ANNUAL REVIEWS

Annual Review of Physiology

Cerebral Vascular Dysfunctions Detected in Human Small Vessel Disease and Implications for Preclinical Studies

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Annu. Rev. Physiol. 2022. 84:409–34

First published as a Review in Advance on October 26, 2021

The *Annual Review of Physiology* is online at physiol.annualreviews.org

https://doi.org/10.1146/annurev-physiol-060821-014521

Keywords

stroke, dementia, small vessel disease, blood–brain barrier, cerebrovascular reactivity, phospholipid flippase

Abstract

Cerebral small vessel disease (SVD) is highly prevalent and 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 and leading to brain damage. These dysfunctions reflect defects that appear to be 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

AD: Alzheimer's disease; a type of dementia typically associated with accumulation of amyloid and tau proteins in the brain

MRI: magnetic resonance imaging; a powerful method to image the brain structure and function, including vascular function, in vivo

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 Small Vessel Disease Important?

SVD is the underlying cause of many strokes, dementias, and mobility disorders, and it 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, are less than 2 cm in diameter when acute, and occur in the subcortical gray or white matter (i.e., the perforating arteriole territories); 15% of strokes are hemorrhagic (2 million per year worldwide), mostly due to SVD (2).

About 47.5 million people are living with dementia worldwide (3). Alzheimer's disease (AD) is the most common dementia, and vascular dementia the second most common, 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 Small Vessel Disease?

Until about 40 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 are visible.

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), or 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 subcortical infarcts and leukoencephalopathy (CADASIL), cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy, or retinal vasculopathy with cerebral leukodystrophy (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 review, unless otherwise stated, SVD means patients with clinical features or neuroimaging lesions of presumed vascular origin attributable to abnormal cerebral perforating vessels.

WMHs: white matter hyperintensities; lesions appearing on brain imaging or at postmortem, indicating damage to the white matter; part of the spectrum of SVD

PVSs: perivascular spaces; spaces around blood vessels in the brain that become increasingly visible on brain MRI with worsening SVD

Supplemental Material >

What Does Small Vessel Disease Look Like Radiologically?

On brain MRI, SVD lesions are small, located in subcortical tissues, and not usually associated individually with specific symptoms; they include white matter hyperintensities (WMHs), lacunes (small fluid-filled cavities), microbleeds, cortical siderosis, perivascular spaces (PVSs), and brain volume loss (21). In acute stroke, MRI may show a recent small subcortical (lacunar) infarct or a brain hemorrhage (21) (**Figure 1**; **Supplemental Figure 1**). Hemorrhages may be small or large when acute (**Supplemental 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).

WMHs are the most common SVD lesions, present to some degree on brain MRI in most people aged over 70 (23). Lacunes and microbleeds also increase with age, although they are less frequent than WMHs and are unusual in the absence of WMHs, and they may represent later stages of vascular damage. PVSs surround the perforating vessels and function as fluid and waste clearance channels via the so-called glymphatic system connecting to meningeal lymphatics (see the section titled Glymphatic and Meningeal Lymphatic Systems for Waste Drainage). In SVD, PVSs around perforating vessels may be enlarged and hence visible on conventional MRI. All of these SVD lesions are interrelated and tend to increase together, such that patients with many WMHs are also likely to have many PVSs and some lacunes or microbleeds; PVSs may predate SVD lesion formation and hence be present when other SVD lesions are sparse.

Having more SVD lesion types means more brain damage because collectively the lesion types increase adverse outcomes beyond those of individual lesion types (10, 24), so it is useful to consider the "total SVD burden" (25, p. 1228). In the long term, damage following lacunar infarcts, WMHs, 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 (ourselves included) have shown recently that any of the SVD lesion types—i.e., WMHs (29), lacunes, or microbleeds (30)—can shrink or even resolve completely (PVSs 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 such as 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), because lesions (and symptoms) can wax, wane, and progress differently (**Figure 1**; **Supplemental 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.

