine [1,119]. However, by analogy with sporadic forms of vascular dementia, stenosis and vasomotor dysfunction [43,44] have been of much interest in CADASIL. Lumen size is slightly decreased but marked fibrosis of the vessel walls has been noted [68]. Nonetheless, vascular occlusions are on the whole scarce or absent [17,61,105]. This is mostly consistent with what occurs in sporadic aging-related SVD where lumen are not entirely stenosed although subcortical vessels may bear microthrombi or microemboli [65]. Brulin et al. [17], measured capillary and arteriole lumen diameter by electron microscopy and found no significant stenoses but observed numerous flattened capillaries and arterioles implicating hypotonicity as profound entity in CADASIL. In contrast, Miao et al. [84,85] observed

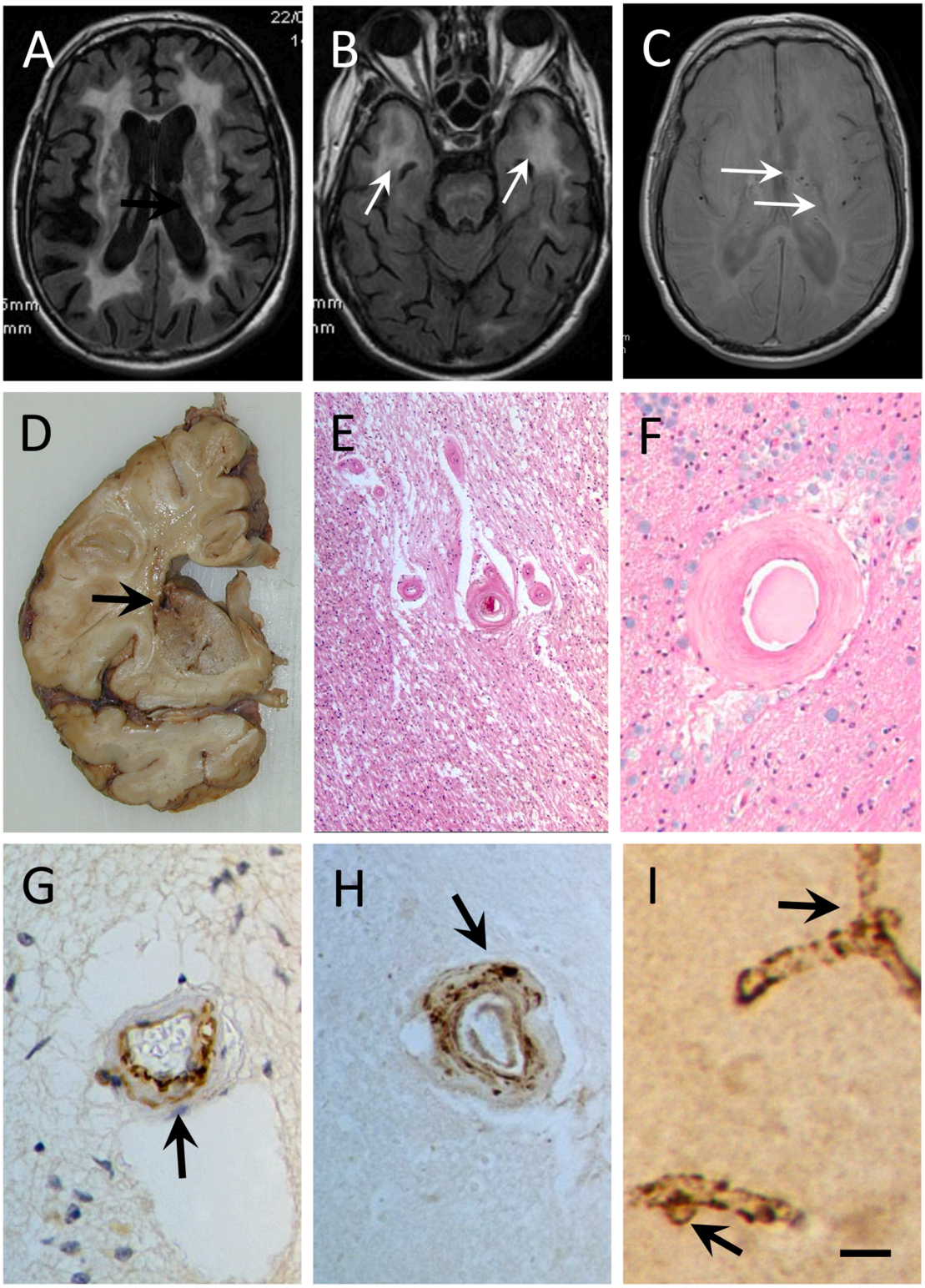

Fig. 1. Neuroimaging and neuropathological aspects of CADASIL. A-C, Axial plane MR images of a 68-year-old CADASIL patient with the *NOTCH3 Arg141Cys* mutation. A, MR FLAIR image showing periventricular and deep WM hyperintensities. B, MR FLAIR image showing characteristic WM hyperintensities in the anterior temporal poles (arrows). C, MR Gradient Echo images demonstrating several subcortical (deep) microbleeds (arrows). D-I, Pathological hallmarks of CADASIL. D, A coronal block cut at the level of the lenticular nuclei showing a lacunar infarct in the caudate (arrow). E-F, vessels showing severe arteriolosclerosis with hyalinisation and WM rarefaction (F). G-H, Arterioles with severely disrupted vSMCs immunostained by α-smooth muscle actin (G) and artery specific protein, medin (H), respectively. I, Capillaries lined by N3ECD immunopositive punctate deposits. Pericytes are revealed as round cellular deposits (arrows). Capillaries staining in both gray and white matter included arterioles and many capillaries (confirmed by GLUT-1 and α-actin staining). CADASIL cases showed clear distinct staining patterns with N3ECD antibody compared to aging controls. Magnification bar =10 μm, applies to panels E, F, G, H and I.

