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► **To cite this version:**

Anne Joutel, Hugues Chabriat. Pathogenesis of white matter changes in cerebral small vessel diseases: beyond vessel-intrinsic mechanisms. *Clinical Science*, 2017, 131 (8), pp.635-651. 10.1042/CS20160380 . inserm-02067812

**HAL Id: inserm-02067812**

**<https://inserm.hal.science/inserm-02067812>**

Submitted on 14 Mar 2019

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# **Title: Pathogenesis of white matter changes in cerebral small vessel diseases: beyond vessel-intrinsic mechanisms**

**Short title: White matter changes in cerebral small vessel diseases**

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**Word count:** 7929

**Key words:** hypoperfusion, cerebrovascular reactivity, blood brain barrier, oligodendrocyte precursor cells

**Abbreviations:** BBB, blood brain barrier; CBF, cerebral blood flow; CSF, cerebrospinal fluid; CVR, cerebrovascular reactivity; DTI, diffusion tensor imaging; MRI, magnetic resonance imaging; MTI, magnetization transfer imaging; OPCs, oligodendrocyte precursor cells; SHRSP, spontaneously hypertensive stroke prone; SVDs, small vessel diseases; VLMCs, vascular and leptomenigeal cells; WM, white matter; WMHs, white matter hyperintensities

## **Abstract**

Cerebral small vessel diseases (SVDs) are a leading cause of age and hypertension-related stroke and dementia. The salient features of SVDs visible on conventional brain magnetic resonance images include white matter hyperintensities (WMHs) on T2-weighted images, small infarcts, macrohemorrhages, dilated perivascular spaces, microbleeds, and brain atrophy. Among these, WMHs are the most common and often the earliest brain tissue changes. Moreover, over the past two decades, large population- and patient-based studies have established the clinical importance of WMHs, notably with respect to cognitive and motor disturbances. Here, we seek to provide a new and critical look at the pathogenesis of SVD-associated white matter (WM) changes. We first review our current knowledge of WM biology in the healthy brain, and then consider the main clinical and pathological features of WM changes in SVDs. The most widely held view is that SVD-associated WM lesions are caused by chronic hypoperfusion, impaired cerebrovascular reactivity, or blood brain barrier leakage. Here, we assess the arguments for and against each of these mechanisms based on population, patient, and experimental model studies, and further discuss other potential mechanisms. Specifically, building on two recent seminal studies that have uncovered an anatomical and functional relationship between oligodendrocyte progenitor cells and blood vessels, we elaborate on how small vessel changes might compromise myelin remodeling and cause WM degeneration. Finally, we propose new directions for future studies on this hot research topic.

## BACKGROUND

Cerebral small vessel diseases (SVDs) represent different pathological processes that affect the structure or function of small arteries, veins, or capillaries in the brain. SVDs are involved in one-third of ischemic strokes and more than 90% of cases of intracerebral hemorrhage—the most devastating type of stroke [1]. Although long overshadowed by the attention paid to Alzheimer’s disease, SVDs contribute significantly to cognitive decline and dementia in the elderly [2]. The vast majority of SVDs appear to result from a complex mix of genetic and cardiovascular risk factors, the most important of which are age and hypertension. Among the important advances in the field of SVDs has been the identification of genes involved in Mendelian forms of adult-onset SVDs, which share a number of clinical and pathological features with their sporadic counterparts [3–7]. Among these, CADASIL (Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy), caused by highly stereotyped mutations in the NOTCH3 receptor, has emerged as the most common hereditary cause of SVDs and is now recognized as a genetic paradigm of these diseases [8]. Despite the profound impact of SVDs on human health, apart from the management of vascular risk factors, no treatment specific for SVDs is currently available.

Whereas cerebral small vessels are difficult to investigate in humans, their pathological consequences are easily recognized on images obtained by conventional magnetic resonance imaging (MRI). These include not only small infarcts, intracerebral hemorrhages, but also white matter hyperintensities (WMHs), defined as patchy or confluent hyperintensities without tissue cavitation on T2-weighted or Flair images; microbleeds, corresponding to small foci of chronic blood products in the brain tissue; abnormally enlarged perivascular spaces, which become visible on high resolution MR images; and brain atrophy. Among these imaging markers, WMHs are the most common and often earliest tissue lesions that can be observed in vivo. More sophisticated imaging techniques that are particularly sensitive to white matter (WM) tract integrity, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI), have recently allowed the detection of subtle WM damage before it becomes visible on conventional MR images, suggesting that WMHs may represent only the tip of the iceberg [9].

Over the past two decades, WMHs have been the subject of numerous studies. We have learned that the prevalence of WMHs is strongly related to age and hypertension. In the general population, WMHs are detected in ~90% of individuals older than 60 years of age [10,11], and in approximately 30% of these cases, WMHs are moderate or severe [12]. WMHs can also expand with aging and/or hypertension; thus, both baseline and changes in blood pressure are significant predictors of their degree of progression over time [13]. The baseline volume of WMHs also appears to be one of

the strongest predictors of their future spread [14]. Finally, both the severity and progression of WMHs are independently related to major clinical outcomes in elderly subjects, and particularly impact both cognitive and motor disturbances [15]. Hence, quantitation of WMH volumes, especially in the context of aging or hypertension, has been proposed as a potential surrogate marker for future intervention trials in SVDs [16].

The most widely held view is that SVD-associated WM lesions are caused by chronic hypoperfusion, impaired cerebrovascular reactivity, or blood brain barrier (BBB) leakage [2]. However, is there any clear evidence for these different mechanisms? This review aims to provide an original and critical look at this issue and suggests future research directions. For this purpose, we first set the scene by reviewing the most recent knowledge of WM biology. We next briefly discuss the main features of SVD-associated WM changes, assuming that age-related WMHs are part of this spectrum. We then critically examine the classical mechanisms put forward in the literature to explain the development of WMH. Finally, we propose alternative mechanisms to account for these lesions; specifically, we discuss the hypothesis that dysfunction of oligodendrocyte lineage cells is related to changes in small vessels.

## WHITE MATTER BIOLOGY IN THE HEALTHY BRAIN: SETTING THE SCENE

The WM, which occupies roughly half of the brain in humans and about 14% in rodents, mediates the essential connectivity between the different gray matter regions throughout the brain [17]. The axon and its associated myelin sheath are morphologically and metabolically coupled and constitute a functional unit. Myelin is essential for the rapid propagation of action potentials along nerve fibers, and achieves this by electrically insulating axons and restricting action potentials to short segments of unmyelinated axons (i.e., nodes of Ranvier). Moreover, both oligodendrocytes—the myelin-forming cells—and myelin itself provide the key trophic support to axons needed for their long-term integrity and survival [18].