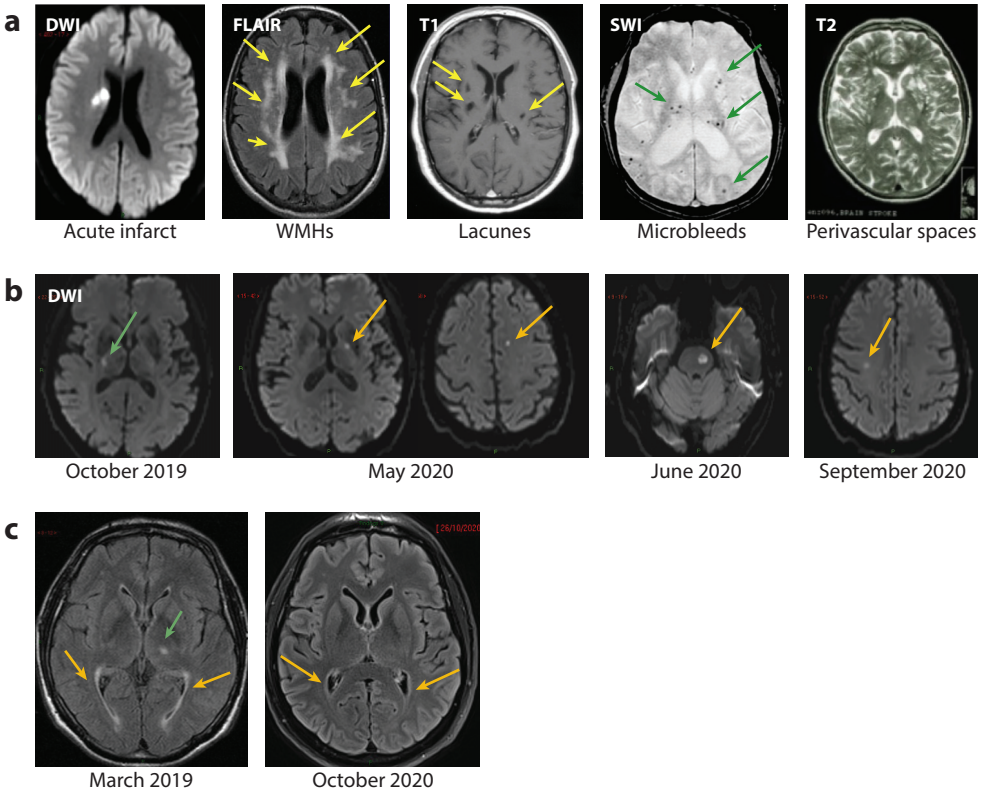

Figure 1

Appearances of key SVD lesions commonly seen on brain MRI. (*a*, *left to right*) DWI (very sensitive to acute ischemia) shows a small acute subcortical (lacunar) infarct (*bright white area*) in the white matter adjacent to the right lateral ventricle; FLAIR image shows diffuse WMHs (*yellow arrows*); T1-weighted image shows several lacunes in the basal ganglia (*yellow arrows*); a gradient echo (SWI; blood-sensitive) sequence demonstrates numerous microbleeds (*green arrows*); T2-weighted image demonstrates numerous visible perivascular spaces (*small white dots* and *lines*). (*b*) Sequential DWIs from initial presentation with a pontine small subcortical infarct (October 2019; *green arrow*) and on routine follow-up imaging performed 7 (May 2020), 8 (June 2020), and 11 (September 2020) months later. At each time point, one or more new acute small subcortical infarcts were seen on diffusion imaging (*orange 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*, *green arrow*) and 18 months later (*right*) in a patient in whom the WMHs shrank visibly over the 18 months (compare *orange arrowed areas*). See **Supplemental Figure 1** for SVD-related hemorrhage and appearance of cases in panels *b* and *c* on other MRI sequences. Abbreviations: DWI, diffusion-weighted image; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; SVD, small vessel disease; SWI, susceptibility-weighted imaging; WMH, white matter hyperintensity.

Supplemental Material >

WHAT CAUSES SMALL VESSEL DISEASE?

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 unraveling 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 the 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 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.

SNP: single nucleotide polymorphism; where a nucleotide in the genome is altered

Caveats of Experimental Models of Small Vessel Disease

Preclinical SVD models generally focus on vessel pathology rather than the lesions generated, perhaps because rodents have sparse white matter compared to gray 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 gray matter, the complexity of cortical folding in humans, and different vascular anatomy (33). Sporadic human SVD typically occurs in mid- or late life, but older lab rodents are challenging as a test platform. Diseases in mid- to late life frequently occur on a background of exposure to multiple risk factors such as hypertension, hyperlipidemia, smoking, lifelong poor diet, lack of exercise, or 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 and is critically dependent on its blood supply, and cells that form blood vessels must be closely integrated with other cell types in the brain. Endothelial cells (ECs) and pericytes talk back and forth to each other and to astrocytes, oligodendrocytes, and microglia and then 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. First, some individuals appear predisposed from early life to developing SVD (35) because 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). Because SVD predominantly affects white matter, the link with cognitive ability may reflect white matter tract integrity and connections that underpin human intelligence (36).

The balance of susceptibility versus resilience is underpinned by genetic studies of SVDs that show that multiple modest variants in DNA (37) in the form of single nucleotide polymorphisms (SNPs), or altered gene expression (38), are associated with worse SVD lesions and their clinical expression (39). Furthermore, family studies indicate that SVD lesions (WMHs and PVSs) 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 among these is CADASIL due to mutations in the *NOTCH-3*

BP: blood pressure

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., *COL4* 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 SVD research and is 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 sociopolitical interventions. Hence, the influence of early life factors including cognitive ability, education, and socioeconomic status should be considered in future research because their impact on SVD risk is large (35).

