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## Review

# The Effects of Impaired Cerebral Circulation on Alzheimer's Disease Pathology: Evidence from Animal Studies

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**Abstract.** Persistent systemic hypoxia, a direct consequence of alterations in vascular function, can compromise the brain by increasing the risk of developing dementias such as Alzheimer's disease (AD). Vascular contributions to cognitive impairment and AD in aged individuals are common, and several vascular risk factors for AD are linked to hypoxia. Clinical evidence confirms that structural and functional changes characteristic of AD pathology also occur following hypoxic-ischemic events such as stroke and traumatic brain injury. Studies with transgenic and non-transgenic mouse models reliably show that hypoxia increases the levels of amyloid- $\beta$  peptides that form the characteristic plaques in AD brains. Moreover, some studies suggest that vascular lesions also promote tau phosphorylation, modulate apolipoprotein E expression, and have more profound effects in aged animals, but additional evidence is needed to establish these findings. Although the mechanisms underlying hypoxia-related effects remain unclear, controlled animal studies continue to reveal mechanistic aspects of the relationship between hypoxia and AD pathology that are necessary for therapeutic developments. The present review summarizes evidence from rodent studies regarding the effects of hypoxia on AD-related pathology and evaluates its impact on understanding human disease.

**Keywords:** Amyloid- $\beta$ , apolipoprotein E, cerebral amyloid angiopathy, cerebral hypoxia, ischemia, tau protein

## INTRODUCTION

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases associated with aging. The majority of AD cases manifest as a late onset sporadic form, accounting for more than 95% of cases, but genetically the disease is divided into familial and sporadic cases [1]. Familial AD is caused by mutations in the amyloid- $\beta$  protein precursor (A $\beta$ PP) and presenilin 1 and 2 genes [2]. Risk factors for

sporadic AD include age, ApoE  $\epsilon$ 4 polymorphism, hypercholesterolemia, hypertension, diabetes mellitus, stroke, brain trauma, and obesity, among others [1]. The two main pathological hallmarks of AD are accumulation of amyloid- $\beta$  (A $\beta$ ) plaques in brain tissue and in the walls of the small brain arteries and hyperphosphorylated tau filaments that aggregate as neurofibrillary tangles (NFTs). A $\beta$  plaques and NFTs lead to cell and synaptic dysfunction and ultimately result in cognitive and functional deterioration. AD is frequently accompanied by vascular pathology, and various mouse models of AD have been employed in investigations of how alterations in vascular function impact AD-related processes, primarily those related

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44 to the expression of A $\beta$ . According to the amyloid  
45 hypothesis, formation of A $\beta$  plaques is one of the main  
46 influences on AD pathogenesis, and disease processes  
47 are believed to result from an imbalance between  
48 A $\beta$  production and clearance [3]. Hypoxia, a direct  
49 consequence of cerebral hypoperfusion, increases A $\beta$   
50 production and reduces its clearance [4], and may  
51 trigger mechanisms that contribute to the cognitive  
52 impairment in AD patients. Moreover, hypoxia also  
53 induces microglia activation which results in the pro-  
54 duction of inflammatory cytokines and subsequent  
55 structural damage and neuroinflammation [5–7].

56 Currently, the proposed classification criteria for AD  
57 consist of core clinical features with evidence of patho-  
58 physiological processes, which include biomarkers of  
59 brain A $\beta$  protein and downstream neuronal degenera-  
60 tion or injury [8]. Moreover, the term mild cognitive  
61 impairment has been coined to denote the early stages  
62 of cognitive decline that precede AD dementia [9].  
63 The clinical features of vascular dementia, which are  
64 attributed to vascular-related brain lesions, are more  
65 variable than in AD dementia with respect to neuropsy-  
66 chological profiles, clinical phenotypes, and disease  
67 onset [10]. The diagnosis of vascular dementia is com-  
68 plicated further by the use of various clinical criteria  
69 [10–12]. Additionally, a wide range of vascular lesions  
70 produce cognitive impairment in vascular dementia  
71 [10]; thus, cognitive decline is not reliably associated  
72 with vascular pathology nor are there consensus crite-  
73 ria for pathological features of vascular dementia.

74 Vascular contributions to cognitive impairment and  
75 AD in aged individuals are common, and several vascu-  
76 lar risk factors for AD are linked to hypoxia. Vascular  
77 pathology coexists in at least one-third of AD cases  
78 [13, 14], and a growing body of clinical-pathological  
79 research suggests that vascular factors play a role in the  
80 pathogenesis of AD [15]. Studies with transgenic and  
81 non-transgenic rodents provide supporting evidence  
82 that hypoxia promotes A $\beta$  accumulation by enhancing  
83 A $\beta$  production and reducing its clearance. Moreover,  
84 some studies suggest that vascular lesions also pro-  
85 mote tau phosphorylation, but additional evidence is  
86 needed to establish this link. Currently the use of ani-  
87 mal models to investigate the factors linking cerebral  
88 blood flow and AD pathology is the best approach for  
89 uncovering the mechanisms underlying the impact of  
90 neurovascular alterations on AD. In the present review,  
91 we summarize evidence from transgenic (Table 1)  
92 and non-transgenic (Table 2) rodent studies regard-  
93 ing the effects of hypoxia on AD-related pathology  
94 and evaluate its impact on understanding human  
95 disease.

## 96 CLINICAL-PATHOLOGICAL LINKS 97 BETWEEN AD AND HYPOXIA

98 With aging, the human body becomes less efficient  
99 at delivering oxygen to cells and tissues, and therefore  
100 entire organs are compromised. The brain is particu-  
101 larly susceptible to hypoxia which can result in varying  
102 degrees of neural failure and structural damage [16].  
103 Several cardiovascular and respiratory disorders are  
104 associated with neurodegenerative pathologies includ-  
105 ing AD, Parkinson's disease, and Huntington's disease  
106 [17]. The link between hypoxia and neurodegeneration  
107 is based on the oxygen supplies that are required for  
108 proper nervous system function. The brain consumes  
109 about 20% of the body's oxygen and receives up to  
110 20% of the cardiac output [17, 18]. Under normal  
111 conditions oxygen is transported to brain tissue through  
112 microvessels by diffusion, and rapid localized delivery  
113 of oxygen occurs in response to increases in neuronal  
114 activity [19]. Imaging studies suggest that oxygen lev-  
115 els vary widely among different regions of the brain  
116 even in the resting state [18]. Further, although it  
117 is not clear whether cerebral hypoperfusion is a cause  
118 or a consequence of AD, various neuroimaging stud-  
119 ies in AD individuals confirm a reduction in cerebral  
120 blood flow (CBF) from early to late stages of AD pro-  
121 gression [20]. Vascular risk factors for hypoperfusion  
122 such as ischemic stroke, atherosclerosis, hypertension,  
123 diabetes and cardiac disease can lead to cognitive  
124 impairment by triggering hemodynamic changes in the  
125 brain microcirculation and impairing optimal delivery  
126 of oxygen and glucose to the brain [21]. Hypoperfusion  
127 also contributes to arterial stenosis by reducing CBF,  
128 preventing microemboli from being washed out of the  
129 arteries and restricting the transport of key nutrients  
130 [21].