an increase in lumen diameter in the lenticular nuclei. However, hypertension, diabetes, hypercholesterolemia, hyperhomocysteinemia, or risk factors such as smoking and alcohol abuse in CADASIL patients would dramatically increase vessel wall thickness because the disconnection of aSMCs or pericytes allows passage and accumulation of oversecreted extracellular material leading to an obvious narrowing of the lumen diameter. This was observed in several skin arterioles (MMR, *unpublished observations*) and similar changes appear in the brain (Fig. 2A-C).

4. The capillary and barriers in CADASIL

In principle, four types of capillaries may be identified in different body organs. Our experience shows that these are sinusoid, gap, tight and BBB of capillaries (Table 1). The latter, BBB capillaries bear additional sieve-like features associated with protection of the brain. Typical CADASIL lesions do not occur in tissues endowed with sinusoid-type capillaries with fenestrated endothelium devoid of pericytes. Thus, the liver, the kidney, as well as the endocrine glands and other tissues with sinusoid capillaries appear to be free of any effects of characteristic CADASIL pathology. The only known CADASIL patient with renal involvement also had IgA renal disease [76]. However, kidney arteries and arterioles with characteristic aSMCs show dramatic vascular changes in CADASIL [105] but not necessarily associated with overt renal dysfunction. Tissues, such as the skin, with capillaries consisting of pericytes plus endothelium with gap junctions, generally show no obvious tissue lesions other than the capillaries themselves and arterial wall alterations.

Organs formed by tissues with capillaries not endowed with endothelial tight-junctions and pericytes present little or no apparent abnormalities although functionally the local microcirculation may be affected [5]. This is true of cardiac and skeletal muscle per se and peripheral nerves that exhibit no distinct morphological modifications. Typical of the CNS, capillaries with tight-junction endothelium and specialized pericytes forming the BBB exhibit the most significant parenchymal lesions leading to distinct neurological sequelae in CADASIL. Histopathological evidence suggests that the type of capillaries in tissues rather than the arterial lesion is the determining factor in CADASIL. This is unlike the lesions in hypertension [15,77], hypercholesterolemia, hyperhomocysteinemia and diabetes. The first transgenic mice model of CADASIL (*TgNotch3Arg90Cys*) provided confirmation of this fact because without involvement of capillaries there were no apparent brain lesions observed even at 24 months of age [108] and in the absence of N3ECD or GOM.

Despite the occurrence of consistent alterations in the BBB capillary, the distribution of lesions in the CNS in CADASIL is heterogeneous. This may be also explained by the specific structural organization of the brain. The cortex is mildly involved even though the capillaries show pericyte damage and GOM deposition [105]. In contrast, the deep white matter, the basal ganglia and the brain stem display typical lesions of myelin pallor with rarefaction of the hemispheric WM and lacunes. Cerebral blood flow is centripetal, and the cortex receives blood supply before the white matter. Capillary density varies according to the activity and metabolic demands of the particular brain region. Microvascular density in the gray matter of the brain is three to seven times greater than in the WM, and sensory centers are more richly supplied than motor centers [46,73].

The impact of other barriers such as the blood-retinal barrier and the blood-CSF barriers in the pathogenesis of CADASIL is not clear. Previous studies have shown arteriolar narrowing and arteriovenous nicking occurs in more than 80% of symptomatic CADASIL patients[53]. However, retinal infarcts or vascular occlusions were not evident in the retina, but a patient did exhibit ischemic injury in the optic nerve head that caused acute visual loss [110]. On the other hand, retinal capillary blood flow was mild to moderately reduced but this did not seem to cause major ischemic injury [54]. This suggests mild changes occur in the blood-retinal barrier in CADASIL, but further ultrastructural evidence would be needed to make firm conclusions whether the cellular make up behind the barriers are affected. With reference to the blood-CSF barrier, which is formed by distinct tight-junctions between epithelial cells within the choroid plexus, there is virtually no information on anomalies in these unique cells, which alter with age [25,75]. Although various proteins including inflammatory-immune system markers are evident in the CSF of CADASIL patients [33,42,126], the quality and volume do not appear to be grossly altered suggesting the secretion of CSF is similar to that in sporadic SVD. However, the arteriolar network within the choroid plexus is endowed with vSMCs which would similarly degenerate and accumulate N3ECD (RK, unpublished observations).

