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The role of brain vasculature in neurodegenerative disorders

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Abstract

Adequate supply of blood and structural and functional integrity of blood vessels is key to normal brain functioning. On the other hand, cerebral blood flow (CBF) shortfalls and blood-brain barrier (BBB) dysfunction are early findings in neurodegenerative disorders in humans and animal models. Here, we first examine molecular definition of cerebral blood vessels, and pathways regulating CBF and BBB integrity. Then, we examine the role of CBF and BBB in the pathogenesis of Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and multiple sclerosis. We focus on AD as a platform of our analysis because more is known about neurovascular dysfunction in this disease than in other neurodegenerative disorders. Finally, we propose a hypothetical model of AD biomarkers to include brain vasculature as a factor contributing to the disease onset and progression, and suggest a common pathway linking brain vascular contributions to neurodegeneration in multiple neurodegenerative disorders.

Introduction

Neurons work hard. To keep the 86 billion neurons in the human brain working properly requires an adequate supply of blood, which is accomplished through a vast, well-regulated vascular network of arteries, arterioles, capillaries, venules and veins reaching approximately 400 miles in length[1,2]. Several cell types work in concert to regulate cerebral blood flow (CBF) and maintain blood-brain barrier (BBB) integrity. This assortment of cells, collectively called the neurovascular unit (NVU), is comprised of endothelial cells forming the inner layer of the vessel walls, mural cells along the vessels that help regulate vascular tone (pericytes and vascular smooth muscle cells; SMCs), astrocytes whose endfeet cover most of the vasculature, and neurons[1,2]. The NVU cellular composition varies along the vascular tree, with rubber band-like SMCs wrapping vessels at the arterial and arteriole level, pericytes along capillaries, and "stellate" SMCs along venules[3] (Figure 1a).

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A continuous endothelium monolayer forms the BBB, which maintains cerebrovascular integrity through continuous cross-talk between endothelium, mural cells, astrocytes and neurons. The BBB restricts paracellular and transcellular endothelial transport of most blood-derived macromolecules, blood cells (e.g., leukocytes) and microbial pathogens between blood and brain, and is present at all levels of the vascular tree from arteries and arterioles to capillaries to venules and veins[4,5]. The largest surface area of the BBB (> 85%) is provided by the capillary endothelium[6]. Numerous transport systems expressed mainly in brain capillary endothelium and venule endothelium[7] facilitate or actively shuttle molecules across BBB, as reviewed elsewhere[4].

Here, we first examine molecular definition of cerebral blood vessels, cell types and zonation in the brain vasculature. Next, we review key pathways along the vascular tree regulating CBF and BBB integrity. Then, we discuss the role of CBF and BBB in the pathogenesis of neurodegenerative changes in humans in different neurodegenerative disorders concentrating on recent neuroimaging data. We focus on Alzheimer's disease (AD) because more is known about vascular dysfunction in this disease than in other neurodegenerative disorders. Finally, we propose an updated hypothetical model of AD biomarkers[8] to incorporate brain vascular changes as an important factor of the disease onset and progression, and suggest a common pathway linking brain vascular changes to neurodegeneration in multiple neurodegenerative disorders.

Molecular definition of cerebral blood vessels

Endothelial zonation.

The concept of vascular 'zonation' landmarks was recently introduced based on single cell RNA-sequencing of brain vascular cell types isolated from different murine models suggesting molecular and functional phenotypic differences along the vasculature[7] (Figure 1b). Single endothelial cells isolated from *Cldn5*-GFP reporter mice with green fluorescent protein (GFP) expression driven by claudin-5, an endothelial tight junction protein, and transcriptional data allowed endothelial cell clustering into groups based on transcriptomic (dis)similarity. For example, arterial vascular zonation could be identified with arterial-specific endothelial markers, *Bmx* [encoding BMX non-receptor tyrosine kinase], *Efnb2* [encoding ephrin B2], *Vegfc* [encoding vascular endothelial growth factor C], *Sema3g* [encoding semaphorin 3G], and *Gkn3* [encoding gastrokine-3], and venous vascular zonation with venous-specific endothelial markers, *Nr2f2* [encoding nuclear receptor subfamily 2 group F member 2] and *Slc38a5* [encoding a sodium-dependent amino acid transporter][7]. Analysis of the gradual arterial-to-venous (A-V) zonation reveals endothelial genes that peaked in the middle, representing the capillary enriched genes *Mfsd2a* [major facilitator superfamily domain-containing protein 2a (MFSD2a)] and *Tfrc* [transferrin receptor][7]. MFSD2a is an essential omega-3 fatty acid transporter[9] required for BBB formation and omega-3 fatty acid transport function[10], and transferrin receptor is important for brain delivery of iron[4]. For the first time, this novel approach enables studies of transcriptional expression with respect to vascular zonation revealing molecular and functional differences along the brain vascular tree. For example, transcription factors predominated at the arterial endothelium, whereas transporters predominated at the capillary

and venous endothelium. Some endothelial cells did not fit the A-V zonation pattern exhibiting high expression of ribosomal protein transcripts, which indicates that protein synthesis occurs throughout the A-V axis[7].

Mural cell pattern.

In contrast to the endothelium with gradual zonation, mural cells exhibited a segregated zonation pattern. Isolated from *Pdgfrb*-GFP; *Cspg4*-DsRed mice, mural cells transcriptionally separated into two distinct groups including pericytes and venous SMCs (vSMCs), and arterial and arteriolar SMCs (aSMCs)[7]. Clustering analysis revealed pericyte-enriched genes, *Pdgfrβ* [encoding platelet-derived growth factor receptor-β], *Cspg4* [encoding chondroitin sulfate proteoglycan neuron-glial antigen 2 (NG2)], *Anpep* [encoding N-aminopeptidase CD13], *Rgs5* [encoding regulator of G protein signaling 5], *Abcc9* [encoding SUR2 subunit of K^+-ATP channel], *Kcnj8* [encoding Kir6.1], *Vtn* [encoding vitronectin], and *Ifitm1* [encoding interferon-induced transmembrane protein 1], consistent with reported literature[7,11]. aSMCs enriched genes included *Acta2* [encoding alpha smooth muscle actin, α-SMA], *Tagln* [encoding transgelin], *Myh11* [encoding myosin heavy chain 11], *Myl9* [encoding myosin light chain 9], *Mylk* [encoding myosin light chain kinase], *Sncg* [encoding synuclein gamma], *Cnn1* [encoding calponin-1], and *Pln* [encoding phospholamban][7] (Figure 1c).

In contrast to aSMCs, pericytes express barely detectable levels of *Acta2* encoding α-SMA[7], a protein that plays a key role in cell contractile apparatus, which would argue against pericyte role in contractility and CBF regulation. However, using strategies that allow rapid filamentous-actin (F-actin) fixation or prevent F-actin depolymerization, it has been recently shown that pericytes on mouse retinal capillaries, including those in intermediate and deeper plexus, express α-SMA[12]. Junctional pericytes were more frequently α-SMA-positive compared to pericytes on linear capillary segments. Additionally, short interfering α-SMA-siRNA suppressed α-SMA expression preferentially in high order branch capillary pericytes, confirming the existence of a smaller pool of α-SMA in distal capillary pericytes that is quickly lost by depolymerization[12]. Recent RNA-seq studies also indicated that pericytes express moderate-to-robust levels of other contractile proteins including *Des* (desmin) and *Cnn2* (calponin-2)[7] and *Myl9* (myosin light chain 9)[11]. How and whether these contractile proteins contribute to the contractile apparatus in pericytes remains to be determined. Nevertheless, beyond expression of contractile proteins, more functional studies are needed to determine how exactly pericytes contribute to CBF regulation, as discussed in greater detail below.

Pathways regulating CBF along the vascular tree

The NVU of the mammalian brain is functionally integrated to regulate CBF responses to neuronal stimulation in a process called neurovascular coupling or functional hyperemia[1,2], which ensures a rapid increase in CBF and oxygen delivery to activated brain regions. Mural cells can depolarize and contract, or hyperpolarize and relax in response to different stimuli, to either constrict or dilate blood vessels, respectively, which in turn decreases or increases blood flow. SMCs regulate arteriolar and arterial vessel diameter[1]. Most *in vivo* rodent

studies in the brain and retina, as well as studies using cortical and cerebellar slices and retinal explants suggest that pericytes regulate blood flow at the capillary level[13–20]. However, this has not been confirmed by some *in vivo* studies in the mouse cortex[21,22], leaving this subject still as a matter of controversy. An exact explanation for these conflicting reports remains unclear at present, but could reflect technical challenges or underlying properties of the studied models that should be clarified by future studies. As pointed out in a recent review[1], the evidence to date also does not rule out a role of capillaries (and pericytes) in the retrograde propagation of intramural vascular signals from capillary level upstream[1,23,24]. Given that the vast majority of brain vasculature is composed of capillaries (~90% in mouse cortex)[13,25], capillaries and pericytes are well positioned to regulate CBF throughout the brain parenchyma. Figure 2 illustrates key cellular and molecular pathways regulating CBF.

