



Published in final edited form as:

*Nat Rev Neurol.* 2015 August ; 11(8): 457–470. doi:10.1038/nrneurol.2015.119.

## Clearance systems in the brain—implications for Alzheimer disease

**Jenna M. Tarasoff-Conway, Roxana O. Carare, Ricardo S. Osorio, Lidia Glodzik, Tracy Butler, Els Fieremans, Leon Axel, Henry Rusinek, Charles Nicholson, Berislav V. Zlokovic, Blas Frangione, Kaj Blennow, Joël Ménard, Henrik Zetterberg, Thomas Wisniewski, and Mony J. de Leon**

New York University School of Medicine, 660 First Avenue, New York, NY 10016, USA (**J.M.T.-C., E.F., L.A., C.N.**). University of Southampton, Faculty of Medicine, Institute for Life Sciences, Southampton General Hospital, Southampton, Hampshire SO16 6YD, UK (**R.O.C.**). New York University School of Medicine, 145 East 32<sup>nd</sup> Street, New York, NY 10016, USA (**R.S.O., L.G., T.B., H.R., B.F., T.W., M.J.d.L.**). Zilkha Neurogenetic Institute at Keck School of Medicine of University of Southern California, 1501 San Pablo Street Los Angeles, CA 90089, USA (**B.V.Z.**). The Sahlgrenska Academy at the University of Gothenburg, S-431 80 Mölndal, Sweden (**K.B., H.Z.**). Université Paris-Descartes, 12 Rue de l'École de Médecine, 75006 Paris, France (**J.M.**)

### Abstract

Accumulation of toxic protein aggregates—amyloid- $\beta$  ( $A\beta$ ) plaques and hyperphosphorylated tau tangles—is the pathological hallmark of Alzheimer disease (AD).  $A\beta$  accumulation has been hypothesized to result from an imbalance between  $A\beta$  production and clearance; indeed,  $A\beta$  clearance seems to be impaired in both early and late forms of AD. To develop efficient strategies to slow down or halt AD, it is critical to understand how  $A\beta$  is cleared from the brain.

Extracellular  $A\beta$  deposits can be removed from the brain by various clearance systems, most importantly, transport across the blood–brain barrier. Findings from the past few years suggest that astroglial-mediated interstitial fluid (ISF) bulk flow, known as the glymphatic system, might contribute to a larger portion of extracellular  $A\beta$  (e $A\beta$ ) clearance than previously thought. The meningeal lymphatic vessels, discovered in 2015, might provide another clearance route. Because these clearance systems act together to drive e $A\beta$  from the brain, any alteration to their function could contribute to AD. An understanding of  $A\beta$  clearance might provide strategies to reduce excess  $A\beta$  deposits and delay, or even prevent, disease onset. In this Review, we describe the clearance systems of the brain as they relate to proteins implicated in AD pathology, with the main focus on  $A\beta$ .

---

Correspondence to: M.J.d.L. mony.deleon@nyumc.org.

#### Competing interests

K.B. and H.Z. are co-founders of Brain Biomarker Solutions.

The other authors declare no competing interests.

#### Author contributions

M.J.d.L., J.M.T.-C., R.O.C., R.S.O., T.B., H.R., C.N., B.V.Z., K.B., H.Z. and T.W. researched data for article. M.J.d.L., J.M.T.-C., R.O.C., B.V.Z., H.Z. and T.W. wrote the article. M.J.d.L. and J.M.T.C. provided substantial contributions to discussion of the content. All authors participated in reviewing and editing of the manuscript before submission.

## Introduction

Alzheimer disease (AD) is the most common type of dementia and comprises early-onset AD (EOAD) and sporadic or late-onset AD (LOAD).<sup>1–3</sup> EOAD affects a minority of AD patients, whereas LOAD afflicts over 95% of patients with AD.<sup>4–6</sup> Both EOAD and LOAD are characterized by excessive accumulation of toxic forms of amyloid- $\beta$  (A $\beta$ ), which has been hypothesized to result from an imbalance between its production and clearance.<sup>7–9</sup> Emerging evidence suggests that A $\beta$  clearance is impaired in both early-onset and late-onset forms of AD.<sup>10,11</sup> Specifically, carriers of EOAD-associated presenilin mutations show both increased A $\beta$  production<sup>10,12</sup> and decreased A $\beta$  clearance,<sup>10</sup> whereas individuals with LOAD exhibit decreased A $\beta$  clearance only.<sup>11</sup>

Failure of A $\beta$  clearance is increasingly recognized in the pathogenesis of AD. It is critical to understand how A $\beta$  is cleared from the brain, and to find new ways of investigating this process in carefully phenotyped patients and healthy controls. Because A $\beta$  deposition can be increased in presymptomatic individuals years or even decades before the hallmark symptoms of AD manifest,<sup>20</sup> an understanding of A $\beta$  clearance might eventually provide strategies to reduce excess A $\beta$  deposits and delay, or even prevent, disease onset.

Soluble A $\beta$  can be removed from the brain by various clearance systems, including enzymatic degradation and cellular uptake, transport across the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB), interstitial fluid (ISF) bulk flow, and cerebrospinal fluid (CSF) absorption into the circulatory and lymphatic systems.

In the early 2000s, mouse studies demonstrated that the majority (75%) of extracellular A $\beta$  (eA $\beta$ ) is cleared by the BBB, with only a minority (10%) being cleared by ISF bulk flow.<sup>14,15</sup> However, two-photon imaging studies from the past few years have suggested that ISF bulk flow—facilitated by astroglial aquaporin-4 (AQP4) channels and named the glymphatic (glial + lymphatic) system—contributes to a larger portion of eA $\beta$  clearance than previously thought.<sup>16,17</sup> Furthermore, the discovery of meningeal lymphatic vessels suggests yet another potential clearance route.<sup>18</sup> Although the relative contributions of each of these systems to overall clearance are unknown, they act together to drive eA $\beta$  from the brain, meaning that alterations in any given system can contribute to the altered pathophysiology and accumulation of lesions in AD.

In this Review, we aim to describe the brain's clearance systems that are related to removal of toxic accumulation of proteins in AD. Here, 'clearance' is defined broadly as the removal of any substance, such as A $\beta$ , from the brain. We focus on A $\beta$ , given its ability to form aggregates within the extracellular space, but also briefly cover tau, which needs to be investigated in parallel with A $\beta$ .

## Background

### Risk factors for AD

When characterized by autosomal dominant inheritance, EOAD is related to mutations in the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) or amyloid precursor protein (*APP*) genes.<sup>2–4</sup> However, epidemiological data suggest that only a minority of EOAD cases demonstrate

autosomal dominant transmission, leaving the genetic association of the majority of EOAD cases unexplained.<sup>2,19</sup>

Various factors have been reported to positively and negatively modulate the risk of LOAD. Specifically, the greatest overall risk factor for LOAD is ageing;<sup>20</sup> for example, in the USA, over 40% of individuals above the age of 85 years have been diagnosed with AD.<sup>21–24</sup> The strongest identified genetic risk factor for LOAD is the apolipoprotein E (APOE)  $\epsilon 4$  allele (*APOE\** $\epsilon 4$ ),<sup>25,26</sup> although genome-wide association studies have linked LOAD to several other genetic variants, such as *TREM2* (triggering receptor expressed on myeloid cells 2),<sup>27</sup> clusterin (*CLU*),<sup>28</sup> and phosphatidylinositol-binding clathrin assembly protein (*PICALM*).<sup>28,29</sup> Known environmental risk factors for LOAD include cardiovascular disease, and factors conferring a risk of cardiovascular disease, such as diabetes mellitus and hypertension. Head trauma, physical and mental inactivity, and sleep impairment are additional risk factors for LOAD.<sup>13,30–35</sup>

### Pathological changes

AD is characterized by specific neuropathological and biomarker changes. The gross pathological changes consist of brain atrophy, particularly in the hippocampal formation, temporal lobes and parietotemporal cortices, accompanied by cortical thinning, enlarged ventricles and white matter abnormalities, as evident on MRI.<sup>36,37</sup> Microscopic changes include accumulation of A $\beta$  into parenchymal senile plaques (also known as neuritic plaques) or in the walls of cerebral capillaries and arteries (known as cerebral amyloid angiopathy, or CAA), as well as aggregation of hyperphosphorylated tau into intracellular neurofibrillary tangles (NFTs) and neuropil threads.<sup>36,38</sup> The severity of CAA, the NFT load, and the magnitude of synapse loss—but not the number and extent of amyloid plaques—correlate well with the degree of cognitive decline.<sup>39–44</sup> These AD-related neuropathological changes presumably occur decades before symptom onset, because they are also found in individuals with mild cognitive impairment (MCI), and in people with no cognitive symptoms.<sup>20</sup>

### *In vivo* AD biomarkers

Recent advances now enable several AD-related brain changes to be detected *in vivo*: <sup>18</sup>F-FDG-PET detects decreases in glucose metabolism,<sup>45,46</sup> and MRI detects brain atrophy, as well as diffusion and perfusion abnormalities, which are most prominent in the vulnerable hippocampal formation and cortical regions.<sup>47–49</sup> The pathological accumulation of A $\beta$  and tau proteins in the brain can be inferred by analysing their levels in the CSF, with longitudinal changes having been described and modelled.<sup>50–53</sup> Specifically, A $\beta$  accumulation into extracellular plaques is marked by decreased CSF levels of A $\beta_{1-42}$ , and tau accumulation into NFTs is marked by increased CSF levels of total tau and hyperphosphorylated tau.<sup>51,54</sup> In addition, PET can be used to assess A $\beta$  brain accumulation directly,<sup>50</sup> and PET for tau is currently under investigation.<sup>52,55</sup>

## Clearance systems

The removal of soluble waste from the brain occurs via various overlapping clearance systems, which can be classified according to the compartment from which the waste is directly cleared, and the compartment into which the waste is directly cleared. Protein waste can be cleared from the intracellular compartment, or from the extracellular compartment, which comprises the ISF that surrounds neurons and the CSF that surrounds the brain. These proteins can then be removed by enzymes or cellular uptake, exported into the blood or lymph, or recirculated in the CSF (Table 1). The relative contributions of each of the various clearance systems are currently unknown; the prevailing view is that the BBB clearance predominates, though recent studies involving perivascular CSF circulation challenge this view, making clearance systems of the brain an important area for future research.

