**Title:** Cerebral vascular dysfunctions detected in human small vessel disease and implications for preclinical studies

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**Abstract** (150 words)

Cerebral small vessel disease (SVD) is highly prevalent, is a common cause of ischemic and hemorrhagic stroke and dementia, yet the pathophysiology is poorly understood. Its clinical expression is highly varied and prognostic implications are frequently overlooked in clinics, thus treatment is currently confined to vascular risk factor management. Traditionally SVD is considered the ‘small vessel’ equivalent of large artery stroke (occlusion, rupture), but data emerging from human neuroimaging and genetic studies refute this, instead showing microvessel endothelial dysfunction, impacting on cell-cell interactions, leading to brain damage. These dysfunctions reflect defects that appear inherited and secondary to environmental exposures including vascular risk factors. Interrogation in preclinical models shows consistent and converging molecular and cellular interactions across the endothelial-glial-neural-unit that increasingly explain the human macroscopic observations, and identify common patterns of pathology despite different triggers. Importantly, these insights may offer new targets for therapeutic intervention focused on restoring endothelial-glial physiology.

INTRODUCTION

This review focuses on pathophysiological mechanisms identified in humans that contribute to brain damage seen in cerebral small vessel disease (SVD), using evidence from humans with covert, stroke-related or cognitive impairments due to SVD and corresponding evidence from experimental models.

*Why is SVD important?*

SVD is the underlying cause of many strokes, dementias and mobility disorders, and is increasingly common with population aging. Of the approximately 17 million strokes per year worldwide,(1) 80% are ischemic of which 25% are small vessel (or ‘lacunar’) type, less than 2cm in diameter when acute, and occur in the subcortical grey or white matter, i.e., the perforating arteriole territories. Fifteen percent of strokes are hemorrhagic, 2 million per year world-wide, most due to SVD.(2)

About 47.5million people are living with dementia worldwide.(3) Alzheimer’s disease (AD) is the commonest and vascular dementia the second commonest dementia, of which SVD is the dominant pathology.(3) SVD lesions are also seen on brain imaging in young onset inherited forms of AD (where they may predate symptoms of AD by several years(4)) and in late onset AD (where they worsen cognitive function beyond that due to AD pathology alone(5)). Since most dementias occur in older people, and mixed AD and vascular pathologies are common,(6) it is reasonable to estimate that SVD is a major contributor to at least 45% of dementias worldwide.(3)

Having a high burden of SVD lesions found incidentally on brain imaging, so-called ‘covert SVD’, trebles the future risk of stroke, doubles the risk of dementia or death,(7) and increases the risk of delirium.(8) Similarly, in patients with recent stroke, a high SVD lesion burden trebles the risk of recurrent stroke and doubles the risk of dementia, dependency and death.(9, 10)

*What is SVD?*

Until about forty years ago, knowledge of SVD in humans depended on postmortem findings. Since the late 1970s, awareness of SVD has expanded with better brain imaging methods, particularly magnetic resonance imaging (MRI). It is important to recognize that, although some larger perforating arterioles and venules can be visualized on high field strength (7T) MRI,(11) most small vessels are not visible *themselves* on conventional MRI, only the *consequences* of the SVD on the brain.

Clinically, ‘SVD’ can be a radiological term, referring to the presence of lesions of several types visible on brain imaging. It can also be a clinical term for presentation with a lacunar ischemic stroke, or some hemorrhagic strokes. As a pathological term, ‘SVD’ usually means abnormal-appearing perforating vessels(12, 13) and surrounding tissue. Arteriole appearances are described as ‘arteriolosclerosis’, ‘lipohyalinosis’, ‘fibrinoid necrosis’, ‘segmental arteriolar disorganization’,(14) ‘periarteriolar inflammation’,(15) ‘amyloid angiopathy’.(16) Capillaries and venules are also abnormal, including dilation and tortuosity in venules near the lateral ventricles.(17) Other specific vessel appearances occur in rare monogenic disorders such as Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy (CADASIL), cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) or retinal vasculopathy with cerebral leukodystrophy (RVCL).(18)

SVD research has been hampered through widely varied, poorly defined nomenclature for SVD lesions.(19, 20) Recent efforts have improved terminology in human imaging(21) and pathology(12) research but caution is required when reading the literature to avoid confusion about what aspect of ‘SVD’ is being discussed. In this article, unless otherwise stated, ‘SVD’ means patients with clinical features or neuroimaging lesions of presumed vascular origin attributable to abnormal cerebral perforating vessels.

*What does SVD look like radiologically?*

On brain MRI, SVD lesions are small, located in subcortical tissues, not usually associated *individually* with specific symptoms, and include white matter hyperintensities (WMH), lacunes (small fluid filled cavities), microbleeds, cortical siderosis, perivascular spaces (PVS), and brain volume loss.(21) In acute stroke, MRI may show a recent small subcortical (‘lacunar’) infarct, or a brain hemorrhage (Figure 1, Supplement Figure 1).(21) Hemorrhages may be small or large when acute (Supplement Figure 1). In subacute and chronic phases, small subcortical infarcts may cavitate (i.e. become a lacune), remain looking like a WMH, or disappear.(22)

WMH are the commonest SVD lesions, present to some degree on brain MRI in most people aged over 70.(23) Lacunes and microbleeds also increase with age although are less frequent than WMH, unusual in the absence of WMH, and may represent later stages of vascular damage. Perivascular spaces surround the perforating vessels and function as fluid and waste clearance channels via the so-called ‘glymphatic system’ connecting to meningeal lymphatics (vide infra). In SVD, PVS around perforating vessels may be enlarged and hence visible on conventional MRI. All of these SVD lesions are inter-related, tending to increase together, such that patients with many WMH are also likely to have many PVS and some lacunes or microbleeds; PVS may predate SVD lesion formation and hence be present when other SVD lesions are sparse.

Having more SVD lesion types means more brain damage, since *collectively* the lesion types increase adverse outcomes beyond those of individual lesion types,(10, 24) so it is useful to consider the ‘total burden of SVD’.(25) In the long term, damage following lacunar infarcts, WMH or lacunes may propagate along projection fibers(26) leading to progressively more white matter damage, focal thinning of the overlying cortex, Wallerian degeneration,(27) and ultimately focal and global brain volume loss (atrophy).(28)

SVD has long been considered as gradually progressive and permanent, several groups (including ourselves) have shown recently that any of the SVD lesion types, i.e. WMH,(29) lacunes, or microbleeds,(30) can shrink or even resolve completely (PVS may also change but data are currently lacking). Reasons for reduction versus worsening are unclear. Possibly, lesions at early stages of development may resolve more readily than established lesions. Regardless, SVD prevention and treatment are likely to be most effective in early disease, underscoring the importance of early detection.