Briefly, neurons signal the vasculature through transmitters adenosine triphosphate (ATP) and adenosine that act on their receptors - purinergic P2X and P2Y for ATP (also expressed on other CNS cell types[7,11]) and adenosine A2A for adenosine - on SMCs and pericytes causing depolarization and cell contraction that reduces blood flow, or hyperpolarization and cell relaxation that increases blood flow, as shown *in vivo* in cortex and retina, and *ex vivo* in cortical slices and whole mount retina[2,13,26]. Noradrenaline contracts SMCs *ex vivo* in isolated retina and *in vivo* in cortical arterioles[23] and pericytes *ex vivo* in rat cerebellar slices and isolated retina[14], whereas neuropeptide Y contracts cortical arterioles *in vivo*[27]. Neuronal nitric oxide (NO) can hyperpolarize and relax SMCs and pericytes increasing blood flow *in vivo* and *ex vivo*[2,15,20]. Although it has been initially suggested that NO can act by blocking 20-hydroxyeicosatetraenoic acid (20-HETE) production, this mechanism may play a more significant role in autoregulation of vascular tone than neurovascular coupling[28]. In contrast, a recent study suggested that dilation of arterioles depends on NMDA receptor activation and Ca^{2+}-dependent NO generation by interneurons, whereas the dilation of capillary pericytes in mouse cortex is mediated by local Ca^{2+} elevations in glial processes and endfeet along capillaries[16] although regional differences may exist[15–17,20,29]. These findings indicate that different signaling cascades regulate CBF at the capillary and arteriole levels. Neuronal activity elevates extracellular potassium ($[K^+]$) directly and/or via release from astrocytes (as an effect of neuronal ATP stimulation, see below), which acts on various K^+ and Ca^{2+} channels to alter pericyte and SMCs tone, leading to alterations in blood flow as shown in brain and retina *in vivo*[2,29] (Figure 2).

Astrocytes also respond to neuronal ATP through P2X or P2Y receptors by increasing intracellular calcium concentration ($[Ca^{2+}]$) (similar to microglia and oligodendrocytes[7,11]), as shown in live and whole mount retina[26], which triggers production of arachidonic acid (AA) and its vasoactive metabolites (20-HETE; prostaglandin E2, PGE2; and epoxyeicosatetraenoic acids, EETs) as shown *in vivo* in cortex and retina and *ex vivo* in cortical and cerebellar slices and retinal explants[2,15,16,29], and secretion of K^+. Mural cells generate 20-HETE from AA causing cell depolarization and blood flow reduction, while PGE2 hyperpolarizes SMCs and pericytes (via PGE2 receptor EP4, EP4R) increasing vessel diameter, as shown *in vivo* in cortex and retina and *ex vivo* in cortical and cerebellar slices and retinal explants[2,15,16,29]. The role of astrocytes in CBF regulation has been, however, a matter of controversy, stemming from conflicting data as to whether astrocyte $[Ca^{2+}]$ can

increase in response to stimulus, and whether if any [Ca^{2+}] increase can occur rapidly enough to influence neurovascular coupling[29] (Figure 2). Additional studies are needed to resolve this controversy.

Endothelial cells respond to acetylcholine (ACh) from the blood stream via muscarinic ACh receptors[30,31], and blood flow shear stress to generate NO[23], which increases blood flow by relaxing pericytes and SMCs, as described above. ACh also causes NO-independent endothelial hyperpolarization[23]. Shear stress triggers production of AA and AA-derived metabolites (EETs; prostacyclin, PGI2), which act on SMCs to increase blood flow[23]. Extracellular [K^+] elevations can hyperpolarize endothelium at both the capillary and arteriole level, which can propagate in a retrograde direction via gap junctions shared between endothelial cells and endothelial-mural cell junctions, leading to relaxation and dilation of upstream vasculature to increase blood flow[1,2,23]. These endothelial pathways have been described *ex vivo* in various rodent aortic and brain arteriole ring preparations, and *in vivo* in cortex and cerebellum, but further validation studies in brain are needed.

Expression of all major receptors, ion channels and other key proteins in SMCs, pericytes, endothelial cells and astrocytes contributing to CBF regulation, as shown in Figure 2, has been supported by recent single cell RNA-seq analysis of the brain vasculature in murine models[7].

Pathways regulating BBB integrity along the vascular tree

Formation and maintenance of the BBB is accomplished through expression of tight junctions (e.g., claudins, occludin, zona occludens) and adherens junctions (e.g., vascular endothelial (VE)-cadherin, platelet endothelial cell adhesion molecule (PECAM-1), connexins) connecting neighboring endothelial cells, as well as the paucity of trans-endothelial bulk flow transcytosis[4]. Gap junctions between endothelial cells, pericytes and astrocytes contribute to cerebrovascular integrity. Upholding endothelial barrier is essential for specialized transport properties and functions of BBB, as reviewed elsewhere[4]. Figure 3 summarizes key cellular and molecular pathways underlying BBB establishment and maintenance.

Studies using pericyte-deficient murine models with reduced endothelial-derived platelet-derived growth factor-BB (PDGF-BB) bioavailability or deficient PDGF receptor-β (PDGFRβ) signaling in pericytes, have suggested that pericytes regulate BBB formation and maintenance at the level of brain capillaries[5,32–35]. PDGF-BB is a ligand for PDGFRβ on pericytes, which controls pericyte survival, proliferation, and migration[32–36] (Figure 3, *left*). Endothelial-secreted Notch ligands signal via Notch3 receptor in pericytes to promote pericyte survival[5]. Similarly, pericyte-derived Notch ligands bind to endothelial Notch1 receptor, which promotes N-cadherin synthesis stabilizing BBB[5]. Vascular endothelial growth factor-A (VEGF-A) signals VEGF receptor-2 (VEGFR2) in capillary endothelium reinforcing cell survival[5]. Transforming growth factor-β (TGFβ) and TGFβ receptor-2 (TGFβR2) signaling between pericytes and endothelial cells regulates endothelial proliferation and differentiation and pericytes proliferation and migration[5] (Figure 3, *left*).

Ephrin type B receptor 2 (EphB2) expressed by arterial endothelial cells regulates SMC recruitment to the developing vessels contributing to cerebrovascular integrity, which is indirectly indicative of BBB stability[4,37]. Loss of EphB2 disrupts mural cell coverage causing BBB breakdown[37]. Ablation of astrocytic laminin leads to BBB breakdown[38]. Apolipoprotein E (APOE) isoforms, secreted by astrocytes regulate BBB integrity by signaling the low-density lipoprotein receptor-related protein-1 (LRP1) on pericytes[39]. Additionally, neuron-secreted Wnt, a ligand of frizzled (FZD) receptor on endothelium, promotes endothelial cell differentiation during brain vasculogenesis[40,41].

Expression of all major receptors and key proteins in endothelial cells, pericytes, SMCs and astrocytes regulating BBB integrity, as shown in Figure 3, has been supported by recent single cell RNA-seq analysis of the brain vasculature in murine models[7]. Besides contributing to BBB formation, these pathways continue to play an important role in BBB maintenance in the adult and aging brain.

Gap between basic, translational and human studies of the brain vasculature

The molecular and cellular mechanisms regulating CBF and BBB integrity, as discussed in the first half of this Review, have been extensively examined in animal models generating important basic physiological data. However, these mechanisms have not been studied in great detail in models of neurodegenerative diseases that show early neurovascular dysfunction[6], nor in humans with early CBF and BBB deficits that are consistently observed in different neurodegenerative diseases[2,4,6,42–45]. Whether the pathways and molecular makeup of the human brain vasculature can reproduce findings in animal models remains unclear, illustrating a major gap between animal models and human studies. Nevertheless, knowledge from animal studies offers a roadmap to examine the link between neurovascular dysfunction and neurodegeneration in humans.

Neurovascular dysfunction is increasingly recognized in AD[1,2,4,42–48], besides classical pathological hallmarks of the disease including amyloid-β (Aβ) plaques, hyperphosphorylated tau neurofibrillary tangles, and neuronal loss[8]. Neuropathological studies show that cerebrovascular pathology contributes to dementia and clinically diagnosed AD[46] and lowers the threshold for dementia due to AD[47]. Similarly, vascular risk factors also lower the threshold and act synergistically with Aβ burden to promote cognitive decline[49]. Neurovascular dysfunction is also seen as an early event in AD, influenced by genetic, lifestyle and environmental factors, as demonstrated by vascular biomarkers studies[42,44,45,50], and discussed in sections to follow.

In addition to AD, the brain vasculature has been implicated in the pathogenesis of frontotemporal dementia, Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and other neurodegenerative conditions such as HIV-induced neurocognitive disorder, and chronic traumatic encephalopathy[1,5,45]. Here, we focus on AD because more is known about neurovascular dysfunction in this disease than in other neurodegenerative disorders, but also discuss the emerging role of neurovascular dysfunction in PD, HD, ALS and MS. We concentrate on

CBF and BBB neuroimaging biomarkers that have advanced the field in recent years establishing early role of brain vasculature in multiple neurodegenerative disorders.

Cerebral blood flow in Alzheimer's disease

CBF reductions, impaired cerebrovascular reactivity and impaired hemodynamic responses are increasingly recognized in early stages of AD and across normal aging-to-mild cognitive impairment (MCI)-to-AD spectrum (Table 1). However, no evidence to date shows that CBF deficits are causal to AD. Below, we examine studies showing that CBF is a useful biomarker in preclinical AD.