131 The obstruction of blood resources to regions of the  
132 brain such as that which occurs following stroke rep-  
133 resents one of the most damaging forms of hypoxia  
134 and can lead to severe pathological consequences [17].  
135 Neuroimaging studies with positron emission tomog-  
136 raphy (PET) and single photon emission computerized  
137 tomography (SPECT) provide evidence that stroke  
138 produces region-specific hypoperfusion that results in  
139 the brain receiving just enough blood supply to support  
140 tissue viability but not enough to support cognitive or  
141 neurological function [22, 23]. In addition, the acute  
142 pathogenesis of stroke involves the activation of pro-  
143 inflammatory mediators that may exacerbate tissue  
144 damage in the long term [24, 25]. Various studies have  
145 confirmed that stroke victims are significantly more  
146 likely to develop AD in the years following stroke [26].

Table 1  
Studies employing AD transgenic mouse models to examine links between low oxygen brain levels and AD pathology

Model	Characteristics (age, gender, strain) <sup>1</sup>	Treatment/Approach	Effects on AD-related pathology [ref]
Triple-transgenic mice (3xTg-AD)	15 months, male	Temporal occlusion of the bilateral common carotid arteries (12 min)	Decreased total tau and AT270; increased pAKT and GSK3 $\beta$ three months after injury [60].
	3 months, male	Temporal occlusion of the bilateral common carotid arteries (4 min)	Elevated A $\beta$ <sub>42</sub> and oligemia for >3 weeks; robust increase in BACE1; reduced tau [58].
	5–7 months, both genders, homozygous	Experimental TBI with cortical impact by an electromagnetic device to produce mild, mild-moderate, and moderate injuries	Intra-axonal A $\beta$ accumulation in the pericontusional fimbria; increased tau immunoreactivity in regions with moderate injury; increased total tau in contralateral CA1 [105].
Transgenic ArcA $\beta$ mice	4 and 24 months, both genders, expressing human A $\beta$ PP 695 with both Swedish and Arctic mutation	CE- $\mu$ MRA was used to assess cerebral artery and vein diameters	Reduction of functional intracortical microvessels; accumulation of A $\beta$ and fibrinogen in small and medium sized vessels but not in large arteries in 24-month-old mice [68].
Transgenic mice (ApoE, A $\beta$ PPsw and Tg2576)	15 months, A $\beta$ PPsw mice expressing endogenous murine ApoE and A $\beta$ PPsw, mice expressing human ApoE $\epsilon$ 3 and ApoE $\epsilon$ 4 isoforms (knock-in mice Tg2576)	Development of amyloid plaques and CAA	No A $\beta$ deposition at 15 months after CAA with parenchyma plaque depositions in A $\beta$ PPsw mice expressing ApoE $\epsilon$ 4 and ApoE $\epsilon$ 3; elevated levels of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> and increased A $\beta$ <sub>40:42</sub> ratios in young animals expressing ApoE $\epsilon$ 4 [71].
	3–4 and 16–17 months, male, homozygous targeted replacement mice expressing human ApoE $\epsilon$ 3 (TRE3) and ApoE $\epsilon$ 4 (TRE4) genes	Intracerebral injections of human A $\beta$ 1-40	Increased A $\beta$ deposits in hippocampus in TRE4 relative to TRE3 in both 3- and 6-month-olds [88].
Transgenic mouse models using A $\beta$ PP and BACE genes	25–26 months, female heterozygous A $\beta$ PP51/16 mice, female heterozygous A $\beta$ PP23 mice, male heterozygous A $\beta$ PP23	CAA association with alterations in microvascularisation	Severe CAA in thalamic vessels in A $\beta$ PP23 mice compared to A $\beta$ PP51/16 and wt; CAA-related capillary occlusion within the thalamus in A $\beta$ PP23 but not in A $\beta$ PP51/16 or wt mice [70].
	Two modified animal strains, A $\beta$ PP $-/-$ and BACE $-/-$	Global cerebral ischemia performed by bilateral clamping of the common carotid arteries (12 min)	A $\beta$ PP $-/-$ and BACE $-/-$ mice presented greater risk of mortality and reduced CBF under hypoxic conditions; serum response factor and calsequestrin significantly altered in both strains [61].
	6, 11, and 20 months, male A $\beta$ PP23 transgenic mice	Magnetic resonance angiography used to evidence cerebral arterial hemodynamics	A $\beta$ PP23 mice of 11 and 20 months presented flow voids in the internal carotid arteries, with vessel elimination and deformation [67].
	2 months, mice overexpressing a mutant form of human A $\beta$ PP, Swedish and Indiana (A $\beta$ PP <sub>sw/ind</sub> -Tg mice) Aged A $\beta$ PP <sup>sw/0</sup> mice overexpressing human A $\beta$ PP	Chronic cerebral hypoperfusion with BCAS using microcoils Inhibition A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> with RAGE specific blocker, FPS-ZM1, and induced cellular stress	Impaired learning in BCAS-operated A $\beta$ PP <sub>sw/ind</sub> -Tg mice; reduced neural density correlated with low cognitive performance [65]. FPS-ZM1 inhibited RAGE mediated influx of circulating A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> ; inhibited $\beta$ -secretase activity and A $\beta$ production; blocked RAGE activity at the BBB [50].

Table 1  
(Continued)

Model	Characteristics (age, gender, strain) <sup>1</sup>	Treatment/Approach	Effects on AD-related pathology [ref]
	8 months, female, A $\beta$ PP23 transgenic mice	Hypoxia produced in chamber at 8% O <sub>2</sub> for 16 h/day for 1 month	Upregulation of BACE1 promoter activity; increased A $\beta$ PP processing and A $\beta$ generation, $\beta$ -secretase cleavage of A $\beta$ PP and A $\beta$ deposition; impaired memory [64].
	6 months, A $\beta$ PP/PS1 double transgenic mice	Hypoxia produced by enclosure in airtight jar	Decreased memory and cognitive function; increased senile plaques and levels of tau phosphorylation [62].
	10 weeks, males A $\beta$ PP/PS1 double transgenic mice	Hyperoxia produced in normobaric chamber at 40% O <sub>2</sub> for 8 h/day	Reversed deficits in spatial learning and memory; decreased A $\beta$ deposition and neuritic plaque formation in cortex and hippocampus [66].
Transgenic CD-1 mice	6–8 weeks	Treatment with 3 intraperitoneal injections of LPS from <i>Salmonella typhimurium</i>	Inhibitions of CSF bulk flow, impairment of central and peripheral clearance of A $\beta$ , and increased vascular sequestration of A $\beta$ [82].
Transgenic PDA $\beta$ PP mice	4 months, mice containing the familial AD mutation V $\rightarrow$ F at A $\beta$ PP position 717 (PDA $\beta$ PP)	Brain trauma induced by impacting a 3-mm diameter impounder onto the cortex through a 5-mm craniectomy	Increased A $\beta$ , increased hippocampal neuronal death and memory impairment, but no increase in A $\beta$ plaque formation [104].
Transgenic A $\beta$ PP-YAC mice	Both genders, heterozygous	Brain injury by controlled cortical impact	Significant motor and memory deficits in WT and A $\beta$ PP-YAC mice 7 days post brain injury [103].