5. The critical role of the pericyte

There is much evidence to suggest that pericyte loss or damage is key to CADASIL. Ruchoux and Maurage [106,109] had first described endothelial changes in muscle and skin biopsies in patients diagnosed

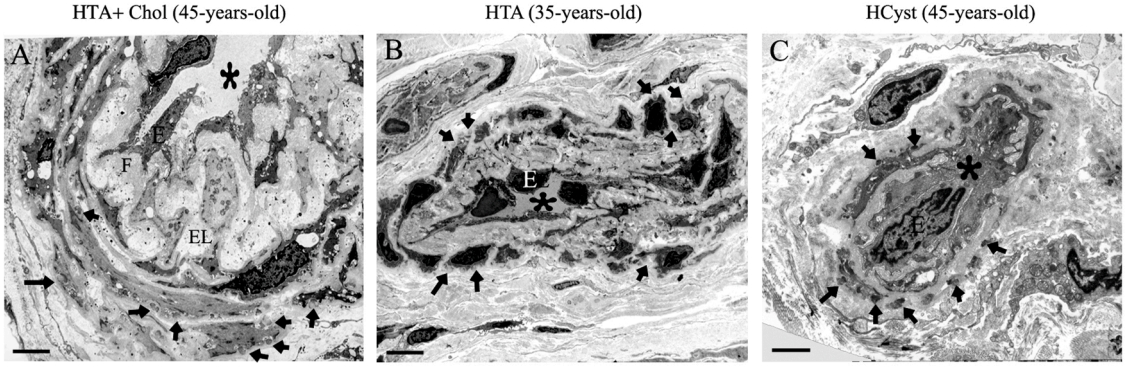

Fig. 2. Comparison of skin arterioles in CADASIL patients with vascular risk factors. A, A 45-year-old patient presenting a high level of LDL cholesterol (Chol) and hypertension (HTA). B, A 35-year-old hypertensive patient. C, A 45-year-old patient with hyper-homocysteinemia (HCyst). The lumen (star) were dramatically narrowed and stenoses were obvious. In (A) remaining fragments of aSMCs surrounded by electrolucent material corresponding to elastine (EL) are overloaded with calcium deposits. Fibrilline (F) formed a large network under the endothelium. aSMCs were fragmented and many GOMs (arrow) were observed in the remaining external layers. In (B), notice the disorganization of the media. Many GOM deposits (arrow) were observed surrounding them. The vessel wall appears to be made up with a cloudy material, some debris and rare calcium deposits. The patient presented HTA and elevated homocysteinemia (~12 μmol/l). In (C), the vessel was completely collapsed, and its wall was replaced with a cloudy material associated with remaining thin aSMC fragments. Numerous GOM deposits (arrow) were observed in this cloudy material. The patient presented severe hyperhomocysteinemia (24 μmol/l). Scale bars = (A) 3 mm; (B) 6 mm; (C) 3 mm. E, Endothelium. (Original magnification (A) 2784x, (B) 1293x, (C) 2784x).

Table 1
Semi-quantitative summary of tissue lesions and organ damage in CADASIL according to capillary type.

Tissues	Arteries	Capillaries	Organ Lesions				
	vSMCs	Endothelium	Pericytes	Endothelium type			
				Sinusoid	Gap	Tight	BBB	BBB Damage
Endocrine Tissues	++	+		X				0
Liver	++	+		X				0
Kidney	++	+		X				0
Pancreas	++	+		X				0
Skin	++	+	5–10%		X			0
Skeletal muscle	++	+	5–10%			X		+
Cardiac muscle	++	+	5–10%			X		+
Retina	++	+	95–100%				X	++
Brain (Cortex)	++	+	60–75%				X	+++
Brain (WM)	++	+	50%				X	++++
Brain (deep gray matter)	++	+	50%				X	++++

Symbols show 0, no lesions; +, slight lesions; ++, moderate lesions; +++, severe lesions; ++++, most severe lesions. Percentages indicate the frequency of presence of pericytes and variation of their number depending on the tissue. The retina harbors a 1:1 ratio with endothelial cells. X, indicates type of capillary.

with CADASIL in the late 90s. As stated above, however, these were not necessarily accompanied by any specific tissue changes. Irrespective, these findings paved the way for studies on endothelial permeability in CADASIL. Utilizing an in vitro BBB model, Ruchoux et al. [107] demonstrated that pericytes are required to increase the BBB permeability, suggesting that pericyte damage plays an important role in permeability impairment that occurs during the first decades of life in CADASIL patients. More recent studies have highlighted the intimate interactions existing between endothelial cells and pericytes, indicating that impairment in one capillary associated cell type will inevitably affect other elements within the neurovascular or gliovascular unit [6,29,45,70,83]. In accord with this, in an in vitro culture study, Dente et al. [30] showed that addition of pericytes at ratios ranging from 10:1 to 5:1 increased the barrier compared to endothelial cells alone. Co-cultures at a 1:1 ratio exhibited the best barrier, significantly better than any other culture ratios. Thus, alterations in pericytes could occur because of aberrant NOTCH3 mediated interactions with the endothelium [29].