CBF reductions.

Early studies found that individuals with greater CBF velocity, as shown by transcranial Doppler measurements in the middle cerebral artery, are less likely to develop dementia or hippocampal and amygdalar atrophy[51]. Similar, individuals with MCI or early AD develop early CBF reductions in the posterior cingulate gyrus and precuneus, as shown by arterial spin labelling (ASL)-magnetic resonance imaging (MRI) [2,43,52–54]. Progression of MCI to AD is associated with more global[55–60] and severe, up to 40%, CBF reductions[61,62], which includes other CNS regions besides the posterior cingulate gyrus and precuneus, such as bilateral parietotemporal, frontal and occipital cortex, parahippocampal gyrus, hippocampus and entorhinal cortex (Table 1). Studies using two-dimensional phase contrast MRI confirmed CBF reductions in MCI[63] and full blown AD[64].

A large cohort longitudinal study from the Alzheimer's Disease Neuroimaging Initiative database suggested that changes in vascular biomarkers (e.g., cerebrospinal fluid (CSF) heart-type fatty acid-binding protein, cortisol, and apolipoprotein A that are all associated with cardiovascular disorders[42]) and decreased CBF by 2D ASL occur before detectable changes in classical Aβ and tau CSF AD biomarkers[42]. Ideally, the CBF findings in this study[42] should be confirmed using more sensitive methods such as those that use contrast agents (e.g., dynamic susceptibility contrast (DSC), DCE perfusion) to achieve better signal to noise ratio compared to ASL, which is less optimal and noisy. Reduced CBF in parietotemporal cortex and basal ganglia in MCI was also shown to occur before brain atrophy by DSC-MRI studies[65]. ASL imaging and Pittsburgh compound B (PiB) amyloid positron emission tomography (PET) imaging revealed widespread and Aβ-independent CBF reductions in cognitively normal *APOE4* carriers with strongest genetic risk for AD[66]. [^{15}O]-labeled water-PET longitudinal study showed widespread decline of CBF occurs over time in cognitively normal *APOE4* carriers[67]. More longitudinal studies are needed to better understand the relationship between CBF and Aβ and tau pathological changes.

Impaired cerebrovascular reactivity.

Impaired cerebrovascular reactivity, reflecting diminished vasodilation of cerebral vessels in response to a CO_2 inhalation challenge, was found early in AD compared to cognitively normal controls using transcranial Doppler[68] and the blood oxygenation level dependent (BOLD)-functional MRI (fMRI)[69]. However, the evidence that this is etiologic is lacking, and while it is possible that these effects could also be downstream to Aβ-mediated

vasculopathy, neither of these two studies investigated the presence of cerebral amyloid angiopathy (CAA). CAA is characterized by deposition of Aβ in the wall of small and mid-sized cerebral and leptomeningeal arteries, veins and cerebral capillaries, and is related to AD[70]. Impaired cerebral blood vessels reactivity to CO_2 has also been shown in the hippocampus of young, non-demented *APOE4* carriers compared to non-carriers by BOLD-fMRI in response to a memory task[71], and by reduced CBF velocity in the middle cerebral artery using transcranial Doppler[72] (Table 1).

Impaired neurovascular coupling.

BOLD-fMRI provides a measure of neurovascular coupling by detecting an increase in blood oxygen delivery and CBF in response to neuronal stimulation[43]. The physiological basis of the BOLD signal has not been fully elucidated and current models include contributions from different hemodynamic and blood parameters[43]. Most fMRI studies treat the BOLD response as an indirect qualitative measure of neuronal activity and interpret BOLD signal differences as changes in neuronal activity. However, the BOLD signal reflects local changes in deoxyhemoglobin content, which in turn exhibits an intricate dependence on changes in CBF, cerebral blood volume, and the cerebral metabolic rate of oxygen consumption ($CMRO_2$). Factors that disturb the connection between CBF and $CMRO_2$ (e.g., altered cerebrovascular structure, reduced blood vessel elasticity, atherosclerosis, reduced resting state CBF, decreased resting $CMRO_2$, reduced vascular reactivity to chemical modulators) may therefore significantly change the BOLD signal during aging and AD, even when neuronal activity is unchanged, which should be taken into account in data interpretation[43]. Reduced CBF response to visual stimuli and memory encoding tasks were detected in the hippocampus, parahippocampal gyrus, precuneus and posterior cingulate cortex in MCI patients[73], and in the hippocampus in AD patients[73,74]. Changes in the hemodynamic response in the visual cortex were also found in patients with CAA[75] that associate with cerebrovascular dysfunction[45]. The alterations in BOLD signal activity during AD progression appear to be regionally specific, and depend on the nature of cognitive tasks, indicating that they indeed may reflect the pathophysiological changes in neurovascular coupling (Table 1). However, two important caveats have limited the application and interpretation of BOLD studies in AD including head motion artifacts and often limited ability of subjects with neurodegenerative diseases to perform tasks.

Animal studies.

Most animal CBF studies have been done in transgenic AD *APP* models of Aβ amyloidosis. These studies have shown that Aβ has vasoactive and vasculotoxic effects on brain vessels, as recently reviewed[2]. Briefly, Aβ vasoactive effects were shown on cerebral blood vessels both *in vitro* and *in vivo* in transgenic mouse models overexpressing human *APP* mutations[1,2], including impaired CBF responses to vasodilators[76], and dysfunctional neurovascular coupling[77]. Thus, preventing Aβ deposition may clearly abolish Aβ-dependent effects on CBF reductions and dysregulation. A recent study in *APP* mice expressing human Swedish, Dutch and Iowa mutations has demonstrated, however, that counteracting the harmful effects of Aβ after vascular depositions is not effective in reversing the neurovascular dysfunction owing to the mural cell damage that is caused by aging and massive Aβ deposition[78].

Several studies reported that CBF dysregulation develops early in experimental models of mural cell or endothelial dysfunction, which can lead to neuronal dysfunction and loss, independent of Aβ, as recently reviewed[2]. For instance, pericyte-deficient mutant mice develop early CBF reductions in grey[33] and white[79] matter in the absence of Aβ pathology, and aberrant CBF responses in the presence of initially normal neuronal activity, endothelial vasodilation, and astrocyte coverage of the blood vessels[13]. Thus, CBF reductions and dysregulation in mouse models can develop independent of Aβ and precede neuronal dysfunction, similar as suggested by human studies (Table 1). In humanized *APOE4* transgenic mice, early CBF reductions precede neuronal dysfunction independently of Aβ[39,80] similar as in human *APOE4* carriers, as discussed above.

Blood-brain barrier in Alzheimer's disease

In parallel to early CBF changes, BBB breakdown and dysfunction have been shown in early stages during AD pathophysiological progression (Table 2). Below, we examine studies indicating that BBB is a useful biomarker in preclinical AD.

BBB permeability.

Blood-to-brain leakage of an intravenously administered gadolinium-based contrast agent, as measured with dynamic contrast-enhanced (DCE)-MRI and quantified with the Patlak analysis, has recently enabled detection of subtle, regional changes in BBB permeability during the normal aging-to-MCI-to-AD spectrum[43,44]. These studies have shown an increase in BBB permeability in the hippocampus and its CA1 and dentate gyrus subfields during normal aging[44], which is further accelerated in individuals with MCI prior to brain atrophy or detectable changes in Aβ and tau CSF biomarkers[44]. Individuals with MCI and early AD develop, with disease progression, increased BBB permeability in other brain regions including cortex, deep gray matter and white matter, all prior to dementia[81,59,82]. During MCI, the increased BBB permeability correlated with pericyte injury, as measured via CSF PDGFRβ pericyte biomarker, further supporting the presence of early BBB breakdown and capillary damage[44]. Interestingly, vascular risk factors such as atherosclerosis, cardiac arrhythmia, and coronary disease did not influence BBB permeability[59], suggesting that BBB breakdown during preclinical and early AD is not related to concomitant vascular conditions, but is rather an early independent biomarker contributing to AD pathophysiology. Future longitudinal studies are needed to precisely define the role of BBB breakdown in cognitive impairment and loss of structural and functional connectivity during AD progression and elucidate the cellular and molecular mechanisms through which it promotes AD pathology.

Microbleeds.

Microbleeds caused by blood leakage into brain parenchyma from damaged blood vessels due to loss of BBB integrity can be visualized by T2*-MRI sequences as hyposignals reflecting perivascular accumulation of blood-derived hemosiderin deposits[43]. Several studies indicate appearance of microbleeds during normal aging[83–88], which is further accelerated in individuals with MCI[83–85,89], early AD[83,85,86,88–90], and AD[85]. Microbleeds reflect cerebral small vessel disease, which is observed in approximately 50% of all

dementia cases worldwide[48,91], and is associated with worse cognitive performance[85] and white matter hyperintensities[87,88]. *APOE4* status accelerates microbleed prevalence in a majority of studies[86–88], but not all[84]. However, at present there is no evidence that microbleeds are etiologically important in causing the AD pathology of plaques and tangles, and/or that they cause AD.

