Alzheimer's disease: A matter of blood–brain barrier dysfunction?

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The blood–brain barrier (BBB) keeps neurotoxic plasma-derived components, cells, and pathogens out of the brain. An early BBB breakdown and/or dysfunction have been shown in Alzheimer's disease (AD) before dementia, neurodegeneration and/or brain atrophy occur. However, the role of BBB breakdown in neurodegenerative disorders is still not fully understood. Here, we examine BBB breakdown in animal models frequently used to study the pathophysiology of AD, including transgenic mice expressing human amyloid-β precursor protein, presenilin 1, and tau mutations, and apolipoprotein E, the strongest genetic risk factor for AD. We discuss the role of BBB breakdown and dysfunction in neurodegenerative process, pitfalls in BBB measurements, and how targeting the BBB can influence the course of neurological disorder. Finally, we comment on future approaches and models to better define, at the cellular and molecular level, the underlying mechanisms between BBB breakdown and neurodegeneration as a basis for developing new therapies for BBB repair to control neurodegeneration.

Introduction

The blood–brain barrier (BBB) is formed by a tightly sealed monolayer of brain endothelial cells, which keeps neurotoxic plasma-derived components, RBCs, leukocytes, and pathogens out of the central nervous system (CNS; Zlokovic, 2011). The capillary length in mouse and human brain is 0.6 and 650 km, respectively, which accounts for >85% of total cerebral blood vessel length, providing the largest endothelial surface area for solute transport exchanges between blood and brain, and vice versa (e.g., ∼120 cm^2/g of brain; Zlokovic, 2008; Pardridge, 2015). Endothelium allows free, rapid diffusion of oxygen and carbon dioxide across the BBB. Specialized endothelial transport systems carry energy metabolites, nutrients, and regulatory molecules across the BBB from blood to brain and metabolic waste products and potentially neurotoxic molecules from brain to blood. This includes solute carrier-mediated transport of carbohydrates (e.g., glucose), amino acids, vitamins, hormones, nucleotides, and monocarboxylic acids (e.g., lactate); receptor-mediated transport (RMT) of peptides and proteins; a major facilitator transporter of essential omega-3 (ω-3) fatty acids; ATP-binding cassette active efflux of xenobiotics and drugs; and multiple ion transporters. The BBB transport systems and cellular junctions have been recently reviewed elsewhere (Zhao et al., 2015a).

The mean distance between the BBB and neurons is ∼8 µm, allowing rapid diffusion of molecules across the brain interstitial space from capillaries to neurons, and vice versa (Pardridge, 2015). The BBB controls the composition of neuronal internal milieu, which is essential for proper neuronal and synaptic functioning (Zhao et al., 2015a). The BBB-associated mural cells (pericytes) play a key role in the formation and maintenance of the BBB (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010; Sweeney et al., 2016). BBB breakdown has been shown in Alzheimer's disease (AD; Zlokovic, 2011) and is associated with cerebral blood flow (CBF) reductions and impaired hemodynamic responses (Iadecola, 2013, 2017; Kisler et al., 2017a). Recent imaging and biomarker studies suggest an early BBB breakdown and vascular dysregulation in AD detectable before cognitive decline and/or other brain pathologies (Montine et al., 2014; Montagne et al., 2015, 2016; Snyder et al., 2015; Sweeney et al., 2015; Iturria-Medina et al., 2016; van de Haar et al., 2016a; Kisler et al., 2017a). Neuropathological studies also support early contribution of cerebral vessel disease to AD (Toledo et al., 2013; Arvanitakis et al., 2016).

Here, we briefly summarize key findings from human studies demonstrating BBB breakdown and dysfunction in AD and examine BBB breakdown in transgenic models frequently used to study pathophysiology of AD. We concentrate on animal models carrying major autosomal-dominant AD (ADAD) mutations in human amyloid-β precursor protein (*APP*) and presenilin 1 (*PSEN1*) genes (Zhao et al., 2015a),

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Abbreviations used: 2DG, 2-deoxy-D-glucose; 2DG-6P, 2-deoxy-D-glucose-6-phosphate; Aβ, amyloid-β; AD, Alzheimer's disease; ADAD, autosomal dominant AD; ALS, amyotrophic lateral sclerosis; APC, activated protein C; Apoe, apolipoprotein E; APP, amyloid-β precursor protein; BBB, blood–brain barrier; CBF, cerebral blood flow; CNS, central nervous system; CypA, cyclophilin A; ECS, extracellular space; GFAP, glial fibrillary acidic protein; JAM-C, junctional adhesion molecule-C; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; Pdgfrβ, platelet-derived growth factor receptor β; PET, positron emission tomography; Pgp, P-glycoprotein; PICALM, receptor for advanced glycation end products; RAGE, receptor for advanced glycation end products; RMT, receptor-mediated transport; TR, targeted replacement.

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Tau gene, and apolipoprotein E (*APOE*) ε4 allele, a major genetic risk factor for sporadic, late-onset AD (Verghese et al., 2011; Liao et al., 2017). We also briefly discuss findings in pericyte-deficient models, because pericytes maintain BBB integrity and they degenerate in AD (Sweeney et al., 2016) and AD models (Park et al., 2013b; Sagare et al., 2013). Other models associated with BBB breakdown, such as *PGRN* (progranulin)-deficient mice modeling loss-of-function mutations in the *PGRN* gene linked to frontotemporal dementia (Jackman et al., 2013), deficiency in endothelial sphingosine-1-phosphate receptor 1 (Yanagida et al., 2017), and vascular risk factors such as diabetes and hypertension (Girouard and Iadecola, 2006; Iadecola, 2013; Nelson et al., 2016), have been summarized elsewhere.

We focus on CNS capillaries that maintain the largest surface area of the BBB. Additionally, we examine changes in other vascular segments, including arterioles and small arteries, which when disrupted also contribute to a loss of blood-to-brain vascular integrity. We discuss the role of BBB breakdown and dysfunction in the neurodegenerative process, pitfalls in BBB measurements, and how targeting the BBB can influence the course of a neurological disorder. We also suggest future cellular and molecular studies to investigate the underlying mechanisms between BBB breakdown and neurodegeneration as a basis for developing new therapeutic approaches for BBB repair to control neurodegeneration.

BBB breakdown in AD

Recent neuroimaging studies in individuals with mild cognitive impairment (MCI) and early AD have shown BBB breakdown in the hippocampus (Montagne et al., 2015) and several gray and white matter regions (van de Haar et al., 2016a,b, 2017), respectively, before brain atrophy or dementia. These neuroimaging studies used advanced dynamic contrast-enhanced magnetic resonance imaging (MRI) to determine BBB permeability to the contrast agent Gadolinium. Increased CNS cerebral microbleeds reflecting loss of cerebrovascular integrity have also been shown by MRI studies in 25% of individuals with MCI and 45–78% of individuals with early AD before dementia (Brundel et al., 2012; Heringa et al., 2014; Yates et al., 2014; Shams et al., 2015; Poliakova et al., 2016; Shams and Wahlund, 2016).

The BBB breakdown in AD has been confirmed by more than 20 independent postmortem human studies showing brain capillary leakages and perivascular accumulation of blood-derived fibrinogen, thrombin, albumin, immunoglobulin G (IgG) and hemosiderin deposits, pericyte and endothelial degeneration, loss of BBB tight junctions, and RBC extravasation, as recently reviewed (Nelson et al., 2016). An early cerebrovascular disorder (Toledo et al., 2013; Arvanitakis et al., 2016), vascular dysregulation (Iturria-Medina et al., 2016), ischemic vascular damage from comorbidities and vascular risk factors (Iadecola, 2013; Faraco et al., 2016; Nelson et al., 2016), and small vessel disease of the brain (Wardlaw et al., 2013; Snyder et al., 2015; Hachinski, 2016) may introduce additional vascular components contributing to BBB breakdown in AD.