These last two points underpin two important concepts. First, although SVD may cause *focal* symptoms like stroke and individual lesions may appear discrete, SVD is a *global* brain disease with *global* effects on cognitive and physical dysfunction.(31) Second, SVD is a *relapsing and remitting* disorder, radiologically(29, 30) and with evidence emerging clinically,(32) since lesions (and symptoms) can wax, wane, and progress differently (Figure 1; Supplement Figure 1), perhaps reflecting different stages of pathology, and should not be viewed as uniform ‘lesions’. This picture should provoke a search for subtle but far-reaching pathophysiological mechanisms in which vessel and brain damage can build up gradually, perhaps with early stages where tissue damage can reverse, but if not corrected, can lead to permanent damage.

WHAT CAUSES SVD?

Features of SVD were described on pathology in the mid-1800s,(15) yet the causes, vessel abnormalities, and how these damage the brain, remain poorly understood. Key problems in unravelling SVD pathophysiology are the inability to see human small vessels in vivo, the submersion of clinical manifestations into undifferentiated ‘stroke’ or ‘dementia’, and the time lag between lesion development in life and opportunity to study the affected vessels at postmortem when end-stage damage may not reflect early pathogenic mechanisms. Consequently, there are several theories about the causes of vascular abnormalities in SVD and of how they damage the brain. Most theories consider SVD as a *focal* disorder causing progressive *permanent* damage, drawing heavily on causes of large artery stroke (atheroma, embolism) and focal arteriolar occlusion. Hence, many models of lacunar stroke or SVD reflect such concepts, and our lack of understanding of the early mechanisms, and the many potential triggers for the disease.

*Caveats of experimental models of SVD.*

Preclinical ‘SVD’ models generally focus on vessel pathology rather than the lesions generated, perhaps since rodents have sparse white compared to grey matter and clinical manifestations can be subtle. Despite extensive use of MRI to study brain disease in humans, there has been less use of MRI in rodents,(33) although equivalent features from human MRI are visible in rodents and would help confirm model relevance.(34) Obvious differences between humans and rodents, of particular importance when studying a predominantly subcortical disease, are the larger proportion of white to grey matter and complexity of cortical folding in humans, and different vascular anatomy.(33) Sporadic human SVD typically occurs in mid or later life but older lab rodents are challenging as a test platform. Diseases in mid-late life frequently occur on a background of exposure to multiple risk factors such as hypertension, hyperlipidemia, smoking, lifelong poor diet, lack of exercise, socioeconomic stress, yet exposure of rodents to these environmental risk factors would be difficult and perhaps unethical.

The brain and its blood supply constitute a complex system, and in preclinical research, it is usual to reduce complex systems into smaller components. To cleanly study a mechanism may be an advantage, but also a disadvantage if research on a ‘dissected’ component (e.g., hypertension, or one cell type) remains too narrow. Clearly, the brain functions as a highly connected whole, is critically dependent on its blood supply, and cells that form blood vessels *have to be* closely integrated with other cell types in the brain. Endothelial cells (EC) and pericytes ‘talk’ back and forth to each other and to astrocytes, oligodendrocytes, microglia, thence to neurons. To study any cell, pathway, or anatomical structure in isolation risks missing the ‘big picture’, despite revealing another piece of the complicated puzzle making up SVD. Accumulating clinical research suggests that most sporadic SVD may be the result of multiple minor defects in aggregate, with interpersonal variation in these defects, leading to a common, or at least similar, clinical/radiological/pathological phenotype, rather than a single process going badly wrong.

*Lifetime and genetic influences*

Human studies increasingly point to most ‘sporadic’ cases of SVD being a mix of susceptibility versus resilience, together with environmental and risk factor exposures. Firstly, some individuals appear predisposed from early life to developing SVD,(35) since large scale meta-analyses show that lower cognitive ability and lower educational exposure in youth are independently associated with worse SVD in later life.(35) Since SVD predominantly affects white matter, the link with cognitive ability may reflect white matter tract integrity and connections which underpin human intelligence.(36)

The balance of susceptibility versus resilience is underpinned by genetic studies of SVDs which show that multiple modest variants in DNA(37) in the form of single nucleotide polymorphisms (SNPs), or altered gene expression,(38) associate with worse SVD lesions and their clinical expression.(39) Furthermore, family studies indicate that SVD lesions (WMH and PVS) are highly heritable.(40, 41)

There is also a lengthening list of rare familial monogenic SVDs with known gene defects,(18) some with rodent models. Foremost amongst these is CADASIL (Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy) due to mutations in the *Notch-3* gene on Ch19, for which there are mouse models.(42, 43) Other monogenic disease models are being developed,(44) plus models incorporating gene variants known to cause rare familial SVDs when severe (e.g. *Col4A* and related deficiencies) while milder variants occur in sporadic SVDs.(45) These models enable the downstream effects of these genetic mutations to be unpicked, even though genetic causes of SVD are rare. SNPs in some of these causal genes are also seen in sporadic SVD (e.g. Col4,(45)), providing further evidence of the many routes to the common end pathway of SVD. Despite evidence of lifetime and genetic influences, the susceptibility-resilience part of the equation is not widely considered in human SVDs research, and rarely factored into rodent models.(46) Cognitive ability in youth, educational attainment and socioeconomic status at different life epochs can all be estimated in later life using relatively simple tasks. Furthermore, education and socioeconomics are modifiable through socio-political interventions. Hence, the influence of ‘early life factors’ including cognitive ability, education and socioeconomic status should be considered in future research since their impact on SVD risk is large.(35)

Observations from the whole human perspective

*Vascular risk factors in SVD.*

Typical risk factors for sporadic SVD are increasing age, hypertension, smoking, diabetes and hyperlipidemia. These common and (mostly) modifiable risk factors have received the most attention to date, undoubtedly worsen SVD, and good control is important for many health reasons. However, looked at from the perspective of ‘*how much of the visible SVD brain damage is accounted for by these common risk factors?*’ the answer is ‘*not much’* since all common vascular risk factors combined only accounted for 2% of the variance in WMH,(47) while at the same time explaining 65% of the variance in large artery atheromatous disease. Hence, while hypertension, smoking, diabetes, may be important causes or accelerators of SVD, they only account for a small proportion of the severity of SVD. Consistent with this, it has proved difficult to demonstrate in randomized controlled trials that reduction in blood pressure (BP) either delays worsening or reverses the development of SVD. Only one trial so far found that a median three years of sustained intensive (systolic BP <120mmHg) versus guideline-based (systolic BP <140mmHg) BP reduction reduced WMH *progression*, but only by a small amount (difference in WMH progression between intensive vs guideline BP control: -0.54cm3, 95%CI -0.87 to -0.02, roughly a tenth of a teaspoon, for a starting WMH volume of 4.5cm3, approximately a teaspoon),(48) with no definite reduction in incident dementia.(49) In contrast, similarly rigorous antihypertensive treatment in a much larger trial did not reduce recurrent small vessel (lacunar) stroke or prevent cognitive decline.(50) The difficulty in demonstrating much effect of BP reduction on SVD progression in RCTs contrasts with the expectation that if hypertension is the main cause of SVD, then better control of hypertension should prevent development or worsening of SVD. More confusingly, in our observational study of patients with mild small vessel-related stroke, those who spontaneously managed better control of their BP over one year of follow-up showed more *regression* of WMH than those with poorer BP control.(29) The reasons for these discrepancies are not known but it is clear that conventional risk factors are not the sole cause of SVD lesions and, while risk factors should always be treated according to best practice, improved risk factor control alone is unlikely to prevent worsening of SVD brain damage.