AD, Alzheimer's disease; ApoE  $\epsilon$ 3, apolipoprotein E  $\epsilon$ 3; ApoE  $\epsilon$ 4, apolipoprotein E  $\epsilon$ 4; A $\beta$ PP, amyloid- $\beta$  protein precursor; A $\beta$ PP<sub>sw/ind</sub>-Tg mice, transgenic mice with A $\beta$ PP and two mutations, Swedish and Indiana; A $\beta$ PP 695, amyloid- $\beta$  protein precursor 695; AT270, tau phosphorylated at Thr<sup>181</sup>; BACE1, beta-site amyloid- $\beta$  protein precursor cleaving enzyme 1; BCAS, bilateral common carotid artery stenosis; CAA, cerebral amyloid angiopathy; CBF, cerebral blood flow; CE- $\mu$ MRA, contrast-enhanced magnetic resonance microangiography; CSF, cerebrospinal fluid; C99, membrane-bound peptide generated from A $\beta$ PP; F, phenylalanine; FPS-ZM1, High affinity RAGE specific inhibitor; GSK3 $\beta$ , glycogen synthase kinase 3 beta; LPS, lipopolysaccharides; pAKT, serine/threonine-specific protein kinase; PS1, Presenilin 1; RAGE, receptor for advanced glycation end products; TBI, traumatic brain injury; wt, wildtype; V, valine; YAC, yeast artificial chromosome; 3xTg-AD, Triple-transgenic mice for AD. <sup>1</sup>Information provided when available.

Table 2  
Studies employing non-transgenic animals to examine links between low oxygen brain levels and AD pathology

Animal model	Characteristics (age, gender) <sup>1</sup>	Treatment/Approach	Effects on AD-related pathology [ref]
Mongolian gerbils	Male	BCO, 10 min to produce global ischemia	Loss of 90% of the CA neurons 24 to 72 h after ischemia; decreased A $\beta$ PP and A $\beta$ immunostaining at 24 h after ischemia; increased A $\beta$ PP and A $\beta$ after 48 h that overlapped with increased ApoE expression and glial fibrillary acidic protein [80].
Sprague-Dawley rats	3–20 months, male	Reversible occlusion of the cerebral middle artery to produce focal cerebral ischemia	Upregulation of A $\beta$ PP and A $\beta$ fragments; presence of A $\beta$ PP and A $\beta$ in large round cells between macrophages from blood and/or brain in the infarct region (core and penumbra). Focal accumulation of A $\beta$ PP and A $\beta$ in adult rats [73].
	23 weeks, male	2VO	Deficits in memory after 30 days that worsened after 180 days in aged but not young adult rats; decreased cytochrome oxidase activity mostly in hippocampus and accumulation of A $\beta$ oligomers in the CA1 area after 180 days 2VO [77].
Wistar rats	Male	4 groups: bilateral A $\beta$ intracerebroventricular injection, BCCAO, sham, and A $\beta$ toxicity and BCCAO	Impaired spatial memory in A $\beta$ toxicity-BCCAO group compared to A $\beta$ toxicity and BCCAO groups alone; exacerbated AD pathology in A $\beta$ toxicity-BCCAO group compared to A $\beta$ toxicity group [72].
	10 months, male	ME, occlusion of both external carotid arteries temporarily and then released 500 non-radioactive microspheres into the left common carotid artery	Brain injury associated with A $\beta$ accumulation and tau pathology by microvessel injury; promoted neuropathology similar to NFTs and aberrant eNOS expression and protein tyrosine nitration in microvascular endothelial cells consistent with A $\beta$ -amyloid accumulation [74].
	6–9 months, male	2VO to produce progressive neuronal damage and cholinergic dysfunction	Histologically observed infarction in the cortex of 28.6% and 42.9% in the striatum; neural loss 4 months after 2VO in CA1 hippocampus; rarefaction of white matter found 4 months after 2VO [78].
	10 months, male	2VO	Impaired learning and memory; downregulated synaptophysin in hippocampus; downregulated MAP-2 expression; upregulated GAP-43 mRNA [76].
	11 months, male	tMCAO	Maximal increase of ApoE expression in the core 7 days after tMCAO detection and in periischemic region at 7 and 21 days; increased ApoE mRNA in glial cells but not in neurons in periischemic region [89].

ApoE, apolipoprotein E; A $\beta$ PP, amyloid- $\beta$  protein precursor; AVF, arteriovenous fistula; BCCAO: permanent occlusion of bilateral common carotid arteries; BCO, bilateral carotid occlusion; CBF, cerebral blood flow; GAP-43, growth associated protein 43; MAP-2, microtubule associated protein 2; ME, microsphere embolism; mRNA, messenger ribonucleic acid; NFTs, neurofibrillary tangles; NO, nitric oxide; tMCAO, transient middle cerebral artery occlusion; 2VO, Permanent occlusion of bilateral common carotid arteries. <sup>1</sup>Information provided when available.

147 In addition to stroke, a reduction in the levels of oxygen  
148 that reach the brain is often a consequence of microin-  
149 farcts. A recent study of postmortem brains showed  
150 that chronic microinfarcts and particularly multiple  
151 microinfarcts elevated the likelihood of dementia [27].  
152 Microinfarcts located in cortical regions of the brain  
153 were associated with greater risk for dementia than  
154 those in subcortical regions [27]. Moreover, subjects  
155 with multiple microinfarcts exhibit greater overall cog-  
156 nitive impairment [27]. Clinical-pathological evidence  
157 shows that individuals with AD neuropathology and  
158 white matter or basal ganglia infarcts have a 20-fold  
159 increased risk of developing dementia compared to AD  
160 individuals without infarcts [28, 29].

161 In studies with AD patients, clinical evidence shows  
162 that hypoxia increases the levels of A $\beta$ PP and A $\beta$  in  
163 the vasculature of the brain [17]. Cardiac arrest, an  
164 extreme form of hypoxia, causes a massive increase in  
165 A $\beta$  in blood [30]. Experimental studies support clinical  
166 observations showing that ischemia promotes the  
167 upregulation of A $\beta$ PP resulting in an increase in A $\beta$   
168 accumulation and ultimately in the production of A $\beta$   
169 plaques [31–33]. Increases in A $\beta$  are believed to pro-  
170 duce neurotoxicity by causing perturbations in Ca<sup>2+</sup>  
171 homeostasis, which can lead to a number of dysfunc-  
172 tions in cellular processes including neurotransmitter  
173 release and gene expression [34]. Chronic hypoxia  
174 has been shown to potentiate whole cell voltage-gated  
175 Ca<sup>2+</sup> flows and produce overexpression of A $\beta$  in vari-  
176 ous cell types [17]. There is a growing body of evidence  
177 that disturbances in calcium homeostasis provide a  
178 mechanistic link between hypoxia and AD pathology,  
179 although it remains to be established how calcium alter-  
180 ations account for AD pathogenesis [35].