OBSERVATIONS FROM THE WHOLE HUMAN PERSPECTIVE

Vascular Risk Factors in Small Vessel Disease

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 and 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," as all common vascular risk factors combined accounted for only 2% of the variance in WMHs (47) while at the same time explaining 65% of the variance in large artery atheromatous disease. Hence, while hypertension, smoking, and diabetes may be important causes or accelerators of SVD, they account for only 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 $<$ 120 mm Hg) versus guideline-based (systolic BP $<$ 140 mm Hg) BP reduction reduced WMH progression, but only by a small amount (difference in WMH progression between intensive versus guideline BP control: -0.54 cm^3, 95% CI -0.87 to -0.02, roughly one-tenth of a teaspoon, for a starting WMH volume of 4.5 cm^3, 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 randomized controlled trials 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 small vessel disease. The most common SVD lesions—WMHs, 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 computed tomography, or reduced T1, increased diffusion, or T2 or fluid-attenuated inversion recovery 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 (54) meticulous dissections showed arteriolar changes that 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, and areas of luminal narrowing but also dilatation. 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 it does not explain 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 WMHs are coassociated 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 cardioembolic sources are rare (58). In community-dwelling older subjects, carotid stenosis did not associate with WMHs or cognitive decline, cross-sectionally or longitudinally (47, 59), and the association with cortical thinning affected vertebrobasilar 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).

WMHs 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 WMHs had lower resting CBF, but excluding studies with poorly age-matched controls and patients with dementia removed the association between WMHs 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.

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

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 WMHs (64, 68), consistent with the concept of "transit time heterogeneity," where blood may shunt from arterioles to venules, leading to relative tissue hypoxia (69, p. 303). Abnormal arterioles, with luminal

CBF: cerebral blood flow

BBB: blood–brain barrier; a structure composed of endothelial cells, glial cells, and membranes that controls movement between the brain's vasculature and interstitium

Supplemental Material >

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CSF: cerebrospinal fluid; liquid that bathes the brain, exchanges nutrients, supports the brain in cranial cavity, and cushions against trauma

CVR: cerebrovascular reactivity

NO: nitric oxide; a gas and potent vasodilator produced by vascular cells

dilation, could increase cerebral blood volume as found in WMHs (70) and delay transit time (**Supplemental 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 small vessel disease. 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 BBB. Further, because pulsatility and vasomotion are important drivers of cerebrospinal fluid (CSF) flow, we also briefly discuss the glymphatic system and meningeal lymphatics for waste drainage and central nervous system fluid homeostasis.

Cerebral Vasoreactivity

CBF changes continuously to match demand to supply of nutrients and to 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% CO_2 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 that the magnitude of CVR response in white matter was lower, and the time to respond was longer, in patients with worse WMHs (72) (**Figure 2**; **Supplemental Figure 2**), particularly with worse periventricular WMHs, suggesting that failure of vasodilation has the most impact at their deepest limits (72). Reduced CVR magnitude was also associated with increased visible PVS and increased intracranial vascular pulsatility, providing further evidence of links between dilated dysfunctional PVS and SVD lesions (72) (**Supplemental Figure 2**).

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 to 0.2 Hz) variations in arterial/arteriolar smooth muscle tone, thought to be endothelial dependent, in which ~0.1 Hz may be a distinct signature frequency of arterioles (73) and may be important in autoregulation. Vasomotion has been demonstrated as ultraslow hemodynamic oscillations at ~0.1 Hz in pial surface vessels during open brain tumor surgery in an awake patient and related to the same frequency (~0.1 Hz) observed on the raw functional blood-oxygen-level-dependent (BOLD) functional MRI (fMRI) signal (74). In anatomical terms, vasomotion implicates the meta-arterioles (between the arterioles and capillaries) and precapillary sphincters that open or close 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 nitric oxide (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 β-amyloid (Aβ) deposition interacted with vascular smooth muscle cells (77). There is little to no information on vasomotion in SVD: The abnormal BOLD fMRI response in patients with cerebral amyloid angiopathy (78) may be relevant, although the relationship of any vasoreactivity signals to vasomotion is poorly understood.