In some robust ultrastructural studies, Dziewulska and Lewandowska [38] independently examined microvessels in eight autopsy brains and five skin-muscle biopsies of 13 CADASIL patients aged 38–73 years, half of whom had genetic confirmation. In agreement with our observations, these authors found degeneration and loss of pericytes in capillary vessels in every case. Pericytes were shrunken, with vacuolated cytoplasm containing large vesicular structures and complexes of enlarged, abnormal mitochondria. [57] Degenerative changes were also observed within endothelial-pericyte connections, especially involving peg-and-socket junctions. These observations collectively provide strong morphological support for the central role of pericytes in the pathogenesis of CADASIL. This phenomenon was observed very early in CADASIL before development of other pathological changes in vessel walls [38].

It is not unlikely that degrees of pericyte-endothelial disconnection will depend on densities of pericytes per capillary length or endothelial cells. Thus, high pericyte-endothelium ratios such as that in the retina [30] suggests the occurrence of relatively milder lesions in patients with CADASIL [52,53,95,102]. Moreover, only pericyte involvement resulting in impaired BBB capillary permeability could explain the absence of lesions in the *TgNotch3Arg90Cys* mice [108], the lack of renal dysfunction in CADASIL patients, the absence of significant retinal parenchymal lesions, the heterogenous distribution of brain lesions and the absence of angiogenesis in a chronically hypoxic environment.

An important question is how does mutant NOTCH3 or it's signaling impact on pericytes? This is basically not known but both N3ECD deposits and GOM are associated with pericyte cell bodies (Fig. 1) and membrane folds even in the WM [38,137]. However, this is not apparent in all transgenic mouse models [4,108]. Irrespective, it is still unclear whether GOM or the N3ECD is also produced by pericytes [27] although there is ample evidence that pericytes express NOTCH3 [57,131,132]. Previous studies in cell model systems have further reported equivocal findings including that Notch3 deficiency reduces pericyte coverage [74] and mutant NOTCH3 impairs pericyte differentiation [132] or not affect pericyte platelet-derived growth factor receptor-β expression in vSMCs but only their morphology [57].

6. Progression of vessel pathology

To better understand the pathogenesis of the microvascular lesion, we have followed the evolution of vessel lesions in CADASIL correlating: i) skin biopsies, ii) the TgNotch3Arg90Cys mice and iii) late sequelae of brain lesions. Fig. 3 schematically illustrates the postulated evolution of brain arterial and capillary lesions. The earliest lesion is a progressive loss of anchorages of aSMCs and pericytes to adjacent extracellular matrix and cells. This process leads to an early increase in the subendothelial space (Fig. 3A, E). We also noted similar changes at 10 months in the TgNotch3Arg90Cys mice expressing an archetypal CADASIL mutant *NOTCH3* (Fig. 4, cf. A-B and C) [108].

The loss of anchorage was also observed in life in an 8-year-old girl whose father harbored a *NOTCH3* mutation (Fig. 4A-B). In skin and skeletal muscle, at this stage, pericytes and aSMCs exhibit an irregular holly-leaf shape and fragmentations (Fig. 4A-B) with the parenchyma largely preserved. Despite all the above-mentioned changes, the lumen is perfectly preserved (Fig. 4). Early changes are followed by a dramatic evolution in CADASIL vessels according to age, with striking increase in loss of anchorages along with fragmentation of pericytes and aSMCs (Fig. 5A-C). Moreover, some holes and indentations occur at the periphery of the pericytes and aSMCs filled with dense highly-osmiophilic, fine GOM deposits (Fig. 5A-C). GOM was initially found around 20 years of age.

In the brain, the walls of arteries undergo similar transformation (Fig. 6). Rupture of anchorages likely separates aSMCs leading to loss of cohesion within the vessel wall and increased vessel-wall thickness without true lumen decrease or stenosis (Fig. 6A). In capillaries, the rupture of anchorage may lead to progressive loss of the areas called "peg-socket contacts" [31] although BBB dysfunction [125] does not appear to be a constant feature in CADASIL [12,101]. New insights into the molecular mechanisms of endothelial–pericyte interactions have suggested the complexity of the endothelial-pericyte communication that is dependent on endothelial-pericyte contact, as well as on astrocyte-endothelial contacts in the CNS [12]. Independent of disruption in pericyte contacts, it is likely that other pathways including caveolae within endothelial cells contribute to the disintegration of neurovascular unit components with expected impact on localized tissue prefusion and uncoupling. For example, Chow et al. [26] reported that