***Myelin appearance and structure.*** Myelin sheaths are formed as a multi-layered stack of self-associating plasma membranes that originate from oligodendrocytes and are tightly wrapped around the axon in a spiral fashion. The innermost lamella, which is not compacted, consists of a cytoplasmic compartment (inner tongue) facing the axonal internode along its length and connected to the oligodendroglial soma by nanometer-wide “myelinic channels”. The outermost lamella also retains some cytoplasm (outer tongue), which connects the segmental length of myelin to the cell body of the parent oligodendrocyte (Figure 1). Adjacent to the nodes of Ranvier, where the axonal membrane is exposed to the extracellular space and action potentials are generated, are the paranodes. At this level,

the spiraled lamellae of myelin, which are not compacted, gradually terminate and contain cytoplasmic processes called paranodal loops [18].

Compared with other plasma membranes, myelin is a poorly hydrated structure with only 40% water and a very high lipid content (70–80% of its dry weight). It is an extraordinarily stable structure that likely owes its stability to its unusual lipid composition, which includes high levels of saturated long-chain fatty acids [19]. Another feature of myelin is its relative metabolic stability after completion of myelination, characterized by slow turnover. In this context, the half-life of cholesterol in myelin has been estimated to be longer than 7–8 months [20], and components of the myelin sheath have been shown to be the most long-lived proteins in the mouse [21].

Assessing the fine structural abnormalities of WM tracts requires electron microscopy analysis, a technique that is particularly demanding because of the risk of artifactual shearing defects, particularly because of the high lipid content of myelin. Myelin, and especially the close cytoplasmic-rich structures, appears much better preserved with the use of high-pressure freezing followed by freeze substitution than with conventional aldehyde fixation [22].

***Oligodendrocyte precursor cells.*** Oligodendrocytes develop from glial progenitor cells, called oligodendrocyte precursor cells (OPCs). During embryonic development, OPCs emerge from discrete regions of the ventricular germinal zone of the brain, following a ventral-to-dorsal wave. From these areas, OPCs proliferate and migrate widely throughout the brain before differentiating into myelin-forming oligodendrocytes. Genetic-lineage tracing experiments in mice have provided new evidence that OPCs remain largely committed to the oligodendrocyte lineage in the healthy brain as well as in disease states [23]. Maturing OPCs pass through different stages of differentiations that can be sequentially grouped into committed oligodendrocyte precursors, newly formed oligodendrocytes, myelin-forming oligodendrocytes and mature oligodendrocytes, based on morphological criteria and their repertoires of expressed genes [24].

Notably, a significant proportion of OPCs remains in a precursor state in the adult brain. They are nearly uniformly distributed throughout both the grey and WM, and their numbers remain more or less constant, representing about 5% of all cells in the central nervous system. Using in vivo two-photon imaging in the upper layers of the adult mouse cortex, Bergles and colleagues showed that OPCs are highly dynamic and maintain exclusive territories through self-repulsion mechanisms. The loss of a single OPC through cell death or differentiation is rapidly compensated by the appearance of a new and immediately adjacent OPC [25].

***Myelin assembly.*** Once an oligodendrocyte is engaged in the axon myelination process, its plasma membrane undergoes dramatic changes, resulting in as much as a 6,500-fold increase in

membrane area. Seminal work from Simons and colleagues recently revealed that the future myelin sheath grows and wraps around the axons in two coordinated motions that occur fairly simultaneously: the wrapping of the leading edge at the inner tongue around the axon that lies beneath the preceding layer, and the longitudinal expansion of the myelin sheath towards nodal regions (Figure 1) [26]. Yet, it is still unclear how oligodendrocytes recognize the axons that need to be myelinated and how they establish stable contacts.

Another critical process in myelin biogenesis is compaction of the multiple membrane layers, which must be coordinated with myelin growth. Myelin compaction starts in the outermost layers and progresses inward; this process critically requires the removal of molecules that prevent compaction as well as polymerization of myelin basic protein (MBP), which acts as a “zipper” to close adjacent plasma membrane bilayers [27]. Critical for the conversion of MBP molecules from the soluble phase to the polymerized state is the double-phenylalanine motif in the amphiphatic helix formed by amino acid residues 85–92 [28]. Notably, Simons and colleagues recently showed that a MBP monoclonal antibody raised against an epitope adjacent to this double-phenylalanine motif (QD9 clone), previously known to specifically stain degenerated myelin [29], actually recognizes MBP molecules in their depolymerized, and thus nonfunctional, state [30]. Indeed, this peculiar epitope is masked when MBP molecules are polymerized (normal compacted myelin) and becomes unmasked when MBP molecules are depolymerized and converted into their dispersed phase—that is, when myelin undergoes uncompaction or degradation.

In the brain, a single oligodendrocyte usually myelinates different axons and several internodes per axon [18]. This may have important implications in pathology, as the destruction of only a few oligodendrocytes may result in extensive demyelination.

***Dynamics and plasticity of myelination and oligodendrocyte generation in the healthy adult brain.*** In the mouse, the vast majority of oligodendrocytes are produced during the first month after birth, and myelination is almost completed at 2 months in most brain regions [31]. Nevertheless, nearly all OPCs throughout the brain continue to divide during adulthood for at least 8 postnatal months. The cell-cycle duration depends on the brain region, and is shorter in the corpus callosum and longer in the cortex. Notably, the cell division cycle of OPCs increases with age, indicating that the ability to form new oligodendrocytes decreases with age [32]. Importantly, Richardson and colleagues showed in the mouse that, during the first year of life, up to 40% of OPCs can differentiate into mature oligodendrocytes and form myelin sheaths, even within almost fully myelinated WM tracts [32]. Notably, adult-born oligodendrocytes produce much shorter, and consequently more internodes than early-born oligodendrocytes. Hence, experimental studies in the mouse suggest that myelin is

continuously remodeled in the adult and that oligodendrocyte turnover contributes significantly to this process, either through replacement of oligodendrocytes that died or by intercalating new myelin internodes among existing ones [32].

Remarkably, oligodendrogenesis and myelination can also be triggered by neuronal activity in the mouse. Monje and colleagues showed that optogenetic stimulation of the premotor cortex in the mouse promotes OPC proliferation and differentiation, increases myelination within the deep layers of the premotor cortex and subcortical WM, and improves motor function [33]. Conversely, short periods of social isolation negatively impact myelination of the prefrontal cortex, which is involved in social functions [34,35].

Various MRI methods, and particularly DTI, have revolutionized our ability to follow myelination within the human brain in vivo. Data indicate that the myelination process peaks during childhood. The myelination starts in the brainstem, develops progressively within the whole brain at least until the third decade of life, and ends within the frontal areas involved in higher-level executive functions [36,37]. Frisén and colleagues have established that, in the corpus callosum, the number of OPCs and oligodendrocytes stabilizes at about 5 and 10 years of age, respectively, whereas the volume of the WM reaches its maximum at 17 years of age, followed by a slow, gradual decline during aging [38]. Using a clever approach, in which the age distribution of DNA and proteins was estimated by integration of radiocarbon derived from nuclear bomb testing during the Cold War, these authors provided further evidence that the oligodendrocyte population in the human corpus callosum is much more static than that in the mouse, with a replacement rate of 1 in 300 oligodendrocytes per year and a generation rate at least 100-fold lower than that in mice. Notably, they also observed that myelin is exchanged at a higher rate, suggesting that, in contrast with rodents, oligodendrocyte turnover contributes minimally to myelin remodeling in humans. However, it is worth noting that these data were based solely on an analysis of the corpus callosum and that the same study found a higher replacement rate of oligodendrocytes in the grey matter, leaving open the possibility that the scale of myelin and oligodendrogenesis turnover may differ considerably depending on brain region. Moreover, in non-human primates, Peters and Sethares found evidence of age-related myelin remodeling, demonstrating replacement or intercalation of new, shorter myelin segments in some, but not all, WM tracts [39].