### Degradation clearance

Degradation clearance is the enzymatic breakdown of proteins in the brain, and entails both extracellular and intracellular degradation. Extracellular degradation of ISF proteins mainly consists of degradation by proteases expressed and secreted by cells such as astrocytes.<sup>56,57</sup> ISF proteins can also be taken up from the extracellular space to be degraded intracellularly in neurons or glia, including phagocytic microglia and astrocytes.<sup>58–60</sup> Intracellular degradation of proteins occurs via the ubiquitin–proteasome pathway, the autophagy–lysosome pathway, and the endosome–lysosome pathway.<sup>56</sup>

### Blood–brain barrier clearance

Interstitial proteins can be cleared into the blood directly at the BBB through specialized transport systems located in the brain endothelium.<sup>61–64</sup> The BBB endothelial cells are connected by tight junctions and have two functionally distinct sides: the luminal side facing the blood circulation, and the abluminal side facing the brain parenchyma.<sup>65</sup> In addition to the BBB, the brain is also protected by the so-called ‘glial barrier’ (also known as the glia limitans) that surrounds the BBB and consists of astroglial endfeet processes that cover the majority of the parenchymal vasculature, with the remaining area consisting of intercellular astrocytic endfeet clefts forming gap junctions.<sup>66</sup> The BBB and glial barrier are part of the neurovascular unit, which comprises various components, including cerebral microvascular endothelium, basement membrane, contractile pericytes (which share the capillary basement membrane with the endothelium), smooth muscle cells (which invest the endothelium of precapillary arterioles), astroglia, and neurons.<sup>67</sup>

Transport at the neurovascular unit across the glial barrier and BBB depends on the solubility, molecular weight and diameter of the protein.<sup>68,69</sup> The relatively large size of the intercellular clefts (20 nm)<sup>70,71</sup> implies that the glial barrier is permeable to nearly all proteins.<sup>66</sup> Given the size of AD-related proteins, monomeric A $\beta$ <sub>1–40</sub>, A $\beta$ <sub>1–42</sub> and tau, should be able to pass freely through astrocytic endfeet clefts at the glial barrier.<sup>72</sup> However, endothelial tight junctions at the BBB prevent free passage of A $\beta$  and tau into the blood, so they must instead be transported across the endothelium by specialized transporters, which, as described below, have been identified for A $\beta$ , but not for tau.

## Interstitial fluid bulk-flow clearance

ISF proteins can also be cleared directly into the CSF via ISF bulk flow<sup>73</sup> that enters the CSF sink (described below) or the perivascular space (sometimes referred to the paravascular space—we use the term ‘perivascular space’ to describe the region surrounding the parenchymal vasculature).

**Cerebrospinal fluid sink clearance**—In parts of the body other than the CNS, lymphatic vessels run in parallel with the circulatory system to clear waste from the ISF in the form of lymph. Lymphatic vessels have recently been described in the meninges surrounding the mouse brain,<sup>18</sup> but the brain parenchyma itself is devoid of such vessels, leading to the long-held assumption that CSF serves as a ‘lymph equivalent’ to clear waste from the CNS.<sup>74,75</sup> Apart from transport across the endothelium, the removal of ISF from the brain parenchyma was traditionally believed to occur by diffusion<sup>76</sup> or ISF bulk flow into the CSF sink, which comprises the ventricles and subarachnoid space.<sup>77</sup> Given that diffusion is dependent on molecular size, diffusion into the CSF sink has been proposed to be too slow for the highly metabolic and large human brain.<sup>77</sup> As such, ISF bulk flow, which is independent of molecular size, has been proposed as the predominant pathway for movement of large molecules into the CSF sink.<sup>77</sup> ISF bulk flow was initially thought to course through the brain in a diffuse manner, but later evidence (described below) suggests the existence of definite pathways.<sup>78</sup>

**Perivascular clearance**—Support for an anatomically specific bulk-flow system came from a study of perivascular circulation.<sup>79</sup> Following infusion of horseradish peroxidase into the lateral ventricles or subarachnoid space of anaesthetized cats and dogs, CSF within the subarachnoid space flowed freely through the Virchow–Robin space—a histologically defined space where the subarachnoid space meets the perivascular space. From the Virchow–Robin space, CSF travelled into the periarterial spaces that surround penetrating arteries, moving along specific pathways in the same direction as blood flow.<sup>79</sup> CSF was also shown to move from the perivascular space into the interstitial ISF.<sup>79</sup> This perivascular circulation hypothesis challenged the traditional model of one-way flow of ISF into the CSF sink.<sup>77,80,81</sup>

Mouse model<sup>82</sup> and human<sup>83</sup> studies involving fluorescent soluble tracers and confocal microscopy have demonstrated that following intracerebral injection, ISF solutes diffuse and enter perivascular drainage pathways along the basement membrane of capillary and arterial walls separating smooth muscle cells,<sup>84</sup> then move towards the leptomeningeal arteries at the surface of the brain and, ultimately, to cervical lymph nodes (Figure 1).<sup>84</sup> This pathway was named the perivascular drainage pathway, and was deemed to be the lymphatic drainage of the brain.<sup>44</sup>

The perivascular circulation hypothesis<sup>79</sup> was recently confirmed and expanded on by a study in mice.<sup>16</sup> Following tracer injection into the CSF at the cisterna magna, two-photon microscopy was used to visualize in real time the flux of CSF in living mice through a closed cranial window.<sup>16</sup> As already hypothesized decades ago,<sup>74</sup> CSF was found to act like lymph: it flushed out interstitial substances in a process facilitated by glial cells, prompting

the authors to name it the glymphatic system (Figure 1).<sup>16</sup> This evidence corroborated prior findings that CSF, driven by arterial pulsation, flows into the periarterial space, following the course of the arterial vascular smooth muscle basement membrane to reach the basal lamina of the brain capillary bed, and entering the interstitium at all levels of this perivascular route.<sup>79</sup> The work also confirmed that ISF moves by bulk flow.<sup>77</sup> Moreover, the study extended these findings to include a role for astroglial AQP4 channels. These channels were found to mediate CSF transport from the periarterial space across the pial–glial membrane into the interstitium, where it mixes with ISF. The pia has been shown to be relatively permeable:<sup>16,85</sup> tracers injected into the subarachnoid space rapidly enter the perivascular space and brain parenchyma.<sup>16</sup> CSF–ISF movement from the interstitium into the perivenous space of deep draining veins runs ventromedially towards ventricular and deep white matter structures.<sup>85</sup> Hydrostatic pressure of periarterial bulk flow has been speculated to drive CSF water through the AQP4 channels, which is followed by astrocytic passage of molecules both through clefts and across astrocytes to maintain osmotic balance, although the mechanism has not been fully elucidated.<sup>16</sup> Any remaining CSF components course along the capillary basal lamina.<sup>87,79</sup>

It is unclear whether the perivascular drainage pathway and the glymphatic pathway are in fact distinct pathways, or whether they simply reflect transport along the same pathway captured under differing physiological or experimental conditions. In another study using two-photon imaging, fluorescent tracers were injected directly into the mouse brain via an open skull. Periarterial tracer accumulation was observed, although the direction of the flow was not discerned.<sup>88</sup> Opening of the skull is suggested to be a confounding factor in many experiments involving circulation of CSF, because skull removal can lead to inflammation and mechanical injuries to the cortical surface, or disturb local blood perfusion, BBB permeability and brain homeostasis.<sup>89–92</sup> Regardless, as recently suggested, the perivascular drainage and glymphatic pathways are not mutually exclusive: both could be active depending on the conditions, and the pathway in use could even be different between vessels or within the same vessel at different times (Figure 1).<sup>86</sup>

### Cerebrospinal fluid absorption clearance

Following clearance from the ISF into the CSF, proteins must be cleared from the brain. Circulating CSF can be absorbed directly into the circulatory or lymphatic systems.

**Circulatory clearance**—Although other CSF production sites have also been suggested,<sup>93–95</sup> the majority of CSF seems to be produced at the BCSFB by the choroid plexus, a vascular unit of capillaries comprised of fenestrated endothelium and covered by choroid plexus epithelium (modified ependymal cells with tight junctions), located in the ventricles. The BCSFB serves not only as a CSF production site, but also as a ventricular CSF solute clearance site.<sup>96,97</sup> According to the traditional view, following CSF production by the choroid plexus, CSF circulates within the subarachnoid space, from where it is primarily cleared from the brain at arachnoid villi (also known as arachnoid granulations)—one-way valve structures leading to the dural venous sinuses.<sup>98</sup>

**Lymphatic clearance**—As described above, circulating CSF within the perivascular space can be cleared from the brain to cervical lymph nodes.<sup>84,16</sup> Another clearance route for circulating CSF to cervical lymph nodes is along perineural spaces, extensions of the subarachnoid space surrounding nerves.<sup>99,100</sup> Furthermore, the recent development of a method for mounting of whole meninges, such that mouse meninges can be examined intact on a single slide, led to the discovery of meningeal lymphatic vessels, which might provide another clearance route for circulating CSF proteins.<sup>18</sup> These meningeal lymphatic vessels might also provide a more conventional path for immune cells to exit the CNS, and dysfunction of these vessels might have important implications for neurological disorders associated with altered immune responses.<sup>18</sup>

## Clearance of amyloid- $\beta$

### Amyloid- $\beta$ aggregation

A $\beta$  is produced during neuronal activity<sup>101</sup> from amyloid precursor protein (APP), a membrane protein that acts as a signalling receptor.<sup>101,102</sup> In nonpathological conditions, APP is cleaved by  $\alpha$ -secretase, which precludes formation of A $\beta$ , and the resulting carboxy-terminal fragment is then cleaved by  $\gamma$ -secretase.<sup>103</sup> The resulting products do not aggregate.<sup>104</sup>

If APP is first cleaved by  $\beta$ -secretase 1 (also known as BACE1) instead of  $\alpha$ -secretase, the subsequent  $\gamma$ -secretase cleavage will result in soluble monomeric A $\beta$ . The most common soluble monomeric isoforms of A $\beta$  are A $\beta_{1-40}$  (<80%), A $\beta_{1-38}$  (<20%) and A $\beta_{1-42}$  (10%).<sup>105</sup> A $\beta_{1-40}$  is prone to be deposited in the vasculature, as seen in CAA.<sup>15,106</sup> A $\beta_{1-38}$  is less likely to aggregate in either the vasculature or the brain than the other isoforms.<sup>107,108</sup> A $\beta_{1-42}$  has two additional amino acids, making it more hydrophobic than A $\beta_{1-40}$ ;<sup>109</sup> thus, it is capable of forming insoluble aggregates.<sup>110</sup> The tendency of A $\beta_{1-42}$  to form hard-to-clear aggregates is particularly increased when the concentration of A $\beta_{1-42}$  is high, and at a lower pH.<sup>111-114</sup>

The different forms of A $\beta$  are in a dynamic equilibrium, and dense amyloid plaques can slough off soluble monomeric A $\beta_{1-42}$ , which can then reform into aggregates.<sup>115</sup> Reflecting the equilibrium, in a longitudinal study of individuals carrying EOAD-linked presenilin mutation, CSF A $\beta$  levels were initially reduced owing to aggregation of A $\beta_{1-42}$  into plaques.<sup>10</sup> Impaired clearance can, thus, result from A $\beta$  aggregation—especially aggregation of A $\beta_{1-42}$ —rather than from an intrinsic defect in the clearance system. Nonetheless, A $\beta$  clearance systems can also become dysfunctional, as discussed below (Figure 2 and Table 2).