*Static measures of vascular dysfunction in SVD*

The commonest SVD lesions, white matter hyperintensities, lacunes and acute lacunar strokes, are typically referred to as ‘ischemic’. This partly reflects similarities in their appearances on brain imaging to the signal changes resulting from large artery ischemic strokes. However, low attenuation on CT, or reduced T1, increased diffusion, T2 or FLAIR MRI signal are not specific to ischemia but occur in inflammatory lesions such as multiple sclerosis, vasculitis, infection, or hypoglycemia.(51, 52)

Acute lacunar infarcts are the stroke manifestation of SVD, but only a few acute lacunar infarcts have been studied pathologically, mostly very late after the stroke onset.(13, 53). Fisher’s meticulous dissections showed arteriolar changes which he described as ‘segmental disorganization’ of the arteriole wall with disruption of the endothelium and usual arteriolar wall layers, infiltration by inflammatory cells, fibrinogen, other debris, areas of luminal narrowing *but also dilatation*.(54) Intriguingly, perforating arteriole occlusion was rare, but perhaps any acute occlusion had spontaneously resolved.

Possibly, arteriolar occlusion occurs as a terminal event where the disrupted inflamed arteriole wall could trigger platelet activation, thrombosis, and acute lacunar infarction. This would explain acute ‘ischemic’ changes in patients with an acute lacunar syndrome, but not why acute lacunar infarcts are often around the middle of an arteriole(55) rather than affecting the whole arteriolar territory beyond the point of obstruction, or what initiated the arteriolar wall damage.

Nonetheless, there are rodent SVD models based on mechanisms thought to induce ischemia, such as placing coils around the carotid arteries to reduce the lumen, or injecting vasoconstrictors such as endothelin into the brain parenchyma.(46) It is, therefore, interesting that the placement of bilateral carotid artery coils , intended to reduce cerebral blood flow (CBF), causes arteriolar changes resembling those in human SVD, including transient blood-brain barrier (BBB) leakage soon after coil application,(56) and later arteriolar wall thickening and inflammation.(34)

In humans, carotid stenosis and WMH are co-associated rather than causative. In patients with lacunar stroke, there is no difference in ipsilateral (i.e. causative) versus contralateral (i.e. incidental) carotid stenosis(57) and cardio-embolic sources are rare.(58) In community-dwelling older subjects, carotid stenosis did not associate with WMH or cognitive decline, cross-sectionally or longitudinally,(47, 59) and the association with cortical thinning affected vertebro-basilar and carotid territories equally.(60) Associations between intracranial artery atheroma and SVD features were also generalized rather than localized to lesion-affected vessels.(61, 62)

*Resting cerebral blood flow (CBF)*

WMH are often called ‘ischemic’ and attributed to ‘low CBF’. However, evidence for low CBF predating SVD lesions in humans is limited. Cross-sectionally, patients with worse WMH had lower resting CBF, but excluding studies with poorly age-matched controls and patients with dementia removed the association between WMH and low CBF.(63, 64) Low CBF predates symptom onset in AD by several years,(65) and separately predicts accelerated brain volume loss,(66) potentially confounding studies of SVD and CBF.

Amongst seven longitudinal studies of WMH and CBF, the largest studies finding low baseline CBF did not predict worsening WMH(64) but that worse baseline WMH predicted falling CBF.(67) Unfortunately, many studies did not account for vascular risk factors, exaggerating any apparent difference in CBF between the patients with WMH and controls. Also, CBF is normally rather lower in white than grey matter (Supplement Figure 2), and reduced CBF may be confined to small areas, limiting detection of subtle CBF reductions in white matter with current imaging techniques.

*Transit time heterogeneity*

Maintenance of adequate brain blood flow and oxygenation throughout daily activities depends on autoregulation, whereby CBF is maintained through altered cerebral blood volume and transit time: once exhausted, CBF falls. Transit time is prolonged in patients with worse WMH(64, 68) consistent with the concept of ‘transit time heterogeneity,’ where blood may shunt from arterioles to venules, leading to relative tissue hypoxia.(69) Abnormal arterioles, with luminal dilation, could increase cerebral blood volume as found in WMH,(70) and delay transit time (Supplementary Figure 2). Or, relative hypoxia could increase blood volume through dilation of the small arterioles and recruitment of capillaries that remain able to react.(70)

*Dynamic measures of vascular dysfunction in SVD*

Failure to maintain adequate CBF in response to a challenge may be a better indicator of vascular health than resting CBF, i.e., dynamic cerebrovascular function: cerebral vasoreactivity, pulsatility, vasomotion, and maintenance of the normal blood-brain barrier. Further, because pulsatility and vasomotion are important drivers of cerebrospinal fluid (CSF) flow we will also briefly discuss the glymphatic system and meningeal lymphatics for waste drainage and CNS fluid homeostasis.