181 Risk factors for cardiovascular disease have pro-  
182 vided further insight into the relationship between  
183 hypoxia and AD pathogenesis. Hypertension is a risk  
184 factor for AD, and there are several reports that blood  
185 pressure increases in patients with AD years before  
186 the onset of the disease [36]. Chronic hypertension is  
187 often accompanied by additional vascular abnormali-  
188 ties that may threaten an optimal blood supply to the  
189 brain and increase the risk for dementia [36]. However,  
190 because hypertension is also associated with various  
191 risk factors for AD, including hypercholesterolemia,  
192 atherosclerosis, and obesity [37], a causal link between  
193 hypertension and AD pathology has not been estab-  
194 lished. Exploration of vascular risk factors in patients  
195 with AD is compulsory, and ongoing prospective  
196 studies should offer further evidence for developing  
197 preventive and therapeutic treatments. Even in the pro-  
198 cess of normal aging there are marked changes in the

199 vascular system that are associated with changes in  
200 cognitive function [38]. Vascular structure and func-  
201 tion are affected adversely over the course of aging by  
202 stiffening of the arteries and luminal dilatation [39].  
203 An early study [40] showed that there are important  
204 substances in the microvasculature that play a major  
205 role in the interactions between the blood-brain barrier  
206 (BBB), astrocytes, and neurons (Fig. 1). Accordingly,  
207 structural changes related to microvascular pathology  
208 have been shown to be greater in demented compared  
209 to non-demented elderly subjects [40]. Thus, live eval-  
210 uations of microvascular pathology offer a promising  
211 approach to the development of useful biomarkers for  
212 early detection and characterization of AD pathology  
213 [41]. Together, clinical-pathological evidence brings  
214 up several fundamental questions, namely, whether  
215 vascular risk factors are causally related to the devel-  
216 opment of dementia, and if so, whether early diagnosis  
217 and treatment of these pathologies could delay or pre-  
218 vent the progression of dementia. Currently, the best  
219 approach to these questions is by direct manipulations  
220 of oxygen supply and subsequent evaluations of behav-  
221 ioral and neuropathological hallmarks of AD using  
222 animal models.

## 223 MOLECULAR SIGNALING PATHWAYS 224 LINKING AD AND HYPOXIA

225 The molecular signaling pathways related to the  
226 two main forms of neuropathology of AD, accumu-  
227 lation of A $\beta$  and NFTs, have been characterized in  
228 numerous studies and will only be briefly discussed.  
229 Readers are referred to recent reviews [2, 42]. The A $\beta$   
230 peptide is released in brain by proteolytic processing  
231 of A $\beta$ PP. Several insults such as hypoxia (Fig. 1A)  
232 can promote elevation of A $\beta$  peptides, and genetic  
233 and environmental factors are believed to contribute  
234 to a chronic imbalance between A $\beta$  production and  
235 clearance in AD. The A $\beta$  peptide is the principal ele-  
236 ment in the extracellular plaques seen in AD brains,  
237 and insoluble forms of the peptide are produced via  
238 sequential cleavage of A $\beta$ PP by two proteases, first by  
239  $\beta$ -site A $\beta$ PP cleavage enzyme 1 (BACE1) followed by  
240  $\gamma$ -secretase and production of A $\beta$  peptides. Hypoxia  
241 activates transcription factor hypoxia-inducible factor  
242 1 $\alpha$  (HIF-1 $\alpha$ ), which binds to and upregulates BACE1  
243 (Fig. 1A), also promoting A $\beta$  peptide production [43].  
244 Toll-like receptor 4 (TLR4), a pattern recognition  
245 receptor mainly expressed in immune cells, is asso-  
246 ciated with hypoxic episodes in tissues like brain,  
247 heart, kidney, and lung [7, 44]. TLR4 is found to