The identification of peripheral macrophages (Hultman et al., 2013) and neutrophils (Zenaro et al., 2015) in the brain in human AD suggests BBB breakdown to circulating leucocytes and their influx into the brain. A novel matrix metalloproteinase radioactive positron emission tomography (PET) ligand was recently used to visualize leukocyte penetration across the BBB and infiltration into the brain in patients with multiple sclerosis (Gerwien et al., 2016). Similar studies, however, have not yet been performed in AD patients, and it remains unclear whether leukocyte infiltration is a cause or a consequence of BBB breakdown.

Multiple studies with [^{18}F]Fluoro-2-deoxy-D-glucose (FDG-PET) in individuals with MCI and early AD showed impaired regional brain uptake of glucose before brain atrophy, neurodegeneration, or conversion to AD, suggesting reduced glucose brain utilization caused by diminished glucose transport across the BBB via endothelial-specific glucose transporter 1 (GLUT1), as recently reviewed (Nelson et al., 2016). 2-Deoxy-D-glucose (2DG) was originally developed by Sokoloff as a surrogate molecule to study brain glucose uptake and utilization (Sokoloff et al., 1977). Similar to glucose, 2DG is transported across the BBB by GLUT1, and after crossing the BBB, it is taken up by different cell types in the brain, including neurons and astrocytes (Cunnane et al., 2011). 2DG is rapidly phosphorylated by intracellular hexokinase (Sols and Crane, 1954) and converted in the brain into 2DG-6-phosphate (2DG-6P; Sokoloff et al., 1977; McDougal et al., 1990). However, unlike glucose-6-phosphate, which is metabolized further in the glycolytic pathway, 2DG-6P is not a substrate for glucose-6-phosphate isomerase and therefore cannot be converted into fructose-6-phosphate, a necessary step to enter the glycolytic pathway or Krebs cycle, as shown by multiple independent studies (Sols and Crane, 1954; Sokoloff et al., 1977; McDougal et al., 1990; Rokka et al., 2017). For example, studies in the mouse brain using enzymatic assays indicated that 1 h after 2DG systemic administration, ∼90% is converted into 2DG-6P and 10% remains as 2DG, with no other significant metabolites found (McDougal et al., 1990). This has been confirmed by another study in the mouse brain using thin-layer chromatography and digital autoradiography, which indicated that after 1 h of [^{18}F]-DG administration, 97% of the [^{18}F]-DG is converted into [^{18}F]-DG-6P (Rokka et al., 2017). Similarly, at longer time points, such as 90 min after [^{19}F]-DG administration to rats, 90% of the [^{19}F]-DG in the brain is converted into [^{19}F]-DG-6P and/or its epimers that are not metabolized further, whereas 10% remains as [^{19}F]-DG (Southworth et al., 2003). Because of minimal glucose-6-phosphatase activity in the brain (Hers and De Duve, 1950; Sokoloff et al., 1977) and low 2DG-6P membrane permeability, 2DG-6P remains trapped in brain cells (Huang and Veech, 1985; Southworth et al., 2003) and is eliminated very slowly from the brain.

P-glycoprotein (Pgp) dysfunction in BBB active efflux transport of xenobiotics and drugs in individuals with early

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AD has also been shown using verapamil (Pgp ligand)-PET (van Assema et al., 2012; Deo et al., 2014). Implications of BBB breakdown and dysfunction in BBB transport systems for development of brain pathology based on findings in AD models are discussed next.

BBB breakdown in *APP* transgenic models

Transgenic models expressing mutations in human *APP* gene linked to early ADAD have been frequently used to study the pathophysiology of AD and/or develop treatments to control AD-related cerebral β-amyloidosis. Here, we examine BBB breakdown in *APP* transgenic models and its relationship with Alzheimer's amyloid-β (Aβ) pathology, neurodegeneration, and behavioral deficits (Table 1).

Several independent studies have shown BBB breakdown in $APP^{Sw/0}$ mice harboring the Swedish double *APP* mutation (KM670/671NL) driven by the hamster prion gene promoter (Hsiao et al., 1996). This mutation increases abnormal cleavage of cellular APP by β-secretase, causing Aβ overproduction. The BBB breakdown has been demonstrated by capillary leakages of blood-derived fibrinogen, IgG, and albumin; BBB leakage of intravenously administrated Evans blue; and immunohistological and electron microscopy studies showing degeneration and loss of brain capillary pericytes, endothelial cells, vascular smooth muscle cells (VSMCs), loss of endothelial tight junction proteins, and cerebral microhemorrhages (Kumar-Singh et al., 2005; Paul et al., 2007; Biron et al., 2011; Park et al., 2013b; Sagare et al., 2013; Table 1). Previous studies have shown that leakage of molecules across the BBB could be caused by a loss and/or misalignment of the tight junction proteins and/or enhanced bulk flow fluid transcytosis across the BBB (Zhao et al., 2015a). Additionally, pericyte degeneration has been shown to lead to BBB disruption at the level of brain capillaries (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010). VSMC degeneration is associated with the breakdown of vascular integrity at the level of small arterioles and arteries, often causing microhemorrhages (Holtzman et al., 2000; Bell et al., 2009). Brain endothelial degeneration is typically seen with advanced damage to the BBB at the capillary level (Zlokovic, 2008).

Studies examining the time course between BBB breakdown and other pathologies indicated that BBB breakdown develops early in $APP^{Sw/0}$ mice (at 1, 3, and 6 mo of age; Ujiie et al., 2003; Paul et al., 2007; Sagare et al., 2013) before Aβ deposition, cerebral amyloid angiopathy (CAA), and/or behavioral deficits that develop later (at 7–10, 9–12, and 6–9 mo of age, respectively; Domnitz et al., 2005; Webster et al., 2014). The underlying mechanisms of early BBB disruption in $APP^{Sw/0}$ mice remain elusive but likely involve direct vasculotoxic effects of oligomeric Aβ and/or other APP-mediated toxic effects on vasculature.

The role of BBB breakdown in the development of brain pathologies has been supported by findings showing that loss of pericytes causing an accelerated BBB breakdown also accelerates parenchymal Aβ deposition and CAA and leads to tau pathology and neuronal loss, which is not seen in $APP^{Sw/0}$ mice but has been shown in double-transgenic $APP^{Sw/0}$; $Pdgfr\beta^{+/-}$ (platelet-derived growth factor receptor β) mice with accelerated pericyte loss (Sagare et al., 2013).

Aberrant expression of BBB transporters and/or receptors in cerebral microvessels has also been shown in $APP^{Sw/0}$ mice and other *APP* models. This includes low levels of low-density lipoprotein receptor–related protein 1 (LRP1), a major clearance receptor for Aβ toxin at the BBB (Deane et al., 2004). Its diminished expression in brain capillaries precedes onset of Aβ pathology and behavioral deficits in $APP^{Sw/0}$ mice and triple-transgenic APP^{SwDI} mice carrying three human *APP* mutations (Swedish, Dutch, and Iowa) under the control of the mouse Thy1 neuronal promoter (Deane et al., 2004). Genetic approaches have demonstrated that increasing or diminishing LRP1 levels in cerebral blood vessels slows down or accelerates Aβ pathology and CAA in $APP^{Sw/0}$ and APP^{SwDI} mice, respectively (Bell et al., 2009; Table 1). Genetic deletion of LRP1 from brain endothelium also accelerates Aβ pathology in *5xFAD* mice (Storck et al., 2016) carrying three *APP* mutations (Swedish, Florida, and London) and two Presenilin 1 human mutations (*PSEN1 M146L* and *PSEN1 L286V*; Oakley et al., 2006) causing ADAD (Karch et al., 2014).

Expression of the receptor for advanced glycation end products (RAGE), a major Aβ influx receptor at the BBB, is increased in brain microvessels in $APP^{Sw/0}$ and *5xFAD* mice, which accelerates reentry of circulating Aβ into the brain, causing Aβ accumulation, CBF reductions, and a neuroinflammatory response (Deane et al., 2003) and BBB breakdown (Kook et al., 2012), respectively. Blockade of RAGE slows down Aβ pathology and behavioral deficits in PD-h*APP* mice expressing a mutant form of human *APP* encoded by a minigene containing several substitutions from ADAD (V717F, K670M, and N761L) under the control of platelet-derived growth factor B (PDGF-B) chain promoter (Deane et al., 2003; Table 1).