**Myelin aging.** Peters and colleagues have extensively analyzed the effects of aging on cerebral WM in non-human primates (rhesus monkeys) using electron microscopy [40]. No reliable data are available in humans owing to major limitations related to the preparation of cerebral tissue samples. These authors found that the two most common age-related defects consisted of focal splitting of

myelin lamellae, which can lead to a double myelin sheath profile and focal vacuolation (sometimes protruding on one side) within the myelin sheath itself. These myelin defects were detected in about 5% of nerve fibers in old monkeys, whereas only a few axons exhibited degeneration. There is also evidence of associated phagocytosis of myelin debris by activated microglial cells. Remarkably, these processes are highly reminiscent of those reported in different mouse models of antibody-mediated or toxic demyelinating diseases, suggesting that they may represent a common pathological feature associated with myelin degeneration [30]. Further studies in these latter mouse models suggest that myelin breakdown arises from disassembly of the MBP network, that is, from the conversion of polymerized MBP molecules to their soluble and non-adhesive state, leading to uncompactation of myelin [30]. Moreover, Simons and colleagues recently provided evidence that, in the healthy mouse, myelin fragments are gradually released from aging myelin sheaths and are subsequently cleared by microglia [41].

***Trophic support of axons.*** Studies of genetic mouse models and human pathology offer compelling evidence that axonal survival is dependent on intact oligodendrocytes, and that axonal degeneration can occur in chronically demyelinating lesions, even in the absence of inflammation. For example, mice lacking 2',3'-cyclic nucleotide phosphodiesterase in oligodendrocytes or the major proteolipid of myelin develop widespread, long-term axonal degeneration, despite the fact that their myelin sheaths are either ultrastructurally normal or show only minor abnormalities [42,43]. Recent studies suggest that oligodendrocytes are metabolically coupled to the adjacent axonal compartments. Glycolysis products (lactate or pyruvate) supplied by oligodendrocytes are transported to myelinated axons via the monocarboxylate transporter 1 (MCT1), which is expressed in internodal myelin, and then metabolized in axonal mitochondria for aerobic ATP generation [44,45]. Recent work from Nave and colleagues further suggests that the mechanism by which glycolysis in oligodendrocytes is matched to different axonal energy needs involves the release of glutamate through axonal activity [46]. Hence, glutamate release stimulates oligodendroglial NMDA receptors, which in turn mobilize additional glucose transporters into oligodendrocytes and myelin, thereby modulating their glucose uptake.

***Syncytium panglial.*** Oligodendrocytes are interconnected with each other and with astrocytes through abundant gap junctions, forming a functional syncytium [47]. Ependymocytes, the cells that line the brain ventricles and are in contact with the cerebrospinal fluid, are also extensively coupled to astrocytes and are part of this syncytium. It has been suggested that a major function of this so-called panglial syncytium is to buffer the potassium ions ( $K^+$ ), released by active axons, that accumulate in oligodendrocytes, and to release  $K^+$  and associated osmotic water into the capillaries [47]. Astrocytic endfoot processes, which ensheath the cerebral vasculature, are considered to be an important output

of this syncytium [47]. In support of this, changes in molecular components of this syncytium, particularly at the level of astrocytic endfoot processes, were found to ultimately cause myelin degeneration. For example, inactivation of the gene encoding Kir4.1, a weakly rectifying K<sup>+</sup> channel expressed in astrocyte endfeet as well as oligodendrocytes, causes myelin vacuolation in mice [48]. Yet, it is unknown whether specific inactivation of Kir4.1 in astrocytes is sufficient to produce the same phenotype. Also, mutations of the gene encoding MLC1, a protein that is mainly expressed in astrocytic endfeet, cause megalencephalic leukoencephalopathy with subcortical cysts (MLC), a human disease characterized by myelin vacuolation [49,50].

**WM blood supply and energy budget.** In humans, the blood supply to the brain WM is supplied almost entirely by medullary arteries. These arteries, which are long and thin, especially in the frontal lobe, arise at right angles from pial arteries, run perpendicularly through the cortical layers, and enter the WM to reach paraventricular regions. As they penetrate the WM, medullary arteries elaborate several long side branches with pedicels, dividing into many branches. Contrary to the common belief, periventricular arterial anastomoses among terminals of the deep WM arteries have been identified [51]. In contrast, WM tracts of the subcortical U-fiber region are supplied by shorter arteries, including subcortical arteries and short side-branches of medullary arteries [52,53]. In the mouse, the long medullary arteries are almost absent and the deep WM is supplied almost entirely by capillaries (AJ, personal observation).

Data from the rodent optic nerve and corpus callosum obtained through both experimental and computational studies indicate that the central WM consumes approximately 35% of the energy used by the grey matter—33-40  $\mu\text{mol}$  of glucose/100 g/min in the WM versus 50-200  $\mu\text{mol}$  of glucose/100 g/min in the GM—, a figure that likely reflects the far smaller density of synapses and lower energy used per synapse in the WM [54,55]. Such findings are in keeping with the observation that the density of capillaries in both human and murine cerebral WM is roughly 2- to 3-times lower than that in the cortex [53]. Importantly, computational studies contradict the common belief that, by reducing axon capacitance, myelination reduces the energy expenditure required by axon tracts to restore the resting ion concentration after each action potential. Indeed, available data suggests that this saved energy is outweighed by the energy needed for myelin biogenesis and maintenance of the oligodendrocyte resting potential [55]. Therefore, it seems that the main function of myelin is instead to allow faster nerve conduction.

## **SVD-ASSOCIATED WM CHANGES: THE MAIN FEATURES**

WMHs are commonly classified according to their location—periventricular versus deep/subcortical—and their extent, which can range from small caps or thin rims close to the ventricles

or punctuate foci in the deep WM to confluent periventricular WMHs extending into the deep/subcortical WM. Because of the physiological importance of the WM, as discussed above, it is not surprising that myelin damage, with or without associated axonal lesions, can impair conduction and thus sensory, motor, or cognitive functions.