### Degradation clearance

Intracellular A $\beta$  (iA $\beta$ ) can be degraded by proteasomes via the ubiquitin–proteasome pathway in neurons,<sup>116</sup> lysosomal cathepsin enzymes,<sup>117</sup> proteases (such as insulin-degrading enzyme, a thiol metalloendopeptidase that degrades monomeric A $\beta$ ) and insulin.<sup>118</sup> Extracellular A $\beta$  can also be degraded by proteases, such as neprilysin (a membrane-anchored zinc metalloendopeptidase that degrades the A $\beta$  monomers A $\beta_{1-40}$  and A $\beta_{1-42}$ , and A $\beta$  oligomers),<sup>119</sup> matrix metalloproteinases 2, 3 and 9,<sup>120</sup> glutamate

carboxypeptidase II,<sup>121</sup> endothelin-converting enzyme,<sup>122</sup> tissue plasminogen activator,<sup>123</sup> plasmin,<sup>120</sup> angiotensin-converting enzyme,<sup>120</sup> and insulin-degrading enzyme.<sup>124</sup> In addition, eA $\beta$  can be degraded following glial phagocytosis. Specifically, ISF A $\beta$  can be taken up by microglia and astrocytes, whereas perivascular A $\beta$  can be degraded by vascular smooth muscle cells, perivascular macrophages, and astrocytes (Figure 2).<sup>125</sup>

Degradation clearance of A $\beta$  is affected by four main factors: enzyme expression and activity, ligand affinity and competition, activation of cellular uptake, and initiation of intracellular degradation pathways (Table 1), all of which become impaired with ageing and in AD. First, expression of neprilysin is decreased in AD,<sup>126</sup> especially in regions with high A $\beta$  loads such as the hippocampus and temporal gyrus.<sup>127</sup> Although overall matrix metalloproteinase 2 expression is increased in AD,<sup>58</sup> its activity is reduced in astrocytes that surround A $\beta$  plaques.<sup>128</sup> Second, both A $\beta$  and insulin are ligands that compete for degradation by insulin-degrading enzyme; thus, hyper-insulinaemia can reduce clearance of A $\beta$ , which might partly explain the link between type 2 diabetes mellitus and AD.<sup>13</sup> Third, plaques activate the immune effectors of the CNS—microglia and astrocytes<sup>129</sup>—inducing both phagocytosis of A $\beta$ , which facilitates clearance from the extracellular space, and production of neurotoxic inflammatory cytokines.<sup>130</sup> A $\beta$  that has undergone cellular uptake can then be degraded, for example via the autophagy–lysosome pathway<sup>131</sup> or be released back into the extracellular space,<sup>130</sup> as found in the brains of patients with AD.<sup>132,133</sup> Last, in AD, A $\beta$  degradation via the endosome–lysosome pathway is increased relative to lysosomal degradation:<sup>134</sup> endocytic activity is elevated, resulting in accumulation of autophagic vacuoles, presence of lysosomal cathepsin enzymes in A $\beta$  plaques, and abnormally enlarged endosomes containing A $\beta$ , leading to generalized proteasome dysfunction.<sup>134,135</sup>

### Blood-brain barrier clearance

**Mechanisms of amyloid- $\beta$  influx and efflux**—A $\beta$  is transported from the interstitial space across the BBB and into blood, and vice versa (Figure 3).<sup>123</sup> Specifically, local soluble A $\beta$  is transferred from the interstitium to the brain by LDL receptor (LDLR) family members such as LRP1, and ATP-binding cassette transporters (ABC transporters).<sup>14,136</sup> Some evidence suggests that LRP1 is the main transporter for A $\beta$  efflux at the BBB, whereas other studies have demonstrated its role to be quite minor.<sup>137–139</sup>

The main ABC transporter responsible for A $\beta$  efflux is ABCB1 (also known as P-glycoprotein 1 or MDR1), which directly exports A $\beta$  into the circulation. ABCA1, which is located on the abluminal side of the brain endothelium,<sup>140</sup> does not directly bind and extrude A $\beta$ ,<sup>141</sup> but mediates A $\beta$  clearance in an ApoE-dependent manner.<sup>142</sup> The precise mechanism by which abluminal ABCA1 mediates A $\beta$  clearance is unknown, although this transporter has been proposed to induce ApoE lipidation, which facilitates ApoE–A $\beta$  interaction in the perivascular space, making A $\beta$  more accessible to transport by LRP1 or ABCB1.<sup>143</sup> Clearance of A $\beta$  through the BBB is also mediated by  $\alpha$ 2-macroglobulin ( $\alpha$ 2M),<sup>14</sup> and LDLR-related protein 2 (LRP2, also known as megalin) when LRP2 forms a complex with clusterin (also known as ApoJ).<sup>14,136</sup> In addition, insulin-degrading enzyme has been



proposed to have a role in A $\beta$  clearance through the BBB, which might explain why BBB clearance is sensitive to insulin.<sup>144</sup>

Free A $\beta$  can be transported from the circulation into the interstitium via RAGE (advanced glycosylation end product-specific receptor).<sup>136,145</sup> Soluble transporters (also known as sequestering agents)—such as the soluble form of RAGE (sRAGE),<sup>14</sup> anti-A $\beta$  IgG,<sup>14</sup> serum amyloid P component (SAP),<sup>14</sup> and the soluble form of LRP (sLRP), which binds 70–90% of plasma A $\beta$ —bind to soluble A $\beta$  and inhibit its binding to RAGE, thereby preventing A $\beta$  from entering the interstitium.<sup>146</sup>

**Factors impairing amyloid- $\beta$  clearance in AD**—Clearance of A $\beta$  through the BBB is affected by transporter expression and activity, ligand affinity and competition, and vascular integrity (Table 1). In AD, these factors are impaired in a number of ways. First, expression of the blood efflux transporters LRP1<sup>123</sup> and ABCB1<sup>147</sup> is decreased, whereas expression of the blood influx transporter RAGE is upregulated.<sup>123</sup>

Second, oxidative changes in AD are linked to changes in sLRP that reduce its affinity for A $\beta$ , potentially facilitating A $\beta$  influx into the interstitium by RAGE.<sup>123</sup> Inflammation, a common feature of AD, can affect ligand affinity by making the pH more acidic, which promotes hyperphosphorylation of tau and induces conformational changes in A $\beta$  that hinder its clearance.<sup>148,149</sup> ApoE is a cholesterol transporter that competes with A $\beta$  for efflux by LRP1 from the interstitium into the circulation;<sup>150</sup> competition for shared receptors is the primary mechanism by which ApoE mediates A $\beta$  clearance.<sup>151</sup> The strongest genetic risk factor for AD is *APOE\** $\epsilon$ 4<sup>152</sup> (*APOE\** $\epsilon$ 4>*APOE\** $\epsilon$ 3>*APOE\** $\epsilon$ 2<sup>151</sup>), which codes for an ApoE isoform that is less efficient at mediating A $\beta$  clearance than are the other ApoE isoforms.<sup>153</sup>

Third, ApoE4 is also associated with lower antioxidant activity than other ApoE isoforms,<sup>154,155</sup> and it mediates BBB breakdown through a proinflammatory pathway involving cyclophilin A in pericytes.<sup>156</sup> These findings are in line with evidence suggesting that increased oxidative stress<sup>157</sup> and loss of vascular integrity contribute to ageing<sup>158</sup> and AD,<sup>159</sup> as demonstrated by accelerated breakdown of the BBB and the neurovascular unit.

### Interstitial fluid bulk-flow clearance

ISF bulk-flow clearance removes ISF—which contains eA $\beta$ —from the interstitium via ISF bulk flow into the CSF sink and perivascular space.<sup>16,44</sup> Here, we will discuss perivascular clearance of A $\beta$  specifically via the perivascular drainage and glymphatic pathways.

**Perivascular drainage**—A $\beta$  is cleared along perivascular drainage pathways.<sup>83</sup> In both AD<sup>44,160</sup> and CAA<sup>44</sup> (commonly associated with AD<sup>84</sup>), perivascular drainage of A $\beta$  is impaired. Known factors affecting perivascular drainage of A $\beta$  include *APOE\** $\epsilon$ 4, deposition of immune complexes, arterial age, and—possibly—arterial pulsation (Table 1). The presence of ApoE4 is associated with reduced perivascular drainage of A $\beta$ ,<sup>161</sup> which in turn is linked to deposition of immune complexes.<sup>162</sup> Perivascular drainage of A $\beta$  fails as arteries age;<sup>163</sup> this failure is associated not only with loss of homeostasis<sup>164</sup> and elevated levels of soluble A $\beta$  in the brain, but also with accumulation of A $\beta$  in arterial walls (as seen

in CAA), which increases the risk of intracerebral lobar haemorrhages.<sup>165</sup> One of the main complications following immunization against A $\beta$  is the solubilization of A $\beta$  from plaques and entrapment in perivascular drainage pathways, which worsens CAA.<sup>162,166</sup> It is possible that arterial pulsation drives perivascular drainage of ISF solutes,<sup>88,167</sup> and that morphological changes associated with age-related arteriosclerosis result in failure of perivascular drainage.<sup>168</sup> Of note, a high-fat prenatal maternal diet has recently been reported to result in a failure of A $\beta$  clearance along cerebrovascular basement membranes. This failure was exacerbated if the high-fat diet had been lifelong, suggesting a role for epigenetic changes and diet in AD pathogenesis.<sup>169,170</sup>

**Glymphatic clearance**—Recent mouse studies suggest that the AQP4-dependent glymphatic pathway is an important clearance system for driving the removal of soluble A $\beta$  from the interstitium. In mice, A $\beta$  is cleared along perivascular pathways, and A $\beta$  clearance was reduced by 55–65% in *Aqp4* knockout mice compared with wild-type mice.<sup>16,171</sup> Furthermore, glymphatic clearance was reduced by 40% in aged relative to young mice,<sup>17</sup> suggesting that the glymphatic pathway is impaired with age, which, as mentioned above, is the primary risk factor for LOAD.

Potential factors affecting glymphatic ISF bulk flow include molecular size, arterial pulsation, AQP4 expression and localization, and sleep (Table 1). Following subarachnoid injection, larger tracer molecules are slower to enter the parenchyma than are smaller tracers, and soluble perivascular A $\beta$  can cross the 20 nm astrocytic endfeet clefts.<sup>16</sup>

Arterial pulsation is critical for perivascular circulation and transport of CSF into the interstitium.<sup>79,172,173</sup> Recirculating A $\beta$ -rich CSF within the periarterial space, might be taken up by vascular smooth muscle cells, particularly in the presence of glymphatic stasis (caused by reduced arterial pulsation) that could facilitate protein misfolding and aggregation.<sup>81,174–176</sup> This is one mechanism by which A $\beta$  might accumulate in the periarterial space, as seen in CAA, and the resulting A $\beta$  accumulation might block perivascular pathways, further reducing glymphatic clearance.<sup>87</sup>

In *Aqp4* knockout mice, interstitial clearance is reduced by about 70%, resulting in a 55–65% reduction in A $\beta$  clearance.<sup>16,171</sup> In AD, AQP4 expression could be decreased, given that in cultured mouse cortical astrocytes, interstitial A $\beta_{1-42}$  reduces AQP4 expression,<sup>177</sup> which can lead to additional accumulation of plaque-forming A $\beta_{1-42}$ .<sup>178</sup> In traumatic brain injury (TBI)—a risk factor for AD—reactive gliosis is increased.<sup>178</sup> Initially, AQP4 expression is increased in TBI, but long-lasting AQP4 mislocalization from perivascular endfeet to the astrocytic soma occurs, resulting in reduced perivascular AQP4 availability, which can reduce A $\beta$  clearance.<sup>66,171</sup> Both TBI and AD are associated with perivascular inflammation,<sup>16,66</sup> and these changes might partly explain the link between these conditions.<sup>13</sup>