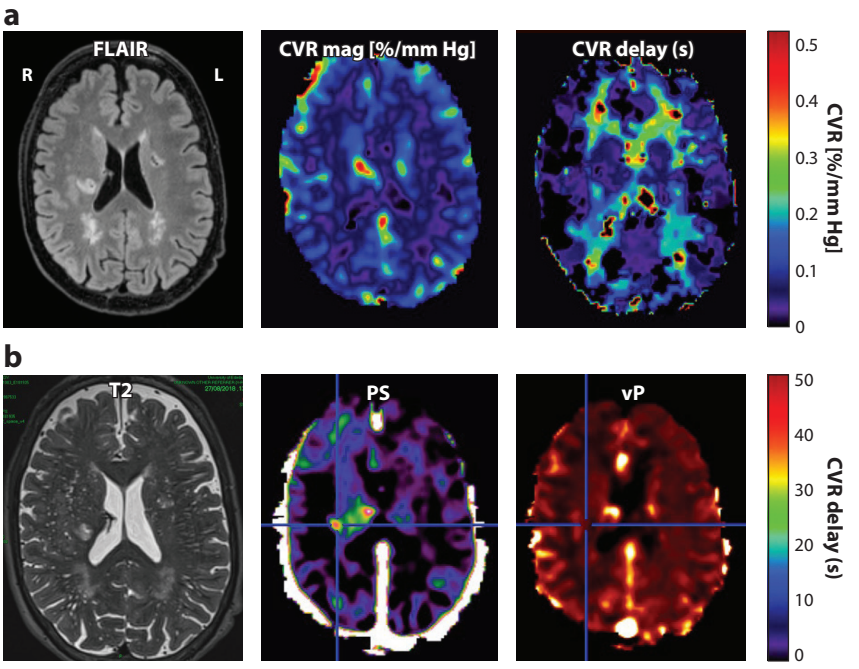


Figure 2

Measures of cerebrovascular function and SVD. (*a*) CVR magnitude (mag) is reduced and delay prolonged in areas affected by WMH and adjacent tissues in a patient with severe WMH (*left*; FLAIR); CVR magnitude reduced (*middle*, *very dark blue areas*), and delay (*right*, *turquoise* and *red areas*) images show that white matter is widely affected (see scales on right). (*b*) The same patient, who has many visible PVSs (*left*; T2), has diffuse, subtly increased permeability (*middle*; PS) in white matter, with a focal area of strongly increased permeability in a lacune (crosshairs), and diffusely increased blood volume (*right*; vP). See **Supplemental Figure 2** for examples of cerebral blood flow and CVR in SVD and graphs of CVR magnitude changes with worsening of WMH and PVS. Abbreviations: CVR, cerebrovascular reactivity; FLAIR, fluid-attenuated inversion recovery; PS, permeability surface area product, a measure of blood–brain barrier leakage; PVS, perivascular space; SVD, small vessel disease; vP, plasma volume fraction; WMH, white matter hyperintensity. CVR images courtesy of Emilie Sleight, University of Edinburgh; blood–brain barrier images courtesy of Cameron Manning, University of Edinburgh.

Supplemental Material >

Vascular and Cerebrospinal Fluid Pulsatility

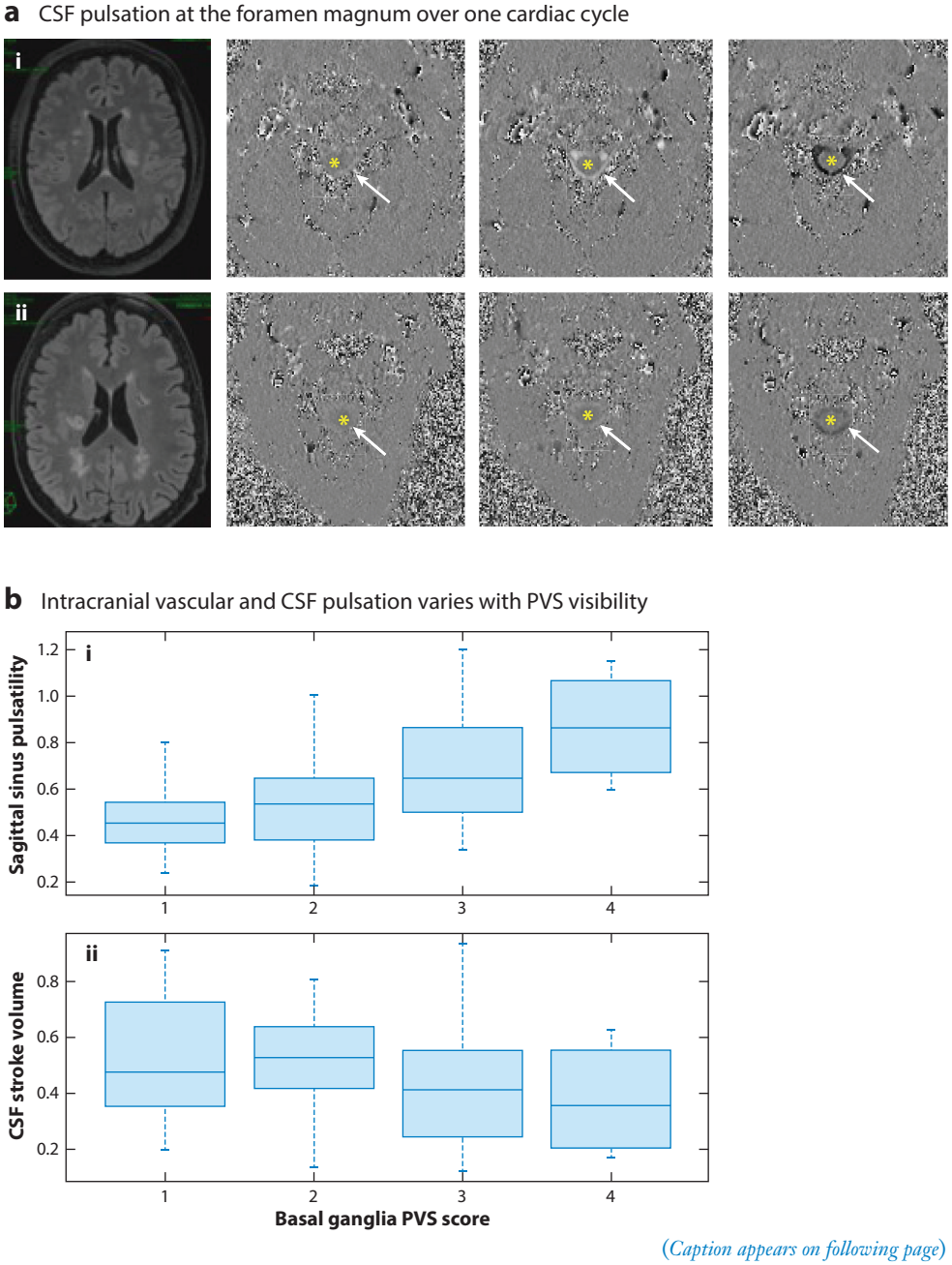
Normal internal carotid artery elasticity is important for damping systemic arterial pulse pressure to avoid brain damage. Pulsatility reflects the elasticity of the vasculature and is related to cardiac rhythm; it also drives glymphatic system transport (see the section titled Glymphatic and Meningeal Lymphatic Systems for Waste Drainage).