***Clinical importance of WMHs.*** Over the past two decades, large observational, cross-sectional and longitudinal studies in the general population or among healthy individuals or symptomatic patients have provided meaningful insights into the clinical importance of WMHs. Baseline WM lesion load is associated with an increased risk of incident stroke, depression, cognitive decline, dementia and death, as well as with gait difficulties and urinary urgency at the cross-sectional level [15,56,57]. Progression of WMHs is particularly associated with a faster decline in global cognitive function, as well as in executive performances and information-processing speed [15]. These findings are consistent with the idea that WM damage primarily results in cognitive slowing and that suboptimal conduction velocity caused by myelin alterations may impair the speed of information processing [17]. However, interpretation of the relationship between WMHs and neurological manifestations is often complicated by the presence of comorbid brain pathologies in elderly people. Taking advantage of the fact that WMHs in CADASIL patients occur early and pre-date any other neuroimaging lesions or clinical manifestations (apart from migraine with aura) [8], Jouvent and colleagues investigated a cohort of 66 CADASIL patients with isolated WMHs to address this issue [58]. This study confirmed that WMHs are associated with mild cognitive alterations that are mainly restricted to executive and attention dysfunctions. Notably, the data revealed that isolated WMHs are not associated with severe disability, suggesting that additional lesions are necessary to cause significant disability and/or dementia [58]. Moreover, in a recent study of younger CADASIL patients at an early stage of their disease, only time-measured tasks—determined using a computerized neuropsychological battery—were found to be altered; their reaction time was also significantly increased [59]. These findings support an association of early WMHs with slowed processing speed and a reduction in cerebral connectivity network efficiency.

***Heterogeneity of WMHs.*** WMHs exhibit large inter-individual variability in terms of regional distribution, severity, rate of progression, and clinical consequences [60]. A few examples highlighting this heterogeneity of WMH are presented below. First, the recent identification of distinct monogenic forms of SVDs has revealed that the distribution of WMHs may differ substantially among different hereditary SVDs. For instance, early extensive WMHs in the anterior temporal lobe are highly characteristic of CADASIL, whereas WMHs are mild or absent in this region in both CARASAL (Cathepsin A-Related Arteriopathy with Strokes and Leukoencephalopathy) [7,61], and PADMAL (Pontine Autosomal Dominant Microangiopathy with Leukoencephalopathy) [6,62]. In contrast with other

ischemic hereditary SVDs, the severity of WMHs in CARASAL has been reported to be disproportionately extensive compared with lacunar infarcts, which are very rare [7,61]. Second, longitudinal analyses of WMHs in elderly community-dwelling volunteers have shown that early, confluent WMHs, but not punctate WMHs, are actually progressive during follow-up [14]. Third, the nature and severity of clinical manifestations associated with WMHs may largely depend on the severity of underlying microstructural lesions. Previous DTI studies have demonstrated varying degrees of microstructural tissue loss in similar WMHs, sometimes at the same location over time [63,64]. Mild lesions in WM that appear normal on conventional MR images can also be detected using such refined MRI techniques. Finally, the nature and severity of clinical manifestations associated with WMHs may also depend on the location of lesions. Using voxel-based lesion mapping, Duering et al. [65] showed that reduced processing speed was strongly associated with frontal interhemispheric and thalamic projection WM fiber tracts.

***Histopathology of WM changes.*** Reports based on archival autopsy tissues from patients with SVDs have highlighted several patterns of WM alterations. The authors of these studies reported that these patterns include myelin loss in Luxol Fast blue staining or MBP immunostaining— noting that the MBP marker has demonstrated low post mortem stability [66]—, vacuolation of the WM (also called spongiosis), axonal damage (as evidenced for example by the presence of dephosphorylated neurofilaments), as well as cavitations. Importantly, all these lesions usually spare the subcortical U-fibers. Mild gliosis with clasmatodendritic astroglia, which refers to cytoplasmic swelling and vacuolation of the astrocyte soma with beading and fragmentation of their processes, especially in the deeper layers, as well as dilated perivascular spaces, have also been reported [67,68]. However, autopsy specimens may represent the end-stage disease pathology.

The histopathological correlates of WMHs have also been investigated using postmortem MRI [69]. Yet, interpretation of these studies is complicated by the wide heterogeneity of subjects analyzed, which encompassed healthy elderly subjects, patients with Alzheimer’s disease, and patients with other neurological diseases. Moreover, it should be stressed that the duration of formalin fixation of the brain influences T1 and T2 relaxation times, which decline during the first week before stabilizing after 3–4 weeks, and thus could affect the reliability of such data [70]. Nevertheless, an emerging implication of these studies is that discontinuities in the ependymal lining of lateral ventricles with subependymal gliosis and varying WM fiber loss are consistent findings in elderly individuals with periventricular WMHs. Punctate WMHs in the deep WM more commonly correspond to dilated perivascular spaces with mild myelin loss. More extensive WMHs are associated with increasing severity of WM changes, with loss of myelin, axons and oligodendrocytes, and a finding of spongiosis [60,69]. Interestingly, in some subjects with prominent perivascular WMHs, corresponding

histopathological analyses found widened perivascular spaces, although without evidence of demyelination [71]. Also, a correlative pathological and MRI study in a small cohort of CADASIL patients suggested that WMHs in the temporal pole are related to the numerous enlarged, fluid-filled perivascular spaces, but with associated degeneration of myelin [72].

Experimentally, a detailed microstructural characterization of early-stage WM changes has been recently carried out in the well-established TgNotch3<sup>R169C</sup> mouse model of CADASIL, which recapitulate the pre-symptomatic stage of the disease, including the presence of age-related WM lesions [73]. The main cerebral WM changes in these mutant mice are microvacuoles in myelin sheaths (especially at the inside of the myelin sheath) that are associated with focal degradation and uncompactation of myelin, as evidenced by immunostaining using an anti-MBP monoclonal antibody raised against the epitope adjacent to the double-phenylalanine motif (SMI94 clone) (Figure 2). Half the damaged myelin sheaths lacked an axon, whereas the other half still contained an apparently intact axon, suggesting that axonal injury is secondary. There was a significant, but mild, microglial reaction, with occasional myelin debris either in contact with or internalized by microglial cells [74]. Notably, these lesions are highly reminiscent of those observed in models of degenerating myelin, and especially of age-related myelin breakdown, as described above [30,40,41]. Future MRI and DTI analyses of these mice should provide important insights into the neuroimaging correlates of these lesions.

## **PATHOPHYSIOLOGY OF SVD-ASSOCIATED WM CHANGES: CHALLENGING THE DOGMA**

Chronic cerebral hypoperfusion, reduced cerebrovascular reactivity, and compromised BBB integrity are usually considered the master players in SVD-associated WM changes. If these changes play a causal role, one might expect them to predate WHMs or predict the development of future WMHs. These changes might also be expected to occur not only in areas of WMHs, but also in the unaffected WM. In this section, we review the arguments for and against each of these mechanisms based on studies in elderly subjects with WMHs, in patients with sporadic SVD or CADASIL—the latter of which constitute a very homogeneous group of individuals with pure SVD—as well as in experimental models.