Microbleed etiology and topography are related, with CAA causing lobar microbleeds in AD and hypertensive arteriopathy causing deep infratentorial microbleeds[91]. Hypertension positively associates with microbleed size and prevalence[85,87] particularly in infratentorial regions, whereas other vascular risk factors such as diabetes and hyperlipidemia do not associate with microbleeds[86]. Some studies indicate that microbleeds predominate in deep infratentorial regions during early preclinical AD and MCI stages[84,87] supporting the view that they may precede amyloid pathology, determined by no difference in CSF Aβ42 in MCI subjects with and without microbleeds[84], but are later seen in lobar regions[85,86,88,90] reflecting CAA etiology during AD progression (Table 2). CAA further contributes to BBB breakdown, infarcts, white matter changes, and cognitive impairment leading the detection of dementia earlier[92]. Of note, studies using different magnetic field strengths indicate that the 7T magnet has approximately 3 times greater ability to detect smaller microbleeds compared to 3T magnet[89]; this has implications for future research to determine the true quantity and size of microbleeds that appear throughout the normal aging-MCI-AD spectrum.

Glucose transport.

Numerous ^{18}F-fluoro-2-deoxyglucose (FDG)-PET studies show decreased glucose brain uptake in early stages of AD prior to amyloid and tau changes detected by amyloid-PET and/or Aβ42 and phosphorylated-tau CSF analysis, and/or brain atrophy[93–98]. Cognitively normal individuals with increased genetic risk for AD such as *PSEN1* mutation carriers[99,100] and *APOE4* carriers[96] or positive AD maternal history[94] also display decreased FDG brain uptake as an initial pathophysiological event. Longitudinal studies indicate that FDG brain uptake is further diminished in AD in individuals that converted into AD either from cognitively unimpaired stage or MCI[93,97,101–103]. These reductions in brain uptake of glucose in AD patients are thought to reflect pathological hypometabolism resulting from neurodegeneration[8,104]. However, there is evidence from animal models that brain uptake of 2-deoxyglucose (2DG) depends on the BBB glucose transporter-1 (GLUT1). In particular, deletion of a single *Slc2a1* allele encoding GLUT1 at the BBB in mice diminishes ^{14}C-2DG brain uptake followed by BBB breakdown, CBF reductions and dysregulation, capillary rarefaction, and eventually neuronal dysfunction and loss[105]. In humans, a few kinetic FDG-PET studies demonstrated reductions in FDG BBB transport in AD[106–108]. These reductions in BBB transport may be explained by the fact that the reduced expression of GLUT1 was found in the brain capillaries of AD patients in post-mortem studies[109–112] (Table 2). Thus, in our opinion reduced FDG-PET does not reflect only glucose neuronal hypometabolism but can also reflect reduced transport of glucose across the BBB. Future studies in animal models and humans using glucose, FDG and glucose analogs that track only BBB transport (e.g., 3-O-[^{11}C]-methyl glucose)[113], and in addition to PET more sensitive analytical methods such as nuclear magnetic resonance spectroscopy should further explore brain

glucose transport and metabolism in AD and the therapeutic potential of GLUT1 BBB transporter.

Diminished P-glycoprotein function.

P-glycoprotein is an active efflux transporter expressed at the BBB luminal endothelium[4]. Laboratory studies indicate that Aβ is cleared from brain-to-blood via abluminal LRP1-dependent efflux followed by luminal P-glycoprotein transport[4,114]. Individuals with early AD develop widespread reductions in P-glycoprotein BBB function in the parieto-temporal, frontal, occipital and cingulate cortices, and hippocampus, as shown using PET with the radiolabeled P-glycoprotein substrate, ^{11}C-verapamil[115,116]. Diminished P-glycoprotein BBB function occurred independently of reduced CBF[115] and prior to brain atrophy[116]. In addition to P-glycoprotein, other BBB transporter changes including increased levels of the receptor for advanced glycation endproducts (RAGE), a major Aβ influx transporter at the BBB, and decreased levels of LRP1, a major Aβ efflux transporter at the BBB, were found in cerebral blood vessels of AD patients[45].

Animal studies.

Consistent with human studies, studies in different models relevant to AD pathophysiology also indicate the presence of BBB breakdown and dysfunction. This topic has been comprehensively reviewed recently[6], and will not be examined in great detail here. Briefly, animal studies using transgenic models with various mutations in human *APP* gene, have demonstrated perivascular accumulation of blood-derived proteins (e.g., fibrinogen, immunoglobulin G (IgG), albumin), vascular leakage of circulating exogenous tracers, loss of tight junction proteins, loss of pericyte coverage, pericyte and endothelial degeneration, and microbleeds, altogether indicating BBB breakdown[6,117]. Although most studies did not examine the time course of BBB changes in relation to other brain pathologies, those that did indicated that BBB breakdown occurs early prior to amyloid accumulation, behavioral deficits or brain degenerative changes[6,117,118]. Simultaneously, BBB transporter dysfunction occurs, including decreased luminal P-glycoprotein function, decreased LRP1-mediated Aβ clearance, increased RAGE-mediated Aβ influx, which all accelerates Aβ accumulation in *APP* models[114,119–121]. Decreased GLUT1 BBB expression also contributes to BBB breakdown and Aβ pathology by transcriptionally downregulating LRP1[105], thus corroborating evidence from human BBB studies.

Different *PSEN1* models (e.g., *PSEN1* knockouts and *PSEN1* mutations driven by neuronal promoters) exhibit microbleeds and endothelial degeneration indicative of BBB breakdown[122,123], occurring prior to brain Aβ and CAA pathology[122]. AD models with tau mutations show also BBB leakage of exogenous tracers, IgG deposits, microbleeds, and leukocyte infiltration despite no evidence of brain Aβ or CAA accumulation[124]. Finally, *APOE* knockout mice and mice with targeted replacement of human *APOE4* gene show BBB breakdown and dysfunction (e.g., loss of BBB GLUT1 and increased RAGE)[39,125] prior to development of behavioral deficits, synaptic changes and neuronal dysfunction[39].

Cerebral blood flow and blood-brain barrier in other neurodegenerative disorders

Early CBF and BBB changes are also found in other neurodegenerative disorders including PD, HD, MS and ALS (Table 3).

CBF reductions.

Decreased CBF was found throughout the cortical mantles and precuneus in PD patients with a range of motor and cognitive impairments using ASL-MRI[126]. A recent study showed that CBF reductions were also detectable in non-demented patients with PD[127], suggesting that perfusion abnormalities may be an early biomarker upstream of cognitive impairment and neurodegeneration. Similarly, perfusion deficits were found in the cortex in early HD prior to cognitive dysfunction[128]. Using contrast MRI, early CBF reductions were detected in white matter lesions and in normally appearing white matter in relapsing-remitting MS patients with and without cognitive impairment[129,130]. ALS patients also develop early perfusion deficits in fronto-parietal cortex, subcortical grey matter as well as white matter in the absence of cognitive decline[131], which is independent of brain atrophy[132].

Impaired cerebrovascular reactivity.

Global impaired vasodilation of cerebral vessels in response to CO_2 has been shown in PD using transcranial Doppler[133] and BOLD-fMRI[134]. Decreased cerebrovascular reactivity was also reported in MS[135]. Whether altered cerebrovascular reactivity occurs prior to motor and cognitive impairments remains, however, unknown.

BBB permeability.

DCE-MRI studies indicated increased regional BBB permeability (i.e., K_{trans} values) in the basal ganglia in PD[136], caudate nucleus in HD[137], and white matter in MS[44,129,138–140] prior to cognitive decline[44,136,137,139]. Increased K_{trans} BBB permeability values indicated perivascular lesion growth in MS with minimal motor symptoms[139], suggesting loss of BBB integrity could be an early event driving MS pathophysiology.

Microbleeds.

Brain microbleeds are found in cortical gray matter, basal ganglia, corpus callosum, and internal and external capsules in PD[141–143], as well as in deep layers of motor cortex in ALS[144], occurring prior to cognitive decline[141,144]. However, it remains unclear whether microbleeds precede motor symptoms in PD and ALS. Moreover, the prevalence and regional distribution of microbleeds in HD and MS is presently unknown.

Diminished P-glycoprotein function.

[^{11}C]-verapamil-PET scans indicated diminished P-glycoprotein efflux transport function at the BBB in the mid-brain in PD subjects with no cognitive impairment[145]. Whether or not P-glycoprotein or other BBB transporters play a role in HD, ALS, or MS, is currently elusive.

CNS leukocyte infiltration.

PET studies using a radiolabeled matrix metalloproteinase (MMP) inhibitor ligand indicated increased MMP activity in MS lesions suggesting enhanced leukocyte infiltration across the BBB into the brain of cognitively normal MS patients[146].

Animal studies.

CBF and BBB dysfunctions have also been shown in animal models of PD, HD, ALS, and MS, as recently examined elsewhere[147,148].