$APP^{Sw/0}$ mice develop an early GLUT1 BBB dysfunction that reduces brain glucose uptake (Niwa et al., 2002). Moreover, diminished GLUT1 expression in brain endothelium leads to BBB breakdown and transcriptionally inhibits LRP1. This accelerates Aβ pathology and leads to secondary neurodegenerative changes, loss of neurons, and brain atrophy, as shown in double-transgenic $Slc2a1^{+/-}$; $APP^{Sw/0}$ mice with GLUT1 endothelial-specific haploinsufficiency (Winkler et al., 2015). Loss of brain endothelial Pgp efflux transporter also diminishes LRP1 expression at the BBB and leads to brain accumulation of Aβ, as shown in triple-transgenic $APP^{Sw/0}$; $mdr1a/b^{-/-}$ (Pgp-null) mice (Cirrito et al., 2005). Additionally, Pgp endothelial dysfunction diminishes active efflux at the BBB, leading to brain accumulation of potentially neurotoxic xenobiotics such as environmental pollutants, food additives, pesticides, and drugs (Mokgokong et al., 2014).

Recent studies have shown that brain endothelial deficiency in the phosphatidylinositol-binding clathrin assembly

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Table 1. **BBB breakdown in *APP*, *PSEN1*, *Tau*, and pericyte-deficient transgenic models**

Mouse line, construct, promoter	Findings	Age	Region	Reference	Brain Aβ	CAA	Neurodegenerative and behavior changes	Reference
Tg APP models								
*APP**Sw/0*** **(Tg2576)**, *hAPP695*, *K670M/N671L*, hPrP	Fibrin perivascular deposits, BBB leakage of Evans blue	6 and 12 mo; not studied before 6 mo	Cortex, hippocampus	Paul et al., 2007	7–10 mo	9–12 mo	6–9 mo	Hsiao et al., 1996; Domnitz et al., 2005; Kumar-Singh et al., 2005; Webster et al., 2014
	Loss of BBB tight junctions,[a] LRP1 endothelial expression, PDGFRβ$^+$ pericytes, VSMCs	18 mo; not studied before 18 mo	Cortex	Park et al., 2013a				
	IgG perivascular deposits, loss of CD13$^+$ pericytes, endothelial degeneration	1, 3, 6, and 9 mo	Cortex, hippocampus	Sagare et al., 2013				
	IgG perivascular deposits, albumin perivascular deposits, microhemorrhages,[b,c] endothelial degeneration, pericyte degeneration, VSMC loss, swollen astrocytic end-feet	17 and 25 mo; not studied before 17 mo	Cortex, hippocampus, thalamus	Kumar-Singh et al., 2005				
	BBB leakage of Evans blue, BBB leakage of exogenous tracers	4 and 10 mo	Cortex	Ujiie et al., 2003				
	Loss of BBB tight junctions[a,d]	18 and 24 mo; not studied before 18 mo	Cortex, hippocampus	Biron et al., 2011				
	Loss of LRP1 endothelial expression	4 and 6 mo	Cortex, hippocampus, thalamus	Deane et al., 2004				
	Increased RAGE vascular expression	9 mo; not studied before 9 mo	Cortex, hippocampus	Deane et al., 2003				
	Genetically increased LRP1 expression in brain microvessels	16 and 24 mo; not studied before 16 mo	Cortex	Bell et al., 2009	Slowed down	Slowed down	Not studied	Bell et al., 2009
*APP**Sw/0**; Pdgfrβ**+/–***, accelerated loss of pericytes	IgG perivascular deposits, loss of CD13$^+$ pericytes	1, 3, 6, and 9 mo	Cortex, hippocampus	Sagare et al., 2013	Accelerated, +tau pathology and loss of neurons	Accelerated	Accelerated	Sagare et al., 2013
*APP**Sw/0**; Slc2a1**+/–***, *GLUT1* endothelial haploinsufficiency	Fibrin perivascular deposits, IgG perivascular deposits, and loss of BBB tight junctions,[a,d] LRP1 endothelial expression, GLUT1 endothelial expression	2 wk and 1, 8–10, 12, and 16 mo	Cortex, hippocampus	Winkler et al., 2015	Accelerated, +loss of neurons	Not studied	Accelerated	Winkler et al., 2015
*APP**Sw/0**; mdr1a/b**–/–***, loss of *Pgp* endothelial function	Loss of Pgp endothelial expression, BBB leakage of Pgp substrate, loss of LRP1 endothelial expression	2 and 3 mo	Striatum, cortex, hippocampus	Cirrito et al., 2005	Accelerated	Not studied	Not studied	Cirrito et al., 2005

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Table 1. **BBB breakdown in *APP*, *PSEN1*, *Tau*, and pericyte-deficient transgenic models** *(Continued)*

Mouse line, construct, promoter	Findings	Age	Region	Reference	Brain Aβ	CAA	Neurodegenerative and behavior changes	Reference
*APP**Sw/0**; Picalm+/–*, *PICALM* endothelial haploinsufficiency	Loss of PICALM endothelial expression	3 mo	Cortex, hippocampus	Zhao et al., 2015b	Accelerated	Accelerated	Accelerated	Zhao et al., 2015b
APP V717F **(PDAPP)**, *hAPP* full-length, *V717F*, *PDGFb*	Fibrin perivascular deposits, BBB leakage of Evans blue	6 and 12 mo; not studied before 6 mo	Cortex, hippocampus	Paul et al., 2007	6–9 mo	10–12 mo	6 mo	Paul et al., 2007; Webster et al., 2014
PD-hAPP, *hAPP* minigene, *V717F/K670M/N671L*, PDGFb	Blockade of RAGE	9 mo	Cortex, hippocampus	Deane et al., 2003	Slowed down	Not studied	Not studied	Deane et al., 2003
*APP**Swl*** **(TgCRND8)**, *hAPP695*, *K670M/N671L/V717F*, hPrP	Fibrin perivascular deposits, BBB leakage of Evans blue	6 and 12 mo; not studied before 6 mo	Cortex, hippocampus	Paul et al., 2007	3 mo	6–7 mo	3 mo	Domnitz et al., 2005; Webster et al., 2014
	Fibrin perivascular deposits	6 mo; not studied before 6 mo	Cortex	Chen et al., 2017				
*APP**SwDI***, *hAPP770*, *K670M/N671L/E693Q/D694N*, mThy1	Albumin perivascular deposits	6 mo; not studied before 6 mo	Dorsal subiculum	Kruyer et al., 2015	3–6 mo	6 mo	3–6 mo	Davis et al., 2004; Deane et al., 2004
	Loss of LRP1 endothelial expression	4 and 6 mo	Cortex, hippocampus, and thalamus	Deane et al., 2004				
	Genetically decreased LRP1 expression in brain microvessels	16 and 24 mo; not studied before 16 mo	Cortex	Bell et al., 2009	Accelerated	Accelerated	Not studied	Bell et al., 2009
*APP**Sw*** **(APP23)**, *hAPP751*, *K670M/N671L*, mThy1	Microhemorrhages,[b,c] leukocyte infiltration	16 and 30 mo; not studied before 16 mo	Cortex, thalamus	Beckmann et al., 2011	6 mo	12 mo	3 mo	Webster et al., 2014
*TetO-APP**Swl***, *hAPP695*, *K670M/N671L/V717F*, tetracycline-responsive promoter	Microhemorrhages,[b,c] increased BBB permeability to iodinated contrast agent	14 mo; not studied before 14 mo	Cortex, hippocampus, thalamus	Tanifum et al., 2014	2–6 mo	14 mo	Not studied	Tanifum et al., 2014
*APP**SwArc*** **(ArcAβ)**, *hAPP695*, *K670M/N671L/E693G*, mPrP	Microhemorrhages[e] and increased BBB permeability to gadolinium contrast agent	9 and 21 mo; not studied before 9 mo	Cortex, hippocampus, olfactive bulb	Klohs et al., 2011, 2013	6 mo	9–15 mo	6 mo	Klohs et al., 2012
*APP**Sw/0**; PSEN1**Δ E9***, *m/hAPP695 K595N/M596L* × *hPSEN1dE9*, mPrP	Microhemorrhages[b,c]	9 mo; not studied before 9 mo	Cortex	Kelly et al., 2015	6–7 mo	6 mo	6 mo	Duff et al., 1996; Webster et al., 2014
	Fibrin perivascular deposits	10 mo; not studied before 10 mo	Hippocampus, periventricular zone	McManus et al., 2017				
*APP**Sw/0**; PSEN1**M146L***, *hAPP695 K670M/N671L* × *PSEN1 M146L*, hPrP	Microhemorrhages,[b,c] albumin perivascular deposits, immunoglobulin extravasation, endothelial degeneration	5 and 11 mo	Cortex, hippocampus, thalamus	Kumar-Singh et al., 2005	3–6 mo	10 mo	3 mo	Kumar-Singh et al., 2005