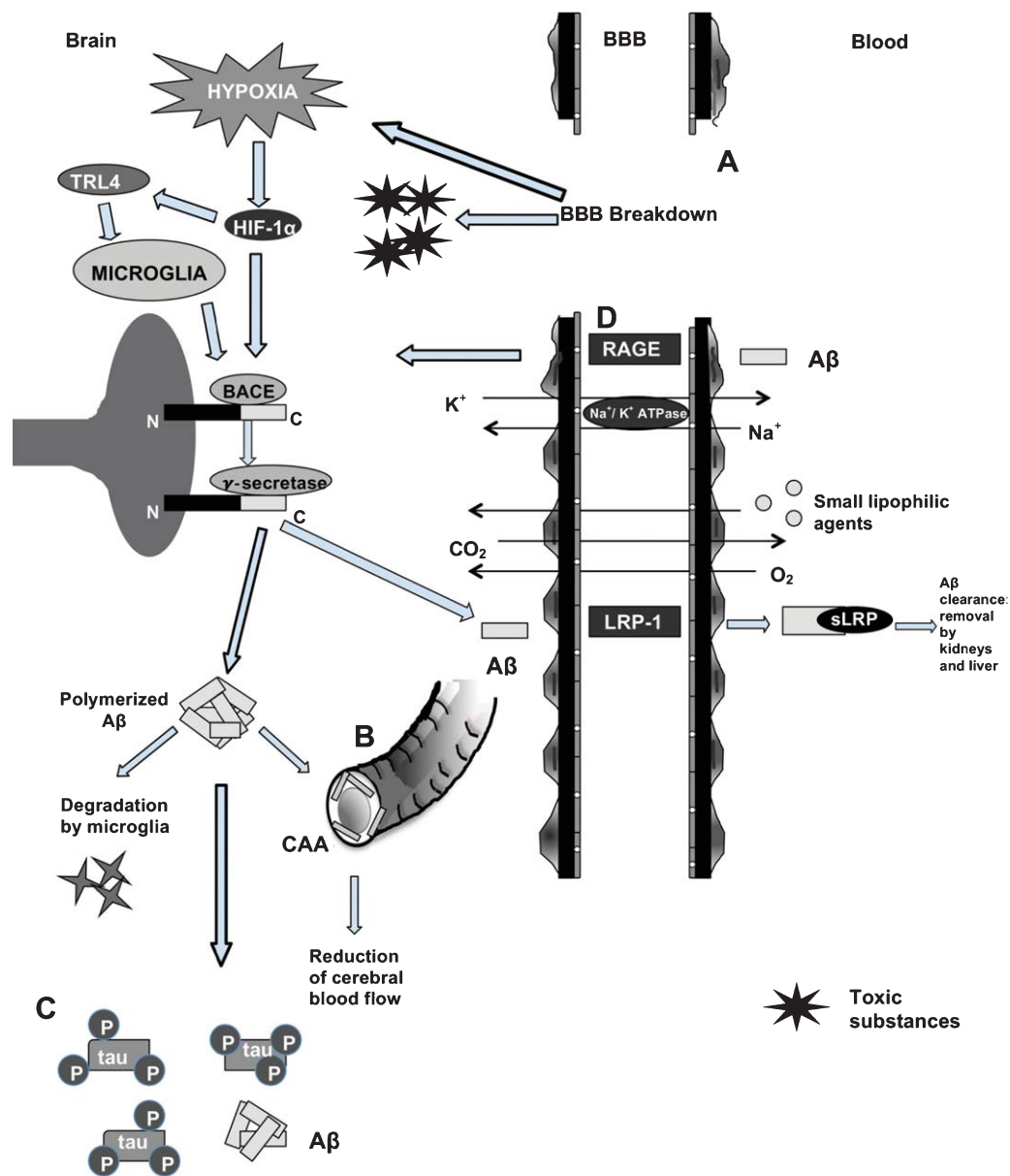


Fig. 1. A) Blood-brain barrier (BBB) breakdown caused by pericyte detachment leads to an accumulation of neurotoxic substances in the brain as well as a reduction in oxygen supply. This activates hypoxia-inducible factor (HIF-1 $\alpha$ ), which binds to and upregulates  $\beta$  amyloid cleaving enzyme-1 (BACE). HIF-1 $\alpha$  promotes the expression of toll like receptor-4 (TLR4) and subsequent microglia activation with release of cytokines that upregulates BACE. Sequential cleavage of the amyloid- $\beta$  protein precursor (A $\beta$ PP) by BACE and  $\gamma$ -secretase results in the amyloid- $\beta$  (A $\beta$ ) peptide [44, 69]. Accumulation of A $\beta$  results in the polymerization of A $\beta$  into plaques that are one of the hallmarks of AD. The plaques may be degraded by microglia or accumulate in the brain parenchyma and walls of small brain arteries leading to a reduction of blood flow [69, 109, 110]. B) Cerebral amyloid angiopathy (CAA) also results from the accumulation of A $\beta$ , which leads to capillary occlusion in the brain and a reduction of blood flow as well as local loss of neurons, microglial activation and microhemorrhage [69]. C) A $\beta$  plaques may lead to hyperphosphorylation of tau protein, which contributes to neurofibrillary tangles and an increase in basement membrane thickness surrounding cortical microvessels [62]. D) A $\beta$  is transported between the brain and blood through two main receptors: the receptor for advanced glycation end products (RAGE) and the low-density lipoprotein receptor-related protein 1 (LRP-1) [50]. RAGE, located on the luminal side of the endothelium mediates the influx of A $\beta$  into the brain. LRP-1, located on the abluminal endothelial cell membrane mediates efflux of free A $\beta$  from the brain interstitial fluid into the blood. Soluble LRP (sLRP) is formed in the liver by cleavage of LRP through  $\beta$ -secretase [87]. It binds to A $\beta$  and is then removed by the liver and kidney. In this way, soluble LRP functions as a peripheral sink for A $\beta$  [111]. LRP-1 also binds directly to A $\beta$ PP affecting endoproteolytic processing of A $\beta$ PP and increasing production of A $\beta$  [87]. Small lipophilic agents as well as O $_2$  and CO $_2$  pass through the BBB by simple diffusion whereas ions require ATP-dependent transporters such as (Na $^+$  and K $^+$ ) ATPase [112].



248 be overexpressed in macrophages and microglia via  
 249 HIF-1 $\alpha$  under hypoxia, mediating brain inflammation  
 250 and hypoxic-ischemic-related diseases [5, 44]. Acti-  
 251 vated microglial cells release several inflammatory  
 252 mediators such as cytokines, reactive oxygen species,  
 253 complement components, and nitric oxide that pro-  
 254 mote upregulation of BACE1 (Fig. 1A) and ultimately  
 255 increase A $\beta$  peptide production [7, 44, 45].

256 The brain uses several routes to clear A $\beta$  from  
 257 the brain. One of the principal routes is by the low  
 258 density lipoprotein receptor-related protein (LRP),  
 259 specifically LRP-1, a major cell surface A $\beta$  clear-  
 260 ance receptor located on vascular smooth muscles cells  
 261 that allows the transport of A $\beta$  peptides through the  
 262 BBB [46] (Fig. 1D). LRP-1 is also found in neurons  
 263 where it mediates A $\beta$ -induced oxidative stress and  
 264 intraneuronal transport, causing mitochondrial dys-  
 265 function [47, 48]. A $\beta$  is also cleared from the brain  
 266 by A $\beta$  chaperones such as ApoE isoforms (ApoE2,  
 267 ApoE3, or ApoE4), by microglia and perivascular  
 268 brain macrophages, by direct enzymatic degradation  
 269 of A $\beta$  in the brain, and by passive drainage of A $\beta$  into  
 270 the perivascular space [46, 49]. The influx of periph-  
 271 eral A $\beta$  to the brain is mediated by the receptor for  
 272 advanced glycation end products (RAGE) (Fig. 1D).  
 273 RAGE acts as a cell surface receptor that binds A $\beta$   
 274 in BBB, neurons, and microglia [50]. Because of  
 275 its diverse localization, RAGE contributes to vari-  
 276 ous aspects of AD pathology, including A $\beta$ -induced  
 277 inflammatory response, oxidative stress, and intraneu-  
 278 ronal mitochondrial dysfunction [50]. At later stages  
 279 of AD progression, tau protein, a soluble microtubule-  
 280 associated protein, becomes hyperphosphorylated and  
 281 forms intracellular NFTs. In AD, NFTs compromise  
 282 intracellular transport and the structural integrity of  
 283 neurons [2, 51]. Hypoxia-induced alterations in AD  
 284 metabolism may drive NFT formation (Fig. 1C). Both  
 285 senile plaques and NFTs are used as markers for the  
 286 definitive diagnosis of AD in postmortem brain.

#### 287 *Hypoxia increases A $\beta$ production through its* 288 *effects on A $\beta$ signaling pathways*

289 The bulk of experimental evidence linking hypoxia  
 290 to AD pathology indicates that hypoxia exerts powerful  
 291 modulatory effects on the A $\beta$  signaling pathway. Stud-  
 292 ies of postmortem brain tissue have found that mild  
 293 and severe ischemic episodes are associated with ele-  
 294 vated levels of A $\beta$ PP [52] and aggregation of A $\beta$ <sub>1-40</sub>  
 295 and A $\beta$ <sub>1-42</sub> [53]. Similar studies using immunohisto-  
 296 chemical evaluations of axonal pathology have shown  
 297 an increase in A $\beta$ PP and A $\beta$  following severe head

298 injury and cerebral ischemia [54–56]. Likewise, ani-  
 299 mal studies *in vivo* have demonstrated increased levels  
 300 of A $\beta$ PP and A $\beta$  and upregulation of BACE1 under  
 301 hypoxic conditions [57, 58]. Significantly, only a sin-  
 302 gle, mild temporal occlusion (4 min) of the common  
 303 carotid arteries in adult (3 months) 3xTg-AD mice was  
 304 sufficient to produce acute elevations in A $\beta$  levels by  
 305 enhancing BACE1 that were sustained for at least 3  
 306 weeks [58]. Also, mild hypoperfusion produced a long-  
 307 lasting reduction in tau, presumably through autophagy  
 308 and ubiquitin-proteosomal pathway activation within  
 309 the affected brain region [58]. Hypoperfusion altered  
 310 phosphorylated tau proteins [58] that have been impli-  
 311 cated in the long-term formation of NFTs in AD  
 312 patients [58, 59]. In much older 3xTg-AD mice (15  
 313 months), a single but longer-lasting (12 min) occlu-  
 314 sion of the bilateral common carotid arteries did not  
 315 affect A $\beta$  levels, but rather enhanced A $\beta$ PP phos-  
 316 phorylation and insoluble tau levels at three months  
 317 post-ischemia [60]. The same effects were produced in  
 318 wild-type controls, suggesting that these parameters of  
 319 global ischemia promote changes in AD-related path-  
 320 ways in this strain of aged mice regardless of genetic  
 321 profile [60]. Other studies with knock-out transgenic  
 322 mice confirm A $\beta$ PP involvement in responses to vas-  
 323 cular insults [61]. Global cerebral ischemia, performed  
 324 by transient bilateral clamping of the common carotid  
 325 arteries in mice lacking either A $\beta$ PP or BACE1 genes  
 326 (A $\beta$ PP  $-/-$  and BACE  $-/-$ ) increased the risk of  
 327 mortality and reduced CBF compared to wild-type lit-  
 328 termates [61]. Moreover, two molecules involved in  
 329 vascular regulation, serum response factor and calse-  
 330 questrin, were also significantly altered in both strains  
 331 [61]. Thus, studies with A $\beta$ PP and BACE1 knockouts  
 332 suggest a beneficial role for A $\beta$ PP and its cleavage  
 333 fragments in the regulation of blood flow and the  
 334 adaptation to ischemic insults. Taken together, results  
 335 with transgenic mice confirm A $\beta$ PP involvement in the  
 336 brain's response to hypoperfusion, whereas hypoxic  
 337 insults produce variable effects on A $\beta$  and tau levels.