***Chronic cerebral hypoperfusion.*** SVD-associated microvascular alterations may impede the efficient delivery of blood, especially in the deep WM, where long penetrating arteries mediate perfusion and perfusion pressure is lowest. The observed sparing of cortical U-fibers, which are supplied by shorter arteries, is consistent with such a mechanism. Several cerebral blood flow (CBF) and perfusion studies employing a variety of techniques have shown that resting CBF within WMHs is lower than that in normal-appearing WM in elderly subjects [75,76]. A recent meta-analysis of 28 cross-

sectional CBF studies in 1161 elderly individuals showed that CBF was globally reduced in subjects with more WMHs [77]. In CADASIL patients, MRI bolus tracking has also shown that resting CBF is significantly reduced within areas of WMHs compared with that in age-matched controls [78].

Although all of these studies have shown reduced CBF in WMHs, it remains unclear whether hypoperfusion is a causative factor or a secondary response to the reduced metabolic demand of injured WM. Yet, most cross-sectional CBF studies in elderly subjects with WMHs did not use age-matched controls or did not match for important confounding factors, such as associated brain lesions (e.g., lacunar infarcts) or vascular risk factors, such as age and hypertension. Hence, in their systematic review, Wardlaw and colleagues [77] concluded that, after excluding studies of demented patients or those without age-matched samples, the association between low CBF and high WMH burden was tenuous, except with respect to mean global brain CBF and CBF within the centrum semiovale. In support of this conclusion, a recent cross-sectional study conducted on community-dwelling elderly individuals who were cognitively healthy also showed that higher WMH volume was associated with lower perfusion within the WMH, but not with decreased perfusion in the surrounding, normal-appearing WM or grey matter, arguing against a general cerebral perfusion deficit playing a causal role in WMHs [75]. Whereas a small longitudinal study (40 subjects) suggested that low CBF at baseline in normal-appearing WM might precede the development of new WMHs on follow-up imaging [79], a much larger longitudinal study that included 575 patients with manifest arterial disease showed that reduced baseline global CBF, adjusted to brain tissue mass, was actually not associated with the progression of WMHs, and that larger baseline periventricular and deep WMHs predated falling CBF [80]. In CADASIL patients, CBF was also found to be unchanged in normal-appearing WM as well in the cortex [78].

Experimentally, the bilateral common carotid artery stenosis (BCAS) mouse model, which replicates chronic cerebral hypoperfusion, develops vacuolation and glial activation of the WM without significant gray matter lesions [81]. However, this experimental model, which has been widely used as a proof of concept that moderate hypoperfusion can cause WM damage, actually has several important limitations. First, the degree of CBF reduction in this model, initially estimated to be about 15–20% based on laser Doppler flowmetry at the brain surface [81], proved to be much more pronounced using arterial spin-labeling MRI. Indeed, cortical and subcortical parenchymal CBF was decreased to approximately 50% of baseline level 1 and 14 days post-surgery and recovered to approximately 70% on day 28 [82]. Second, the surgery required to introduce the microcoils needed to induce BCAS is associated with an initial further reduction in CBF that is often underappreciated. Finally, it has been shown that the WM in these mice also displays an increase in BBB permeability after 3 days that continues to 14 days post-surgery [83]. On the other hand, in the TgNotch3<sup>R169C</sup> mouse

model of CADASIL, the combination of information gathered in the past on resting CBF with the recent re-analysis of WM integrity, by immunostaining for MBP with the clone SMI94 as described above, indicates that WM lesions largely predate WM hypoperfusion. Specifically, the reduction in CBF in WM tracts, measured by quantitative autoradiography, becomes significant only after 20 months of age [73], whereas degradation and uncompactation of myelin is detectable as early as 6 months of age [84].

In summary, there is as yet no clear evidence that chronic moderate hypoperfusion plays a causal role in the initial development of WM lesions in SVDs. Instead, both human and experimental studies suggest that hypoperfusion is mainly a consequence of decreased metabolic demand and not the cause of tissue damage.

***Reduced cerebrovascular reactivity (CVR).*** Because the brain has little capacity for energy storage, the cerebral circulation has evolved adaptive mechanisms that ensure that the brain receives an adequate supply of blood under a range of conditions. Specifically, increases in cellular activity normally increase CBF, which serves to support enhanced glucose and oxygen demands. Also, despite the fact that the brain is subjected to wide variations in arterial pressure during daily activity, the cerebral vasculature is able to autoregulate and maintain CBF relatively constant over a substantial range of arterial pressures [85]. CVR is defined as the change in CBF in response to a vasodilatory stimulus and represents the dilatory ability of brain vessels, especially under the situations mentioned above. In the context of SVDs, resting CBF might be relatively preserved or compensated, whereas CVR could be impaired.

Sam et al. examined CVR in a cohort of 45 to 75 patients with moderate to severe WMHs in response to a standardized steep change in end-tidal partial pressure of carbon dioxide by monitoring blood oxygen level-dependent MRI signals [86,87]. These studies, which lacked normal controls, simultaneously and repeatedly assessed the integrity of WM using conventional MRI and DTI. The results of these studies showed that CVR at baseline was lower in WMHs than in normal-appearing WM. Importantly, CVR was lower and vascular responses were slower in normal-appearing WM transitioning to WMH than within unchanged normal-appearing WM [86,87]. However, it should be pointed out that the normal-appearing WM transitioning to WMH already exhibited significant microstructural changes, as evidenced by altered mean diffusivity and fractional anisotropy. CVR was also assessed in two small cohorts of patients with CADASIL (15–25 in each study) and age-matched controls in response to acetazolamide, a potent vasodilator; CVR was analyzed regionally with MRI bolus tracking [78] and globally with MRI phase contrast [88]. Chabriat et al. reported that CVR was decreased in areas of WMHs, but was unchanged in normal-appearing WM [78]. On the other hand, MRI results from Liem et al. suggested that the lower global CVR at baseline in CADASIL patients is

associated with a larger increase in WMHs at a 7-year follow-up [88]. Therefore, although different human studies provide strong evidence that CVR is altered in SVDs, whether altered CVR predates structural WM changes and is a key contributor to them remains unsettled.

There is ample experimental evidence from both mice and rats that age and hypertension can cause changes in both the structure and functions of the cerebral microvasculature that impede CVR [89,90]. However, because the WM has not been previously thoroughly examined, the relationship between impaired CVR and WM lesions in these models remains essentially unknown. CVR has also been extensively analyzed in the TgNotch3<sup>R169C</sup> mouse model of CADASIL. In this model, CBF responses to neural activity (functional hyperemia), lowered blood pressure (CBF autoregulation), and topical application of vasodilators are strongly attenuated as early as 6 months of age, when WM lesions become detectable [84]. Notably, recent studies in TgNotch3<sup>R169C</sup> mice have demonstrated that genetic reduction of TIMP3 (tissue inhibitor of metalloproteinase 3), an extracellular matrix protein that abnormally accumulates within the wall of brain vessels in CADASIL, normalizes CVR and, conversely, that genetic overexpression of TIMP3 mimics the CVR alterations observed in these mutant mice [84,91]. Remarkably, normalization of CVR in TgNotch3<sup>R169C</sup> mice proved to have no effect on the burden of WM lesions, assessed by quantifying the number of myelin debris [74], and genetic overexpression of TIMP3 was not found to be associated with WM lesions [84] (Joutel, unpublished data). Hence, although CVR is an early alteration in CADASIL mice, available data strongly question the proposed mechanism that impaired CVR is a key contributor to WM changes, especially at the early stage of the disease.