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Table 1. **BBB breakdown in *APP*, *PSEN1*, *Tau*, and pericyte-deficient transgenic models** *(Continued)*

Mouse line, construct, promoter	Findings	Age	Region	Reference	Brain Aβ	CAA	Neurodegenerative and behavior changes	Reference
*APP**SwFILo**; PSEN1**M146L/L286V*** **(5xFAD or Tg6799)**, *hAPP695*, *K670M/N671L/I716V/V717I*, mThy1, *PSEN1 M146L/L286V*, mThy1	IgG perivascular deposits	9 and 10 mo; not studied before 9 mo	Cortex	Park et al., 2017	2 mo	Not studied	4–5 mo	Oakley et al., 2006; Webster et al., 2014
	Loss of LRP1 endothelial expression	7 mo; not studied before 7 mo	Cortex	Storck et al., 2016				
	Loss of GLUT1 endothelial expression, increased RAGE vascular expression, increased MMP-9 vascular expression, loss of BBB tight junctions[a,d,f]	8 mo; not studied before 8 mo	Cortex	Kook et al., 2012				
Tg PSEN1 models								
PSEN1−/−	Microhemorrhages,[e] endothelial degeneration	E18.5	Neocortex	Wen et al., 2005	Not studied	Not studied	Not studied (perinatal lethality)	Wen et al., 2005
*PSEN1**M146V***	Microhemorrhages,[e] basement membrane degeneration	10 and 37 mo	Cortex, hippocampus	Gama Sosa et al., 2010	24 mo	No CAA	Not studied	Chen et al., 2000
Tg tau models								
*TetO-Tau**P301L*** **(rTg4510)**, *hMAPT P301L*, tetracycline-responsive promoter	BBB leakage of Evans blue, IgG perivascular deposits, microhemorrhages,[e] leukocyte infiltration	9 and 12 mo	Cortex and hippocampus	Blair et al., 2015	No Aβ pathology; Tau pathology 12 mo	None	Not studied	Blair et al., 2015
Tg pericyte-deficient models								
Pdgfrβ+/−	Loss of pericytes, IgG perivascular deposits, fibrin perivascular deposits, thrombin and plasmin brain extravasation, loss of BBB tight junctions,[a,d] brain extravasation of exogenous tracers	1, 6, 8, 14, and 16 mo	Cortex, hippocampus	Bell et al., 2010	Not studied	Not studied	6–9 mo, +neurodegenerative changes, loss of neurons	Bell et al., 2010; Kisler et al., 2017b
*Pdgfrβ**F7/F7***	Loss of pericytes, IgG perivascular deposits, fibrin perivascular deposits, thrombin and plasmin brain extravasation, loss of BBB tight junctions,[a,d] brain extravasation of exogenous tracers	6 and 8 mo; not studied before 6 mo	Cortex, hippocampus	Bell et al., 2010	Not studied	Not studied	6–9 mo, +neurodegenerative changes, loss of neurons	Bell et al., 2010
	Fibrin perivascular deposits	1, 2, 3, 4, 8, and 12 mo	Cortex, hippocampus, striatum	Nikolakopoulou et al., 2017				

APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; GLUT1, glucose transporter 1; hPrP, hamster prion promoter; IgG, immunoglobulin G; LRP1, low-density lipoprotein receptor-related protein 1; MAPT, microtubule-associated protein tau; MMP-9, matrix metalloproteinase-9; mPrP, mouse prion promoter; mThy1, mouse thymus cell antigen 1 promoter; PDGFRβ, platelet-derived growth factor receptor β; Pgp, P-glycoprotein; PICALM, phosphatidylinositol binding clathrin assembly protein; PSEN1, presenilin 1; RAGE, receptor for advanced glycation end products; VSMC, vascular smooth muscle cell.

[a]Loss of BBB tight junctions as shown by high-resolution confocal microscopy analysis.

[b]Microhemorrhages (hemosiderin deposits) at the capillary level.

[c]Microhemorrhages (hemosiderin deposits) at the arteriolar level.

[d]Loss of BBB tight junctions as shown by immunoblotting of isolated brain capillaries.

[e]The exact vascular location is difficult to determine.

[f]Loss of BBB tight junctions as shown by electron microscopy analysis.

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(PICALM) protein encoded by the *PICALM* gene, a highly validated genetic risk factor for human AD (Harold et al., 2009; Lambert et al., 2009; Huang et al., 2017), accelerates Aβ deposition in $APP^{Sw/0}$ mice, consistent with its critical role in regulating LRP1-mediated Aβ internalization at the abluminal side of the BBB and Aβ transcytosis and clearance across the BBB via Rab5- and Rab11-guided vesicular trafficking (Zhao et al., 2015b; Table 1).

Several studies using other *APP* transgenic models, such as *APP* V717F, $APP^{Sw/I}$ (Swedish, Indiana), APP^{SwDI}, and $APP^{Sw/Arc}$ (Swedish, Arctic), and *APP* mice crossed with *PSEN1* ADAD mutants, confirmed BBB breakdown, including fibrinogen, IgG, and albumin perivascular deposits; BBB leakage of Evans blue and contrast imaging agents; microhemorrhages; leukocyte infiltration; loss of tight junctions; and diminished GLUT1 and LRP1 expression at the BBB (Table 1). Collectively, these data provide strong experimental support for the role of BBB breakdown and dysregulated BBB transport in AD pathophysiology.

BBB breakdown in *PSEN1* transgenic models

PSEN1 ADAD mutations lead to elevated Aβ production (Tanzi, 2012; Potter et al., 2013; Karch et al., 2014; Elbert et al., 2015), neuronal dysfunction (Lee et al., 2010; Karch and Goate, 2015), and major cerebrovascular pathology, including BBB breakdown; pericyte degeneration; Aβ capillary, arteriolar, and arterial deposits; and decreased BBB glucose uptake, as shown by human postmortem studies (Dermaut et al., 2001; Mann et al., 2001; Armstrong, 2008) and imaging studies in the living human brain (Bateman et al., 2012; Benzinger et al., 2013; Fleisher et al., 2015), respectively. Consistent with these findings, *PSEN1*-knockout mice (Wen et al., 2005) and mice expressing *PSEN1* mutations driven by the neuronal Thy1 promoter (Gama Sosa et al., 2010) develop BBB breakdown and microhemorrhages and loss of BBB integrity and reductions in microvasculature, suggesting loss-of-function vascular phenotype in the absence of CAA and/or other Aβ-related pathology. Vascular and BBB changes are also seen in *PSEN1* models crossed with *APP* mice (Kumar-Singh et al., 2005; Kelly et al., 2015; Table 1).

BBB breakdown in *Tau* transgenic mice

BBB leakage of Evans blue, IgG perivascular deposits, microhemorrhages, and leukocyte infiltration indicating BBB breakdown have been shown in *hTau.P301L* mice before significant accumulation of tau pathology (Blair et al., 2015; Table 1) and in the absence of CAA and/or other Aβ pathology. These mice carry a Pro to Leu mutation at codon 301 of the tau gene driven by an inducible Tet-on promoter. In humans, this tau mutation leads to early-onset frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) that is characterized by extensive tau pathology (Lewis et al., 2000). Whether the underlying mechanisms of BBB breakdown involve disrupted tau clearance across the BBB or direct vasculotoxic effects of tau remains unclear at present. Interestingly, *Tau P301L* mice actually outperform wild-type littermates in behavioral tests within the first 2–3 mo of age (Boekhoorn et al., 2006), before the onset of BBB breakdown, raising the possibility that other pathologies, including BBB breakdown, may contribute to dementia and motor symptoms. Because tau pathology is seen in AD, these findings are relevant to AD dementia as well. Future studies should determine the role of BBB breakdown and tau clearance across the BBB in models of dementias caused by tau pathology.