Vascular pathways to neurodegeneration

The first hypothetical model of *in vivo* AD dynamic biomarkers, often called the *Jack model*, was proposed in 2010 and updated in 2013 to include interim evidence and present inter-subject variability in cognitive impairments[8]. This model was intended to summarize literature showing temporal evolution of *in vivo* AD biomarkers relative to each other and to the onset and progression of clinical symptoms. This initial model, however, did not include currently available evidence that vascular dysfunction contributes to AD pathophysiology in a significant way[1]. Therefore, it is time now to update the hypothetical biomarkers model of AD and include the impact of cerebral blood vessels on AD pathophysiology, as previously suggested[42,149]. Figure 4 presents an updated revised model of AD biomarkers to show the role of brain vasculature and early changes in CBF and BBB biomarkers in AD that, according to some studies (see Table 1 and Table 2), are altered prior to cognitive decline, brain atrophy, neurodegeneration, and/or amyloid and tau biomarker abnormalities. The sigmoid shape of the vascular curve reflects growing evidence obtained from imaging, biofluid and post-mortem tissue studies indicating an initial acceleration followed by deceleration of brain vascular changes, which do not plateau, similar as shown for the other biomarkers. However, future longitudinal studies may warrant amendment of the proposed trajectories of the vascular curve as well as of the other biomarkers curves and/or their order of their respective appearance.

As discussed above, AD can be viewed as a model for other neurodegenerative diseases that are beginning to reveal notable vascular contributions to disease pathophysiology such as PD, HD, ALS and MS (Table 3). Figure 5 illustrates the commonalities of vascular dysfunction across neurodegenerative diseases, focusing on specific regions where CBF shortfalls and BBB dysfunction are evident during early stages of each disease.

Conclusions and future directions

An emerging role of brain vasculature in the pathogenesis of human neurodegenerative diseases, particularly AD, has led to increasingly recognized importance of healthy blood vessels for normal brain functioning. A recent single cell transcriptomics approach to examine vascular zonation along the A-V axis in mouse models is a huge conceptual advancement[7], providing important insights to biological processes at different levels of the vascular tree and BBB. The single cell RNA-seq approach holds great promise to help us understand the cellular and molecular basis of CBF and BBB dysfunction in models of neurodegenerative disorders and AD, and by extension in humans with these disorders.

While the molecular atlas of cerebral blood vessels and BBB is being elucidated in animal models, we lack the molecular definition of human brain vasculature, BBB, and perhaps NVU, to generate an atlas of blood vessels in the human brain during health and disease. Comparative knowledge of molecular makeup between humans and animals is essential to bridge this translational gap, and for future studies to take advantage of discoveries in animal models. This has the potential to reveal why regional changes in the brain vasculature may lead to disease-specific neurological phenotypes in different neurodegenerative diseases and inform gene networks and upstream regulators driving the link between cerebrovascular dysfunction and neurodegeneration.

Neuroimaging holds the potential to further examine the regional vascular pathophysiology in the living human brain. Further advances are needed such as development of novel neuroimaging modalities for biomarkers of different vascular cell types including SMCs, pericytes and endothelial cells, markers of vascular zonation, and/or molecular pathways in blood vessels. For example, PET imaging with radiolabeled matrix metalloproteinase inhibitors examines *in vivo* leukocyte infiltration across the human BBB in multiple sclerosis, which can be applied to AD and other neurodegenerative disorders[45]. Similarly, MRI imaging of endothelial adhesion molecules detects the inflammatory phenotype of brain vascular endothelium in the rodent brain and models of different brain diseases[150], which could be extended and developed for the living human brain. Similar types of probes could be developed to interrogate RAGE-mediated Aβ vascular influx, and/or LRP1-mediated Aβ vascular efflux capacity. Longitudinal studies with high field strength 7T (or greater) magnets will provide improved sensitivity to detect early, subtle, regional changes in cerebrovascular dysfunction, which will confirm or amend the temporal course of biomarkers abnormality in AD and other neurodegenerative disorders. The advances in imaging will continue to establish new early vascular biomarkers in the living human brain, hopefully revealing untapped novel targets of disease-modifying therapeutics for multiple neurodegenerative disorders.

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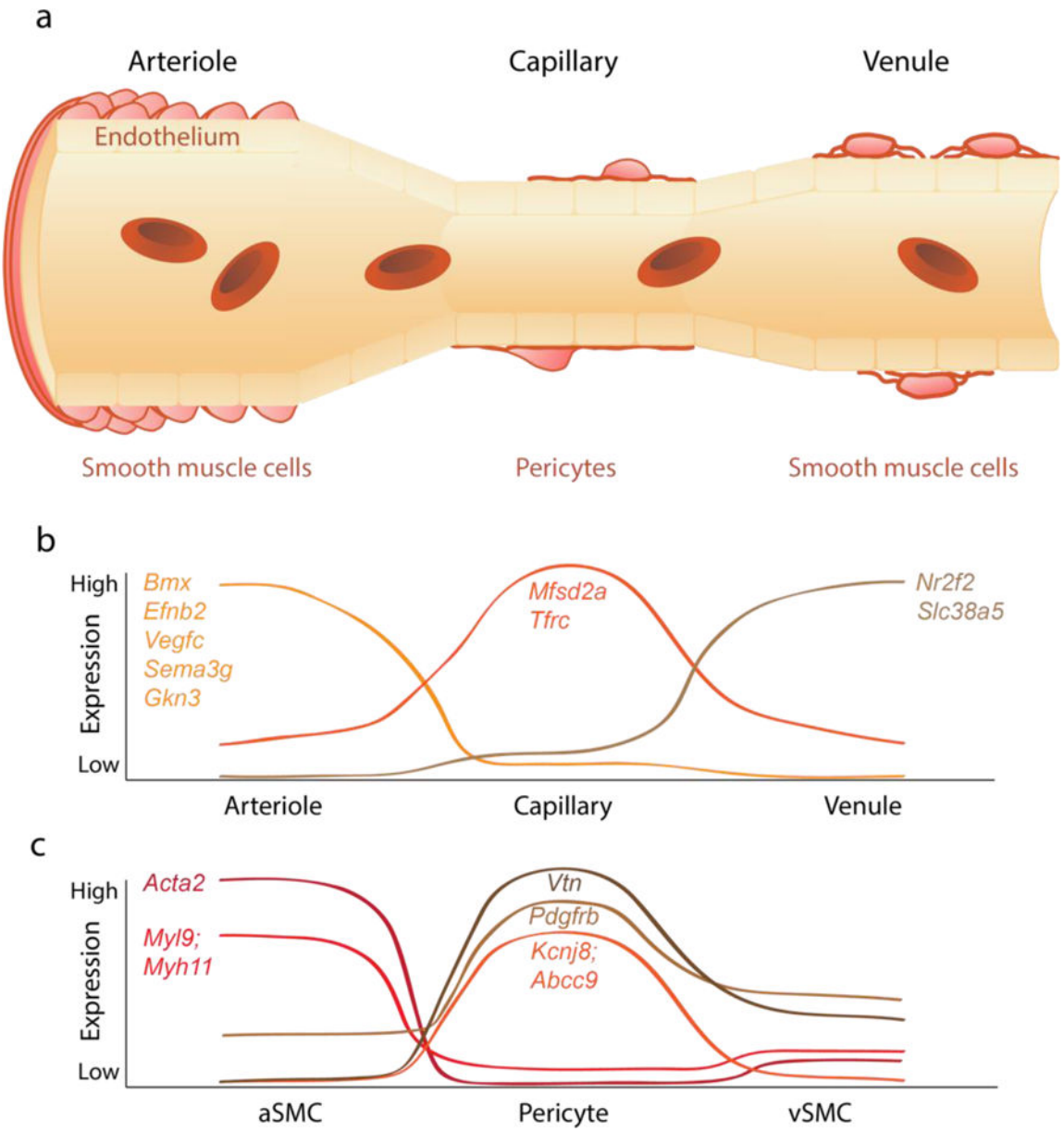

Figure 1. Molecular definition of the blood-brain barrier and cerebral blood vessels.

(**a**) The brain vasculature is a continuum from artery to arteriole to capillary to venule to vein. The blood-brain barrier is formed by a continuous endothelium monolayer surrounded by mural cells. Vascular 'zonation' refers to the molecular and phenotypic changes along the vascular endothelial continuum. Molecularly, the endothelium is a gradual continuum enriched with cell-specific markers at the arterial/arteriolar, capillary and venule/veins levels. Mural cells also cluster at different vascular segments: smooth muscle cells (SMCs) at arterioles and venules and pericytes at capillaries. (**b**) Representative curves showing molecular expression patterns of endothelial cells. The arteriole-specific genes include *Bmx*, *Efnb2*, *Vegfc*, *Sema3g*, and *Gkn3*, capillary-specific genes include *Mfsd2a* and *Tfrc*, and venule-specific genes include *Nr2f2* and *Slc38a5*. (**c**) Representative curves showing molecular expression patterns of mural cells. Arteriole SMCs enriched genes include *Acta2*,

Myl9, and *Myh11*. Capillary pericyte enriched genes include *Vtn, Pdgfrβ*, *Kcnj8*, and *Abcc9*. The molecular characterization is informed from recent single cell RNA-sequencing studies in multiple murine models[7]. Arteriole markers represent averaged artery and arteriole expression. See main text for details.