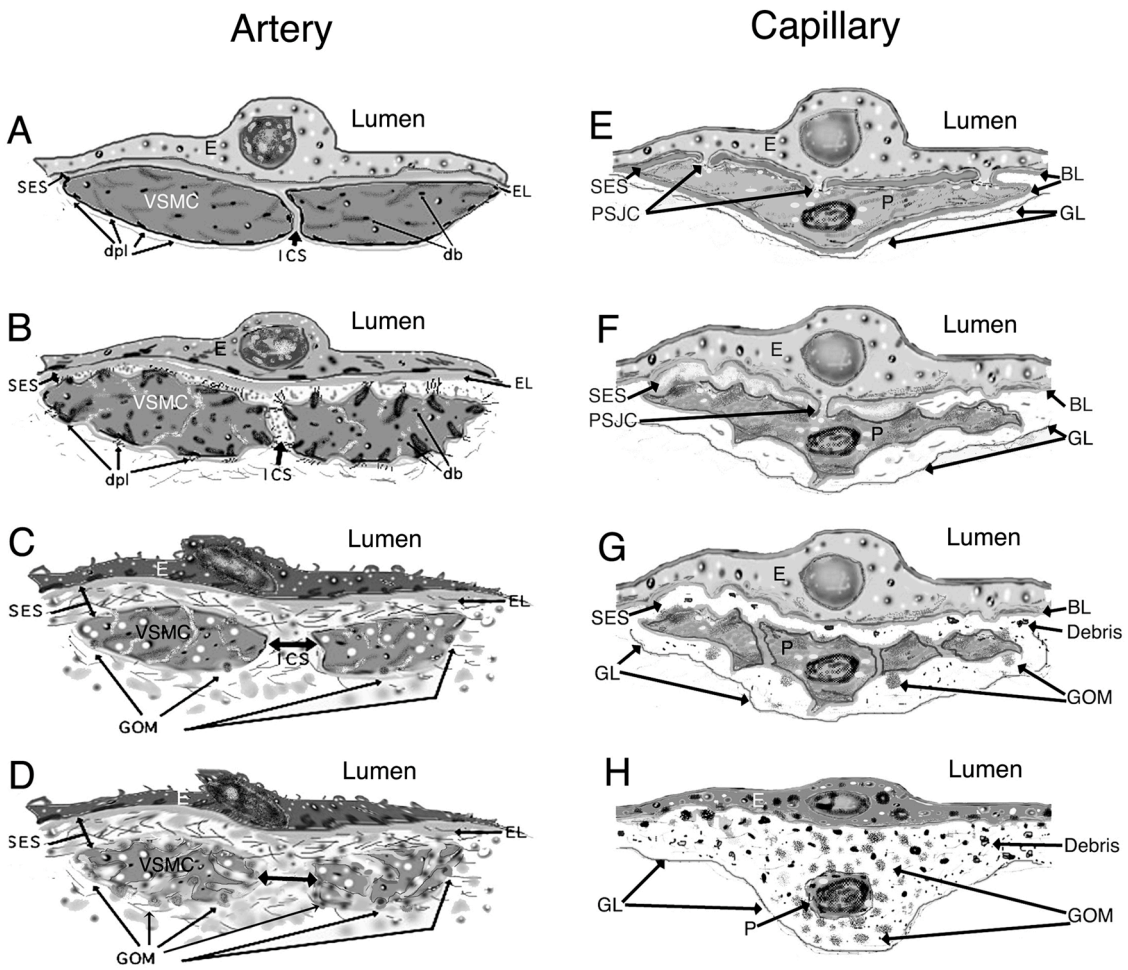

Fig. 3. Schematic representation of arteriolar and capillary changes in CADASIL. A-D, Artery changes in patients and transgenic mice (B, C, D). (A) Normal vessel wall. (B) First modifications observed within the vessel wall of a young CADASIL patient (8-years-old) and of young mice (10-months-old). The sub-endothelial space (SES) and the inter-smooth muscle cell spaces (ICS) were widened. The contractile aspect was obvious with an increase in dense bodies (db) and dense plaques (dpl) and a general holly-leaf-like aspect. C and D, Evolution of aSMC alterations with dramatic separation of the different wall cells and the presence of GOMs (arrow) observed at around 20 years of age in humans and 14 months in transgenic mice. aSMCs showed several fragmentation areas. (E: endothelium; EL: elastica lamina). E-H, brain capillary changes in patients (F, G, H). (E) Normal capillary with peg-socket-junctional complex (PSJC). Notice the endothelium (E) basal lamina (BL) embedding the pericyte (P) and the presence of the glia limitans (GL). F, First modifications observed in young patients: the progressive detachment of the pericyte with stretching of the remaining PSJCs. The pericyte gradually appears to lose the endothelium contacts. G, The endothelium-pericyte contacts were almost all lost. The fragmentation of the pericyte and the loss of cytoplasm were beginning. H, The end-stage where the pericyte nucleus is isolated, surrounded with a rim of cytoplasm. GOM is held back by the glia limitans (GL).