BBB breakdown in *Pdgfrβ-deficient* transgenic mice

Pericyte-deficient transgenic mice, including $Pdgfr\beta^{+/-}$ mice and $Pdgfr\beta^{F7/F7}$ mice with disrupted *Pdgfrβ* signaling, develop an early and progressive BBB breakdown (Bell et al., 2010; Daneman et al., 2010; Nikolakopoulou et al., 2017) and impaired hemodynamic responses (Kisler et al., 2017b) beginning at 1 mo of age. These vascular changes lead to secondary neurodegeneration, loss of cortical and hippocampal neurons, and behavioral deficits at 6–9 mo of age (Table 1). Loss of pericytes in mice with diminished PDGF-BB bioavailability also leads to an early BBB breakdown (Armulik et al., 2010) and calcium deposition in the basal ganglia detectable at 1 yr of age (Keller et al., 2013; Vanlandewijck et al., 2015). Because pericytes degenerate in human AD and other neurodegenerative disorders (Sweeney et al., 2016), these findings support vascular-mediated neurodegeneration independent of Aβ and tau pathology.

BBB breakdown in *APOE* transgenic models

The apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for AD (Verghese et al., 2011; Zlokovic, 2013; Liao et al., 2017). One and two *APOE* ε4 alleles increase risk by ∼3.8- and ∼12-fold, respectively, compared with ε3/ε3 genotype, respectively. The effect of one ε4 allele on AD risk is stronger in females than in males. One copy of *APOE* ε2 allele decreases risk by ∼0.6-fold relative to ε3/ε3 genotype. Additionally, *APOE4* increases the risk of CAA.

APOE exerts its toxic effects on the cerebrovascular system (Zlokovic, 2013) and neurons (Mahley and Huang, 2012) and influences Aβ clearance, amyloid deposition, and tau-related neurodegeneration in an allele-dependent manner (ε4>ε3>ε2; Holtzman et al., 2012; Liao et al., 2017). Despite the fact that *APOE* has such a strong effect on AD risk, we still lack a comprehensive understanding of how the effects of *APOE* on the cerebrovascular system and other aspects of its function contribute to AD. We also do not have an effective ApoE-based therapy for AD.

$Apoe^{-/-}$ mice lacking mouse apolipoprotein E (Apoe) develop BBB breakdown, as shown by accumulation of perivascular IgG, fibrinogen, thrombin, and hemosiderin deposits; leakage of Evans blue, different exogenous tracers, or MRI contrast agents; penetration of *N*-methyl-D-aspartate receptor antibodies; loss of tight junctions; basement membrane degeneration; and loss of perivascular pericytes (Fullerton et al., 2001;

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Table 2. **BBB breakdown in *APOE* transgenic models**

Mouse line	Findings	Age	Region	Reference
Apoe$^{-/-}$	BBB leakage of Evans blue, IgG perivascular deposits	2 and 3 mo	Hippocampus, cerebellum, spinal cord	Fullerton et al., 2001
	BBB leakage of Evans blue, BBB leakage of peroxidase	1.5 and 2 mo	Cortex	Methia et al., 2001
	IgG perivascular deposits, brain leakage of exogenous tracer (sodium fluorescein)	11 mo	Hippocampus, cerebellum	Mulder et al., 2001
	BBB leakage of Evans blue	3 and 4 mo	Cortex, cerebellum	Hafezi-Moghadam et al., 2007
	BBB leakage of Evans blue	6 mo	Cortex, cerebellum	Nishitsuji et al., 2011
	BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, hemosiderin deposits, loss of CD13$^+$/PDGFRβ$^+$ pericytes, loss of BBB tight junctions,[a,b] increased MMP-9 vascular expression	2 wk and 4, 6, 8, 9, and 18 mo	Cortex, hippocampus	Bell et al., 2012
	Fibrin perivascular deposits, loss of PDGFRβ$^+$ pericytes, basement membrane degeneration	9 mo	Cortex	Soto et al., 2015
	BBB leakage of extracted immunoglobulin fractions directed against NMD AR, behavior alterations after injection of immunoglobulin fractions directed against NMDAR	3 and 4 mo	Cortex, hippocampus, cerebellum, brainstem, spinal cord	Hammer et al., 2014; Castillo-Gomez et al., 2016
	IgG perivascular deposits, increased BBB permeability to Gadolinium contrast agent	10 and 11 mo	Periventricular zone, fornix fimbria (hippocampus)	Di Cataldo et al., 2016
TR-*APOE4* and GFAP-*APOE4*	BBB extravasation of exogenous tracers (Dextran and Cadaverine), fibrin perivascular deposits, thrombin perivascular deposits, IgG perivascular deposits, microhemorrhages (hemosiderin deposits),[c] loss of CD13$^+$/PDG FRβ$^+$ pericytes, loss of BBB tight junctions,[a,b] increased MMP-9 vascular expression	2 wk and 4, 6, 8, 9, and 18 mo	Cortex, hippocampus	Bell et al., 2012
TR-*APOE4*	Basement membrane degeneration, loss of GLUT1 endothelial expression, increased RAGE endothelial expression	12 mo	Cortex, hippocampus	Alata et al., 2015
	Microhemorrhages (hemosiderin deposits)[d]	6 and 7 mo	Cortex	Cacciottolo et al., 2016
APOE4 knock-in	BBB leakage of Evans blue	6 mo	Cortex, cerebellum	Nishitsuji et al., 2011

APOE, apolipoprotein E; GFAP, glial fibrillary acidic protein; GLUT1, glucose transporter 1; IgG, immunoglobulin G; MMP-9, matrix metalloproteinase-9; NMDAR, *N*-methyl-D-aspartate receptor; PDGFRβ, platelet derived growth factor β; RAGE, receptor for advanced glycation end products; TR, targeted replacement.

[a]Loss of BBB tight junctions as shown by high-resolution confocal microscopy analysis.

[b]Loss of BBB tight junctions as shown by immunoblotting of isolated brain capillaries.

[c]Microhemorrhages (hemosiderin deposits) at the capillary level.

[d]Microhemorrhages (hemosiderin deposits) at the capillary and arteriolar level.

Methia et al., 2001; Mulder et al., 2001; Hafezi-Moghadam et al., 2007; Nishitsuji et al., 2011; Bell et al., 2012; Hammer et al., 2014; Soto et al., 2015; Castillo-Gomez et al., 2016; Di Cataldo et al., 2016; Table 2). These studies indicate that Apoe is essential for maintaining BBB integrity.

Studies using transgenic mice with targeted replacement of mouse Apoe with each human APOE isoform (TR-*APOE*) or mice expressing each human APOE isoform under control of the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter on an Apoe-null background, have shown that expression of APOE4, but not APOE2 and APOE3, leads to BBB breakdown (Nishitsuji et al., 2011; Bell et al., 2012) and cerebral microhemorrhages (Cacciottolo et al., 2016; Table 2). Loss of endothelial GLUT1 expression and increased expression of RAGE have also been shown in TR-*APOE4* mice, but not TR-*APOE3* or TR-*APOE2* mice (Alata et al., 2015). Studies in human *APOE4* carriers compared with noncarriers have also demonstrated a more pronounced BBB breakdown and pericyte degeneration (Salloway et al., 2002; Zipser et al., 2007; Hultman et al., 2013; Zonneveld et al., 2014; Halliday et al., 2016), diminished regional BBB uptake of glucose (Ossenkoppele et al., 2013; Protas et al., 2013), and early neurovascular dysfunction (Reiman et al., 2004; Sheline et al., 2010; Thambisetty et al., 2010; Hajjar et al., 2015; Suri et al., 2015).