In mice, A $\beta$  clearance during sleep is twice as fast as during awake periods.<sup>179</sup> This increase in A $\beta$  elimination is mediated by a 60% increase in the volume of the extracellular space, which might be modulated by a change in astrocyte cell volume in response to change in adrenergic signalling, as would be expected during sleep.<sup>179–182</sup> This expansion of the

extracellular space was caused by sleep itself rather than circadian rhythms, as it not only occurred during normal sleep, but could also be induced with anaesthesia.<sup>17,179</sup> It should be noted, however, that circadian rhythm disturbances have been reported in patients with AD,<sup>183</sup> and might affect clearance through a different mechanism involving increased oxidative stress caused by decreased expression of circadian clock genes, which are involved in protection from oxidative damage.<sup>184,185</sup>

The recent study describing the glymphatic system demonstrated that accelerated ISF-to-CSF bulk flow was partly responsible for the increase in total A $\beta$  clearance during sleep, representing about 40% of total clearance, which can be calculated from the clearance rate constant data.<sup>179</sup> The remaining 60% is probably attributable to accelerated BBB transport of A $\beta$ , because during these transport clearance measurements, the degradation of AB was minimal, which is in line with previous reports.<sup>178,179</sup> This finding might result from the glymphatic system flushing A $\beta$  toward the BBB during sleep. Thus, sleep could indirectly increase BBB clearance of A $\beta$  through increased glymphatic bulk flow, but it might also directly increase clearance through the BBB via various mechanisms, such as molecular changes (for example, upregulated LRP1), as seen with AD-protective physical and cognitive activity in mice.<sup>187–189</sup> These findings might partly explain why sleep impairment increases the risk of AD.<sup>33,35</sup>

### Cerebrospinal fluid absorption clearance

A $\beta$  in the circulating CSF can be absorbed either through the arachnoid villi<sup>196</sup> and BCSFB<sup>136</sup> into the circulation, or through the perivascular<sup>16,44</sup> and perineural spaces<sup>191</sup>—and possibly the meningeal lymphatics<sup>18</sup>—into the lymphatic system.

CSF absorption clearance of A $\beta$  by the circulatory and lymphatic systems depends on CSF production, BCSFB integrity and transporters, arachnoid villi resistance, and lymphatic absorption of the CSF (Table 1). In ageing and AD, these factors are impaired in a number of ways. First, in ageing, and particularly in AD, CSF production by the choroid plexus is reduced, as shown by decreased water secretion into the ventricles via AQP1 water channels.<sup>192</sup> In AD, the choroid plexus undergoes many structural changes, such as calcification, fibrosis and A $\beta$  deposition, all of which can obstruct CSF production.<sup>193</sup>

Second, these structural changes affect BCSFB integrity, thereby reducing A $\beta$  clearance. Many of the A $\beta$  transporters expressed at the BBB, including LRP1, LRP2, ABCB1 and RAGE, are also found at the BCSFB.<sup>194</sup> LRP1 is likely to have an important role in ventricular A $\beta$  clearance at the BCSFB, given that the overall clearance rate of A $\beta$  from the CSF is fivefold faster than the rate observed via CSF flow through the arachnoid villi.<sup>195</sup> However, the age-related change in expression of many BCSFB transporters follows an opposite pattern to that observed at the BBB for A $\beta$ , such that there is increased efflux and decreased influx transporter expression, which is suggested to be a result of the BCSFB compensating for age-dependent BBB transporter defects.<sup>136</sup>

Third, CSF outflow resistance at the arachnoid villi is increased in AD.<sup>196</sup> This increased resistance is mechanistically similar to normal pressure hydrocephalus,<sup>196</sup> and has been proposed to result from amyloid deposition and fibrosis at the arachnoid villi,<sup>197</sup> resulting in

decreased CSF bulk outflow and, thus, decreased CSF A $\beta$  absorption into the blood. Although no evidence has yet been obtained that CSF A $\beta$  levels initially increase in LOAD, reduced CSF turnover would be expected to result in heavily A $\beta$ -laden recirculating CSF, subsequently resulting in reduced concentration as A $\beta$  is deposited in plaques,<sup>111</sup> in the vasculature as CAA,<sup>175</sup> and in the meninges, thereby increasing outflow resistance at the arachnoid villi.<sup>98</sup>

Last, lymphatic absorption of CSF decreases with age<sup>98</sup>—the primary risk factor for LOAD. In EOAD, by contrast, overproduction of A $\beta$  might result in increased absorption of A $\beta$  by the lymphatic system, as demonstrated in a transgenic mouse model of AD, in which increasing A $\beta$  levels in cervical and axillary lymph nodes mirrored increased A $\beta$  levels in the brain.<sup>198</sup>

## Clearance of tau

Tau—a splicing variant of the microtubule-associated protein tau (MAPT)—is an intracellular neuronal protein that stabilizes axons.<sup>199</sup> Intracellular tau (i-tau) can undergo two transformations that are relevant to its clearance: modification and release. Tau modification is regulated by phosphorylation. i-Tau can undergo nondegradative cleavage by proteolytic enzymes, such as aminopeptidases, thrombin, HTRA1, calpain and caspases.<sup>135</sup> Rather than degrading tau, these enzymes produce proteolytic fragments, which can ultimately be degraded; however, these fragments have an increased propensity to form aggregates, resulting in reduced clearance. In AD, i-tau is hyperphosphorylated, which induces the formation of insoluble NFTs that cannot readily be cleared, and can also be neurotoxic.<sup>200</sup> Neuronal activation (namely, presynaptic glutamate release),<sup>201</sup> neuronal death and increased i-tau concentration or aggregation<sup>202</sup> trigger the release of i-tau into the extracellular space, leading to elevated CSF tau levels.

Tau clearance is less well understood than A $\beta$  clearance, but also seems to be less complex. Transporters that specifically transport tau through the BBB have not been identified, which suggests that tau does not undergo clearance through the BBB, except after brain injury, when BBB permeability is temporarily increased.<sup>203</sup> Instead, tau is thought to be cleared from the brain primarily by degradation, ISF bulk flow, and CSF absorption clearance (Table 2). Recent studies using passive immunization with anti-tau oligomer antibodies have shown that like A $\beta$ , pathological tau can be cleared from the brain by a peripheral sink mechanism, indicating that enhancement of tau clearance might be a therapeutic strategy in AD.<sup>204</sup>

## Degradation clearance

Tau is mainly cleared through intracellular degradation by lysosomes via the autophagy–lysosome pathway, and by proteasomes via the ubiquitin–proteasome pathway.<sup>202</sup> AD-related dysfunction of these pathways has been suggested to result in the accumulation of soluble i-tau.<sup>135</sup> i-Tau can also be degraded by proteases (such as caspases) in response to apoptosis-inducing stressors, and by calpain in response to elevated intracellular calcium concentrations. Phosphorylation of tau by protein kinase A increases its resistance to degradation by calpain; thus, AD-associated hyperphosphorylation of tau has been suggested

to impair tau turnover and result in tau accumulation in the form of NFTs.<sup>205</sup> Following release of i-tau into the extracellular space—a process that could result from neuronal death or stimulation<sup>202</sup>—e-tau can be internalized by other neurons via endocytosis, leading to prion-like spreading of tau pathology.<sup>206</sup> In addition, soluble e-tau might bind to muscarinic type 1 and type 3 receptors, thereby increasing intracellular calcium levels, which might facilitate further release of i-tau.<sup>202</sup> Tau released into the extracellular space is highly stable:<sup>207</sup> its CSF half-life is 12–14 h,<sup>208</sup> compared with about 2 h for A $\beta$ .<sup>209</sup>

### Interstitial fluid bulk-flow clearance

If e-tau is not cleared by endocytosis, it might be cleared via the glymphatic system.<sup>210</sup> Following TBI, glymphatic clearance of ISF solutes was impaired by about 60% in wild-type mice, and to an even greater extent in *Aqp4* knockout mice that displayed NFTs, neuroinflammatory reactive gliosis, and neurodegeneration.<sup>210</sup> These findings support the link between TBI and tau aggregation, with resulting neurodegeneration similar to that seen in AD and chronic traumatic encephalopathy.<sup>210,211</sup> Recirculation of CSF poses an additional challenge to tau clearance: cells closest to the periarterial boundary might internalize tau from the tau-laden recirculating CSF within the periarterial space.<sup>175</sup>

### Cerebrospinal fluid absorption clearance

As for any soluble substance in circulating CSF, tau can be absorbed either into the circulatory system from the arachnoid villi and BCSFB, or from the lymphatic system through the perivascular and perineural spaces. Meningeal lymphatic vessels<sup>18</sup> provide another possible route, although their specific contribution to tau elimination has not been tested.

## Conclusions

Removal of proteins from the brain occurs via various overlapping clearance systems: enzymatic degradation and cellular uptake, transport across the BBB and BCSFB, ISF bulk flow, and absorption of CSF into the circulatory and lymphatic systems (Table 1). The majority of eA $\beta$  is cleared across the BBB, with a minority being cleared by ISF bulk flow (Figure 2 and Table 2).<sup>14,15</sup> However, the recently discovered glymphatic pathway (Figure 1) also seems to be an important contributor to eA $\beta$  clearance, because it can flush A $\beta$  towards the perivascular space, thereby mediating clearance through the BBB (Figure 3) or re-entry into the capillary basement membranes.<sup>16,17</sup> The recently discovered meningeal lymphatic vessels might provide another clearance route,<sup>18</sup> but their role in A $\beta$  and tau clearance has not yet been assessed. In contrast with A $\beta$ , tau does not seem to undergo receptor-mediated BBB clearance; thus, the elimination of tau seems less complex than that of A $\beta$ . However, the mechanisms involved in the clearance of tau from the brain are not completely understood, and further research into tau trafficking could help us better understand its role in AD.

Recent evidence for A $\beta$  accumulation in LOAD points to reduced clearance, as opposed to overproduction, as the main culprit, but which specific clearance systems are defective is unclear.<sup>10,11</sup> The evidence for clearance failure in AD comes from the use of stable isotope

labelling kinetics,<sup>212</sup> which enable measurement of both A $\beta$  production and clearance in humans. However, this technique does not provide information on the specific clearance systems themselves but, rather, provides a composite of production and clearance measures for A $\beta$ . This technological progress has introduced an urgent need for detailed information on the specific A $\beta$  clearance defects in patients with AD. Likewise, a technique to measure overall tau clearance and specific tau clearance defects in AD is needed, because such defects might be expected in light of the reduced CSF production and arachnoid villi defects that are observed in ageing individuals and patients with AD.

Because A $\beta$  deposition can be increased in presymptomatic individuals years or even decades before the hallmark symptoms of AD manifest, an understanding of A $\beta$  clearance might eventually provide strategies to restore clearance mechanisms, so as to eliminate excess A $\beta$  deposits and delay or possibly even prevent disease onset. Whether the observed clearance defect in AD is a cause or a consequence of pathology, or merely coincidental, remains unknown. Regardless, A $\beta$  clearance defects are a consistent finding in patients with AD, and might provide a useful biomarker and indicator of reversible clinical pathology.

## Acknowledgements

The authors acknowledge the following grants: NIH/NIA/NHLBI AG022374, AG13616, AG12101 and AG008051 (to M.J.d.L.), HL118624 (to R.S.O.), HL111724 (to L.G.), AG20245 and AG008051 (to T.W.), and NIH/NINDS NS028642 (to C.N.). K.B. has received funding from the Torsten Söderberg Foundation at the Royal Swedish Academy of Sciences, and H.Z. has received funding from the Swedish Research Council and the Knut and Alice Wallenberg Foundation.