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 Aβ deposition, particularly in persons with WMHs 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 69 years and carotid pulsatility at age 72 years were associated independently with WMH severity at age 72 years, 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), and larger numbers of visible PVSs and more PVSs were



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

CSF pulsatility around the spinal cord at C1–2 level and the relationship to severity of WMH and PVS (72). (*a*, *left*) FLAIR MRI and phase-contrast images at C1–2 level at three evenly spaced time points during one cardiac cycle (for full sequence and detailed anatomical labeling, see **Supplemental Figure 3**). Asterisks indicate the spinal cord, and white arrows indicate CSF around the spinal cord. Note that CSF flows away (*white*) and toward (*black*) the head in systole and diastole, respectively. (*a*, *i*) Image of a patient with moderate SVD, with no progression over one year, and the clear white/black CSF signal indicates good CSF flushing during the cardiac cycle. (*a*, *ii*) Image of the same patient as in **Figure 2*b*** with severe SVD that progressed over one year shows a poor white/black signal indicating poor CSF flushing during the cardiac cycle. (*b*) Summary graphs of sagittal sinus pulsatility (*i*) and CSF stroke volume at C1–2 level (*ii*) with increasing basal ganglia PVS scores (72). A higher PVS score indicates a more visible PVS. Abbreviations: CSF, cerebrospinal fluid; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; PVS, perivascular space; SVD, small vessel disease; WMH, white matter hyperintensity. Foramen magnum images courtesy of Alasdair Morgan, University of Edinburgh.

Supplemental Material >

associated with reduced CSF pulsatility at the foramen magnum (72) (**Figure 3**). CSF bathes and cushions the brain inside the skull but also is critical in flushing out metabolic waste via the glymphatic and meningeal lymphatic systems (85). Hence, the uptake of CSF into the PVS is thought to be 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 PVSs 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 the 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), PVS transport became inefficient with vacillating nanoparticle movement (86). Unilateral internal carotid artery occlusion reduced CSF and solute transport into ipsilateral PVSs (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 rodents (85) and now in humans (89) and are conceptualized as a dynamic three-step process (**Figure 4**): (*a*) bulk flow–driven influx of CSF from the periarterial compartment into interstitial fluid, (*b*) CSF-interstitial fluid mixing in the neuropil driving waste solutes toward perivenous conduits, and (*c*) exit of waste solutes from perivenous conduits to meningeal lymphatic (mlymphatic) vessels draining to cervical lymph nodes (85) (**Supplemental Figure 4**). 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.