Genetic ablation, siRNA silencing, and pharmacological studies in transgenic *APOE4* mice revealed that activation of the proinflammatory cyclophilin A (CypA)–matrix metalloproteinase 9 (MMP-9) pathway in pericytes leads to degradation of tight junction and basement membrane proteins causing BBB breakdown (Bell et al., 2012). Interestingly, neuropathological findings in AD *APOE4* carriers compared with noncarriers also demonstrate elevated levels of CypA and MMP-9 in brain endothelium and pericytes that correlate with elevated IgG and fibrinogen capillary leakages, suggesting activation of the CypA-MMP-9 BBB degrading pathway (Halliday et al., 2016). Cerebrospinal fluid analysis in *APOE4* nonsymptomatic carriers compared with noncarriers confirmed that activation of the CypA–MMP9 pathway correlates with BBB breakdown (Halliday et al., 2013), which has been also corroborated by tissue CypA mRNA analysis (Conejero-Goldberg et al., 2014).

An early and progressive BBB breakdown detectable in TR-*APOE4* and GFAP-*APOE4* mice between 2 and 6 wk of age precedes changes in sensory-evoked neuronal functioning, loss of neuritic density, and loss of pre- and postsyn-

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aptic proteins that develop at 4 mo of age (Bell et al., 2012). Importantly, pharmacologic inhibition of the CypA–MMP-9 BBB pathway in addition to repairing the BBB also slowed down and reversed neurodegenerative changes in *APOE4* and *Apoe*$^{-/-}$ mice. Similarly, genetic inhibition of the CypA–MMP-9 pathway at the BBB reversed neurodegenerative changes in *Apoe*$^{-/-}$ mice (Bell et al., 2012). Because *Apoe*$^{-/-}$ (Raber et al., 1998; Bell et al., 2012; Lane-Donovan et al., 2016) and *APOE4* transgenic mice (Bell et al., 2012; Salomon-Zimri et al., 2014, 2015; Liu et al., 2015) develop behavioral deficits at 4–6 mo of age after BBB breakdown, collectively, these findings suggest that BBB breakdown (Table 2) not only contributes to neuronal changes in these models but also is an important therapeutic target.

Several studies have established that human APOE isoforms differentially regulate brain Aβ clearance (Castellano et al., 2011), as reviewed elsewhere (Verghese et al., 2011; Liao et al., 2017). Consistent with these findings, APOE isoforms differentially regulate Aβ clearance across the BBB (Deane et al., 2008; Zhao et al., 2015b). Studies in animal models have shown that APOE3 and APOE2 mediate Aβ clearance across the BBB via LRP1, whereas APOE4 preferentially binds to very-low-density lipoprotein receptor (VLDR) at the mouse BBB, which clears ligands across the BBB at much slower rate than LRP1, contributing to brain accumulation of Aβ (Deane et al., 2008).

Potential pitfalls in BBB permeability measurements

High-resolution confocal microscopy analysis and multiphoton microscopy imaging of various endogenous and exogenous tracers, studies using molecular biomarkers of BBB cellular breakdown and/or dysfunction, electron microscopy studies, and MRI studies strongly support BBB breakdown in animal models of AD and *Apoe*$^{-/-}$ and *APOE4* transgenic mice (Tables 1 and 2). Some studies, however, did not detect BBB breakdown in *Apoe*$^{-/-}$ and *TR-APOE4* mice using exogenous circulating antibodies (e.g., IgG and anti–β-secretase 1 [BACE1]) but showed significant brain uptake of the bispecific therapeutic anti–transferrin receptor–BACE1 antibody, which uses the Trf receptor to cross the BBB (Bien-Ly et al., 2015). Interestingly, this study also did not detect BBB breakdown in a superoxide dismutase-1 *SOD1*^{G93A} model of amyotrophic lateral sclerosis (ALS) using control IgG and anti–BACE1 antibodies (Bien-Ly et al., 2015). In contrast, multiple independent studies in *SOD1*^{G93A} mice and other *SOD1* ALS mutants reported an early BBB breakdown using different techniques and approaches (Garbuzova-Davis et al., 2007a,b; Zhong et al., 2008, 2009; Nicaise et al., 2009; Miyazaki et al., 2011; Winkler et al., 2014). Here, we briefly discuss potential reasons for discrepancy between different studies and possible factors that could interfere with interpretation of BBB measurements in animal models.

In brief, the use of systemically administered exogenous macromolecules such as nonimmune antibodies to determine BBB permeability requires extensive vascular perfusion of the brain with cold saline at the end of experiment to eliminate antibodies from cerebral blood vessels (Bien-Ly et al., 2015). However, this perfusion step can also remove tracers from the brain, particularly nonfixable ones such as exogenous nonimmune IgG, which tends to underestimate brain uptake values. This could be particularly critical in models with focal and/or rather discrete BBB changes, when tracer concentration is determined in brain homogenates or lysates. To overcome this problem, some studies used tissue-fixable tracers, such as Cadaverine-Alexa Fluor 555 (Armulik et al., 2010; Bell et al., 2012) or lysine fixable dextrans (Ben-Zvi et al., 2014), which after crossing the disrupted BBB remain bound to brain tissue and therefore are easily detectable by either histological or biochemical analysis.

Besides technical challenges, BBB breakdown in models of neurodegenerative disorders (Zlokovic, 2011) and in humans with these disorders such as AD (Montagne et al., 2016; Nelson et al., 2016) typically starts focally (Montagne et al., 2015) and then spreads throughout several gray and white matter regions (van de Haar et al., 2016a,b, 2017). In contrast, BBB breakdown in models of neuroinflammation, such as experimental allergic encephalitis, is more widespread and robust (Wang et al., 2016; Shaw et al., 2017), similar to multiple sclerosis in humans, where BBB breakdown develops more rapidly and is typically an order of magnitude greater than in neurodegenerative disorders such as AD (Montagne et al., 2016). Thus, experimental approaches that rely solely on biochemical analysis of the exogenous tracer's concentration in brain lysates without additionally using tissue imaging analysis might run the risk of missing subtle changes in BBB integrity around brain capillaries first affected by the disease process, which are otherwise detectable by high-resolution confocal or multiphoton microscopy analysis (Paul et al., 2007; Bell et al., 2010, 2012).

It is not completely unexpected, therefore, that studies using systemically administered radiolabeled tracers (e.g., dextran and albumin) and assaying their concentration in different CNS tissue lysates were able to readily detect BBB permeability increases in models of neuroinflammation, but not in models of neurodegenerative disorders (Bien-Ly et al., 2015). Because multiple independent studies by different groups showed BBB breakdown in models of neurodegeneration using imaging techniques (Tables 1 and 2), the question persists whether radiotracer methods alone are sensitive enough to detect focal and less pronounced BBB changes. A recent neuroimaging study in individuals with MCI also suggests that even within a single brain region in humans, as for example in the hippocampus, some subregions may have intact BBB, such as CA3, whereas others such as CA1 and dentate gyrus show BBB breakdown (Montagne et al., 2015). Combining different methods and approaches is therefore highly recommended to ascertain whether given animal models develop BBB breakdown.