## References

1. Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem. Pharmacol.* 2014; 88:640–651. [PubMed: 24398425]
2. Guerreiro R, Hardy J. Genetics of Alzheimer's disease. *Neurotherapeutics.* 2014; 11:732–737. [PubMed: 25113539]
3. Karch CM, Cruchaga C, Goate AM. Alzheimer's disease genetics: from the bench to the clinic. *Neuron.* 2014; 83:11–26. [PubMed: 24991952]
4. Bertram L, Tanzi RE. The genetics of Alzheimer's disease. *Prog. Mol. Biol. Transl. Sci.* 2012; 107:79–100. [PubMed: 22482448]
5. Kim DH, et al. Genetic markers for diagnosis and pathogenesis of Alzheimer's disease. *Gene.* 2014; 545:185–193. [PubMed: 24838203]
6. Bruni AC, Conidi ME, Bernardi L. Genetics in degenerative dementia: current status and applicability. *Alzheimer Dis. Ass. Disord.* 2014; 28:199–205. [PubMed: 24805970]
7. Selkoe DJ. Aging, amyloid, and Alzheimer's disease: a perspective in honor of Carl Cotman. *Neurochem. Res.* 2003; 28:1705–1713. [PubMed: 14584824]
8. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002; 297:353–356. [PubMed: 12130773]
9. Zlokovic BV, Yamada S, Holtzman D, Ghiso J, Frangione B. Clearance of amyloid  $\beta$ -peptide from brain: transport or metabolism? *Nat. Med.* 2000; 6:718–719.
10. Potter R, et al. Increased *in vivo* amyloid- $\beta$ 42 production, exchange, and loss in presenilin mutation carriers. *Sci. Transl. Med.* 2013; 5:189ra77.
11. Mawuenyega KG, et al. Decreased clearance of CNS  $\beta$ -amyloid in Alzheimer's disease. *Science.* 2010; 330:1774. [PubMed: 21148344]

12. Reiman EM, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol.* 2012; 11:1048–1056. [PubMed: 23137948]
13. Alzheimer's Association. 2013 Alzheimer's disease facts and figures. *Alzheimers Dement.* 2013; 9:208–245. [PubMed: 23507120]
14. Zlokovic, BV.; Frangione, B. Transport-clearance hypothesis for Alzheimer's disease and potential therapeutic implications. *Madame Curie Bioscience Database.* 2003. [online], <http://www.ncbi.nlm.nih.gov/books/NBK5975/>.
15. Shibata M, et al. Clearance of Alzheimer's amyloid- $\beta_{1-40}$  peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J. Clin. Invest.* 2000; 106:1489–1499. [PubMed: 11120756]
16. Iliff JJ, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid  $\beta$ . *Sci. Transl. Med.* 2012; 4:147ra111.
17. Kress BT, et al. Impairment of paravascular clearance pathways in the aging brain. *Ann. Neurol.* 2014; 76:845–861. [PubMed: 25204284]
18. Louveau AE, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature.* <http://dx.doi.org/10.1038/nature14432>.
19. Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. *Arch. Neurol.* 2012; 69:59–64. [PubMed: 21911656]
20. Sperling RA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011; 7:280–292. [PubMed: 21514248]
21. Bishop NA, Lu T, Yankner BA. Neural mechanisms of ageing and cognitive decline. *Nature.* 2010; 464:529–535. [PubMed: 20336135]
22. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch. Neurol.* 2003; 60:1119–1122. [PubMed: 12925369]
23. Hetzel, L. 65 Years and Over Population: 2000. Census 2000 Brief. DIANE Publishing; 2008.
24. Alzheimer's Association. 2012 Alzheimer's disease facts and figures. *Alzheimers Dement.* 2012; 8:131–168. [PubMed: 22404854]
25. Potter H, Wisniewski T. Apolipoprotein E: essential catalyst of the Alzheimer amyloid cascade. *Int. J. Alzheimers Dis.* 2012; 2012:489428. [PubMed: 22844635]
26. Raber J, Huang Y, Ashford JW. ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiol. Aging.* 2004; 25:641–650. [PubMed: 15172743]
27. Boutajangout A, Wisniewski T. The innate immune system in Alzheimer's disease. *Int. J. Cell Biol.* 2013; 2013
28. Harold D, et al. Genome-wide association study identifies variants at *CLU* and *PICALM* associated with Alzheimer's disease. *Nat. Genet.* 2009; 41:1088–1093. [PubMed: 19734902]
29. Lambert JC, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat. Genet.* 2013; 45:1452–1458. [PubMed: 24162737]
30. Di Marco LY, et al. Modifiable lifestyle factors in dementia: a systematic review of longitudinal observational cohort studies. *J. Alzheimers Dis.* 2014
31. Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. *Nat. Rev. Neurol.* 2011; 7:137–152. [PubMed: 21304480]
32. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol.* 2011; 10:819–828. [PubMed: 21775213]
33. Picchioni D, Reith RM, Nadel JL, Smith CB. Sleep, plasticity and the pathophysiology of neurodevelopmental disorders: the potential roles of protein synthesis and other cellular processes. *Brain Sci.* 2014; 4:150–201. [PubMed: 24839550]
34. Ju YE, Lucey BP, Holtzman DM. Sleep and Alzheimer disease pathology—a bidirectional relationship. *Nat. Rev. Neurol.* 2014; 10:115–119. [PubMed: 24366271]

35. Spira AP, et al. Self-reported sleep and  $\beta$ -amyloid deposition in community-dwelling older adults. *JAMA Neurol.* 2013; 70:1537–1543. [PubMed: 24145859]
36. Jack CR Jr, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 2013; 12:207–216. [PubMed: 23332364]
37. de Leon, MJ.; Bobinski, M.; Convit, A.; De Santi, S. *Neurobiology of Mental Illness.* 1st edn. Charney, DS.; Nestler, EJ., editors. Oxford University Press; 1999. p. 698-714.Ch. 5
38. Spires-Jones TL, Hyman BT. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron.* 2014; 82:756–771. [PubMed: 24853936]
39. Thal DR, et al. Pathology of clinical and preclinical Alzheimer's disease. *Eur. Arch. Psychiatry Clin. Neurosci.* 2013; 263(Suppl. 2):S137–S145. [PubMed: 24077890]
40. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann. Neurol.* 1990; 27:457–464. [PubMed: 2360787]
41. Blennow K, Bogdanovic N, Alafuzoff I, Ekman R, Davidsson P. Synaptic pathology in Alzheimer's disease: relation to severity of dementia, but not to senile plaques, neurofibrillary tangles, or the *APOE4* allele. *J. Neural Transm.* 1996; 103:603–618. [PubMed: 8811505]
42. Giacobini E, Gold G. Alzheimer disease therapy—moving from amyloid- $\beta$  to tau. *Nat. Rev. Neurol.* 2013; 9:677–686. [PubMed: 24217510]
43. Thal DR, Griffin WS, de Vos RA, Ghebremedhin E. Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. *Acta Neuropathol.* 2008; 115:599–609. [PubMed: 18369648]
44. Weller RO, Subash M, Preston SD, Mazanti I, Carare RO. Perivascular drainage of amyloid- $\beta$  peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain Pathol.* 2008; 18:253–266. [PubMed: 18363936]
45. Mosconi L, et al. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. *Neurology.* 2005; 64:1860–1867. [PubMed: 15955934]
46. Ferris SH, et al. Positron emission tomography in the study of aging and senile dementia. *Neurobiol. Aging.* 1981; 1:127–131. [PubMed: 24279935]
47. de Leon, MJ. *Alzheimer: 100 Years and Beyond Illness.* 1st edn. Jucker, M., et al., editors. Springer; 2006. p. 385-390.
48. de Leon MJ, et al. The radiologic prediction of Alzheimer disease: the atrophic hippocampal formation. *AJNR Am. J. Neuroradiol.* 1993; 14:897–906. [PubMed: 8352162]
49. Frisoni GB, Fox NC, Jack CR Jr, Scheltens P, Thompson PM. The clinical use of structural MRI in Alzheimer disease. *Nat. Rev. Neurol.* 2010; 6:67–77. [PubMed: 20139996]
50. Cohen AD, Klunk WE. Early detection of Alzheimer's disease using PiB and FDG PET. *Neurobiol. Disease.* 2014
51. Blennow K. CSF biomarkers for AD: state of the art and new developments. *Neurobiol. Aging.* 2014; 35:S3–S3. [PubMed: 24973118]
52. Harada R, et al. [ $^{18}\text{F}$ ]THK-5117 PET for assessing neurofibrillary pathology in Alzheimer's disease. *Eur. J. Nucl. Med. Mol. Imaging.* 2015; 42:1052–1061. [PubMed: 25792456]
53. Balasubramanian AB, Kawas CH, Peltz CB, Brookmeyer R, Corrada MM. Alzheimer disease pathology and longitudinal cognitive performance in the oldest-old with no dementia. *Neurology.* 2012; 79:915–921. [PubMed: 22895581]
54. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat. Rev. Neurol.* 2010; 6:131–144. [PubMed: 20157306]
55. Villemagne VL, Fodero-Tavoletti MT, Masters CL, Rowe CC. Tau imaging: early progress and future directions. *Lancet Neurol.* 2015; 14:114–124. [PubMed: 25496902]
56. Kiffin R, Bandyopadhyay U, Cuervo AM. Oxidative stress and autophagy. *Antioxid. Redox Signal.* 2006; 8:152–162. [PubMed: 16487049]
57. Yin K-J, et al. Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid- $\beta$  peptide catabolism. *J. Neurosci.* 2006; 26:10939–10948. [PubMed: 17065436]
58. Wilcock DM, et al. Microglial activation facilitates A $\beta$  plaque removal following intracranial anti-A $\beta$  antibody administration. *Neurobiol. Dis.* 2004; 15:11–20. [PubMed: 14751766]