*Cerebral vasoreactivity (CVR)*

CBF changes continuously to match demand to supply of nutrients and remove waste. Some of these sophisticated mechanisms are only now beginning to be understood.(71) Cerebrovascular reactivity (CVR) can be measured with MRI in a standardized, reproducible, well-tolerated way, independent of neuronal activity and breath-holding, where the subject breathes alternately 6% CO2 in medical air and medical air alone, via an open-ended breathing circuit and a tight-fitting face mask.(72)

There are few studies of CVR in SVDs or other dementias using MRI.(72) We found the magnitude of the CVR response in white matter was lower, and the time to respond was longer, in patients with worse WMH (Figure 2, Supplement Figure 2)(72) particularly worse periventricular WMH, suggesting that failure of vasodilation has most impact at their deepest limits.(72) Reduced CVR magnitude also associated with increased visible PVS and increased intracranial vascular pulsatility, providing further evidence of links between dilated dysfunctional PVS and SVD lesions (Supplement Figure 2).(72)

*Vasomotion*

Vasomotion describes spontaneous oscillations (unrelated to cardiac rhythm) in arterial tone and diameter in multiple vascular beds including the brain, but its importance in pathology is unknown. These are rhythmic, very low frequency (ranging from ~ 0.05-0.2Hz) variations in arterial/arteriolar smooth muscle tone, thought to be endothelial-dependent, in which ~0.1Hz may be a distinct signature frequency of arterioles(73) and may be important in autoregulation. Vasomotion has been demonstrated as ultra-slow hemodynamic oscillations at ~0.1Hz in pial surface vessels during open brain tumor surgery in an awake patient and related to the same frequency (~0.1Hz) observed on the raw functional blood oxygen level dependent fMRI signal.(74) In anatomical terms, vasomotion implicates the meta-arterioles (between the arterioles and capillaries) and precapillary sphincters which are open or closed several times per minute dependent on local oxygen demand,(75) but can also be observed in larger arteries, with fluctuations in vessel diameter comparable to those resulting from cardiac contraction. Several endothelium-derived factors may be important for vasomotion including the NO system,(73) potentially linking to SVD pathology. There is some evidence of altered vasomotion in AD,(76) and a cerebral amyloid angiopathy mouse model where fibrillar Aβ deposition interacted with vascular smooth muscle cells.(77) There is little to no information on vasomotion in SVD: the abnormal functional MRI BOLD response in patients with cerebral amyloid angiopathy(78) may be relevant, although the relationship of any vasoreactivity signals to vasomotion is poorly understood.

*Vascular and CSF pulsatility*

Normal internal carotid artery elasticity is important for damping systemic arterial pulse pressure to avoid brain damage. Pulsatility reflects the elasticity of the vasculature, is related to cardiac rhythm; it also drives glymphatic system transport (vide infra).

Numerous cross-sectional studies show that increased systemic pulse pressure, carotid pulsatility index or pulse wave velocity are independently associated with WMH severity.(79, 80) Increased pulse wave velocity is also associated independently with lower brain volumes (particularly in areas affected in AD) and cortical amyloid-β deposition, particularly in persons with WMH and mild cognitive impairment.(81)

There are limited longitudinal data on vascular stiffness and brain changes. In the Lothian Birth Cohort 1936, increased systemic pulse pressure at age 69y and carotid pulsatility at age 72y were associated independently with WMH severity at age 72y, with no relationship to carotid stenosis.(82) Increased systemic vascular stiffness is also associated with increased visibility of basal ganglia PVS,(83) indicating distension and possibly slowed PVS fluid flow (Figure 3).

Intracranial arterial, venous sinus and CSF pulsatility can be measured using phase contrast MRI in humans.(84) In patients with sporadic SVD, increased pulsatility in the venous sinuses was associated with worse WMH,(84) larger numbers of visible PVS and more PVS were associated with reduced CSF pulsatility at the foramen magnum (Figure 3).(72) CSF bathes and cushions the brain inside the skull, but also is critical in flushing out metabolic waste via the glymphatic-meningeal lymphatic systems.(85) Hence, the uptake of CSF into PVS is thought to be so critical to sustaining brain health, and reduced magnitude of CSF pulsation with each heartbeat at the foramen magnum, seen on MRI in patients with worse SVD, infers less CSF ‘flushing’ within the cranial cavity.(72)

Given the important role of PVS and CSF in clearing metabolic waste, the finding is mechanistically relevant and important in determining relationships between vascular dysfunction, SVD and dementia. Indeed, in rodents, CSF nanoparticle transport in PVS is driven by pulsatile arteriolar flow, normally continuous and smoothly pulsatile; when arterial pulsation was raised acutely by short-term hypertension (increasing vascular stiffness), and PVS transport became inefficient with ‘vacillating’ nano-particle movement.(86) Unilateral internal carotid artery occlusion reduced CSF and solute transport into ipsilateral PVS,(87) suggesting that carotid coil models(56) may induce SVD pathology by increasing carotid artery *stiffness,* not by reducing CBF.

*Glymphatic and meningeal lymphatic systems for waste drainage*

The glymphatic system is a brain-wide perivascular transit passageway for CSF facilitating waste clearance from the brain.(88) Waste and solute drainage via the glymphatic system have been studied in live rodent(85) and now in humans(89) and are conceptualized as a dynamic 3-step process (Figure 4): (1) bulk-flow driven influx of CSF from the peri-arterial compartment into interstitial fluid ; (2) CSF-interstitial fluid mixing in the neuropil driving waste solutes towards peri-venous conduits; and (3) exit of waste solutes from peri-venous conduits to meningeal lymphatic (mlymphatic) vessels draining to cervical lymph nodes (Supplement Figure ).(85) Metabolic waste from the brain, including soluble Aβ, tau(90) and lactate,(91) is transported via g/mlymphatics to draining lymphatics primarily to the deep cervical lymph nodes ,(91, 92) including via nasal lymphatics.(93) Thus, functional mlymphatics and afferent lymphatics draining to the deep cervical nodes are of key importance for brain waste drainage(94) and potentially of importance in neurodegenerative diseases including SVD.

Although the mechanisms controlling g/mlymphatic systems are still incompletely understood, several physiological drivers of glymphatic system transport have been identified of relevance to SVD, including vascular pulsatility (vide supra),(86, 95) vasomotion,(77) respiration,(96) sleep-wake cycle,(97) and circadian light-dark cycle.(98) In preclinical studies, glymphatic efficiency and mlymphatic function decline with aging,(99) in AD mouse(100) and SVD rat models.(86, 101-103) We used dynamic contrast enhanced MRI with gadolinium analyzed with computational fluid dynamic models to characterize CSF flow dynamics and glymphatic transport in ~9-month SHRSP rats, which model SVD.(102) MRI data were processed using our analytical framework based on regularized optimal mass transport theory(102, 104) which calculates trajectories of solute and fluid movement, so-called ‘pathlines’, over a finite tracer circulation time in the CSF and brain parenchyma (Figure 4), from which the total ‘*flux*’ of solute and fluid movement can be extracted, in addition to the mean relative solute *speed*. The total CSF ‘flux’ (but not solute speed) was decreased by 20% in SHRSP compared to the Wistar-Kyoto (WKY) controls, suggesting that CSF flux into the glymphatic PVS was reduced in the SVD rodents thus reducing waste clearance.(102)

*Blood-brain barrier dysfunction*

A key element of vascular function is to protect the cellular microenvironment by controlling entry of fluids, electrolytes, proteins, lipids and cells into the brain. The movement of fluid, molecules and cells across the BBB is complex involving multiple active, passive and endo- and exo-cytotic processes.(105) The normal BBB becomes more permeable to water-soluble substances with aging, shown in humans by numerous studies with gadolinium MRI, which detects fluid movement across the BBB.(106) The hippocampus may be a particular site for age-related BBB leakage.(107) However, note that while plasma-brain transfer of water soluble substances appears to increase with age, transcytosis of proteins via receptor-mediated transport across the BBB decrease with age, thus altering blood components that reach the brain parenchyma through two mechanisms.(105) It is likely that age-related BBB failure reflects multiple elements of ‘wear and tear’: accumulating chronic endothelial cell damage,(108) failure of inter-cellular tight or gap junctions, altered amounts and types of fluid/protein leakage, altered transcytosis,(105) and/or pericyte function,(107) secondary vessel wall and perivascular damage.