**Compromised BBB integrity.** Small vessel changes could also affect one or several major components of the BBB, including endothelial cells and cellular junctions, basement membranes, pericytes and astrocyte endfoot polarity, causing plasma components or cells to leak out of the vessels and produce tissue damage [92].

In a systematic review of the literature, Farrall and Wardlaw [93] reported evidence of increased BBB permeability, mostly assessed by measuring the cerebrospinal fluid (CSF)/serum albumin ratio, during normal ageing as well as in patients with vascular dementia compared with controls. Importantly, they found that the observed increase in BBB permeability was associated with increasing WMH load, although the number of available studies was limited. However, such CSF studies are unable to localize where in the brain the BBB is leaking. By analyzing postmortem tissues from vascular dementia patients and age-matched controls using immunodetection of plasma proteins extravasated from blood vessels, Akiguchi et al. [94] found evidence of BBB leakage in the area of WM lesions, especially in the periventricular and deep WM. However, in another study, BBB leakage was

demonstrated first and foremost in the cortex of SVD patients, whereas the WM in both patients and controls exhibited weaker and more diffuse immunoreactivity around arteries and veins of unclear significance [95]. Furthermore, postmortem examination of CADASIL patients revealed focal breaches of the BBB that occurred at the site of microbleeds or recent infarcts in the cortex or in the deep gray matter, whereas there was no evidence of generalized breakdown of the BBB in the WM [96].

BBB permeability has also been investigated in patients with SVDs using neuroimaging. Dynamic contrast-enhanced MRI appears to be a reliable technique for assessing subtle BBB leakage, provided that data acquisition and analysis, as well as potential sources of bias, are carefully controlled [97]. At least three studies using this approach have highlighted changes in BBB permeability in patients with SVDs [98–100]. In a large cross-sectional study of 197 patients with SVDs (but no control subjects), MR indices of BBB permeability were found to be higher in WMHs than in normal-appearing WM, a difference that was even larger with increasing WMH burden [100]. In contrast, a smaller longitudinal study of 22 patients with SVDs and 12 age-matched controls revealed that most high-permeability voxels were not inside WMHs [99]. They also found very little overlap between higher-permeability WM voxels and voxels transitioning to WMHs on repeated MRIs, noting that a high proportion of high-permeability voxels were located at the edges of WMHs. Hence, the significance of these changes in BBB permeability with respect to WMH pathophysiology is by no means clear-cut.

The spontaneously hypertensive stroke prone (SHRSP) rat is commonly considered an experimental model of SVDs. This model is a substrain of the Japanese strain of spontaneously hypertensive rat (SHR) that develops severe hypertension, with systolic pressure peaking at 220 mmHg between 6 and 12 weeks of age [101]. They also develop subsequent cerebrovascular pathologies, including fibrinoid degeneration of penetrating arterioles, enlarged perivascular spaces and hemorrhages, as well as tissue damage [101]. It is worth noting that such tissue damage consists of spongy cystic tissue destruction predominately in the cortex, but also in basal ganglia and adjacent WM [102,103]. The timing and incidence of such lesions vary depending on the genetic background and, especially, the dietary regimen (high salt or Japanese diet vs. a normal diet). Importantly, a number of studies have reported multifocal BBB leakage in SHRSP animals, highlighting a close spatial and temporal relationship between the extravasation of plasma constituents, brain edema spread, and subsequent tissue spongiosis [102,103]. However, the SHRSP rat is a model of malignant hypertension that can lead to massive BBB leakage (depending on the severity of hypertension), as evidenced by extensive brain edema and tissue spongiosis. This contrasts with the TgNotch3<sup>R169C</sup> CADASIL model, in which we have found no evidence of BBB leakage in the WM using either microstructural analysis or intravenous injection of exogenous tracers [73,74] (AJ, unpublished data).

## OTHER POTENTIAL MECHANISMS OF WM CHANGES

In this section, we discuss a few alternative mechanisms of SVD-related WM changes based on recent knowledge gained about WM in the healthy brain and WM changes in SVD brains, reviewed above.

***Dysfunction of the pial syncytium.*** As discussed in the first section on the biology of WM, molecular defects of the pial syncytium can cause WM damage, possibly through defective ion and water homeostasis [47]. Because the vasculature is ensheathed by astrocytic endfoot processes, which are suspected of being important components of this syncytium, it is conceivable that small vessel changes might disrupt the normal functioning of the syncytium. For instance, vessel changes may compromise the activity of molecules such as Kir4.1 or MLC1, which are enriched in astrocytic endfeet and are important for the function of the syncytium.

***Dysfunction of oligodendrocyte lineage cells.*** Also, as discussed in the first section, myelin undergoes remodeling with age, and although controversial in humans, there is experimental evidence in the mouse and non-human primates that OPCs contribute significantly to this process. Consistent with age being the primary risk factor for WMHs in humans, studies in non-human primates and mice have shown evidence of age-related myelin breakdown. Moreover, our recent microstructural characterization of WM lesions in a CADASIL mouse model revealed that these lesions resemble age-related myelin breakdown, consistent with the possibility that WM lesions in SVDs correspond to premature aging of WM. Collectively, these observations lead us to hypothesize that, in SVDs, and especially upon aging, WM lesions may be related to a failure of myelin remodeling and oligodendrocyte turnover, and by extension, to a failure of OPCs to generate new functional oligodendrocytes and new myelin segments in the adult brain. Below, we provide several lines of evidence of a close relationship between OPCs and brain vessels, and speculate about how small vessel changes might alter the function of OPCs and thus myelin remodeling.