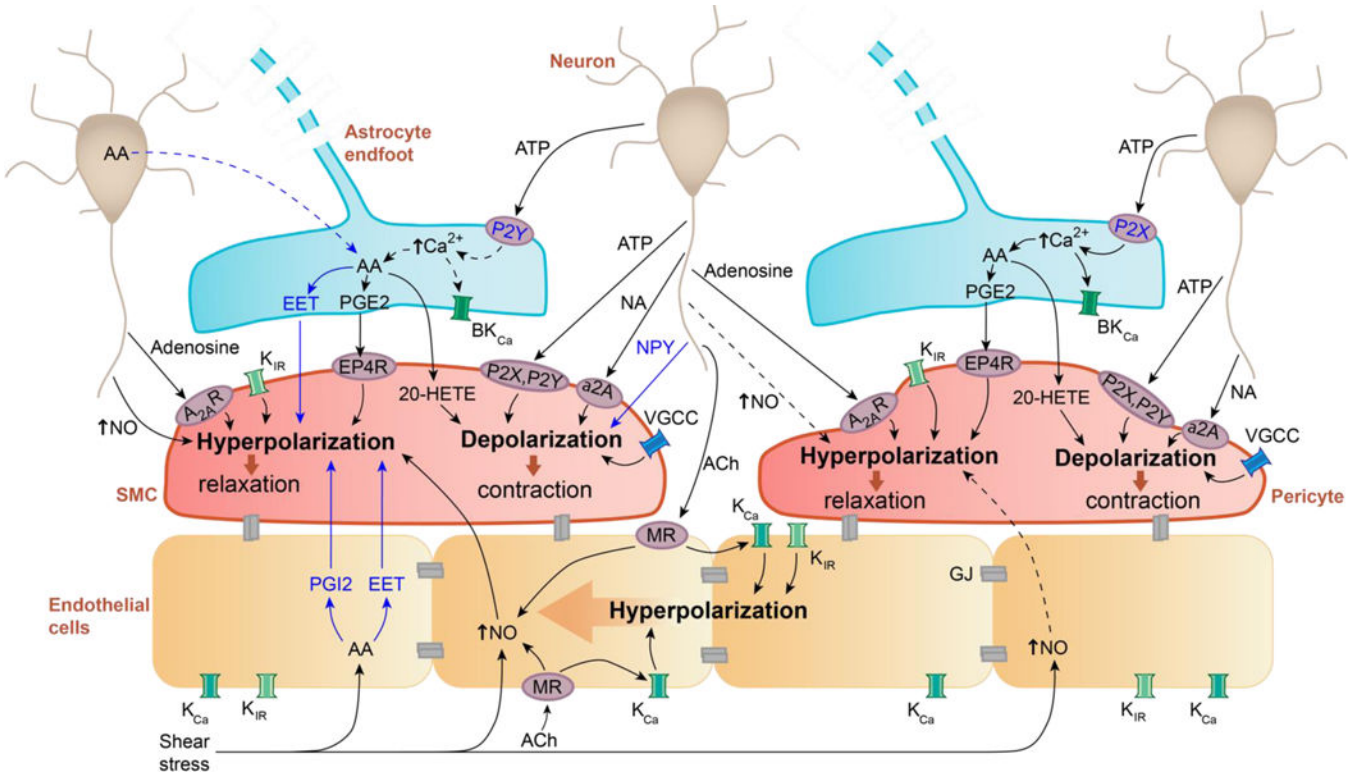

Figure 2. Key cellular and molecular pathways regulating cerebral blood flow.

Neuron-mural cells crosstalk. ATP and noradrenaline (NA) released by neurons act on smooth muscle cells (SMCs) and pericytes through adenosine A_{2A} receptors ($A_{2A}R$) or α2-adrenergic receptors (α2A), respectively, causing cell depolarization and constriction, which reduces blood flow. Adenosine acts via purinergic P2X and P2Y receptors to hyperpolarize SMCs and pericytes, which increases blood flow. Neuropeptide Y (NPY) causes SMCs contraction. In both SMCs and pericytes, nitric oxide (NO) produced by neurons leads to hyperpolarization resulting in blood flow increase. Pericyte response to NO may vary by brain region, indicated by dashed arrows. Extracellular potassium ions (K^+) released during neuronal activation can act on K^+ (inward rectifier, K_{IR}) and Ca^{2+} (Voltage-gated, VGCC) channels in SMCs and pericytes to hyperpolarize and relax the cells, or depolarize and contract cells. *Astrocyte-mural cells crosstalk*. ATP acts on P2X or P2Y receptors on astrocytes, which according to some studies can increase intracellular [Ca^{2+}]. However, the role of arteriolar astrocyte [Ca^{2+}] changes remains debatable (indicated by dashed arrows; see text). [Ca^{2+}] increase triggers production of arachidonic acid (AA) and its metabolites (prostaglandin E2, PGE2, through PGE2 receptor EP4, EP4R; 20-hydroxyeicosatetraenoic acid; 20-HETE; epoxyeicosatetraenoic acids, EETs) that act on SMCs and pericytes to regulate blood flow. Alternatively, neurons may release AA to be further metabolized by astrocytes, indicated by dashed line. *Endothelial-mural cells crosstalk*. Acetylcholine (ACh) released from neurons or blood-derived ACh act on endothelial muscarinic ACh receptors (MRs) to increase endothelial NO production causing hyperpolarization and relaxation of mural cells, which increases blood flow. Shear stress can also increase NO endothelial production as well as production of AA and metabolites EETs and prostacyclin (PGI2) that hyperpolarize and relax SMCs, increasing arteriolar blood flow. Extracellular [K^+] increase

or ACh can activate K_{IR} or calcium-activated K^+ (K_{Ca}) channels on endothelial cells, leading to endothelial hyperpolarization that can propagate via gap junctions (GJs) between endothelial cells in a retrograde direction to increase blood flow. Altogether these findings are informed from various CNS regions and from both *in vivo* and *in vitro* studies; see main text for details.

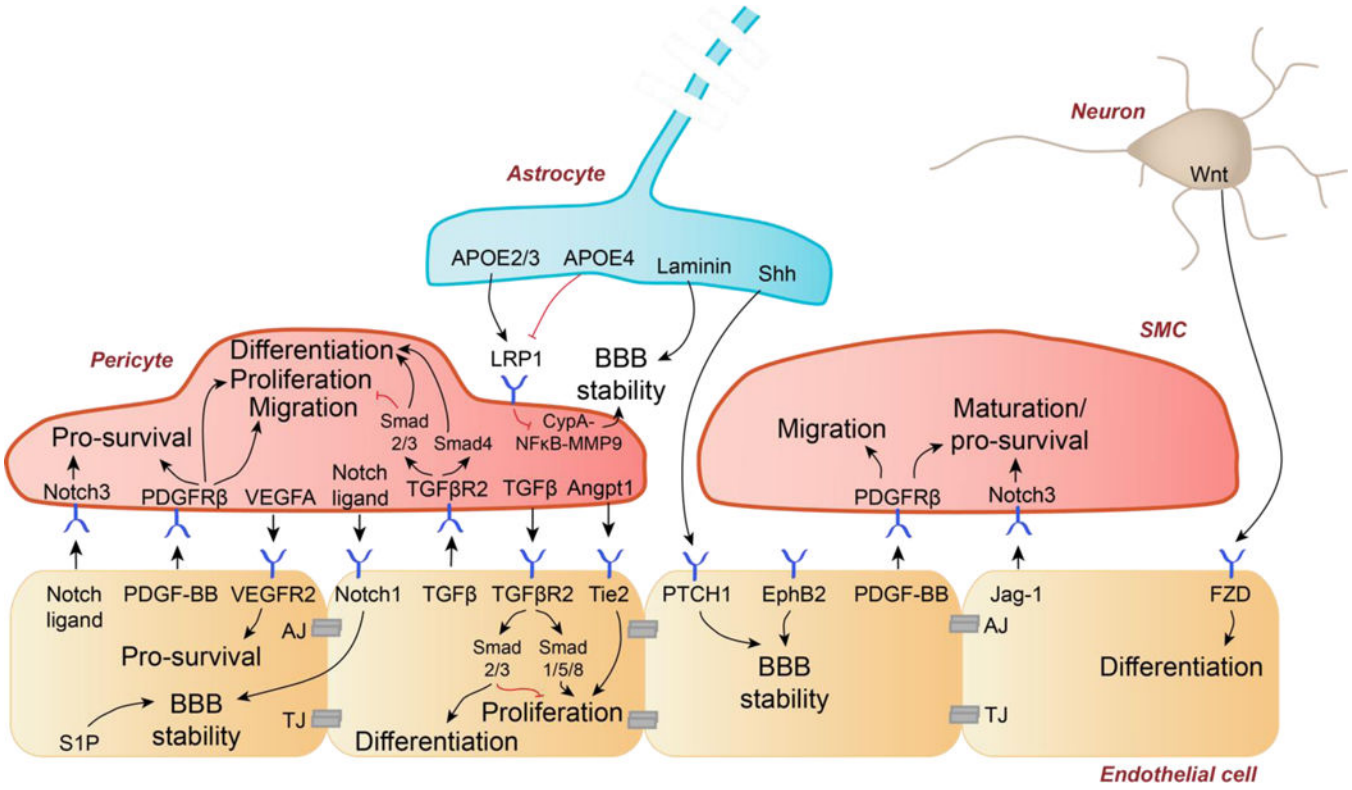

Figure 3. Key cellular and molecular pathways regulating blood-brain barrier integrity.