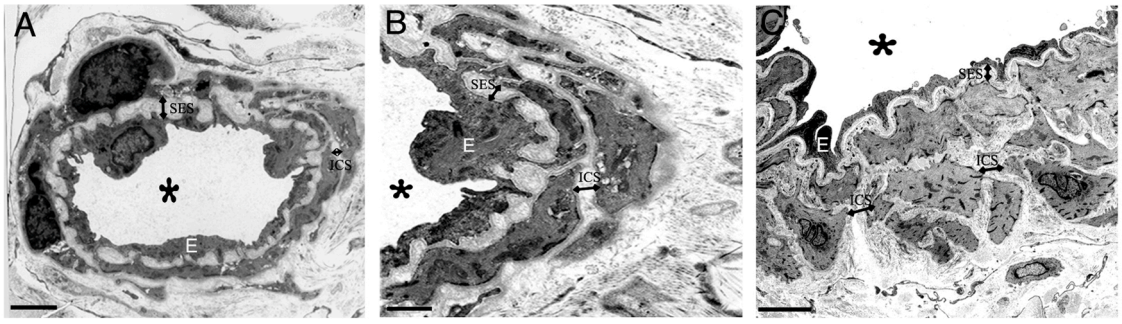

Fig. 4. Arteriolar changes in CADASIL and TgNotch3Arg90Cys mouse. A, B, Skin arteriole from an 8-years-old girl with severe migraine, whose father presented a *Arg90Cys NOTCH3* mutation. The endothelium (E) is highly osmiophilic. The sub-endothelial space (SES) was enlarged with a loss of medio-endothelium junctions. The aSMCs displayed a contractile aspect with numerous fragmentations. The inter-smooth muscle cell spaces (ICS) were enlarged, and inter-cellular junctions were already lost. The lumen (star) size was normal. C, Tail arteriole from a 10-months-old transgenic mouse. Notice the thin and osmiophilic endothelium (E). The sub-endothelial space (SES) and the inter smooth muscle cell space (ICS) are widened. Some aSMCs are already isolated from the others. The contractile aspect was obvious. Scale bars = (A) 4 μm; (B) 2 μm; (C) 8 μm. (Original magnification: (A) 2784x; (B) 6000x; (C) 1670x).

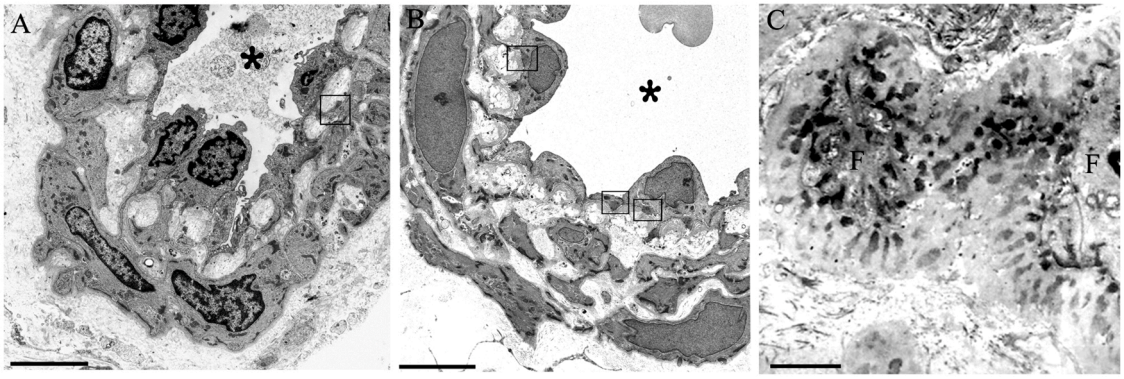

Fig. 5. Evolution of skin vessels lesions in CADASIL. A-B, Two skin arterioles from a 20-year-old patient (A) and a 40-year-old patient (B). C, aSMC fragments from a 53-year-old patient (C). (A, B) The lumen (star) sizes were normal. The endothelium (E) was highly osmiophilic and showed stress bands (square). Sub-endothelial space (SES) and inter-smooth cell space (ICS) were dramatically enlarged. aSMC fragmentation started in the younger patient and was advanced in the oldest one with total loss of junctions between cells. GOM was seldom found in the younger patient but was frequently seen in the oldest one. C, Remaining aSMC fragments (F) recognizable with their circlet of GOMs. Scale bars = (A, B) 500 nm (C) 1 μm. (Original magnification: (A, B) 2784x; (C) 6000x).

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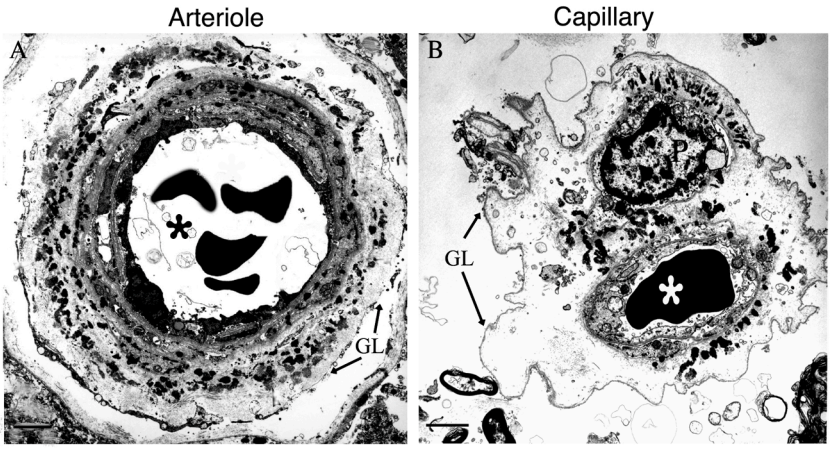

Fig. 6. Brain Arteriolar and Capillary changes in CADASIL. A-B, 57-year-old patient: brain arteriole and capillary from autopsy material. Both lumen (star) sizes were conserved. Numerous GOM deposits are seen spreading out, held by the glia limitans (GL). In the capillary, the pericyte nucleus (P) was surrounded with a thin rim of cytoplasm. The pericyte appears isolated and floating in a widened and empty area surrounded by the glia limitans (GL) retaining numerous GOM deposits. An erythrocyte (star) occupying nearly the whole lumen is also seen in the capillary. Also, note the enlarged perivascular space. Scale bars = (A) 5 μm; (B) 3 μm. (Original magnification: (A) 6650x; (B) 13000x).

endothelial cells mediate vasodilation actively relaying signals from aSMCs via a caveolae-dependent pathway.