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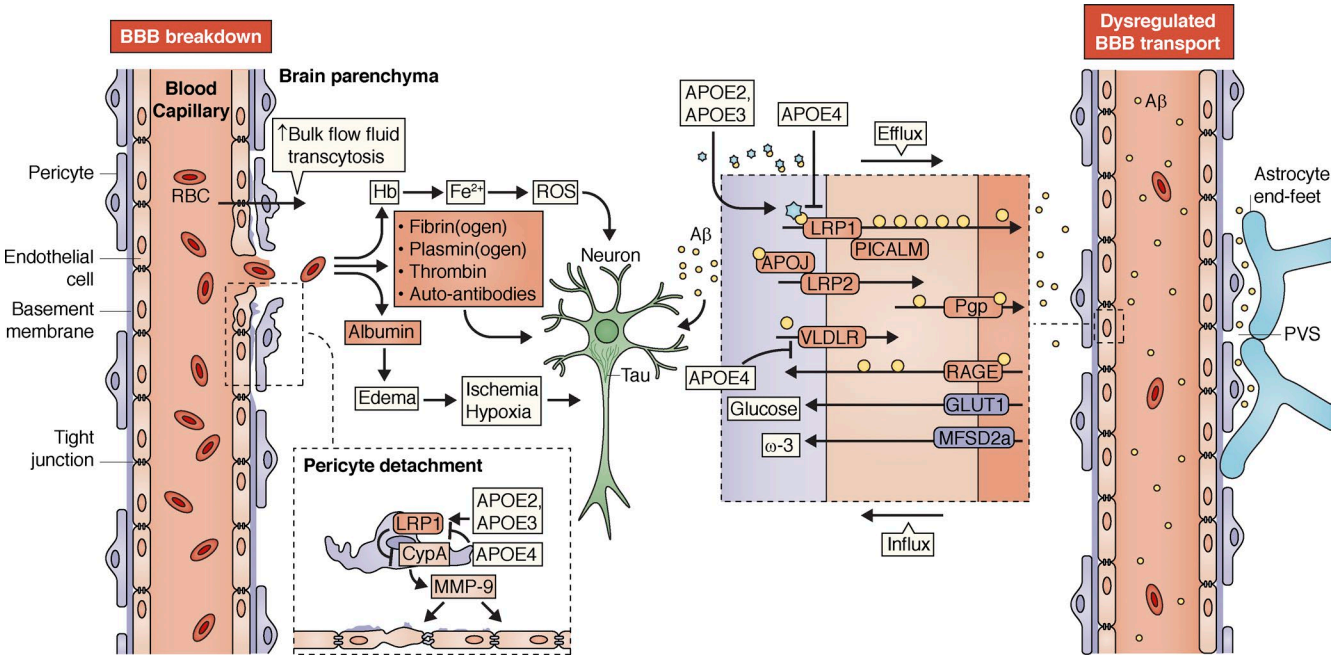

Figure 1. **Contribution of BBB breakdown and dysregulated BBB transport to AD pathophysiology based on findings in animal models, as shown in Tables 1 and 2.** BBB breakdown (left) leads to perivascular accumulation of blood-derived neurotoxic products in the brain, such as red blood cell (RBC)–derived hemoglobin (Hb) and free iron (Fe^{2+}) generating reactive oxygen species (ROS) and oxidant stress to neurons; potentially toxic plasma proteins such as fibrin(ogen), plasmin(ogen), thrombin, and/or autoantibodies, which could lead to neuronal injury, cell death, and inflammatory response; and albumin contributing to the development of edema, hypoperfusion, and tissue hypoxia. Pericyte detachment, degeneration, and loss leads to BBB breakdown. Apolipoprotein E (APOE) isoforms differentially regulate pericyte metabolism and BBB integrity. APOE2 and APOE3, but not APOE4, act via low-density lipoprotein receptor–related protein-1 (LRP1) on pericytes to inhibit the proinflammatory cyclophilin A (CypA)–matrix metallopeptidase-9 (MMP-9) pathway. When activated by APOE4, this pathway leads to MMP-9–mediated degradation of BBB tight junction and basement membrane proteins causing BBB breakdown. Dysregulated BBB transport (right) leads to a loss of equilibrium between Alzheimer's amyloid β (Aβ) efflux and influx across the BBB, which is a key mechanism that maintains brain Aβ homeostasis. Aβ efflux is normally mediated via its receptors on brain endothelium, including LRP1, which works closely with phosphatidylinositol-binding clathrin assembly protein (PICALM) to clear Aβ monomers, oligomers, and aggregates from brain across the BBB; LRP2, which clears Aβ in a form of complexes with APOJ (clusterin); and P-glycoprotein (Pgp), which mediates active efflux of Aβ from brain endothelium to blood. LRP1 and Pgp BBB levels are reduced in AD models before Aβ deposition, which contributes to Aβ accumulation in the brain. Aβ influx from blood to brain is mediated by the receptor for advanced glycation end products (RAGE), which also triggers an inflammatory response. RAGE expression at the BBB is increased in AD models, which contributes to Aβ accumulation in the brain and inflammatory response. *APOE* isoforms differentially regulate Aβ clearance. Aβ complexes with human APOE2 and APOE3 isoforms are cleared across the BBB by LRP1. APOE4 has lower affinity for LRP1 and binds to the very-low-density lipoprotein receptor (VLDLR), which slowly transports its ligands across the BBB, including the APOE4–Aβ complex, causing its accumulation in the brain. BBB *GLUT1* transporter delivers glucose to the brain across the BBB and is down-regulated in AD models. Its reduction accelerates BBB breakdown and Aβ pathology and leads to tau pathology and neuronal loss. BBB *MFSD2a* (major facilitator superfamily domain–containing protein 2) transports essential ω-3 fatty acids into the brain, which is essential for brain development, cognition, and maintenance of BBB integrity. Aβ can also accumulate in the perivascular space (PVS) between astrocyte end-feet and the vessel wall because of inefficient drainage along the perivascular route.

Targeting BBB to control neurodegeneration

Fig. 1 illustrates various pathways showing how BBB breakdown and dysregulated transport systems contribute to development of neurodegenerative changes, accumulation of Aβ and tau pathology and neuronal loss, based on findings in animal models of AD (Tables 1 and 2). In addition to influx of various neurotoxic agents, cells, and pathogens into the brain, BBB breakdown leads to the development of enlarged perivascular spaces (unpublished data), and brain ischemic changes. On the other hand, dysfunction in BBB transport systems leads to the development of Aβ and tau pathology and neuronal loss, as for example in *APP* expressing mice challenged by added vascular hits such as pericyte loss (Sagare et al., 2013), diminished GLUT1 (Winkler et al., 2015) and LRP1 expression (Storck et al., 2016), or increased RAGE expression (Deane et al., 2012) at the BBB. Here, we discuss therapeutic strategies targeting BBB pathways to control neurodegeneration.

Repairing the BBB with activated protein C (APC) and/or its cell-protective analogues (Griffin et al., 2015) not only protects BBB integrity but also slows down the course of a neurological disorder, as shown in the $SOD1^{G93A}$ ALS model (Winkler et al., 2014). APC enhances the integrity of endothelial membranes by activating Rac1-dependent stabi-

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lization of the cytoskeleton and/or down-regulating MMP-9 at the BBB, thus protecting the neuroglial environment from systemic influences (Zlokovic and Griffin, 2011). Whether APC can exert similar beneficial effects in AD models with disrupted BBB (Tables 1 and 2) remains to be seen. Because 3K3A-APC analogue has completed a phase 2 clinical trial for stroke (NCT02222714), this approach holds promise to translate to patients with other neurological disorders, including AD. It also remains to be determined whether inhibition of the CypA–MMP-9 pathway at the BBB with CypA inhibitors can seal the BBB and exert beneficial effects on cognitive function in human *APOE4* carriers, as it does in transgenic *APOE4* mice (Bell et al., 2012; Fig. 1). Again, this approach is attractive, because CypA inhibitors are currently being tested in a phase 3 trial for hepatitis C (NCT01318694).

Besides sealing the BBB, eliminating the consequences of BBB breakdown has been investigated. When the BBB is open, plasma proteins enter the neuroglial space and become neurotoxic; therefore, neutralizing toxic accumulates represents a valuable therapeutic approach for neurodegenerative diseases such as AD that are associated with BBB pathology. In fact, depleting accumulated fibrinogen from the brain with ancrod, a defibrinogenating agent, or by genetic manipulation attenuated both neuroinflammation and vascular pathology in *APP* mice (Paul et al., 2007) and in a model of multiple sclerosis (Davalos et al., 2012). On the other hand, BBB damage causing RBC extravasation and brain accumulation of free neurotoxic iron causing oxidant stress can be successfully controlled by iron chelators and/or antioxidant treatment, as shown in $SOD1^{G93A}$ ALS model (Winkler et al., 2014; Fig. 1).