Subtle increased BBB leakage, beyond that of normal aging, occurs in SVDs and dementia, although the precise causes, cellular phenotype and whether the BBB dysfunction is primary or secondary, remains incompletely understood.(31, 109) The subtle leakage is only just above background noise, requiring very careful quantitative MRI techniques for detection (Figure 2).(110) BBB leakage has been demonstrated in cross-sectional studies in patients with small vessel (lacunar) versus non-small vessel stroke,(111) in WMH versus normal-appearing white matter,(112-114) in normal-appearing white matter with increasing WMH(70, 114) and PVS visibility.(111) Neuropathology also shows blood components (fibrinogen) in WMH as evidence of BBB leakage.(115)

In normal-appearing white matter, BBB leakage increases close to WMH particularly in the perilesional zone.(112, 116) In the few longitudinal studies, increased BBB leakage in white matter predicts long term disability, worsening WMH,(117, 118) and cognitive decline after lacunar stroke(119) and in patients at risk of AD,(120) where the increased BBB leakage may be related to APOE genotype but not to amyloid protein deposition.(107)

BBB leakage occurs in several rodent models relevant to SVD. In the mouse carotid coil model, BBB leakage detected with Evans Blue occurs transiently between days one and seven after surgery, corresponding to pericyte detachment(56) and features consistent with chronic low-grade BBB leak were present many weeks later.(34) The SHRSP develops histological features of BBB leak if allowed to age normally and at younger ages when salt loaded.(121, 122) In mice, pericytes are integral to maintaining the BBB since loss causes BBB leakage, perivascular accumulation of fibrinogen, impaired blood flow, loss of myelin, axons and oligodendrocytes.(123) In humans, a CSF marker of pericyte deficiency, soluble platelet-derived growth factor beta corresponds with increased BBB leak in the hippocampal regions that precedes cognitive decline in those at risk of AD.(120) Consistent with this, reduced CBF in the temporal lobes is one of the earliest findings in those at risk of AD,(65) corresponding with regional brain volume loss.(66) BBB leakage also corresponds with reduced CBF around WMH in patients with SVD.(116) Whether BBB leakage predates, or occurs simultaneously with, failing control of CBF, its transience or chronicity, in any of these conditions, is currently unclear.

Observations at the microscopic perspective

To recap, the human data indicate that SVD is due to microvascular dysfunction manifesting as impaired vasoreactivity, increased pulsatility, BBB leak, and variable blood flow. These vascular dysfunctions are also closely tied to the fluid and waste clearance glymphatic system operating via perivascular spaces and meningeal lymphatics. The problem is that the order of these events in humans is unclear, necessitating use of preclinical models.

The imperfect understanding of the pathogenesis of sporadic SVD has made it difficult to identify relevant rodent models, resulting in several models based on putative mechanisms of sporadic SVDs of varying clinical relevance.(124) Researchers now have a portfolio of different models, from natural breeding experiments,(125) putative induced mechanisms,(56) and rare monogenic variants,(42) each of which can inform on different causes of abnormal vascular-brain homeostasis. However, finding similar results in several apparently different models would be consistent with the ‘messy reality’ of human SVDs.

*Complex models of complex human SVD – a translational story*

Here we return to the SHRSP, a ‘messy’ model of the ‘messy’ condition that is human SVD,(121) in which hypertension is blamed for the cerebrovascular damage, as in human sporadic SVD (Figure 5). To determine why these rats are vulnerable to cerebrovascular disease beyond hypertension, we initially examined SHRSP and control WKY rats in white and deep grey matter brain regions typically affected by human SVD, at ages 5, 16 and 21 weeks, i.e., before BP starts to rise, in recently established, and late stages of hypertension. We found differential gene expression in SHRSPs at 5 weeks of age for genes related to endothelial cell tight junctions, nitric oxide (NO) bioavailability and albumin (all reduced), myelination, matrix proteins and vascular reactivity (impaired), and glial and microglial activity (increased), compared with WKY controls,(125) which we confirmed at the protein level at all three time points.(126) Perhaps surprisingly, many of the differentially expressed genes were represented in ‘inflammatory’ and ‘neurological’ and not the ‘vascular’ pathways which mainly reflect hypertension and atheromatous large artery disease.(125) These gene expression and protein differences could explain: BBB leakage (reduced tight junctions), perhaps augmented by reductions plasma oncotic pressure (reduced albumin, the most abundant plasma protein); impaired vasoreactivity (reduced NO bioavailability and vascular reactivity genes); myelin/axonal impairments suggested by the association of SVD with early life cognitive ability (glial impairments); and long-observed perivascular inflammation (microglial activity, inflammatory pathways). In the 21 week SHRSPs and WKYs, adding salt to diet from age 18 weeks exaggerated many differences, including some gene expression changes in the WKY which reduced some of the between-strain differences.(127)

We closely examined sub-5 week SHRSPs to locate the source of endothelial cell dysfunction, explain glial and inflammatory gene expression and protein differences. Using isolated brain slices and tissue from SHRSP pups, we found increased endothelial cell proliferation (at 3 weeks), fewer claudin-5 tight junctions and less NO (at 4 weeks), accompanied by increased oligodendrocyte precursor cell (OPC) proliferation with impaired maturation and more activated microglia (at 5 weeks, Figure 5).(128) Furthermore, from the neonatal stage onwards, endothelial cells secreted a substance that blocked OPC maturation, which we found to be Heat Shock Protein 90α (HSP90α). From whole genome sequencing, we discovered that the SHRSP harbored a deletion mutation in the *Atp11b* gene which caused a total loss of the ATP11B protein. Knockdown of the *Atp11b/ATP11B* gene in cultured wild-type rodent or human endothelial cells caused a similar dysfunctional phenotype to that of the SHRSP, including: endothelial cell proliferation with fewer tight junctions, reduced NO, increased secretion of HSP90α, and treatment of OPCs with medium conditioned by these cells led to increased OPC proliferation and impaired maturation.