In 2016, two seminal studies uncovered an anatomical and functional relationship between OPCs and blood vessels [24][104]. Using a clustering analysis of transcriptomes of approximately 5000 single cells of the oligodendrocyte lineage from distinct regions of juvenile and adult brains, Castello-Blanco and colleagues [24] identified a novel population of OPCs that they called vascular and leptomenigeal cells (VLMCs). These cells express platelet-derived growth factor receptor- $\alpha$  (PDGFR- $\alpha$ ), one of two common OPC markers, but express very low amounts of the other marker, CSPG4 (chondroitin sulfate proteoglycan 4, also known as neuron-gial antigen 2 [NG2]), and Castello-Blanco and colleagues positioned VLMCs prior to OPCs in the maturation path. Interestingly, VLMCs also express some markers of the pericyte lineage, including vitronectin and TBX18, as well as laminins and

collagens characteristic of basement membranes. Remarkably, VLMCs are localized in the wall of blood vessels [24]. In another study, Fancy and colleagues [104] showed that, during embryonic development, OPCs associate with the abluminal endothelial surface of nearby blood vessels and require intimate contact with the endothelium for their migration and for maintenance of their undifferentiated state. The associated molecular pathway was shown to involve interactions of chemokine (C-X-C motif) receptor 4 (CXCR4), expressed by OPCs, with its ligand, CXCL12, expressed on endothelial cells. Whether this finding also applies to some OPCs in the adult brain remains to be investigated. Nevertheless, Arai and colleagues established that a population of OPCs contacts pericytes in the adult brain in both humans and mice [105]. Moreover, Rowitch and colleagues have uncovered a new mechanism, involving OPC-encoded hypoxia-inducible factor (HIF) and Wnt activities, that couples postnatal WM angiogenesis and the onset of myelination in the mouse forebrain [106]. Specifically, they show that into the relatively hypovascularized early postnatal WM, OPCs are exposed to hypoxia, which activates HIF signaling and downstream Wnt ligand production, which in turn promotes angiogenesis; with increasing oxygen tension in the WM, HIF becomes downregulated, which allows OPC maturation and myelination to take place.

The close relationship between OPCs and brain vessels raises the possibility that small vessel changes may cause dysfunction of these OPCs. Several factors could contribute to this dysfunction. For example, a large number of mitogenic growth factors and their receptors that influence proliferation, migration, survival and differentiation of OPCs, including (but not limited to) members of the PDGF, fibroblast growth factor and transforming growth factor- $\beta$  families, are also produced by endothelial cells or pericytes [23]. Moreover, in vitro studies have shown that endothelial cells can sustain OPC proliferation and survival [107]. In addition, extracellular matrix molecules and their receptors (integrins) also participate in the biology of OPCs [23,108]. Changes in the composition of the extracellular matrix of cerebral blood vessels have been recently highlighted as a recurrent theme in SVDs [109]. Hence, changes in the composition of the extracellular matrix of blood vessels or in the perivascular environment might alter the capacity of OPCs to properly differentiate into oligodendrocytes and form new myelin segments.

***Dysfunction of the glymphatic system.*** As indicated in the Introduction and further discussed in the section on the histopathology of WM changes, widening of perivascular spaces is frequently observed in SVDs and may contribute to myelin degeneration. Recent experimental studies have highlighted a fluid transport system, termed the “glymphatic system”, that is proposed to mediate the clearance of interstitial fluid solutes and serve to remove waste. According to the proposed model, subarachnoid cerebrospinal fluid is driven into perivascular spaces along penetrating arteries and exchanges with surrounding interstitial fluid before being cleared along paravenous drainage pathways

The pulsatility of cerebral arteries and astrocytic endfoot-specific aquaporin 4 (AQP4) play critical roles in this fluid transport [110,111]. Notably, recent studies have shown that aging negatively affects cerebral arterial pulsatility, perivascular AQP4 polarization, and consequently glymphatic function [112]. We speculate that small vessel changes may have similarly profound impacts on glymphatic function, leading to reduced clearance of toxic waste. In such a model, changes in the perivascular environment may contribute to the widening of perivascular spaces as well as the abnormal stagnation of cerebrospinal fluid that may contain waste that is toxic toward the WM.

## **CONCLUSIONS AND FUTURE DIRECTIONS**

Over the last 15 years, much has been learned about the epidemiology, heterogeneity and clinical significance of SVD-associated WMHs, particularly in the context of age-related WMHs. At the same time, our knowledge of basic aspects of myelin structure, biogenesis, and remodeling in the healthy brain has dramatically advanced. Unfortunately, our understanding of the pathogenic mechanisms of SVD-associated WM has simply not kept pace. It has been repeatedly stated in review after review that WM changes are likely caused by chronic hypoperfusion, impaired cerebrovascular reactivity or BBB leakage, and the idea that these are, in fact, the causal mechanisms has spread insidiously. A critical review of previous human studies suggests that chronic hypoperfusion is mainly a consequence—and not the cause—of tissue damage, and that the significance of impaired cerebrovascular reactivity and BBB leakage with respect to WMH pathophysiology is by no means clear-cut. Moreover, experimental studies in a mouse model of CADASIL strongly question whether these defects are key players in the initiation of WM changes.

Part of the issue with human studies is that they generally include very heterogeneous populations of subjects who also frequently exhibit comorbid pathologies. Moreover, age-matched controls are often lacking, some studies lack statistical power, and WM is often analyzed globally. Hence, additional longitudinal studies, with voxel-based mapping and including a sufficient number of patients and controls, are required to clarify the relationship between BBB permeability and WM lesions. Given the heterogeneity of SVDs and WMHs, future studies that focus on homogeneous groups of patients, especially at the early stage of the disease, could be particularly informative. In this context, patients with monogenic forms of pure SVD represent a uniquely valuable study population, notwithstanding potential arguments that the mechanisms underlying WM changes may be specific to these SVDs. Also, an effort should be made to further refine the nature of microstructural alterations underlying WM changes. Another aspect to consider is the potential interaction or synergy among hypoperfusion, CVR alterations, and BBB leakage.

No matter how well designed or sophisticated they are, human studies simply lack the power of experimental manipulation necessary to establish a causal relationship between a particular vessel change and WM changes. This is where studies in experimental animal models achieve their full potential, provided that these models are pertinent, as appears to be indisputably the case for models of hereditary SVDs. In addition, models of pure BBB leakage, which are already available [92], would be invaluable for addressing the key question of whether subtle, chronic dysfunction of the BBB is sufficient to produce WM lesions. However, it should be kept in mind that rodent models have two major limitations: the volume occupied by the WM is much lower in rodents (<15%) than in humans (>50%), and the blood supply is distinct between rodents and humans.

Importantly, the time is ripe for thinking about other potential mechanisms of WM changes in SVDs. As touched on here, there are already some tantalizing clues that warrant further investigation. Hence, the pathogenesis of SVD-associated WM changes is a hot research topic and will likely remain so for some time to come.

**Source of funding**

This work was supported by grants from the Fondation Leducq (Transatlantic Network of Excellence on the Pathogenesis of SVD of the Brain) to AJ and HC, and the European Union (Horizon 2020 Research and Innovation Programme SVDs@target, under grant agreement No 666881) to AJ.