BBB integrity is maintained by tight junction (TJ) and adherens junction (AJ) proteins between endothelial cells and low-level bulk flow transcytosis. *Pericyte-endothelial cells crosstalk*: Notch ligands-Notch3 receptor signaling promotes pericyte survival. Platelet-derived growth factor-BB (PDGF-BB) binds to PDGFRβ on pericytes causing pericyte survival, proliferation, and migration. Vascular endothelial growth factor-A (VEGFA) binds to endothelial VEGF receptor-2 (VEGFR2) mediating endothelial survival. Pericyte-derived notch ligands bind to endothelial Notch1 receptor which mediates BBB stability, as does endothelial sphingosine-1 phosphate (S1P). Transforming growth factor-β (TGFβ) and TGFβ receptor-2 (TGFβR2) signaling occurs bi-directionally between pericytes and endothelial cells. Pericyte-secreted angiopoietin-1 (Angpt1) binds Tie2 receptor on endothelial cells to promote proliferation. *Astrocyte-endothelial cells crosstalk*: Astrocyte-secreted APOE2 and APOE3, in contrast to APOE4, suppresses the pro-inflammatory signaling cyclophilin A-NFkB-matrix metalloproteinase-9 (MMP9) pathway in pericytes to maintain BBB stability. Similarly, astrocyte-produced laminin maintains BBB stability. Astrocyte-secreted sonic hedgehog (Shh) interacts with patched-1 (PTCH1) at the endothelium to further promote BBB stability. *Smooth muscle cell (SMC)-endothelial cells crosstalk*: Ephrin B2 (EphB2) on the endothelium promotes BBB stability. PDGF-BB binds PDGFRβ on SMCs to promote survival and migration. Endothelial-secreted jagged-1 (Jag-1) binds Notch3 to promote SMC maturation and survival. *Neuron-endothelial cells crosstalk.* Neuron secreted Wnt is a ligand of frizzled (FZD) at the endothelium that promotes endothelial cell differentiation.

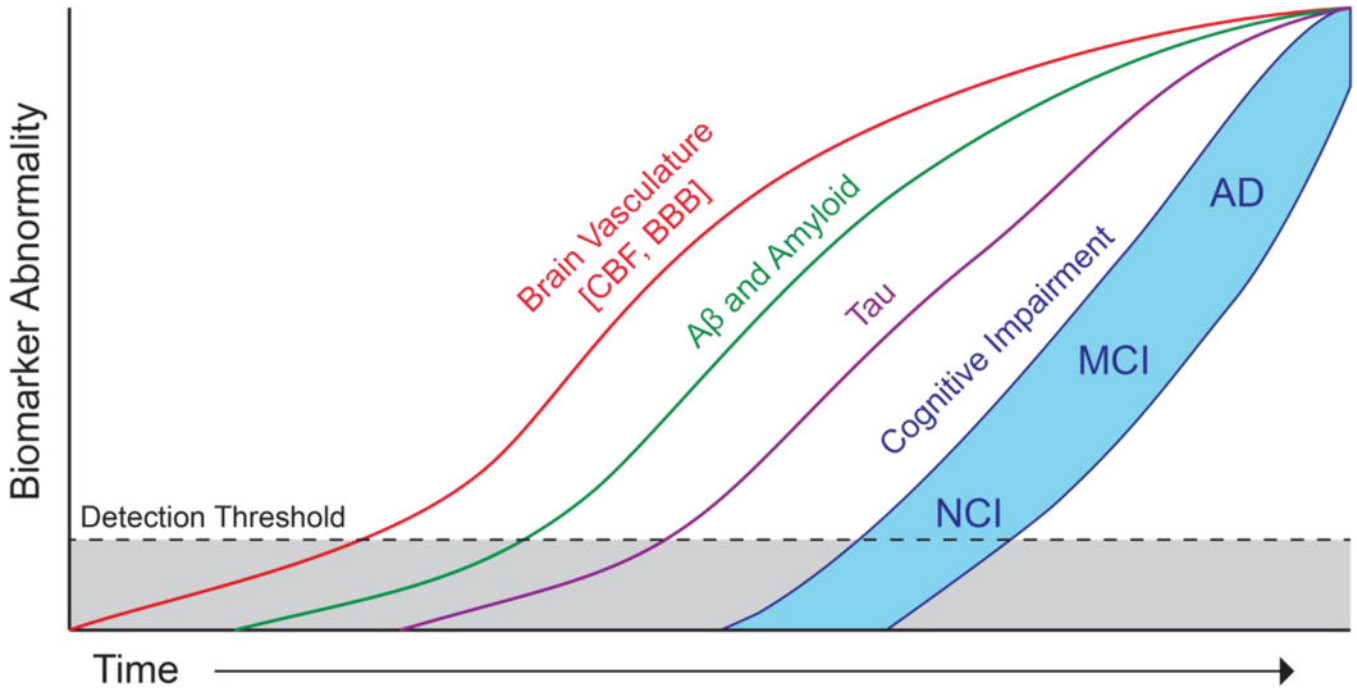

Figure 4. Hypothetical updated Jack model of Alzheimer's disease biomarkers to include the role of brain vasculature.

Hypothetical model of Alzheimer's disease (AD) biomarker changes illustrating that early cerebral blood flow (CBF) and blood-brain barrier (BBB) biomarkers and vascular dysfunction may contribute to initial stages of AD pathophysiological progression from no cognitive impairment (NCI) to mild cognitive impairment (MCI) to AD, which is followed by cerebrospinal fluid and brain changes in Aβ and amyloid, and tau biomarkers. All biomarker curves converge at the top right-hand corner of the plot, that is the point of maximum abnormality. The horizontal axis of disease progression is expressed as time. Cognitive response is illustrated as a zone (blue filled area) with low and high-risk borders. Subjects with high risk of AD-related cognitive impairment are shown with a cognitive response curve that is shifted to the left. In contrast, the cognitive response curve is shifted to the right in subjects with a protective genetic profile, high cognitive reserve and the absence of comorbid brain pathologies.

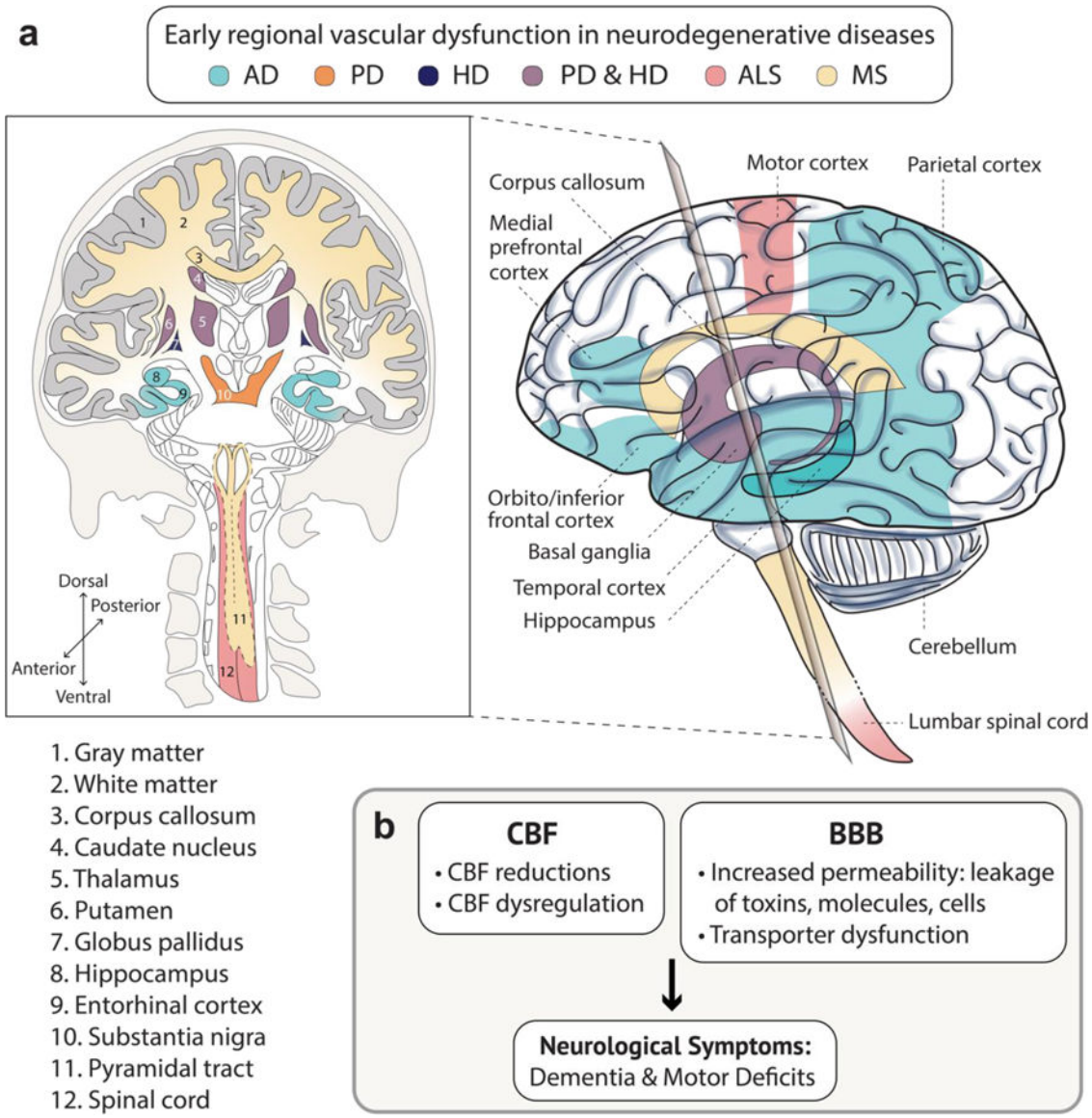

Figure 5. Commonality of an early involvement of brain vasculature in different neurodegenerative disorders.