These changes were partially rescued in intact SHRSPs by treatment between 5 and 12 weeks of age with three drugs known to have endothelial-stabilizing effects via different pathways (perindopril, simvastatin and cilostazol). To varying extents, these drugs reduced endothelial cell proliferation, increased mature tight junctions, decreased OPC proliferation, and increased mature oligodendrocytes leading to less myelin rarefaction. Of note, this pattern of reversal of endothelial and OPC changes occurred in the presence of continued raised BP in the SHRSPs who received simvastatin or cilostazol similarly to the rats whose BP was reduced by perindopril. None of these effects was seen by treatment with hydralazine, which only reduces BP without reversing endothelial dysfunction. These findings demonstrated that a cell-autonomous endothelial dysfunction, completely unrelated to BP, not only impaired endothelial function but also disrupted formation of myelin through OPC maturation block, and activated microglia.(128)

To determine the human relevance, we examined the Cohorts for Heart and Ageing Research in Genomic Epidemiology consortium data for associations between WMH and SNPs in *ATP11B,* finding one intronic SNP associated with WMH.(128) The action of this SNP on *ATP11B* expression remains unknown.

These findings indicate that an intrinsic endothelial cell dysfunction, independent of hypertension, due to a deletion in *Atp11B* and loss of ATP11B protein, causes several key abnormalities highly consistent with human SVD: impaired endothelial cell tight junctions (susceptible to BBB leak(70)), reduction in endothelial nitric oxide synthase and NO (impaired vasoreactivity(72)), blocked maturation of OPCs and impaired myelin formation (vulnerability of white matter to damage determined in early life(35)). OPC maturation failure may also affect myelin maintenance and repair in later life, making affected individuals more liable to accumulate more brain injury faster. Mature myelinating oligodendrocytes provide axons with critical metabolic support (lactate and pyruvate) to maintain the magnitude or duration of neuronal electrical signaling along axons and axon health, the loss of which may explain the fatigue and apathy common in human SVD(32) and long-term neurodegeneration.

*Relevance of ATP11B (Sidebar)*

ATP11B is a member of the P4 subfamily of P-type ATPase proteins, differing from other P4 families by moving phospholipids rather than cations across membranes. ATP11B is a flippase, moving phosphatidylserine and phosphatidylethanolamine from the outer/luminal leaflet of the plasma or organelle membrane to the inner/cytoplasmic leaflet (‘floppases’ perform the opposite maneuver).(129) Asymmetrical inner and outer membranes are thought important for vesicular release and receptor/channel function, as well as exposed phosphatidylserine/phosphatidylethanolamine being ‘eat me’ signals for myeloid cells in apoptosis.(130, 131) Proper flippase function requires a β-subunit transmembrane protein 30A (TMEM30A/CDC50a); transgenic conditional knock-out of *Tmem30a* in mouse Purkinje cells led to P4-ATPase dysfunction causing Purkinje cell stress and apoptosis, astrogliosis and early-onset ataxia.

Several ATPase deficiencies have been described, at least two of which cause severe human disease manifesting as learning difficulties, axonal/neuronal degeneration, AD, obesity, insulin resistance and diabetes, liver disease, sperm anomalies and anemia,(129) indicating strong neural phenotypes and links to risk factors for dementia. High expression of ATP11B in donor lungs pre-transplant predicts primary graft dysfunction(132) and in cell lines predicts resistance to the chemotherapeutic drug cisplatin, the latter due to increased efflux of the drug through increased vesicular formation.(133)

We now link ATP11B to SVD in the SHRSP and humans, but the mechanism is not yet clear. Loss of ATP11B may perturb shuttling of endothelial nitric oxide synthase between the Golgi and the endothelial cell plasma membrane, its location being critical for production of sufficient NO.(128, 134) Loss may increase apoptosis via exposed externalized phosphatidylserine/phosphatidylethanolamine, or affect the function of critical signaling pathways dependent on ordered membranes. These include potassium channel-dependent (Kir2.1) capillary-to-arteriole signaling that mediates very rapid vasodilatory responses increasing CBF in response to altered neuronal activity(71) or the membrane lipid PIP2 (phosphatidylinositol 4,5-bisphosphate) that regulates endothelial cell membrane ion channels.(135) These Kir2.1 potassium channels also determine endothelial cell membrane potential, hence calcium entry into endothelial cells via plasma membrane-calcium ATPase pumps and the sodium–calcium exchanger.(136) In turn, raised intracellular Ca2+ levels increase nitric oxide synthase activity and hence NO release, also important for vasodilation.(137, 138) Flippase-related membrane disruptions may therefore disrupt vasodilatory responses at ion channel and NO levels. Extracellular vesicles from endothelial cells and various blood cells attach to other cells via P-selectin glycoprotein ligand-1 and exposed phosphatidylserine,(139) disruptions to which may increase platelet adhesion, hemostasis and thrombosis, possibly contributing to vascular stasis and late stage thrombosis in SHRSPs and human SVD.

ATP11B expression is not confined to cerebral endothelial cells and blood cells, but relatively ubiquitous (https://www.proteinatlas.org/ENSG00000058063-ATP11B), with potential for compensation by other family members ATP11A and C. A neural phenotype for loss of ATP11B as in the SHRSP also occurs in a transgenic mouse with global *Atp11b* knockout which showed abnormal neural, dendritic and synapse morphology in the hippocampus, with altered distribution of membrane phosphatidylserine and downstream effects on glutamate release, glutamate receptor expression, and intracellular Ca2+ concentration, possibly through the MAPK14 signaling pathway.(140) The MAPK14 pathway is linked with cAMP response element-binding protein (CREB), a component of the dysregulated inflammatory pathways in the SHRSP(125) although it is not known if a similar effect occurs on SHRSP synapses.

*Endothelial cell dysfunction: the unifying pathology in SVDs?*

A similar pattern of endothelial cell dysfunction, with secondary effects on myelin, may perhaps be induced by endothelial injury at any age, e.g., through prolonged poorly treated hypertension, diabetes, or smoking, bringing together these risk factors into a common mechanistic pathway. This also may not be disease specific, as at least in mice, similar endothelial cell gene expression patterns, termed ‘BBB dysfunction modules’, were seen in widely disparate disease models where there is BBB disruption, including large vessel stroke, multiple sclerosis, trauma, and recurrent seizures., where expression change in diseased cerebral endothelial cells invoked a reduced barrier state resembling peripheral endothelial cells.(141) Therefore, there may be a common downstream response to many disparate triggers in these mouse models, and indeed in human diseases.