338 Chronic hypoxia appears to produce more pro-  
 339 nounced and consistent effects on AD pathology.  
 340 In a recent study A $\beta$ PP/PS1 transgenic mice were  
 341 exposed daily to hypoxia treatment for two months  
 342 that produced numerous deficits associated with  
 343 AD pathology, including worsened cognitive deficits,  
 344 increased A $\beta$  accumulation and subsequent forma-  
 345 tion of A $\beta$  plaques, and increased levels of tau  
 346 phosphorylation [62]. Similarly, long-term chronic  
 347 hypoxia treatment was shown to produce more and  
 348 larger A $\beta$  plaques in two strains of transgenic mice  
 349 (A $\beta$ PPSwe + PS1A246E and A $\beta$ PP23) [63]. Different

parameters for generating chronic hypoxia (8% O<sub>2</sub> for 16 h/day) in AβPP23 mice produced similar modulatory effects on Aβ pathways, namely upregulation of BACE1 promoter activity and increases in both BACE1 transcription and expression *in vivo* [64]. This in turn upregulated BACE1 cleavage of AβPP, increased Aβ production, deposits, and plaque formation, and worsened cognitive deficits in transgenic mice. Similar effects on cognitive function were observed in young adult mice (2 months) overexpressing a mutant form of human AβPP (AβPP<sub>sw/ind</sub>-Tg mice). These mice exposed to chronic hypoperfusion via bilateral common carotid artery stenosis using microcoils exhibited greater cognitive deficits and hippocampal neuronal loss compared with controls [65]. Notably, insoluble Aβ was reduced, whereas soluble Aβ was increased, following six months of cerebral hypoperfusion, resulting in a reduction of Aβ deposition and plaque formation, suggesting that the cognitive impairment and neuronal loss associated with stenosis in this transgenic line may be a result of soluble Aβ species. In sum, chronic hypoperfusion produces effects on AD neuropathology that are more consistent with those observed in clinical cases, namely increases in Aβ plaques, tau phosphorylation, and cognitive impairments.

While chronic hypoxia has been shown to produce profound impairments in cognition and brain structure and function, hyperoxia treatment has been shown to have opposite effects. Chronic hyperoxia treatment (40% O<sub>2</sub>; 8 h/day for 4 or 8 weeks) in young adult AβPP/PS1 transgenic mice produced significant improvements in spatial learning and memory and decreased Aβ deposition and plaque formation in cortex and hippocampus [66]. Biochemical analysis of brain tissue indicated that hyperoxia treatment reduced Aβ by inhibiting γ-secretase activity. The results of this study support the application of oxygen therapy as a useful way to reduce the neuropathological changes associated with AD progression, although this possibility requires further study.

Studies examining vascular profiles in AD transgenic mice have found that targeted mutations in these models not only produce forms of AD neuropathology but also various cerebrovascular pathologies. Imaging studies using magnetic resonance angiography to evidence cerebral arterial hemodynamics have shown that adult AβPP23 mice present flow voids in the internal carotid arteries that were observed as late as 20 months of age in large arteries in the circle of Willis. Vessel elimination and vessel deformation were also observed at the site of the flow voids [67]. Imaging techniques

have also uncovered the deposition of Aβ peptides in intracortical vessels and its association with cerebral amyloidosis. A recent study using non-invasive high resolution contrast enhanced magnetic resonance angiography (CE-μMRA) in 4- and 24-month-old arcAβ mice showed an age-dependent reduction in the quantity of intracortical vessels in arcAβ mice compared to littermate controls [68]. Specifically, the number of functional intracortical microvessels was reduced in 24-month-old arcAβ mice compared to wild type controls, whereas no differences were found in four-month-old mice. Moreover, an accumulation of Aβ and fibrinogen, which is associated with vessel stenosis and a reduction in CBF [68], was found in small and medium sized vessels but not in large arteries in 24-month-old arcAβ mice. These results suggest that AβPP23 and arcAβ mice may be suitable models for examining links between AD neuropathology and neurovascular disease.

One of the principal vessel disorders associated with AD is cerebral amyloid angiopathy (CAA), which produces vascular deposits of Aβ similar to the senile plaques in AD (Fig. 1B). One outcome of CAA-related capillary occlusion is disruption of CBF, which leads to Aβ toxicity [69]. Aged AβPP23 mice exhibit CAA-related capillary occlusion in thalamic vessels that is not evident in control transgenic AβPP51/16 or wild type mice [70]. CAA has also been linked with ApoE expression. AβPP<sub>sw</sub> mice expressing endogenous murine ApoE or human ApoE3 and ApoE4 isoforms (knock-in mice Tg2576) develop amyloid plaques as well as CAA [71]. AβPP<sub>sw</sub> mice expressing ApoE4 at 15 months of age showed a change in Aβ deposition that lead to substantial CAA compared to age-matched mice expressing ApoE3 [71], providing evidence that links ApoE expression with Aβ retention in the brain by interfering with Aβ clearance mechanisms. Thus, AD transgenic mice provide evidence that capillary occlusion, which is present in human AD brains, is related also to CAA, pointing to these transgenic lines as useful models for gaining mechanistic insights into neurovascular and AD pathologies.

Additional evidence supporting the association between hypoxia and the development of Aβ pathology has been derived from studies with non-transgenic animals (Table 2). For instance, rats that received bilateral intracerebroventricular injections of Aβ fragments and permanent occlusion of bilateral common carotid arteries showed greater impairments in spatial memory and more extensive AD neuropathology relative to animals that received Aβ toxicity or occlusion alone [72]. Similarly, aged rats exposed to reversible occlusion of

the cerebral middle artery that produced focal cerebral ischemia showed an upregulation of A $\beta$ PP and A $\beta$  fragments [73]. The presence of A $\beta$ PP and A $\beta$  immunoreactivity in the infarct region indicated that concomitant reductions in CBF and cerebral ischemia provide the necessary elements for focal accumulation of A $\beta$ PP and A $\beta$  in adult rats [73]. Importantly, studies show that the effects of hypoxia on AD pathology can persist in time. A mild microsphere embolism in aged rats promoted eNOS expression and protein tyrosine nitration in microvascular endothelial cells, leading to A $\beta$  accumulation in the lesioned area and hyperphosphorylation of tau protein in surrounding neurons [74, 75]. Both A $\beta$  accumulation and hyperphosphorylated tau remained elevated for 12 weeks, indicating long-lasting effects of neurovascular injury on A $\beta$  neurodegeneration [74, 75].