(**a–b**) Region-specific brain vascular dysfunction including cerebral blood flow (CBF) shortfalls (reductions and dysregulation) and/or blood-brain barrier (BBB) breakdown (increased vascular permeability and transporter dysfunction) is a common pathway seen early in multiple neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS), as illustrated schematically in **a** and **b**. See Tables 1, 2 and 3 for details. Specifically, some studies suggest that vascular dysfunction (CBF and/or BBB) in the hippocampus, gray matter and entorhinal cortex in AD may precede dementia, brain atrophy and/or detectable Aβ and tau biomarker changes. Similar, vascular dysfunction in the white matter and corpus callosum in MS, basal ganglia (the caudate nucleus, thalamus, putamen, globus pallidus and substantia nigra) in PD and HD, the spinal cord white matter pyramidal tract in MS, and motor cortex and spinal cord in ALS is found by some studies in

early stages of these disorders prior to progression of neurological symptoms including motor deficits.

Table 1.

Cerebral blood flow deficits in the normal aging-to-mild cognitive impairment-to-Alzheimer's disease spectrum studied with neuroimaging.

Affected CNS regions	Disease Stages				Methods	References	CBF biomarker prior to			References
	NCI	MCI	Early AD	AD			Brain atrophy	Amyloid	Tau	
CBF reductions										
Middle cerebral artery territory			C		TCD	51				
		C	C			56				
		C	C			55				
Precuneus, posterior cingulate, parietotemporal, frontal and occipital cortices, parahippocampal gyrus, hippocampus, caudate nucleus, thalamus			C			59				
			C			57				
		C		C	ASL-MRI	58				
				C		52				
Posterior DMN				C		62	Yes	Yes	—	2,42,43,51,53,65,66
				C		61				
Whole brain		C				66				
	→	→	→			42				
Parietotemporal cortices and basal ganglia		C			DSC-MRI	65				
Internal carotids and vertebral arteries		C		C	PC-MRI	63				
Whole brain		C		C		64				
Orbitofrontal, middle and inferiorfrontal, middle and superior temporal, inferior temporal, and superior parietal cortices	→				[^{15}O]–PET	67				
Impaired cerebrovascular reactivity										
Hippocampus	C				BOLD-fMRI (CO_2-challenge)	71	—	—	—	2,43
Prefrontal, cingulate and insular cortices			C			69				
				C	TCD (CO_2-challenge)	68				
Middle cerebral artery territory		C				72				
Impaired neurovascular coupling										

Affected CNS regions	Disease Stages				Methods	References	CBF biomarker prior to			References
	NCI	MCI	Early AD	AD			Brain atrophy	Amyloid	Tau	
Hippocampal formation		C	C		task-based BOLD-fMRI	[73]	Yes	—	—	[2,43]
Visual cortex	C					[75]				

Abbreviations: C, cross-sectional study; →, longitudinal study; —, not studied; CNS, central nervous system; NCI, no cognitive impairment; MCI, mild cognitive impairment; AD, Alzheimer's disease; CBF, cerebral blood flow; ASL, arterial spin labelling; MRI, magnetic resonance imaging; fMRI, functional MRI; BOLD, blood-oxygenation-level-dependent; DSC, dynamic susceptibility-contrast; PC, phase-contrast; TCD, transcranial Doppler; SPECT, single-photon emission-computed tomography ; PET, positron emission tomography

Table 2.

Blood-brain barrier dysfunction in the normal aging-to-mild cognitive impairment-to-Alzheimer's disease spectrum studied with neuroimaging.

Affected CNS region(s)	Disease Stage				Accelerated with risk factors			Method	Ref.	BBB biomarker prior to			Ref.
	NCI	MCI	Early AD	AD	Age	VRFs	*APOE4*			Brain atrophy	Amyloid	Tau	
Increased BBB permeability to Gadolinium (K_{trans})													
Hippocampus and its subfields		C			Yes	—	—	DCE-MRI	43,44	Yes	Yes	Yes	43,44
Gray matter, normal-appearing white matter, deep gray matter, hippocampus and cortex			C		—	No	—		59,81,82				
Microbleeds													
	C				Yes	Yes	Yes	T2*/SWI-MRI	87	Yes	Yes	Yes	83–85,87,89
Deep infratentorial regions		C			Yes	—	No		84				
		C	C		—	No	—		89				
Lobar and deep infratentorial regions		C	C		Yes	—	—		83				
			C		—	—	—		90				
Lobar regions			→		Yes	No	Yes		86,88				
		C	C	C	Yes	Yes	—		85				
Diminished glucose transport													
Parietal, frontal and/or temporal cortex				C	—	—	—	Dynamic FDG-PET	106–108	—	—	—	NA
Diminished glucose transport and/or metabolism													
Posterior cingulate, parieto-temporal, and entorhinal cortices, hippocampus, whole brain	C*				—	—	—	FDG-PET	99,100	Yes	Yes	Yes	93–98,100
Posterior cingulate and parieto-temporal cortex	C				—	—	Yes		96				
	C#				—	—	—		94				

Affected CNS region(s)	Disease Stage				Accelerated with risk factors			Method	Ref.	BBB biomarker prior to			Ref.
	NCI	MCI	Early AD	AD	Age	VRFs	*APOE4*			Brain atrophy	Amyloid	Tau	
Posterior cingulate, parieto-temporal, occipital and frontal cortices, precuneus, and/or hippocampus		C	C		—	—	—		98				
		C	C	C	—	—	—		95				
				C	—	—	—		52				
		→			—	—	—		103				
Posterior cingulate, parieto-temporal and frontal cortices, hippocampus		→	→	→			—		—				— 97,101
			→				Yes		—				— 102
		→	→	→	—	—	Yes		93				
Diminished P-glycoprotein function													
Parieto-temporal, frontal, occipital and cingulate cortices, hippocampus			C		—	—	—	Verapamil-PET	115,116	Yes	—	—	116

Abbreviations: C, cross-sectional study; →, longitudinal study; —, not studied; CNS, central nervous system; AD, Alzheimer's disease; VRFs, vascular risk factors; APOE4, apolipoprotein E4; NCI, no cognitive impairment; MCI, mild cognitive impairment; DCE, dynamic contrast-enhanced; MRI, magnetic resonance imaging; FDG, fluorodeoxyglucose; PET, positron emission tomography.

Footnotes:

*Accelerated in asymptomatic PSEN1 mutation carriers compared to non-carriers

#Accelerated in individuals with positive maternal history of AD.

Table 3.

Cerebral blood flow deficits and blood-brain barrier dysfunction in other neurodegenerative disorders studied with neuroimaging.

Affected CNS regions	Disease	Study design	Methods	References	Biomarker prior to		References
					Motor symptoms	Cognitive decline	
CBF reductions							
Posterior parieto-occipital cortex, superior temporal gyrus, posterior cingulate, precuneus and cuneus, and middle frontal gyri	PD	C	ASL-MRI	126,127	—	Yes	127
Sensorimotor, paracentral, inferior temporal and lateral occipital cortices, postcentral gyrus, insula and medial occipital areas	HD	C	ASL-MRI	128	—	Yes	128
White matter including lesions	MS	C	DCE-MRI	129	—	—	NA
White matter including lesions, gray matter		C	DSC-MRI	130	—	Yes	130
Frontal and parietal cortices	ALS	C	ASL-MRI	132	—	Yes	131,132
All cortical lobes, and subcortical grey and white matter		→	CT	131			
Impaired cerebrovascular reactivity							
Whole brain	PD	C	ASL-MRI	134	—	—	NA
Middle cerebral artery territory		C	TCD	133	—	—	NA
Default mode, frontoparietal, somatomotor, visual, limbic, dorsal and ventral attention networks	MS	C	ASL-MRI	135	—	—	NA
Increased BBB permeability							
Basal ganglia	PD	C	DCE-MRI	136	—	Yes	136
Caudate nucleus	HD	C		137	—	Yes	137
White matter	MS	C		44,129,138–140	Yes	Yes	44,139
Microbleeds							
Cortical gray and white matter	PD	C	T2*/SWI-MRI	141–143	—	Yes	141
Deep layers of motor cortex	ALS	C		144	—	Yes	144

Affected CNS regions	Disease	Study design	Methods	References	Biomarker prior to		References
					Motor symptoms	Cognitive decline	
Diminished P-glycoprotein function							
Mid-brain	PD	C	Verapamil-PET	145	—	Yes	145
CNS leukocyte infiltration							
White matter	MS	→	MMP inhibitor-PET	146	—	Yes	146

Abbreviations: C, cross-sectional; →, longitudinal study; —, not studied; PD, Parkinson's disease; HD, Huntington's disease; MS, multiple sclerosis; ALS, amyotrophic lateral sclerosis.