59. Hawkes CA, McLaurin J. Selective targeting of perivascular macrophages for clearance of B-amyloid in cerebral amyloid angiopathy. *Proc. Natl Acad. Sci. USA.* 2009; 106:1261–1266. [PubMed: 19164591]
60. Guenette SY. Astrocytes: a cellular player in A $\beta$  clearance and degradation. *Trends Mol. Med.* 2003; 9:279–280. [PubMed: 12900212]
61. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* 2010; 37:13–25. [PubMed: 19664713]
62. Zlokovic BV, Begley DJ, Chain-Eliash DG. Blood-brain barrier permeability to leucine-enkephalin, D-alanine<sup>2</sup> D-leucine<sup>5</sup>-enkephalin and their N-terminal amino acid (tyrosine). *Brain Res.* 1985; 336:125–132. [PubMed: 3891014]
63. Zlokovi BV, Lipovac MN, Begley DJ, Davson H, Raki L. Transport of leucine-enkephalin across the blood-brain barrier in the perfused guinea pig brain. *J. Neurochem.* 1987; 49:310–315. [PubMed: 3585338]
64. Zlokovic BV. Cerebrovascular permeability to peptides: manipulations of transport systems at the blood-brain barrier. *Pharm. Res.* 1995; 12:1395–1406. [PubMed: 8584471]
65. Hermann DM, ElAli A. The abluminal endothelial membrane in neurovascular remodeling in health and disease. *Sci. Signal.* 2012; 5:re4. [PubMed: 22871611]
66. Thrane AS, Thrane VR, Nedergaard M. Drowning stars: reassessing the role of astrocytes in brain edema. *Trends Neurosci.* 2014; 37:620–628. [PubMed: 25236348]
67. Syková E, Nicholson C. Diffusion in brain extracellular space. *Physiol. Rev.* 2008; 88:1277–1340. [PubMed: 18923183]
68. Wong AD, et al. The blood-brain barrier: an engineering perspective. *Front. Neuroeng.* 2013; 6:7. [PubMed: 24009582]
69. Felgenhauer K. Protein filtration and secretion at human body fluid barriers. *Pflügers Arch.* 1980; 384:9–17. [PubMed: 7189872]
70. Mathiesen TM, Lehre KP, Danbolt NC, Ottersen OP. The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. *Glia.* 2010; 58:1094–1103. [PubMed: 20468051]
71. Garai K, Crick SL, Mustafi SM, Frieden C. Expression and purification of amyloid- $\beta$  peptides from *Escherichia coli*. *Protein Expr. Purif.* 2009; 66:107–112. [PubMed: 19233290]
72. Yang L, et al. Evaluating glymphatic pathway function utilizing clinically relevant intrathecal infusion of CSF tracer. *J. Transl. Med.* 2013; 11:107. [PubMed: 23635358]
73. Abbott NJ. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. *Neurochem. Int.* 2004; 45:545–552. [PubMed: 15186921]
74. Milford, H. *Studies in Intracranial Physiology and Surgery.* 1st edn. Cushing, H., editor. Oxford University Press; 1926. p. 1-50. Ch. 1
75. Loukas M, et al. The lymphatic system: a historical perspective. *Clin. Anat.* 2011; 24:807–816. [PubMed: 21544873]
76. Fenstermacher, J.; Patlak, C. *Fluid Environment of the Brain.* 1st edn. Cserr, H., editor. Academic Press; 1975. p. 201-214. Ch. 12
77. Cserr HF. Physiology of the choroid plexus. *Physiol. Rev.* 1971; 51:273–311. [PubMed: 4930496]
78. Cserr H, Cooper D, Suri P, Patlak C. Efflux of radiolabeled polyethylene glycols and albumin from rat brain. *Am. J. Physiol.* 1981; 240:F319–F328. [PubMed: 7223889]
79. Rennels ML, Gregory TF, Blaumanis OR, Fujimoto K, Grady PA. Evidence for a 'paravascular' fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. *Brain Res.* 1985; 326:47–63. [PubMed: 3971148]
80. Pullen RG, DePasquale M, Cserr HF. Bulk flow of cerebrospinal fluid into brain in response to acute hyperosmolality. *Am. J. Physiol.* 1987; 253:F538–F545. [PubMed: 3115117]
81. Szentistvanyi I, Patlak CS, Ellis RA, Cserr HF. Drainage of interstitial fluid from different regions of rat brain. *Am. J. Physiol.* 1984; 246:F835–F844. [PubMed: 6742132]

82. Carare R, et al. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. *Neuropathol. Appl. Neurobiol.* 2008; 34:131–144. [PubMed: 18208483]
83. Preston S, Steart P, Wilkinson A, Nicoll J, Weller R. Capillary and arterial cerebral amyloid angiopathy in Alzheimer's disease: defining the perivascular route for the elimination of amyloid  $\beta$  from the human brain. *Neuropathol. Appl. Neurobiol.* 2003; 29:106–117. [PubMed: 12662319]
84. Hawkes CA, Jayakody N, Johnston DA, Bechmann I, Carare RO. Failure of perivascular drainage of  $\beta$ -amyloid in cerebral amyloid angiopathy. *Brain Pathol.* 2014; 24:396–403. [PubMed: 24946077]
85. Iliff JJ, et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J. Clin. Invest.* 2013; 123:1299–1309. [PubMed: 23434588]
86. Hladky SB, Barrand MA. Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. *Fluids Barriers CNS.* 2014; 11:26. [PubMed: 25678956]
87. Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. The glymphatic system: a beginner's guide. *Neurochem. Res.* <http://dx.doi.org/10.1007/s11064-015-15816>.
88. Arbel-Ornath M, et al. Interstitial fluid drainage is impaired in ischemic stroke and Alzheimer's disease mouse models. *Acta Neuropathol.* 2013; 126:353–364. [PubMed: 23818064]
89. Nimmerjahn A. Two-photon imaging of microglia in the mouse cortex *in vivo*. *Cold Spring Harb. Protoc.* <http://dx.doi.org/10.1101/pdb.prot069294>.
90. Navari R, Wei E, Kontos H, Patterson J. Comparison of the open skull and cranial window preparations in the study of the cerebral microcirculation. *Microvasc. Res.* 1978; 16:304–315. [PubMed: 34775]
91. Bacskai BJ, et al. Imaging of amyloid- $\beta$  deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat. Med.* 2001; 7:369–372. [PubMed: 11231639]
92. Kawamura S, et al. An improved closed cranial window technique for investigation of blood-brain barrier function and cerebral vasomotor control in the rat. *Int. J. Microcirc. Clin. Exp.* 1990; 9:369–383. [PubMed: 2279856]
93. Igarashi H, Tsujita M, Kwee IL, Nakada T. Water influx into cerebrospinal fluid is primarily controlled by aquaporin-4, not by aquaporin-1:  $^{17}\text{O}$  JJVCPE MRI study in knockout mice. *Neuroreport.* 2014; 25:39–43. [PubMed: 24231830]
94. Oreškovi D, Klarica M. The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. *Brain Res. Rev.* 2010; 64:241–262. [PubMed: 20435061]
95. Bering EA Jr. Water exchange of central nervous system and cerebrospinal fluid. *J. Neurosurgery.* 1952; 9:275–287.
96. Johanson CE, et al. Multiplicity of cerebrospinal fluid functions: new challenges in health and disease. *Cerebrospinal Fluid Res.* 2008; 5:10. [PubMed: 18479516]
97. Wraith DC, Nicholson LB. The adaptive immune system in diseases of the central nervous system. *J. Clin. Invest.* 2012; 122:1172–1179. [PubMed: 22466659]
98. Pollay M. The function and structure of the cerebrospinal fluid outflow system. *Cerebrospinal Fluid Res.* 2010; 7:9. [PubMed: 20565964]
99. Bradbury M, Cserr H, Westrop R. Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. *Am. J. Physiol.* 1981; 240:F329–F336. [PubMed: 7223890]
100. Bradbury M, Westrop R. Factors influencing exit of substances from cerebrospinal fluid into deep cervical lymph of the rabbit. *J. Physiol.* 1983; 339:519–534. [PubMed: 6411905]
101. Bero AW, et al. Neuronal activity regulates the regional vulnerability to amyloid- $\beta$  deposition. *Nat. Neurosci.* 2011; 14:750–756. [PubMed: 21532579]
102. Neve RL, McPhie DL. Dysfunction of amyloid precursor protein signaling in neurons leads to DNA synthesis and apoptosis. *Biochim. Biophys. Acta.* 2007; 1772:430–437. [PubMed: 17113271]
103. Chow VW, Mattson MP, Wong PC, Gleichmann M. An overview of APP processing enzymes and products. *Neuromol. Med.* 2010; 12:1–12.
104. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet.* 2006; 368:387–403. [PubMed: 16876668]

105. Zheng L, et al. Intracellular distribution of amyloid beta peptide and its relationship to the lysosomal system. *Transl. Neurodegener.* 2012; 1:19. [PubMed: 23210724]
106. Morris AW, Carare RO, Schreiber S, Hawkes CA. The cerebrovascular basement membrane: role in the clearance of  $\beta$ -amyloid and cerebral amyloid angiopathy. *Front. Aging Neurosci.* 2014; 6:251. [PubMed: 25285078]
107. Mueller-Steiner S, et al. Anti-amyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. *Neuron.* 2006; 51:703–714. [PubMed: 16982417]
108. Moro ML, et al. *APP* mutations in the A $\beta$  coding region are associated with abundant cerebral deposition of A $\beta$ 38. *Acta Neuropathol.* 2012; 124:809–821. [PubMed: 23143229]
109. Glabe C. Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *J. Mol. Neurosci.* 2001; 17:137–145. [PubMed: 11816787]
110. Dawkins E, Small DH. Insights into the physiological function of the beta-amyloid precursor protein: beyond Alzheimer's disease. *J. Neurochem.* 2014; 129:756–769. [PubMed: 24517464]
111. Jarrett JT, Berger EP, Lansbury PT Jr. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry.* 1993; 32:4693–4697. [PubMed: 8490014]
112. Braak H, Zetterberg H, Del Tredici K, Blennow K. Intraneuronal tau aggregation precedes diffuse plaque deposition, but amyloid- $\beta$  changes occur before increases of tau in cerebrospinal fluid. *Acta Neuropathol.* 2013; 126:631–641. [PubMed: 23756600]
113. Grimm MO, et al. Neprilysin and A $\beta$  clearance: impact of the APP intracellular domain in NEP regulation and implications in Alzheimer's disease. *Front. Aging Neurosci.* 2013; 5:98. [PubMed: 24391587]
114. Lührs T, et al. 3D structure of Alzheimer's amyloid- $\beta$ (1-42) fibrils. *Proc. Natl. Acad. Sci. USA.* 2005; 102:17342–17347. [PubMed: 16293696]
115. Schnabel J. Amyloid: little proteins, big clues. *Nature.* 2011; 475:S12–S14. [PubMed: 21760575]
116. Perez FP, et al. Late-onset Alzheimer's disease, heating up and foxed by several proteins: pathomolecular effects of the aging process. *J. Alzheimers Dis.* 2014; 40:1–17. [PubMed: 24326519]
117. Zhang L, Sheng R, Qin Z. The lysosome and neurodegenerative diseases. *Acta Biochim. Biophys. Sin. (Shanghai).* 2009; 41:437–445. [PubMed: 19499146]
118. Querfurth HW, LaFerla FM. Mechanisms of disease. *N. Engl. J. Med.* 2010; 362:329–344. [PubMed: 20107219]
119. Iwata N, et al. Metabolic regulation of brain A $\beta$  by neprilysin. *Science.* 2001; 292:1550–1552. [PubMed: 11375493]
120. Miners JS, et al. A $\beta$ -degrading enzymes in Alzheimer's disease. *Brain Pathol.* 2008; 18:240–252. [PubMed: 18363935]
121. Nalivaeva NN, Beckett C, Belyaev ND, Turner AJ. Are amyloid-degrading enzymes viable therapeutic targets in Alzheimer's disease? *J. Neurochem.* 2012; 120:167–185. [PubMed: 22122230]
122. Eckman EA, Reed DK, Eckman CB. Degradation of the Alzheimer's amyloid  $\beta$  peptide by endothelin-converting enzyme. *J. Biol. Chem.* 2001; 276:24540–24548. [PubMed: 11337485]
123. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat. Rev. Neurosci.* 2011; 12:723–738. [PubMed: 22048062]
124. Qiu WQ, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid  $\beta$ -protein by degradation. *J. Biol. Chem.* 1998; 273:32730–32738. [PubMed: 9830016]
125. Yoon SS, Jo SA. Mechanisms of amyloid- $\beta$  peptide clearance: potential therapeutic targets for Alzheimer's disease. *Biomol. Ther. (Seoul).* 2012; 20:245–255. [PubMed: 24130920]
126. Wang DS, Iwata N, Hama E, Saido TC, Dickson DW. Oxidized neprilysin in aging and Alzheimer's disease brains. *Biochem. Biophys. Res. Commun.* 2003; 310:236–241. [PubMed: 14511676]
127. Yasojima K, McGeer E, McGeer P. Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Res.* 2001; 919:115–121. [PubMed: 11689168]