Chronic bilateral occlusion of the common carotid arteries in rats also reproduces several characteristics of human AD. Chronic hypoperfusion in adult rats has been shown to promote accumulation of oligomeric A $\beta$  and impaired learning and memory that progressed as the period of hypoperfusion increased [76]. Significantly, hypoperfusion caused the downregulation of various proteins important for synaptic plasticity and cognitive function including growth-associated protein-43 (GAP-43), synaptophysin, and microtubule-associated protein-2 (MAP-2) [76]. Similar effects were observed following double ligation of the carotid arteries in adult rats, namely profound deficits in spatial memory in aged but not young rats after 30 days that worsened after 180 days [77]. Hypoperfusion also caused an accumulation of A $\beta$  oligomers in the CA1 region 180 days after surgery and synaptic changes in CA1 that correlated with the structural changes observed in AD progression [77]. Early studies demonstrated that permanent occlusion of bilateral common carotid arteries produced progressive neuronal damage in the hippocampus and white matter, evidenced by increased degeneration from one to four months after cerebral hypoperfusion [78]. Also, hypoperfusion produced long-lasting decreases in acetylcholinergic levels in cortex, striatum, and hippocampus after four months. These results suggest that progressive structural and functional changes in hippocampus and other brain areas play a role in the cognitive decline that occurs in aged persons following chronic hypoperfusion [78]. Moreover, other studies have reported that the observed hypoperfusion-induced deficits in spatial learning are a product of altered energy metabolism in various brain areas in addition to the hippocampus that are responsible for visuo-

motor integration [79]. On the other hand, there is evidence that the nature of structural changes varies post-ischemia. Global forebrain ischemia produced by bilateral carotid occlusion resulted in a loss of 90% of CA1 neurons 24 to 72 hours after ischemia and a decrease in A $\beta$ PP and A $\beta$  at 24 hours following ischemia in aged gerbils [80]. At 48 hours, there was an increase in A $\beta$ PP and A $\beta$  that overlapped with increased ApoE that may provide circumstances that are favorable for the formation of A $\beta$  oligomers after ischemic insults [80]. Together, these results demonstrate that ischemia produces profound and long-lasting effects on brain tissue that are consistent with AD neuropathology, but that the spatial-temporal pattern of these effects varies among studies.

#### *Evidence regarding hypoxia-ischemia effects on A $\beta$ clearance mechanisms*

A $\beta$  is cleared from the brain through receptor-mediated endocytosis by cells in the parenchyma or through the BBB [81], but during a hypoxic episode the A $\beta$  clearance mechanism is impaired. Systemic inflammation is one of the causes of impaired efflux of A $\beta$  from the brain [82]. Young adult mice treated with lipopolysaccharides (LPS) showed several disturbances including inhibition of CSF bulk flow, impairment of central and peripheral clearance of A $\beta$ , and increased vascular sequestration of A $\beta$  [82], which together suggest that inflammatory responses disrupt A $\beta$  transport and clearance that may exacerbate AD pathology. Other A $\beta$  transport molecules that are capable of modulating cerebral blood flow responses and AD pathological processes include LRP1 and RAGE. Inhibition of RAGE, one of the receptors for A $\beta$  in the BBB, has been shown to have positive effects on CBF and AD pathology. In one study, RAGE inhibition was shown to normalize CBF responses and cognitive performance in aged A $\beta$ PP mice [50]. In mice that overexpress human A $\beta$ PP (A $\beta$ PP<sup>sw/0</sup>), a high affinity RAGE specific blocker (FPS-ZM1) inhibited  $\beta$ -secretase activity and A $\beta$  production, reduced A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> levels in the brain, and normalized cognitive performance and CBF in aged animals [50].

Modulation of LRP1 has also been shown to impact AD pathology and vascular processes in the brain. Evidence from studies with young and adult mice indicates that LRP-1 decreases with age [83]. Hepatic A $\beta$  uptake, which accounts for 40–60% of total A $\beta$  uptake, is also attenuated in aged rats, suggesting that A $\beta$  levels increase during the normal aging process as a consequence of insufficient systemic clearance

[84]. Some proteins like receptor-associated protein (RAP)-chaperone facilitate the trafficking of LRP-1 by binding to multiple sites on LRP-1 and competitively blocking all known LRP ligands [83, 85]. It has been reported that increasing RAP concentrations decreases A $\beta$  clearance [86]. Transgenic mice with decreased levels of LRP-1 exhibit greater A $\beta$  accumulation than wild-type mice [86]. The increased levels of A $\beta$  promote proteasome-dependent degradation of LRP-1 that lead to increased A $\beta$  accumulation in a positive feedback loop, suggesting that A $\beta$  peptides compete for the same LRP-mediated efflux system in order to exit the brain [86]. Animal experiments suggest that higher levels of A $\beta$  may completely saturate LRP-1 leading to vascular accumulation of A $\beta$  and subsequent development of cerebrovascular amyloid protein deposits [83]. As such, if the levels of extracellular A $\beta$  exceed the transport capacity of LRP-1 or the transport systems are impaired by downregulation of LRP-1, A $\beta$  could accumulate in brain tissue and vessels [83, 86, 87], producing toxicity and neuronal death.

#### *Evidence linking hypoxia-induced pathology and genetic risk factors for AD*

The ApoE4 polymorphism is a common risk factor in AD and CAA, and recent studies have revealed potential mechanisms linking ApoE4 to both diseases. Studies of postmortem brain tissue have found that mild and severe ischemic episodes are associated with increases in ApoE in the hippocampus [53]. Likewise, transgenic mice (Tg2576) expressing human ApoE4 showed substantial CAA and increased A $\beta_{40:42}$  ratios in brain [71]. A more recent study showed that animals expressing human ApoE4 genes that received intracerebral injections of human A $\beta_{1-40}$  presented significantly greater A $\beta$  deposition in the hippocampus than those expressing ApoE3 at both 3 and 16 months of age, suggesting that ApoE4 disrupts A $\beta$  clearance from the brain [88]. Further, the disruption in A $\beta$  clearance was linked to morphological changes in the vasculature of aged mice [88]. Taken together, these studies suggest an age-dependent effect of ApoE4 expression on the elimination of A $\beta$  from the brain along vascular basement membranes. Similar links between ApoE expression and cerebrovascular processes have been found in studies with non-transgenic animals. Aged Wistar rats exposed to transient middle cerebral artery occlusion showed long-term changes in ApoE immunoreactivity and mRNA expression [89]. After seven days of occlusion the maximal increase of ApoE expression was detected in the core, and

at 21 days increased ApoE was detected in the peri-ischemic region in glial cells but not in neurons. Another source of ApoE expression was macrophages, which was attributed to necrotic tissue clearance after the ischemic insult. Together, studies with transgenic and non-transgenic animals link ischemic episodes with increased ApoE expression, which may disrupt A $\beta$  clearance from the brain in a time-dependent manner post-ischemia.