Mlymphatic: meningeal lymphatic channels that drain along the venous sinuses and meninges

Although the mechanisms controlling g/mlymphatic systems are still incompletely understood, several physiological drivers of glymphatic system transport have been identified that are of relevance to SVD, including vascular pulsatility (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

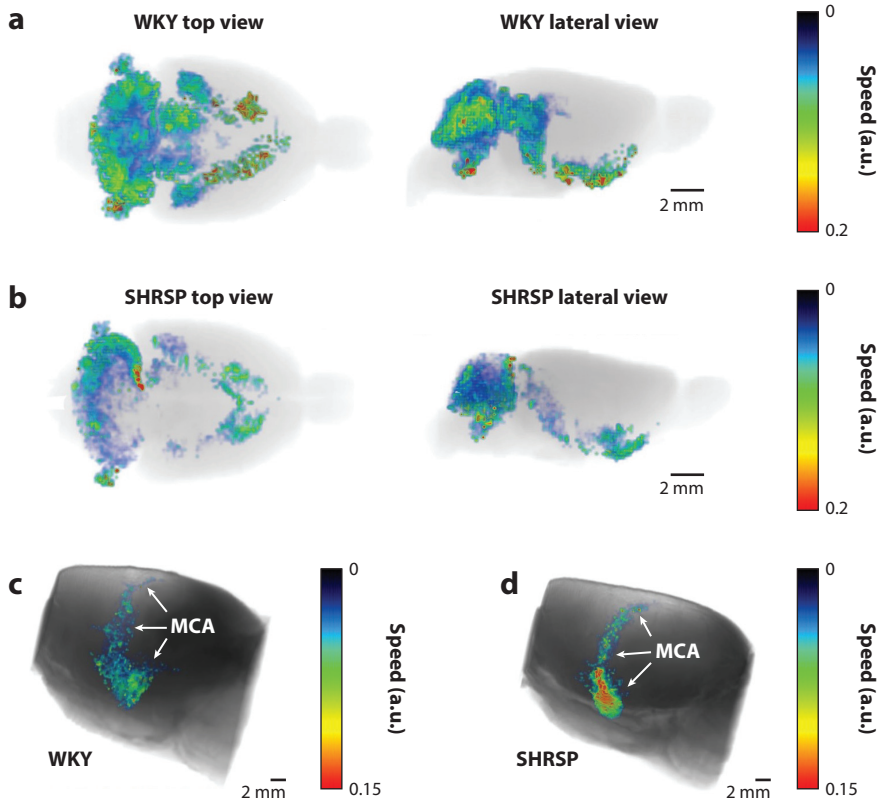


Figure 4

Glymphatic system in the rodent. (*a*, *b*) CSF uptake into PVSs in healthy wild-type (WKY; *a* and *c*) and SHRSP (SVD model; *b* and *d*) rats, visualized following injection of gadolinium into the cisterna magna CSF and MRI over 90 min, analyzed using the optimum mass transport theory (102). Views from above and from lateral to the rat head show CSF moving anteriorly, along the sylvian fissure, and entering the brain from PVSs around the MCA. Note the slower movement in the SHRSP. (*c*, *d*) Solute/fluid speed maps along the MCA show that the CSF contrast is delayed in the SHRSP at the root of the MCA, whereas the WKY rat shows uniform speed and better penetration into the parenchyma. See **Supplemental Figure 4** for images of perivenous interstitial fluid and meningeal lymphatic fluid drainage in the human on MRI. Abbreviations: CSF, cerebrospinal fluid; MCA, middle cerebral artery; MRI, magnetic resonance imaging; PVS, perivascular space; SHRSP, spontaneously hypertensive rat stroke prone; SVD, small vessel disease; WKY, Wistar-Kyoto. Panels *a* and *b* adapted from Reference 102.

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SHRSP: spontaneously hypertensive rat stroke prone; an inbred rat that develops hypertension and brain injury similar to those seen in human SVD

WKY: Wistar-Kyoto rat; a wild-type rat

fluid dynamic models to characterize CSF flow dynamics and glymphatic transport in ~9-month spontaneously hypertensive rat stroke prone (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) rat 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 exocytotic 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 decreases 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 EC damage (108), failure of intercellular tight or gap junctions, altered amounts and types of fluid/protein leakage, altered transcytosis (105), pericyte function (107), and secondary vessel wall and perivascular damage.

APOE: apolipoprotein E; some of the proteins in this group are involved in Alzheimer's disease

PDGFβ: platelet-derived growth factor β; a marker of pericyte 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 remain incompletely understood (31, 109). The subtle leakage is only just above background noise, requiring very careful quantitative MRI techniques for detection (110) (**Figure 2**). 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), and 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 apolipoprotein E (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 leakage were present many weeks later (34). The SHRSP develops histological features of BBB leakage if allowed to age normally and at younger ages when salt loaded (121, 122). In mice, pericytes are integral to maintaining the BBB because loss causes BBB leakage; perivascular accumulation of fibrinogen; impaired blood flow; and loss of myelin, axons, and oligodendrocytes (123). In humans, a CSF marker of pericyte deficiency, soluble platelet-derived growth factor β (PDGFβ), corresponds with increased BBB leakage 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 or 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 leakage, and variable blood flow. These vascular

dysfunctions are also closely tied to the fluid and waste clearance glymphatic system operating via PVSs 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 Small Vessel Disease: 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 gray