128. Lim NK, et al. Investigation of matrix metalloproteinases, MMP-2 and MMP-9, in plasma reveals a decrease of MMP-2 in Alzheimer's disease. *J. Alzheimers Dis.* 2011; 26:779–786. [PubMed: 21694463]
129. Morrone CD, Liu M, Black SE, McLaurin J. Interaction between therapeutic interventions for Alzheimer's disease and physiological A $\beta$  clearance mechanisms. *Front. Aging Neurosci.* 2015; 7
130. Lucin KM, Wyss-Coray T. Immune activation in brain aging and neurodegeneration: too much or too little? *Neuron.* 2009; 64:110–122. [PubMed: 19840553]
131. Cho MH, et al. Autophagy in microglia degrades extracellular beta-amyloid fibrils and regulates the NLRP3 inflammasome. *Autophagy.* 2014; 10:1761–1775. [PubMed: 25126727]
132. Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY. Astrocytes accumulate A $\beta$ 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. *Brain Res.* 2003; 971:197–209. [PubMed: 12706236]
133. Lee CD, Landreth GE. The role of microglia in amyloid clearance from the AD brain. *J. Neural Transm.* 2010; 117:949–960. [PubMed: 20552234]
134. Nixon RA, Cataldo AM. Lysosomal system pathways: genes to neurodegeneration in Alzheimer's disease. *J. Alzheimers Dis.* 2006; 9:277–289. [PubMed: 16914867]
135. Chesser AS, Pritchard SM, Johnson GV. Tau clearance mechanisms and their possible role in the pathogenesis of Alzheimer disease. *Front. Neurol.* 2013; 4:122. [PubMed: 24027553]
136. Pascale CL, et al. Amyloid-beta transporter expression at the blood–CSF barrier is age-dependent. *Fluids Barriers CNS.* 2011; 8:21. [PubMed: 21740544]
137. Ito S, Ohtsuki S, Terasaki T. Functional characterization of the brain-to-blood efflux clearance of human amyloid- $\beta$  peptide (1–40) across the rat blood–brain barrier. *Neurosci. Res.* 2006; 56:246–252. [PubMed: 16926058]
138. Ito S, Ohtsuki S, Kamiie J, Nezu Y, Terasaki T. Cerebral clearance of human amyloid- $\beta$  peptide (1–40) across the blood-brain barrier is reduced by self-aggregation and formation of low-density lipoprotein receptor-related protein-1 ligand complexes. *J. Neurochem.* 2007; 103:2482–2490. [PubMed: 17908238]
139. Ito S, Ueno T, Ohtsuki S, Terasaki T. Lack of brain-to-blood efflux transport activity of low-density lipoprotein receptor-related protein-1 (LRP-1) for amyloid- $\beta$  peptide(1–40) in mouse: involvement of an LRP-1-independent pathway. *J. Neurochem.* 2010; 113:1356–1363. [PubMed: 20367755]
140. Panzenboeck U, et al. ABCA1 and scavenger receptor class B, type I, are modulators of reverse sterol transport at an *in vitro* blood–brain barrier constituted of porcine brain capillary endothelial cells. *J. Biol. Chem.* 2002; 277:42781–42789. [PubMed: 12202492]
141. Akanuma S, et al. ATP-binding cassette transporter A1 (ABCA1) deficiency does not attenuate the brain-to-blood efflux transport of human amyloid- $\beta$  peptide (1–40) at the blood–brain barrier. *Neurochem. Int.* 2008; 52:956–961. [PubMed: 18201804]
142. Fitz NF, et al. Abca1 deficiency affects Alzheimer's disease-like phenotype in human ApoE4 but not in ApoE3-targeted replacement mice. *J. Neurosci.* 2012; 32:13125–13136. [PubMed: 22993429]
143. ElAli A, Rivest S. The role of ABCB1 and ABCA1 in beta-amyloid clearance at the neurovascular unit in Alzheimer's disease. *Front. Physiol.* 2013; 4:45. [PubMed: 23494712]
144. Ito S, et al. Involvement of insulin-degrading enzyme in insulin- and atrial natriuretic peptide-sensitive internalization of amyloid-beta peptide in mouse brain capillary endothelial cells. *J. Alzheimers Dis.* 2014; 38:185–200. [PubMed: 23948938]
145. Deane R, et al. A multimodal RAGE-specific inhibitor reduces amyloid  $\beta$ -mediated brain disorder in a mouse model of Alzheimer disease. *J. Clin. Invest.* 2012; 122:1377. [PubMed: 22406537]
146. Zlokovic BV, Deane R, Sagare AP, Bell RD, Winkler EA. Low-density lipoprotein receptor related protein-1: a serial clearance homeostatic mechanism controlling Alzheimer's amyloid beta-peptide elimination from the brain. *J. Neurochem.* 2010; 115:1077–1089. [PubMed: 20854368]
147. Pahnke J, Langer O, Krohn M. Alzheimer's and ABC transporters—new opportunities for diagnostics and treatment. *Neurobiol. Dis.* 2014; 72:A54–A60.

148. Cai ZY, Yan LJ, Ratka A. Telomere shortening and Alzheimer's disease. *Neuromol. Med.* 2013; 15:25–48.
149. Krstic D, Knuesel I. Deciphering the mechanism underlying late-onset Alzheimer disease. *Nat. Rev. Neurol.* 2013; 9:25–34. [PubMed: 23183882]
150. Kanekiyo T, Xu H, Bu G. ApoE and A $\beta$  in Alzheimer's disease: accidental encounters or partners? *Neuron.* 2014; 81:740–754. [PubMed: 24559670]
151. Verghese PB, et al. ApoE influences amyloid- $\beta$  (A $\beta$ ) clearance despite minimal apoE/A $\beta$  association in physiological conditions. *Proc. Natl Acad. Sci. USA.* 2013; 110:E1807–E1816. [PubMed: 23620513]
152. Liu C-C, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat. Rev. Neurol.* 2013; 9:106–118. [PubMed: 23296339]
153. Wildsmith KR, Holley M, Savage JC, Skerrett R, Landreth GE. Evidence for impaired amyloid beta clearance in Alzheimer's disease. *Alzheimers Res. Ther.* 2013; 5:33. [PubMed: 23849219]
154. Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and  $\beta$ -amyloid peptides. *Nat. Genet.* 1996; 14:55–61. [PubMed: 8782820]
155. Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. *Biochim. Biophys. Acta.* 2000; 1502:139–144. [PubMed: 10899439]
156. Bell RD, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature.* 2012; 485:512–516. [PubMed: 22622580]
157. Li M, Chen L, Lee DH, Yu L-C, Zhang Y. The role of intracellular amyloid  $\beta$  in Alzheimer's disease. *Prog. Neurobiol.* 2007; 83:131–139. [PubMed: 17889422]
158. Winkler EA, Bell RD, Zlokovic BV. Central nervous system pericytes in health and disease. *Nat Neurosci.* 2011; 14:1398–1405. [PubMed: 22030551]
159. Sagare AP, Bell RD, Zlokovic BV. Neurovascular defects and faulty amyloid- $\beta$  vascular clearance in Alzheimer's disease. *J. Alzheimers Dis.* 2013; 33:S87–S100. [PubMed: 22751174]
160. Weller RO, et al. Cerebral amyloid angiopathy: amyloid  $\beta$  accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am. J. Pathol.* 1998; 153:725–733. [PubMed: 9736023]
161. Hawkes CA, et al. Disruption of arterial perivascular drainage of amyloid- $\beta$  from the brains of mice expressing the human APOE  $\epsilon$ 4 allele. *PLoS ONE.* 2012; 7:e41636. [PubMed: 22848551]
162. Carare RO, et al. Immune complex formation impairs the elimination of solutes from the brain: implications for immunotherapy in Alzheimer's disease. *Acta Neuropathol. Commun.* 2013; 1:48. [PubMed: 24252464]
163. Hawkes CA, et al. Perivascular drainage of solutes is impaired in the ageing mouse brain and in the presence of cerebral amyloid angiopathy. *Acta Neuropathol.* 2011; 121:431–443. [PubMed: 21259015]
164. Weller RO, Hawkes CA, Carare RO, Hardy J. Does the difference between PART and Alzheimer's disease lie in the age-related changes in cerebral arteries that trigger the accumulation of A $\beta$  and propagation of tau? *Acta Neuropathol.* 2015; 129:763–766. [PubMed: 25814152]
165. Pezzini A, Padovani A. Cerebral amyloid angiopathy-related hemorrhages. *Neurol. Sci.* 2008; 29:260–263.
166. Sakai K, et al. A $\beta$  immunotherapy for Alzheimer's disease: effects on apoE and cerebral vasculopathy. *Acta Neuropathol.* 2014; 128:777–789. [PubMed: 25195061]
167. Schley D, Carare-Nnadi R, Please C, Perry V, Weller R. Mechanisms to explain the reverse perivascular transport of solutes out of the brain. *J. Theor. Biol.* 2006; 238:962–974. [PubMed: 16112683]
168. Hawkes CA, et al. Regional differences in the morphological and functional effects of aging on cerebral basement membranes and perivascular drainage of amyloid- $\beta$  from the mouse brain. *Aging Cell.* 2013; 12:224–236. [PubMed: 23413811]
169. Hawkes CA, Gentleman SM, Nicoll JA, Carare RO. Prenatal high-fat diet alters the cerebrovasculature and clearance of  $\beta$ -amyloid in adult offspring. *J. Pathol.* 2015; 235:619–631. [PubMed: 25345857]