It is encouraging that in SHRSPs, endothelial dysfunction and effects on OPCs were ameliorated with commonly available drugs. Statins and antihypertensive agents are recommended for stroke prevention, although effects on dementia prevention remain elusive.(142) Antihypertensive drugs with endothelial-stabilizing actions may be better at preventing cerebrovascular disease for a similar degree of BP reduction(143) and trials are ongoing. Furthermore, cilostazol has evidence of benefit in secondary stroke prevention in lacunar stroke(144) and in trials to prevent SVD worsening providing hope for effective future therapies.

Conclusions and next steps

Converging data from human epidemiology, neuroimaging, neuropathology and genetic sources points to microvascular endothelial dysfunction in SVD. This manifests in multiple interconnected ways including molecular changes leading to downstream detrimental effects: on myelin formation, repair and possibly weakened trophic support to axons, increasing white matter vulnerability; weakened BBB prone to leakage; impaired vasoreactivity; impaired vascular and CSF pulsatility; impaired glymphatic transport and waste clearance, all leading ultimately to neurodegeneration. The features in early human life(35) including impaired white matter integrity in young adults,(39) that are associated with SVD in later life, the development of vascular dysfunction (Figure 2), tissue damage (Figure 1), impaired CSF flushing (Figure 3, 4, and Supplement),(72) the vulnerability to tissue damage seen in humans,(9) parallel the rodent model timeline (Figure 5). The microvessel abnormality is not atheromatous, nor primarily occlusive, nor primarily ischemic, although luminal narrowing and thrombotic occlusion and thence ischemia may occur secondarily as vessel damage progresses or due to dysfunctional microvascular flow, as in the rat. However, ischemia *per se* is not the root cause of the endothelial, vessel wall, perivascular or white matter changes.

There are many unresolved questions (see **Future issues**). Do endothelial cells adopt similar gene and protein expression signatures in different humans with SVD, or different SVD models, even if contributing triggers (genetic, environmental or mixed) differ? Does acquired endothelial dysfunction at older ages affect OPC maturation, impede myelin maintenance, repair or trophic support and might this account for apathy?(32) What is the cause of the microglial activation seen in SHRSP and humans and does it modulate the pathology? Apart from reduced NO bioavailability, is the poor vasoreactivity related to impaired endothelial K+ channel signaling to increase flow in response to neuronal activity? Does endothelial dysfunction increase platelet adhesion and in situ thrombosis, or is there a shared mechanism in both endothelial cells and platelets? Does the endothelial dysfunction affect astrocytes, particularly their end feet and fluid clearance function? Could loss of normal flippase function, e.g., through defects in ATP11B or genes with similar functions, account for several human SVDs, as yet unidentified due to their multiplicity? Is endothelial dysfunction confined to the brain, or is it present in other tissues?

Some models exploit mechanisms that do not appear valid in humans and yet they show features seen in human SVD, suggesting that we can learn from the alternative mechanisms by which these models might be operating. A range of flippase knock-out rodent models might illuminate SVDs mechanisms, advance understanding of membrane lipid and protein transport, cell morphology and function.

The implications for SVD prevention and treatment are substantial. Agents that restore endothelial function, improve tight junctions, increase NO, improve vasoreactivity, or unblock OPC maturation arrest, could be effective.(145) Could restoration of flippase function to re-establish correct membrane symmetry and transport function be a legitimate intervention target? A multi-faceted approach, treating brain blood vessels to improve brain parenchymal pathology, will provide therapeutic strategies to benefit patients susceptible to SVD, stroke and dementia.

**Summary Points**

1. Small vessel disease is a common cause of stroke, cognitive decline, dementia and mobility problems.
2. Small vessel disease causes several types of macroscopic brain lesions, all of which are inter-related, plus more widespread diffuse microscopic changes and secondary overt changes remote from visible lesions, indicating that it is a global brain disorder.
3. Small vessel disease lesions are associated with vascular risk factors but SVD is not primarily an atheromatous or thromboembolic occlusive disease.
4. Microvessel luminal narrowing and thrombotic occlusion, thence ischemia, may occur secondarily as vessel damage progresses, but ischemia *per se* is not the root cause of the endothelial, vessel wall, perivascular or white matter changes.
5. Most sporadic SVD is due to cerebral endothelial dysfunction, either innate or acquired, which manifests as impaired vasoreactivity, leakage of the BBB, altered arterial, venous and CSF pulsatility, impaired glymphatic transport and waste clearance.
6. Several factors in early life, including cognitive ability, educational exposure and socioeconomic adversity, increase the risk of SVD in later life, indicating that SVD is not just due to vascular risk factor exposure in mid or later life and pointing to vulnerability established from early life.
7. Endothelial cell dysfunction is present in neonatal rat models of sporadic human SVD long before risk factor exposures, and impede OPC maturation, myelin formation and contribute to white matter vulnerability at older ages.
8. At its most basic level, sporadic SVD is a disorder of the cerebral perforating vessels’ endothelium which disrupts the brain’s energy, fluid and waste management systems, affects myelination thus increasing vulnerability to injury and has a poorly understood inflammatory component.

**Future Issues**

1. Does cell-autonomous or acquired endothelial cell dysfunction underlie most sporadic human SVDs?
2. Is the endothelium-autonomous dysfunction seen in the SHRSP modulated in response to additional insults, such as elevated BP or excess plasma glucose?
3. Does acquired endothelial dysfunction, e.g., due to poorly controlled hypertension, affect OPC maturation and impede myelin maintenance or repair?
4. Does the endothelial dysfunction affect astrocytes’ energy transfer and fluid clearance?
5. Is poor vasoreactivity in SVD related to impaired endothelial K+ channel signaling, which can itself lead to reduced NO, in addition to other causes of NO deficiencies?
6. Does endothelial dysfunction increase platelet adhesion and in situ thrombosis, or is platelet function itself abnormal in SVD?
7. Could flippase, or a related membrane transfer dysfunction, account for human susceptibility to SVDs?
8. Could flippase-modulation prevent SVD?

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**Sidebar**

If dysfunction of a cell membrane phospholipid transport protein (flippase) causes the endothelial cell dysfunction which underpins susceptibility to SVD in the rat, causes similar dysfunction in isolated human cells, and has one intronic SNP associated with SVD lesion burden in a large human cohort, then why have defects in ATPases not shown up more prominently in large-scale human cohort genome-wide association and other analyses to date? While a flippase defect might account for the established vascular NO deficiencies in human SVD and rodent models and that are thought to account for impaired vasoreactivity, e.g. through impaired shuttling of the enzyme endothelial nitric oxide synthase from Golgi apparatus to endothelial cell membrane, it is also interesting to speculate if flippase-like defects could also influence, directly or indirectly, capillary endothelial Kir2.1 channel function, thus impairing the rapid electrical signaling which links increased neuronal activity to rapid hyperemia via retrograde capillary endothelial cell electrical signaling, or its regulator the plasma membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP2), or, via altered intracellular Ca2+, reduce endothelial NO synthase activity, hence NO production and vasodilation. If affected even to a minor degree at any of these points, the capillary ‘endothelial syncytium’ would underperform, rendering the brain vulnerable to damage.