Rupture at sites of anchorage could lead to progressive decrease in pericyte reactivity to endothelium and astrocyte stimulations. This may result in decreased secretory function that becomes more marked with the progressive loss of cytoplasm. Pericytes also secrete vascular endothelial growth factor or VEGF [69,71,139], the major permeability factor and in the absence of adequate stimulation their secretory power may be modified resulting in impaired permeability probably leading to tissue damage with impaired perfusion [128]. Finally, the loss of junctions between endothelium and pericyte leads to progressive separation of the pericyte cell, which appears isolated in the edematous extracellular matrix limited by the glia limitans (Fig. 3H) (Fig. 4B).

Thus, based on morphological observations we propose two stages in CADASIL: First, the progressive impairment and loss of contacts between endothelium and pericyte predicts neurovascular and gliovascular dysfunction. Second, the progressive degeneration of capillary pericytes that impacts on contractile function with apparent BBB damage and diffuse hypo-permeability [107,128]. Concurrently, foci of artery leakage (microbleeds) have been observed as a consequence of the dramatic alteration of the affected blood vessels walls [24,32]. Consistent with there is new imaging evidence [32,68,125] to support previous pathological examination [68] showing cerebral iron deposits, particularly in the lenticular nuclei (Fig. 1C). Vasotonic impairments could be observed early in human patients [3,23,80,82,94,97,124] as well as in transgenic mice [60] but do not appear to be determining factors during the three first decades of life [124]. On the other hand, vasotonic impairments added to the effects of additional vascular risk factors worsen the progression of the disease during the last decades of life in CADASIL patients (Fig. 2).

7. Perivascular spaces and veins

Dilatation of perivascular spaces is a typical feature of CADASIL lesions in the brain (Fig. 1). According to Duvernoy et al. [37] perivascular spaces in the brain parenchyma include the Pfeifer space and the Virchow–Robin space. The Pfeifer space [98] is the periarterial and perivenous circular zone of neural tissue devoid of capillaries; neural tissues in the Pfeifer space depend on the most distal blood supply from capillaries given that capillaries but not arteries, are the site of blood-tissue exchanges. The Virchow–Robin space is an extension of the subarachnoid pial sleeve surrounding deep perforating brain [7,50] and is formed by two pial coats in the basal membrane [99].

In CADASIL, Virchow-Robin spaces are constantly dilated (état criblé, status cribrosus), particularly in the subcortical white matter (66%) and the lentiform nuclei (94%) [28]. Likewise, in CADASIL, Pfeifer spaces around arteries and veins present an incomplete lacunar infarction [77]; interestingly, brain tissues away from the artery present less CADASIL alterations. This observation is fundamental since the cylindrical areas named “Pfeifer spaces” surrounding brain arteries are

involved first, and to a greater extent, in a chronic ischemic process including CADASIL. This observation reinforces our argument that pericytes in capillaries are important in the pathogenesis of the neural lesions in CADASIL.

Pericytes or pericyte-like cells within venules and veins likely also contribute to the pathogenesis of CADASIL. Moody and colleagues [90] demonstrated that periventricular venous collagenosis, which causes venous insufficiency and vasogenic edema is strongly associated with periventricular WMLs [14,16,67]. Sims [118] had reported that pericytes are most abundant on venules suggesting a protecting role for microvessel wall integrity from the mechanical effects of post-arteriolar hydrostatic pressures. In CADASIL, Rafalowska and colleagues [100] noted constant involvement of cerebral veins and interestingly some patients with *NOTCH3* mutations also present with prominent varicose veins [113]. Perturbations in pericytes in cerebral veins may lead to dilatation of veins, increase of post-arteriolar hydrostatic pressures and impair drainage of the interstitial fluid. Changes in pericytes in cerebral veins and venules could also explain the typical WM hyperintensities observed in the temporal poles in CADASIL patients. Yamamoto et al. [138] concluded that these lesions represent enlarged perivascular spaces with degeneration of myelin accompanied by lack of drainage of the interstitial fluid [20].

Overall disintegration of aSMCs and pericytes and damage to capillaries would induce loss of its supporting function like in other vascular pathologies [7,28,50,99] with alterations of the vessel wall, low vascular compliance, elongation and tortuosity equivalent to premature aging of the vessels resulting in état criblé. Loss of autoregulation due to degeneration of pericytes is responsible for the pathogenesis of the ischemia and lacunar infarcts [68,85,113] although diffuse vasogenic brain edema is never present as part of the progressive atrophy occurring in the CADASIL brain [96]. In addition, focal microbleeds corresponding to hemorrhagic leaks from small vessels occur in the brain of CADASIL patients (cf. Fig. 1C) and increase with age and likely with risk factors [32]. In autopsy cases, siderophages were found in the vicinity of small blood vessels of 100 to 300 μm diameter; a significant correlation exists between age and number of microbleeds found after the third decade [32].