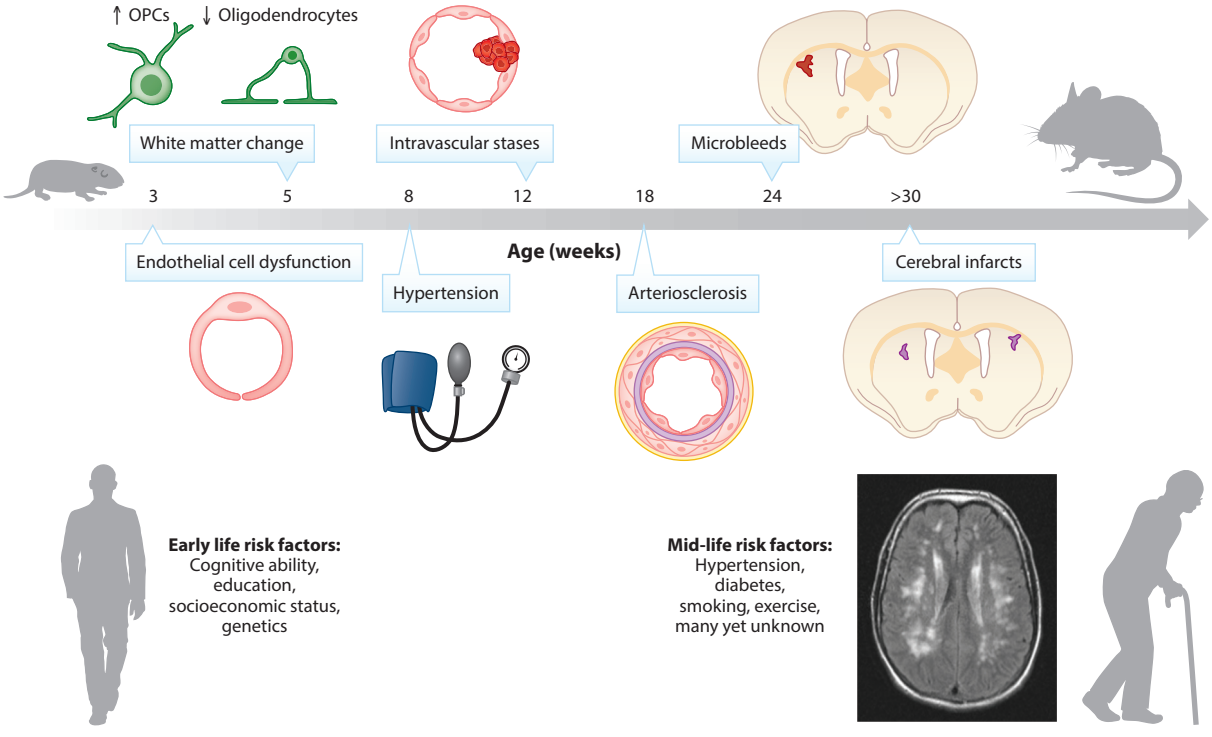

Figure 5

Pathology timeline for the SHRSP, a model of human sporadic SVD (124–126, 128). Endothelial cell dysfunction due to the loss of Atp11b is detectable in neonatal pups, causing OPC maturation block and impaired myelination. Hypertension develops around 8 to 10 weeks; vessel wall changes and tissue damage occur later. Human epidemiology, such as early life risk factors for SVD (35) and genetic and white matter findings (39), shows many similarities. Abbreviations: OPC, oligodendrocyte precursor cell; SHRSP, spontaneously hypertensive rat stroke prone; SVD, small vessel disease.

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 EC tight junctions, 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 the inflammatory and neurological but 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 in 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 the diet from age 18 weeks exaggerated many differences, including some gene expression changes in the WKYs that reduced some of the between-strain differences (127).

We closely examined sub-5-week SHRSPs to locate the source of EC dysfunction and explain glial and inflammatory gene expression and protein differences. Using isolated brain slices and tissue from SHRSP pups, we found increased EC 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) (128) (**Figure 5**). Furthermore, from the neonatal stage onward, ECs 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 that caused a total loss of the ATP11B protein. Knockdown of the *Atp11b/ATP11B* gene in cultured wild-type rodent or human ECs caused a similar dysfunctional phenotype to that of the SHRSP, including EC 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 EC 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 Aging 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 EC 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 EC tight junctions [susceptible to BBB leakage (70)], reduction in endothelial NO synthase and NO [impaired vasoreactivity (72)], and blocked maturation of OPCs

OPC: oligodendrocyte precursor cell; cell that matures into an oligodendrocyte that forms myelin

HSP90α: heat shock protein 90α; protein involved in signaling in endothelial cell dysfunction

ATP11B/*Atp11b*: a protein, or gene, member of a flippase family that moves phospholipids across organelle and cell membranes