Recently identified genetic risk factors for late onset AD can be present at low frequency but can represent almost the same risk as the common sporadic genetic risk factor ApoE. Some of these genetic variants such as TREM2, complement receptor-1 (CR1), and CD33 participate in microglia activation and the subsequent development of A $\beta$  protein deposits [90, 91]. Expression of CD33, a transmembrane protein that encodes a myeloid cell-surface receptor, is a risk factor for AD and has been shown to inhibit the uptake and clearance of A $\beta_{42}$  in microglial cell cultures [92]. Further, studies in transgenic mice show that TREM2 expression is positively correlated with amyloid phagocytosis whereas TREM2 inhibition is related to accumulation of toxic products in brain [93]. Lastly, the presence of the CR1 risk allele or CR1 AD risk variant gene is related to impaired clearance and deposition of A $\beta$  in brain and to an increased rate of cognitive decline [94–96]. Although these three genetic variants (TREM2, CD33, and CR1) occur less frequently than ApoE  $\epsilon$ 4 allele, they represent sporadic genetic risk factors for AD similar to ApoE  $\epsilon$ 4 and implicate microglial impairment as a factor in promoting AD neuropathological processes.

#### *Hypoxia and head injury-produced A $\beta$ pathology*

Clinical studies have shown that head injury generates ischemic changes that induce tau-like pathology and A $\beta$ PP cleavage [34, 97]. A $\beta$  plaques have been seen in 30% of patients who die from traumatic brain injury (TBI) [98]. The accumulation of A $\beta$  after TBI is believed to result from axonal damage [99, 100], which interrupts axonal transport and results in an accumulation of proteins in the axon, including A $\beta$ PP [101, 102]. Several studies have used animal models to examine the pathology of A $\beta$  accumulation following TBI [98, 103–105]. In one study, hippocampal damage and behavioral deficits were seen as a result of TBI in 3xTg-AD mice [105]. There was also an increase in A $\beta$  accumulation in pericontusional white matter and an increase in total insoluble A $\beta$ . Notably, the increase of A $\beta$  in white matter was dependent on the severity of injury [105]. Similarly, a study conducted

656 in a swine model found an increase in A $\beta$ PP and co-  
657 accumulation of A $\beta$  in swollen axons and neuronal cell  
658 bodies, as well as formation of diffuse parenchymal  
659 A $\beta$  plaques in both grey and white matter [106]. Fur-  
660 ther, adult 3xT-AD mice exposed to experimental TBI  
661 showed rapid intra-axonal A $\beta$  accumulation in the area  
662 of the injury, increased tau immunoreactivity in brain  
663 regions following moderate injury, and no A $\beta$  accu-  
664 mulation in areas without injury [105]. Together, the  
665 results of these studies suggest that trauma creates a  
666 unique situation in which all of the necessary enzymes  
667 for A $\beta$  formation co-exist in axons. These results are  
668 consistent with those found in humans, where TBI pro-  
669 duced long-term progressive axonal degeneration and  
670 intra-axonal A $\beta$  accumulation that persisted for years  
671 following the initial trauma [54]. Evidence suggests  
672 that following hypoxia, eventual lysis and breakdown  
673 of damaged axons may be the underlying mechanism  
674 of A $\beta$  release into the parenchyma where it aggre-  
675 gates and causes plaque formation [54]. In related  
676 studies using mice that overexpress normal human  
677 A $\beta$ PP or a mutant form of A $\beta$ PP brain injury produced  
678 an increase in A $\beta$  in brain tissue but not an increase  
679 in plaque formation [103, 104]. In mice overexpress-  
680 ing mutant A $\beta$ PP, the increases in A $\beta$  led also to an  
681 increase in hippocampal neuronal death and memory  
682 impairment [104]. Clearly, brain injury produces alter-  
683 ations in A $\beta$  pathways that promote pathologies similar  
684 to AD, although plaque formation is not reliably pro-  
685 duced in some models.

## 686 CONCLUSION

687 Diagnostic criteria for dementias make a distinction  
688 between the impairment resulting from AD and that  
689 resulting from cerebrovascular insults despite evidence  
690 of extensive overlap between the two [17]. AD and vas-  
691 cular pathologies share several risk factors, and clinical  
692 and experimental research suggests that the burden of  
693 vascular and AD neuropathology may not be inde-  
694 pendent. The present review presented evidence from  
695 transgenic and non-transgenic rodent models linking  
696 AD and cerebrovascular neuropathology. Collectively,  
697 experimental studies with rodents provide strong evi-  
698 dence in favor of hypoxia-induced alterations in A $\beta$   
699 metabolism that may in turn drive the neurotoxicity  
700 marked by NFT formation and subsequent cell death.  
701 First, studies with transgenic mice confirm that oxy-  
702 gen deficiency facilitates AD pathogenesis by altering  
703 A $\beta$ PP expression and driving A $\beta$  overproduction [57,  
704 58, 60–65]. Importantly, chronic hypoperfusion pro-

705 duces pronounced effects on AD neuropathology that  
706 include increases in A $\beta$  plaques [63, 64] and tau hyper-  
707 phosphorylation [62] and worsened cognitive deficits  
708 [64, 65], which are not consistently observed following  
709 mild hypoperfusion. Second, various AD transgenic  
710 manipulations produce vascular pathologies that pro-  
711 vide compelling evidence of links between molecular  
712 pathways common to both AD and neurovascular dis-  
713 ease [67, 68, 70, 71]. Third, studies with non-transgenic  
714 animals indicate that hypoperfusion produces pro-  
715 nounced effects on cognition, A $\beta$  accumulation and tau  
716 hyperphosphorylation [72–78]. Fourth, various stud-  
717 ies point to ischemia-induced time-dependent changes  
718 in brain structure and function [77–80] particularly in  
719 hippocampal areas afflicted in AD progression. Lastly,  
720 various studies suggest that hypoxia effects on A $\beta$   
721 accumulation may reflect disruptions in A $\beta$  clearance  
722 mechanisms [50, 71, 88]. Taken together, hypoxia-  
723 induced effects may cause a significant shift toward  
724 increased A $\beta$  deposition and reduced A $\beta$  clearance  
725 that leads to the neurotoxic cascade and functional  
726 deterioration characteristic of AD neuropathology.

727 The evidence reviewed favors links between  
728 hypoxic insults and AD neuropathology, but variable  
729 findings among studies limits the degree to which  
730 rodent models can reliably reproduce human disease.  
731 Rodent species are amenable to genetic manipulations  
732 and large-scale studies, but differences between the  
733 human and rodent brain and limitations in rodents'  
734 behavioral repertoire represent challenges to trans-  
735 lation. For instance, the evidence reviewed suggests  
736 that hypoxia-induced effects on A $\beta$  pathology are  
737 more consistent across studies than effects on tau  
738 pathology, ApoE, or cognitive impairment. Accord-  
739 ingly, recent reviews have highlighted the limitations  
740 of animal models to produce effective neuroprotec-  
741 tive agents for ischemic stroke in clinical trials and  
742 suggested that nonhuman primate models made be  
743 a more appropriate albeit ethically more challenging  
744 approach [107, 108]. Many biomarker-based studies  
745 have established that the neuropathology associated  
746 with AD, namely A $\beta$  deposits and NFTs, progressively  
747 accumulates in the brain decades before behavioral  
748 symptoms appear [2]. Therefore, it remains a viable  
749 strategy to target the various vascular risk factors for  
750 AD during pre-symptomatic stages in AD develop-  
751 ment. In this regard, the use of rodent models continues  
752 to be the more cost-effective approach to explore the  
753 mechanistic factors linking reductions in CBF to AD-  
754 related neuropathologies and behavioral deficits, and  
755 the continued standardization and refinement of these  
approaches should be pursued.

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