Other approaches, such as using RAGE blockers to inhibit Aβ influx across the BBB and the neurovascular inflammatory response (Deane et al., 2003, 2012), have advanced from animal models to a phase 3 trial in AD (NCT02916056). Targeting BBB clearance in AD is an emerging therapeutic approach to restore the balance between Aβ production and clearance. LRP1 minigene delivery to the BBB by viral vectors facilitates Aβ clearance and mitigates Aβ pathology (Winkler et al., 2015). The PICALM-dependent transcytotic machinery at the BBB can also be targeted therapeutically by gene therapy (Zhao et al., 2015a). Additionally, current Aβ clearance treatments with anti–Aβ antibodies to remove Aβ from brain would benefit from intact Aβ transvascular transport and clearance across the BBB, particularly for the antibodies acting mainly through peripheral Aβ sink action (NCT02008357). Therefore, repairing BBB and Aβ BBB clearance mechanisms is critical for success of these treatments as well.

The vascular drainage pathway along the perivascular spaces of CNS vessels contributes to the clearance of molecules from brain extracellular spaces (ECSs), including Aβ (Tarasoff-Conway et al., 2015; Bakker et al., 2016), and is connected with the cerebrospinal fluid (CSF) compartment and lymphatic vessels within the dura matter of the brain, which drain to the peripheral lymph nodes (Louveau et al., 2015; Engelhardt et al., 2017). Additionally, it has been proposed that convective, "glymphatic" flow of CSF through the ECSs from the para-arterial to the paravenous spaces plays a role in solute transport exchanges in parenchymal ECSs (Iliff et al., 2012; Jessen et al., 2015). However, recent studies have not supported the proposed glymphatic mechanism of convective solute transport in brain parenchyma (Hladky and Barrand, 2014; Spector et al., 2015; Smith et al., 2017) or the convective, pressure-driven fluid flow from para-arterial to paravenous spaces through parenchymal ECSs (Asgari et al., 2015, 2016; Jin et al., 2016; Holter et al., 2017; Smith et al., 2017). The current view states that metabolic waste products and endogenous molecules generated by the brain diffuse away through brain ECSs and are eliminated from the brain by transvascular transport across the BBB (Zlokovic, 2011; Zhao et al., 2015a) and perivascular transport along the vessel walls in the direction opposite the blood flow (Tarasoff-Conway et al., 2015; Bakker et al., 2016), as originally proposed by physiologists decades ago (Milhorat, 1975; Bradbury et al., 1981). This clearance pathway extends to toxic metabolites such as Aβ, which in normal mice contributes to ∼15–20% of Aβ clearance from the brain (Shibata et al., 2000; Xie et al., 2013); the remaining 80–85% is removed from the brain by transvascular transport across the BBB into the blood. Whether ECSs and perivascular clearance of Aβ can be targeted therapeutically in AD and AD models to remove excess Aβ remains to be explored in future studies.

Future directions

Multiple studies have shown BBB breakdown and dysregulated BBB transport in AD models, establishing their roles in neurodegeneration and development of Alzheimer's Aβ and tau pathology. BBB breakdown and dysfunction have also been reported in rare inherited monogenic human neurological disorders with genetic defects affecting exclusively BBB cells, which directly supports the link between BBB breakdown and neurological disorders (Zhao et al., 2015a). For example, loss-of-function mutations in the human *GLUT1* gene encoding the BBB glucose transporter result in GLUT1-deficiency syndrome with early-onset seizures and microcephaly (Wang et al., 2000). $Glut1^{+/-}$ mice not only phenocopy human pathology but also develop BBB breakdown, causing microcephaly and neurodegeneration (Winkler et al., 2015). The role of diminished GLUT1 expression at the BBB in human AD (Nelson et al., 2016) and animal models is still not fully understood; in particular, it is unclear whether pharmacologically reversing GLUT1 expression repairs BBB integrity and reverses neurodegenerative changes, cognitive decline, and behavioral deficits, as suggested by gene therapy studies in $Slc2a1^{+/-}$; $APP^{Sw/0}$ mice with endothelial-specific GLUT1 haploinsufficiency (Winkler et al., 2015).

Loss of tight junction proteins causing BBB breakdown has been found both in human AD and animal models. However, their role in disease process remains elusive. In contrast, mutations in the *OCLN* (occludin) gene encoding the tight junction protein occludin (O'Driscoll et al., 2010) or the

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junctional adhesion molecule-C (*JAM-C*) gene encoding junctional molecule JAM-C (Wyss et al., 2012; Akawi et al., 2013) are causatively related to BBB breakdown, which leads to early-onset seizures, microcephaly, and band-like calcification with simplified gyration in case of *OCLN* mutations or hemorrhages and hydrocephalus, as shown in JAM-C–deficient mice, and/or hemorrhagic destruction of the brain, subependymal, calcification, and congenital cataracts in humans with homozygous mutations of *JAM-C*. Is there any relationship between these findings and findings in complex neurodegenerative disorders such as AD?

Inactivating mutations in major facilitator superfamily domain–containing protein 2a (*MFSD2a*) gene encoding the BBB transporter for essential ω-3 fatty acids cause a lethal microcephaly syndrome (Guemez-Gamboa et al., 2015). *Mfsd2a*-deficient mice exhibit impaired brain uptake of ω-3 fatty acids (Betsholtz, 2014; Nguyen et al., 2014), as well as dysregulated caveolae-mediated transcellular trafficking across the BBB and develop BBB breakdown (Ben-Zvi et al., 2014; Zhao and Zlokovic, 2014; Andreone et al., 2017) resulting in microcephaly, neuronal loss, and cognitive deficits. Essential ω-3 fatty acids found in fish oil help reduce the risk of cardiovascular disease and have beneficial effects on cognition. However, the role of the MFSD2a BBB transporter as a possible therapeutic target in AD models and human AD remains underexplored.

Loss-of-function mutations in the *PDGFRB* gene in pericytes lead to idiopathic primary familial brain calcification and motor and cognitive impairment (Keller et al., 2013; Nicolas et al., 2013). Similarly, $Pdgfb^{ret/ret}$ mice with severe pericyte loss and BBB breakdown develop deep brain calcification (Keller et al., 2013), whereas $Pdgfr\beta^{+/-}$ and $Pdgfr\beta^{F7/F7}$ pericyte-deficient mice develop BBB breakdown, leading to secondary neurodegeneration (Bell et al., 2010). Pericytes degenerate in AD (Farkas and Luiten, 2001; Baloyannis and Baloyannis, 2012; Sengillo et al., 2013; Halliday et al., 2016) and AD models (Tables 1 and 2), but at present, we know very little about whether cell therapies directed at replacing pericytes will have the same beneficial effects in AD models or AD as shown in other models, such as $SOD1^{G93A}$ ALS (Coatti et al., 2017).

Another example is CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). This is an inherited autosomal-dominant ischemic stroke syndrome that progresses to dementia, and it is caused by mutations in the *NOTCH3* gene, which is expressed only in vascular smooth muscle cells and pericytes (Chabriat et al., 2009). Mice carrying the CADASIL Notch3-R169C mutation also show accumulation of NOTCH ectodomain in pericytes, pericyte degeneration, and BBB breakdown (Ghosh et al., 2015). Small-vessel disease, on the other hand, is prominent in AD (Iadecola, 2013, 2017; Wardlaw et al., 2013) and contributes to ∼50% of all dementias worldwide, including AD (Wardlaw et al., 2013; Montine et al., 2014; Snyder et al., 2015; Hachinski, 2016). Thus, it would be interesting to study whether there is a common vascular mechanism predisposing for the development of small ischemic strokes and Alzheimer's pathology in animal models of small-vessel disease.

Finally, as the RNA-sequencing and proteomic studies in animal models have begun to provide new insights into the molecular composition of the BBB and the associated cell types (Lu et al., 2008; Daneman et al., 2010; Zeisel et al., 2015; He et al., 2016), our understanding of the cellular and molecular mechanisms of the BBB transport functions will continue to expand. Hopefully, future large-scale single-cell transcriptomic studies of brain endothelial cells, brain capillary pericytes, and smooth muscle cells on arterioles, arteries, and venules will characterize more precisely cell-specific regional expression and zonation of key endothelial transporters on brain capillary, arteriolar, and venular endothelium and determine to which extent the associated mural cells, including pericytes, contribute to the overall transport of solutes exchanges across the BBB.

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