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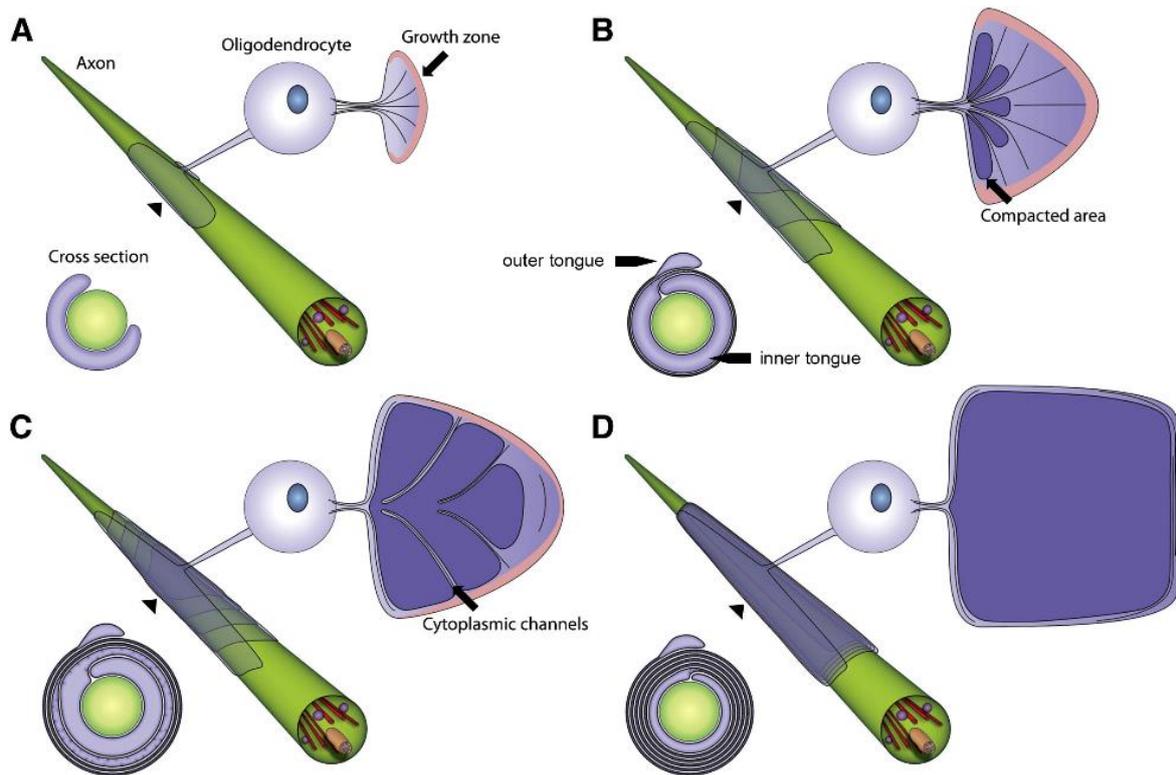
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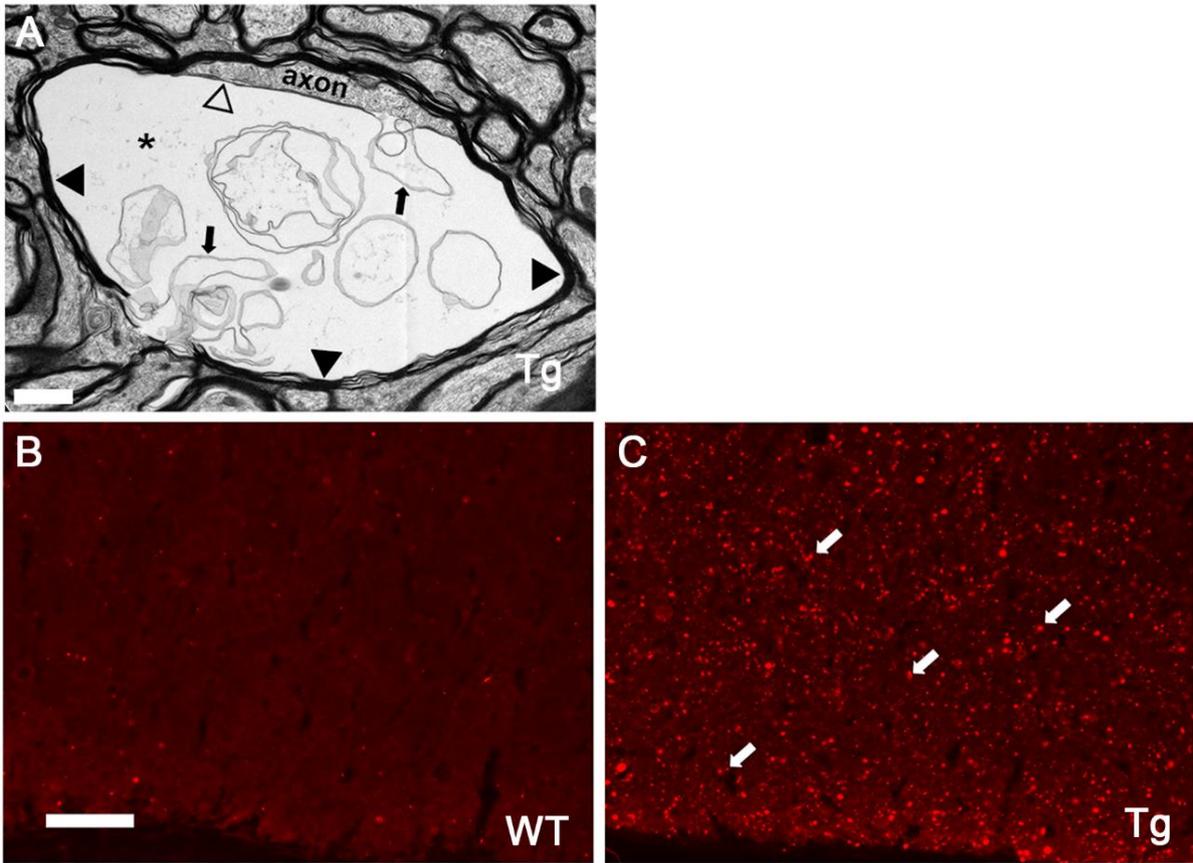
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**Figure 1: Schematic representation of myelin biogenesis in the central nervous system**

(A–D) Shown is a developing myelin sheath in unwrapped, wrapped and cross-section views from the beginning (A) to the completion (D) of myelin biogenesis. In the unwrapped views (top right in each panel), the growth zone is colored in pink, the non-compacted myelin in light blue and the compacted myelin in dark violet. The wrapped views (center in each panel) show the position of the layers when wrapped around the axon (green). Cross-section views (bottom left in each panel) show how myelin membrane layers are compacted during myelin growth. (From Simons and Colleagues [26], modified, with permission from Elsevier # 2014)



**Figure 2: Snapshot of the WM lesions in the TgNotch3<sup>R169C</sup> CADASIL mouse model**

A- Representative electron micrograph of an altered myelin fiber in a TgNotch3<sup>R169C</sup> mouse with a vacuole (star) in the innermost layer of the myelin sheath, containing multiple aberrant myelin sheets (arrows), that separates the axon from its myelin sheath (black arrowheads). Notice the thin myelin sheet (empty arrowhead) at the interface between the axon and the vacuole. (B-C) Representative sections of corpus callosum sections from a control (B) and a TgNotch3<sup>R169C</sup> mouse (C) immunolabeled for MBP using the SMI94 clone; the TgNotch3<sup>R169C</sup> mouse displays numerous hyperintense foci (white arrows). Scale bar represents 1 $\mu$ m (A) and 50  $\mu$ m (B, C).