**Terms and Definitions**

|  |  |
| --- | --- |
| AD | Alzheimer’s disease, a type of dementia typically associated with accumulation of amyloid and tau proteins in the brain |
| APOE | Apolipoprotein E, a protein group, some of which are involved in Alzheimer’s disease |
| ATP11β/*atp11β* | A protein, or gene, member of the cell membrane flippase family, that move phospholipids across organelle and cell membranes and thought to be involved in several diseases. |
| BBB | Blood-brain barrier, a structure composed of ECs, glial cells and membranes that controls entry from the brain vasculature into the brain interstitium and exit back into the vasculature. |
| BP | Blood pressure |
| CBF | Cerebral blood flow |
| CSF | cerebrospinal fluid, a liquid which bathes the brain, exchanges nutrients, supports the brain in the cranial cavity and cushions against trauma.  |
| CVR | Cerebrovascular reactivity |
| HSP90α | Heat shock protein 90 alpha, a protein involved in signalling in endothelial cell dysfunction |
| mlymphatic | Meningeal lymphatic channels that drain along the venous sinuses and meninges. |
| MRI | magnetic resonance imaging, a powerful method to image the brain structure and function, including vascular function, in vivo |
| NO | Nitric oxide, a gas and potent vasodilator produced by vascular cells |
| OPC | Oligodendrocyte precursor cell, the cells which mature into oligodendrocytes that form myelin |
| PDGFβ | Platelet-derived growth factor, a marker of pericyte damage |
| PVS | Perivascular space, a space around the blood vessels in the brain which become increasingly visible on brain MRI with worsening SVD. |
| SHRSP | Spontaneously hypertensive rat stroke prone, an inbred rat which develops hypertension and brain injury similar to that seen in human SVD. |
| SNP | Single nucleotide polymorphism, where a nucleotide in the genome is altered. |
| WKY | Wistar-Kyoto rat, a wild type rat |
| WMH | White matter hyperintensities. Lesions which appear on brain imaging or at post-mortem that indicate damage to white matter and are part of the spectrum of small vessel disease. |

**Figures**

**Figure 1:** Appearances of key SVD lesions commonly seen on brain MRI. A left to right: diffusion-weighted image (DWI; very sensitive to acute ischemia) shows small acute subcortical (lacunar) infarct (bright white area) in the white matter adjacent to the right lateral ventricle; FLAIR image shows diffuse white matter hyperintensities (arrows); T1-weighted image shows several lacunes in the basal ganglia (arrows); gradient echo (SWI; blood-sensitive) sequence demonstrates numerous microbleeds (arrows); T2-weighted image demonstrates numerous visible perivascular spaces (small white dots and lines).

B: Sequential diffusion-weighted images (DWI) from initial presentation with a pontine small subcortical infarct (Oct 2019, green arrow) and on routine follow-up imaging performed seven (May 2020), eight (June 2020) and eleven (Sept 2020) months later. At each time-point, one or more new acute small subcortical infarcts were seen on diffusion imaging (yellow arrows). Only the lesion seen in June 2020 was associated with any stroke-like symptoms.

C: FLAIR imaging at presentation with a small left thalamic infarct (left) and 18 months later (right) in a patient in whom the white matter hyperintensities shrank visibly over the 18 months (compare yellow arrowed areas).

See Supplement Figure 1 for SVD-related hemorrhage, and appearance of cases in B and C on other MRI sequences.

**Figure 2:** Measures of cerebrovascular function and SVD. A) CVR magnitude is reduced and delay prolonged in areas affected by WMH and adjacent tissues in a patient with severe WMH (left, FLAIR), CVR magnitude is reduced (very dark blue areas) and delay (turquoise and red areas) images show white matter is widely affected (scales on right). B, same patient, who has many visible PVS (left, T2), has diffuse subtly increased permeability (PS, middle) in white matter, with a focal area of strongly increased permeability in a lacune (cross-hairs) and diffusely increased blood volume (right, vP). CVR images courtesy Emilie Sleight, BBB images courtesy Cameron Manning, PhD Students, University of Edinburgh. See Supplement Figure 2 for examples of CBF and CVR in SVD and graphs of CVR magnitude changes with worsening of WMH and PVS.

**Figure 3:** CSF pulsatility around the spinal cord at C1-2 level and relationship to severity of WMH and PVS.(72) A, , FLAIR MRI (left) and phase contrast images at C1-2 at three evenly spaced time-points during one cardiac cycle (Full sequence and detailed anatomical labelling please see Supplementary Figure 3). Note CSF flows away (white) and towards (black) the head in systole and diastole respectively. Top row, patient with moderate SVD, no progression over one year, clear white/black CSF signal indicates good CSF flushing during the cardiac cycle; bottom row, same patient as in Figure 2B, severe SVD that progressed over one year, shows poor white/black signal indicating poor CSF flushing during the cardiac cycle.

B, Summary graphs of sagittal sinus pulsatility (upper), and CSF stroke volume at C1-2 level (lower) with increasing basal ganglia PVS scores.(72) A higher PVS score indicates more visible PVS. Foramen magnum images courtesy Alasdair Morgan, PhD Student, University of Edinburgh.

**Figure 4:** Glymphatic system in the rodent. A-C, CSF uptake into perivascular spaces in healthy wild type (WKY, A and C) and the SHRSP (SVD model, B and D) rats, visualized following injection of Gadolinium into the foramen magnum CSF and MR imaging over 90 mins, analyzed using optimum mass transport method.(102) Views from above and from lateral to the rat head show CSF moving anteriorly, along the sylvian fissure and entering the brain from perivascular spaces around the MCA. Note the slower movement in the SHRSP. C, D, solute/fluid speed maps along the MCA show the CSF contrast is delayed in the SHRSP at the root of the MCA while the WKY rat shows uniform speed and better penetration into the parenchyma. Scale bars=2mm. A and B from(102) Please see Supplement Figure 4 for images of perivenous interstitial fluid and meningeal lymphatic fluid drainage in the human on MRI.

**Figure 5:** Pathology timeline for the Spontaneously Hypertensive Rat Stroke Prone (SHRSP), a model of human sporadic SVD.(124-126, 128) EC dysfunction due to loss of ATP11B is detectable in neonatal pups, causing oligodendrocyte precursor cell maturation block and impaired myelination. Hypertension develops around 8-10 weeks, vessel wall changes and tissue damage occur later. Human epidemiology, such as early life risk factors for SVD,(35) genetic and white matter findings,(39) shows many similarities. Images courtesy of Biorender.