8. Implications for other disorders with SVD

Pericyte alterations may be also the potential cause of SVD in the common forms of sub-cortical vascular dementia, as well as in cases of Alzheimer dementia associated with vascular risk factors [103,104]. From our personal observations of 500 skin biopsies from patients with sporadic SVD, aSMCs and pericytes were found to be involved in most cases (MMR, unpublished). Therefore, disturbed circulation and perfusion inducing a chronic hypoxic state appears to play an important role in the pathogenesis of WMLs in the aging brain [41,56].

In Alzheimer's disease, dysfunction of the BBB and alterations of the neurovascular unit [112,140] comprised of aSMCs, pericytes, glia and neurons along with changes in the capillary bed [56,72], are well-accepted components of the neurodegenerative process [122]. As key elements of the BBB, pericyte changes specifically in cellular processes rather than the soma (possibly shruken) have been documented in Alzheimer's disease [34,35,40,56]. This may cause alterations of the endothelial surface of the vascular basement membrane particularly in brain regions affected by Alzheimer pathology [56,88,122]. Moreover, amyloid β (Aβ) induces perivascular cell pathology with degeneration of vSMCs and consequent pericyte injury in the cerebral vasculature of patients with Alzheimer's disease and, in tissue culture, human brain pericytes are more vulnerable to Aβ-induced degeneration than leptomeningeal vSMCs [39,86,129]. Collectively, these observations demonstrate that pericytes may be modified by a number of mechanisms including the presence of potential toxic elements or abnormally proteins as in neurodegenerative diseases.

Alterations of endothelial cells and pericytes occur in transgenic mice including pericyte-deficient mutations such as the Pdgfrβ$^{F7/F7}$ mice [88,121]. Of interest, pericyte coverage on capillaries cause overt BBB disruption to serum proteins confirming the critical role of pericytes on the BBB at the capillary level [133]. However, such leakage or seepage of plasma or even hemosiderin deposition is not consistent in CADASIL and may need confirmation in further studies [101]. Pericytes and perivascular microglial cells are also known to take up Aβ in the vascular wall [121,134,135]. Pericytes are in direct contact with brain interstitial fluid and strongly express the low density lipoprotein receptor-related protein 1 (LRP1) receptor [48], and the Aβ degradation enzyme neprilysin (NEP) [21]. LRP1 and NEP expression are decreased both in pericytes and vSMCs, and in the endothelium of cerebral vessels in Alzheimer's disease [127]. Saint-Pol and colleagues [114] showed that brain pericytes express ATP-binding cassette, sub-family A, member 1 (ABCA1), which mediates cholesterol efflux. Therefore, pericytes interact with Aβ peptides and have a potentially critical role in the removal of amyloid from the brain [47]. There is considerable evidence that LRP and receptor for advanced glycation end products (RAGE) expression are altered in Alzheimer's disease [59]. Increased expression of transcription factors required for pericyte differentiation, such as serum response factor (SRP) and myocardin (MYOCD) occurs in Alzheimer's disease [11], indicating a dysfunction of LRP-mediated clearance of Aβ peptides in cerebral pericytes and other cerebrovascular cells.

9. Therapeutic opportunities

We believe that new avenues for therapeutic research in CADASIL should emphasize alterations of permeability secondary to pericyte loss. Previous trials have focused on vasomotor function in patients [82,97,124] and in transgenic mice [63] but have failed to advance the field since only tissues depending on the BBB appear covertly affected, compared to the other non-affected tissues, even though they all share an abnormal systemic arterial tree [105].

Simpson et al. [117] have shown that WMLs may arise through tissue ischemia but may also reflect the contribution of additional factors like BBB dysfunction. It has become evident that brain pericytes, besides their regulatory activities in brain vessel function, BBB and homeostasis, also possess a number of functions including the capacity for regeneration adopting stem cell properties [71]. This potential property of perictyes, however, is not without controversy and may depend on the milieu with the tissue [18,51]. Exploring the potential plasticity of the pericyte for neurorepair could provide the long-expected treatment for CADASIL [78,91,92]. A therapeutic indication for pericyte protective agents (once developed) could be in the early stages of CADASIL. These patients will need to be treated early, perhaps as early as the first decade of life, before destructive brain lesions proliferate; this can be accomplished with available genetic diagnostic tests but poses ethical problems. Ideally, such treatment should be able to protect the pericytes prolonging their survival for the normal duration of life.

In summary, preservation of pericytes and neurovascular or gliovascular unit functions to retain local perfusion and autoregulation could be a worthwhile area of research for novel treatments. This could not only be implemented in CADASIL but also for SVD with vascular cognitive impairment and vascular dementia, as well as for aging-related diseases such as Alzheimer's disease.

Author contributions

Marie-Magdeleine Ruchoux: acquisition of the original data, analysis and interpretation of the results and, revising the manuscript.

Raj N Kalaria: drafting, revising the manuscript and interpretation of data.

Gustavo Roman: Analysis and interpretation of the results and, revising the manuscript.

Declaration of Competing Interest

The authors declare no competing financial interests

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