RELEVANCE OF ATP11B

If dysfunction of a cell membrane phospholipid transport protein (flippase) causes the endothelial cell (EC) dysfunction that underpins susceptibility to small vessel disease (SVD) in the rat, causes similar dysfunction in isolated human cells, and has one intronic single nucleotide polymorphism 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 nitric oxide (NO) deficiencies in human SVD and rodent models that are thought to account for impaired vasoreactivity (e.g., through impaired shuttling of the enzyme endothelial NO synthase from the Golgi apparatus to the EC membrane), it is also interesting to speculate if flippase-like defects could also influence, directly or indirectly: (*a*) capillary endothelial Kir2.1 channel function, thus impairing the rapid electrical signaling that links increased neuronal activity to rapid hyperemia via retrograde capillary EC electrical signaling; (*b*) its regulator, the plasma membrane lipid, phosphatidylinositol 4,5-bisphosphate; or (*c*) via altered intracellular Ca^{2+}, reduce endothelial NO synthase activity and 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.

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

ATP11B is a member of the P4 subfamily of P-type ATPase proteins, which differs from other P4 families by moving phospholipids rather than cations across membranes (see the sidebar titled Relevance of ATP11B). 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 to be important for vesicular release and receptor/channel function, as well as exposed phosphatidylserine/phosphatidylethanolamine being a so-called eat-me signal for myeloid cells in apoptosis (130, 131). Proper flippase function requires β-subunit transmembrane protein 30A (TMEM30A/CDC50a); transgenic conditional knockout 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 pretransplant 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 NO synthase between the Golgi and the EC 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 PIP_2 (phosphatidylinositol 4,5-bisphosphate) that regulates EC membrane ion channels (135) (see the sidebar titled Relevance of ATP11B). These Kir2.1 potassium channels also determine EC membrane potential and hence calcium entry into ECs via plasma membrane–calcium ATPase pumps and the sodium–calcium exchanger (136). In turn, raised intracellular Ca^{2+} levels increase NO synthase activity and hence NO release, which is also important for vasodilation (137, 138). Flippase-related membrane disruptions may therefore disrupt vasodilatory responses at ion channel and NO levels. Extracellular vesicles from ECs 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 ECs and blood cells but is 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 Ca^{2+} concentration, possibly through the MAPK14 signaling pathway (140). The MAPK14 pathway is linked with cAMP response element-binding protein, 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 Small Vessel Diseases?

A similar pattern of EC 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 EC 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 ECs invoked a reduced barrier state resembling peripheral ECs (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 point to microvascular endothelial dysfunction in SVD. This manifests in multiple interconnected

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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, and 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 (**Figures 3** and **4**; **Supplemental Figures 3 and 4**) (72), and vulnerability to tissue damage (9) parallel the rodent model time line (**Figure 5**). The microvessel abnormality is not atheromatous, nor is it primarily occlusive or primarily ischemic, although luminal narrowing and thrombotic occlusion—and thus 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 the section titled Future Issues). Do ECs 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, or affect 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 ECs 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 that are unidentified as of yet 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 knockout rodent models might illuminate SVD mechanisms and 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 multifaceted 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 (SVD) is a common cause of stroke, cognitive decline, dementia, and mobility problems.
2. SVD causes several types of macroscopic brain lesions, all of which are interrelated, plus more widespread diffuse microscopic changes and secondary overt changes remote from visible lesions, indicating that it is a global brain disorder.

3. SVD 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—and thus 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 blood–brain barrier; altered arterial, venous, and cerebrospinal fluid pulsatility; and 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 late life and pointing to vulnerability established from early life.
7. Endothelial cell (EC) dysfunction is present in neonatal rat models of sporadic human SVD long before risk factor exposures and impedes oligodendrocyte precursor cell (OPC) maturation and myelin formation and contributes 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 and 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 EC dysfunction underlie most sporadic human SVDs?
2. Is the endothelium-autonomous dysfunction seen in the spontaneously hypertensive rat stroke prone modulated in response to additional insults, such as elevated blood pressure 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 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 nitric oxide (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 flippases, or a related membrane transfer dysfunction, account for human susceptibility to SVDs?
8. Could flippase modulation prevent SVD?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Academic grants funding the research managed by the University of Edinburgh include those from the Medical Research Council, UK Dementia Research Institute (which receives funding from the Medical Research Council, Alzheimer's Society, and Alzheimer's Research UK), Fondation Leducq, British Heart Foundation, Stroke Association, Wellcome Trust, Chief Scientist Office, EU Horizon 2020 SVDs@target, NIH (R01NS100366), MS Society UK, and Row Fogo Charitable Trust. We thank Emilie Slight, Cameron Manning, and Alasdair Morgan for assistance with preparation of **Figures 2** and **3** and **Supplemental Figures 2 and 3**.

Supplemental Material >

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