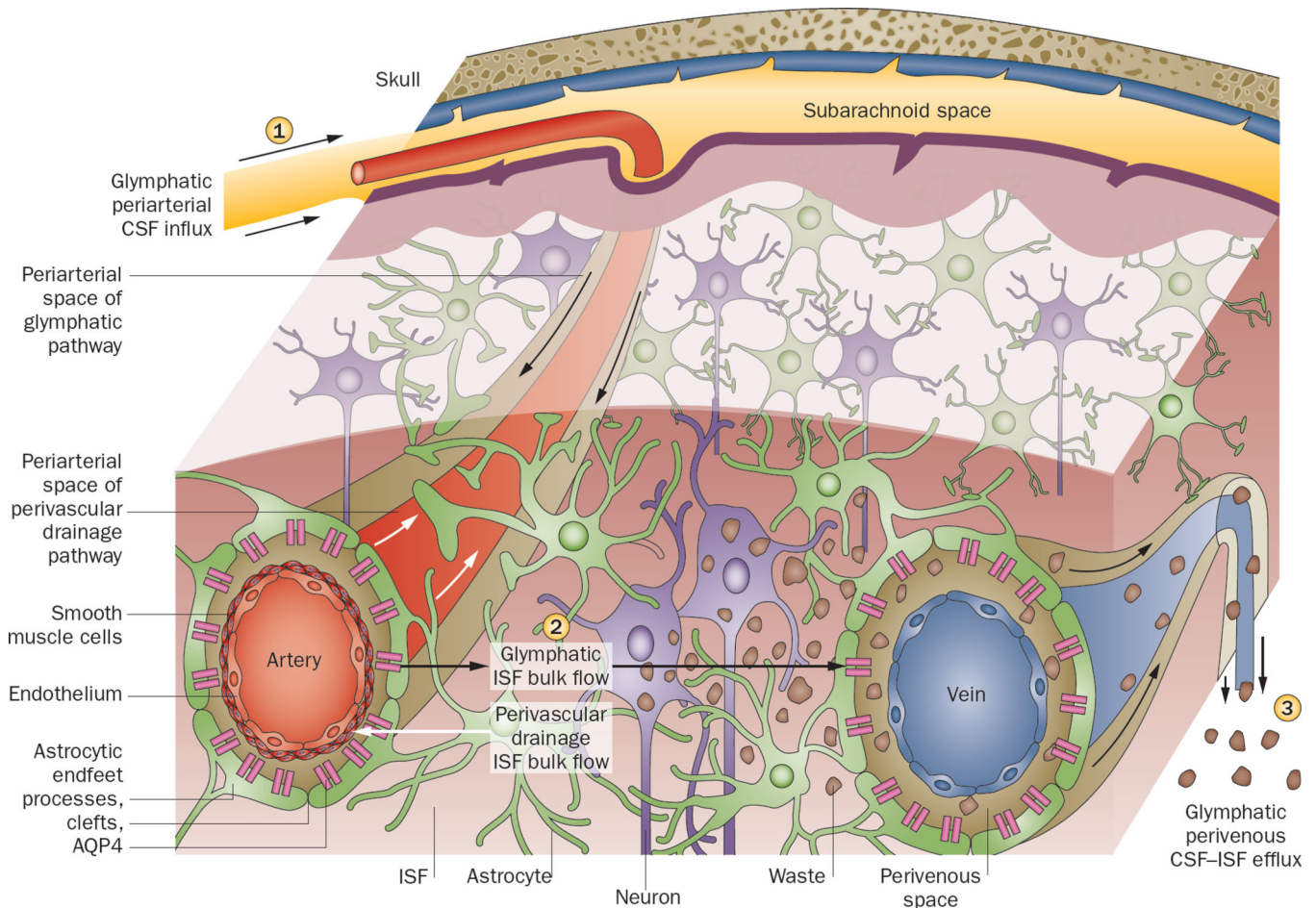
170. Manousopoulou A, et al. Are you also what your mother eats? Distinct proteomic portrait as a result of maternal high-fat diet in the cerebral cortex of the adult mouse. *Int. J. Obes. (Lond.)*. 2015
171. Iliff JJ, Nedergaard M. Is there a cerebral lymphatic system? *Stroke*. 2013; 44:S93–S95. [PubMed: 23709744]
172. Stoodley MA, Brown SA, Brown CJ, Jones NR. Arterial pulsation-dependent perivascular cerebrospinal fluid flow into the central canal in the sheep spinal cord. *J. Neurosurg.* 1997; 86:686–693. [PubMed: 9120633]
173. Iliff JJ, et al. Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. *J. Neurosci.* 2013; 33:18190–18199. [PubMed: 24227727]
174. Maurizi C. Recirculation of cerebrospinal fluid through the tela choroidae is why high levels of melatonin can be found in the lateral ventricles. *Med. Hypotheses.* 1991; 35:154–158. [PubMed: 1890974]
175. Nedergaard M. Garbage truck of the brain. *Science*. 2013; 340:1529–1530. [PubMed: 23812703]
176. Hartl FU, Hayer-Hartl M. Converging concepts of protein folding *in vitro* and *in vivo*. *Nat. Struct. Mol. Biol.* 2009; 16:574–581. [PubMed: 19491934]
177. Yang W, et al. Aquaporin-4 mediates astrocyte response to  $\beta$ -amyloid. *Mol. Cell. Neurosci.* 2012; 49:406–414. [PubMed: 22365952]
178. Ren Z, et al. ‘Hit & Run’ model of closed-skull traumatic brain injury (TBI) reveals complex patterns of post-traumatic AQP4 dysregulation. *J. Cereb. Blood Flow Metab.* 2013; 33:834–845. [PubMed: 23443171]
179. Xie L, et al. Sleep drives metabolite clearance from the adult brain. *Science*. 2013; 342:373–377. [PubMed: 24136970]
180. McKinley J, McCarthy A, Lynch T. Don’t lose sleep over neurodegeneration-it helps clear amyloid beta. *Front. Neurol.* 2013; 4:206. [PubMed: 24391624]
181. Mendelsohn AR, Larrick JW. Sleep facilitates clearance of metabolites from the brain: glymphatic function in aging and neurodegenerative diseases. *Rejuvenation Res.* 2013; 16:518–523. [PubMed: 24199995]
182. O’Donnell J, Ding F, Nedergaard M. Distinct functional states of astrocytes during sleep and wakefulness: is norepinephrine the master regulator? *Curr. Sleep Med. Rep.* 2015; 1:1–8. [PubMed: 26618103]
183. Wu Y-H, Swaab DF. Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer’s disease. *Sleep Med.* 2007; 8:623–636. [PubMed: 17383938]
184. Ju Y-E, et al. Sleep quality and preclinical Alzheimer disease. *JAMA Neurol.* 2013; 70:587–593. [PubMed: 23479184]
185. Musiek ES. Circadian clock disruption in neurodegenerative diseases: cause and effect? *Front. Pharmacol.* 2015; 6:29. [PubMed: 25774133]
186. Deane R, et al. LRP/amyloid  $\beta$ -peptide interaction mediates differential brain efflux of A $\beta$  isoforms. *Neuron*. 2004; 43:333–344. [PubMed: 15294142]
187. Lin TW, et al. Running exercise delays neurodegeneration in amygdala and hippocampus of Alzheimer’s disease (APP/PS1) transgenic mice. *Neurobiol. Learn. Mem.* 2015; 118:189–197. [PubMed: 25543023]
188. Herring A, et al. Environmental enrichment counteracts Alzheimer’s neurovascular dysfunction in TgCRND8 mice. *Brain Pathol.* 2008; 18:32–39. [PubMed: 17924982]
189. Richter H, et al. Wheel-running in a transgenic mouse model of Alzheimer’s disease: protection or symptom? *Behav. Brain Res.* 2008; 190:74–84. [PubMed: 18342378]
190. Marques F, Sousa JC, Sousa N, Palha JA. Blood-brain-barriers in aging and in Alzheimer’s. *Mol. Neurodegener.* 2013; 8:38. [PubMed: 24148264]
191. Picken MM. The changing concepts of amyloid. *Arch. Pathol. Lab. Med.* 2001; 125:38–43. [PubMed: 11151053]
192. Nakada T, Igarashi H, Suzuki Y, Kwee I. Alzheimer patients show significant disturbance in water influx into CSF space strongly supporting  $\beta$ -amyloid clearance hypothesis [abstract S58.0001]. *Neurology*. 2014; 82(Suppl. 1) S58.001.

193. Serot JM, Zmudka J, Jouanny P. A possible role for CSF turnover and choroid plexus in the pathogenesis of late onset Alzheimer's disease. *J. Alzheimers Dis.* 2012; 30:17–26. [PubMed: 22349684]
194. Erickson MA, Banks WA. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. *J. Cereb. Blood Flow Metab.* 2013; 33:1500–1513. [PubMed: 23921899]
195. Fujiyoshi M, et al. Amyloid- $\beta$  peptide(1–40) elimination from cerebrospinal fluid involves low-density lipoprotein receptor-related protein 1 at the blood–cerebrospinal fluid barrier. *J. Neurochem.* 2011; 118:407–415. [PubMed: 21585370]
196. Silverberg GD, Mayo M, Saul T, Rubenstein E, McGuire D. Alzheimer's disease, normal-pressure hydrocephalus, and senescent changes in CSF circulatory physiology: a hypothesis. *Lancet Neurol.* 2003; 2:506–511. [PubMed: 12878439]
197. Silverberg G, Mayo M, Saul T, Fellmann J, McGuire D. Elevated cerebrospinal fluid pressure in patients with Alzheimer's. *Cerebrospinal Fluid Res.* 2006; 3:7. [PubMed: 16737542]
198. Pappolla M, et al. Evidence for lymphatic A $\beta$  clearance in Alzheimer's transgenic mice. *Neurobiol. Dis.* 2014; 71:215–219. [PubMed: 25102344]
199. Shea T, Beermann M. Respective roles of neurofilaments, microtubules, MAP1B, and tau in neurite outgrowth and stabilization. *Mol. Biol. Cell.* 1994; 5:863–875. [PubMed: 7803854]
200. Binder LI, Guillozet-Bongaarts AL, Garcia-Sierra F, Berry RW. Tau, tangles, and Alzheimer's disease. *Biochim. Biophys. Acta.* 2005; 1739:216–223. [PubMed: 15615640]
201. Yamada K, et al. Neuronal activity regulates extracellular tau *in vivo*. *J. Exp. Med.* 2014; 211:387–393. [PubMed: 24534188]
202. Avila J, Simon D, Diaz-Hernandez M, Pintor J, Hernandez F. Sources of extracellular tau and its signaling. *J. Alzheimers Dis.* 2014; 40(Suppl. 1):S7–S15. [PubMed: 24531154]
203. Irazuzta JE, de Courten-Myers G, Zemlan FP, Bekkedal MY, Rossi J 3rd. Serum cleaved tau protein and neurobehavioral battery of tests as markers of brain injury in experimental bacterial meningitis. *Brain Res.* 2001; 913:95–105. [PubMed: 11532253]
204. Castillo-Carranza DL, et al. Passive immunization with tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J. Neurosci.* 2014; 34:4260–4272. [PubMed: 24647946]
205. Litersky JM, Johnson G. Phosphorylation by cAMP-dependent protein kinase inhibits the degradation of tau by calpain. *J. Biol. Chem.* 1992; 267:1563–1568. [PubMed: 1730702]
206. Medina M, Avila J. The role of extracellular tau in the spreading of neurofibrillary pathology. *Front. Cell. Neurosci.* 2014; 8:13. [PubMed: 24478633]
207. Gómez-Ramos A, et al. Characteristics and consequences of muscarinic receptor activation by tau protein. *Europ. Neuropsychopharmacol.* 2009; 19:708–717.
208. David DC, et al. Proteasomal degradation of tau protein. *J. Neurochemist.* 2002; 83:176–185.
209. Cirrito JR, et al. *In vivo* assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid- $\beta$  metabolism and half-life. *J. Neurosci.* 2003; 23:8844–8853. [PubMed: 14523085]
210. Iliff JJ, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *J. Neurosci.* 2014; 34:16180–16193. [PubMed: 25471560]
211. Plog BA, et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. *J. Neurosci.* 2015; 35:518–526. [PubMed: 25589747]
212. Bateman RJ, et al. Quantifying CNS protein production and clearance rates in humans using *in vivo* stable isotope labeling, immunoprecipitation, and tandem mass spectrometry. *Nat. Med.* 2006; 12:856. [PubMed: 16799555]

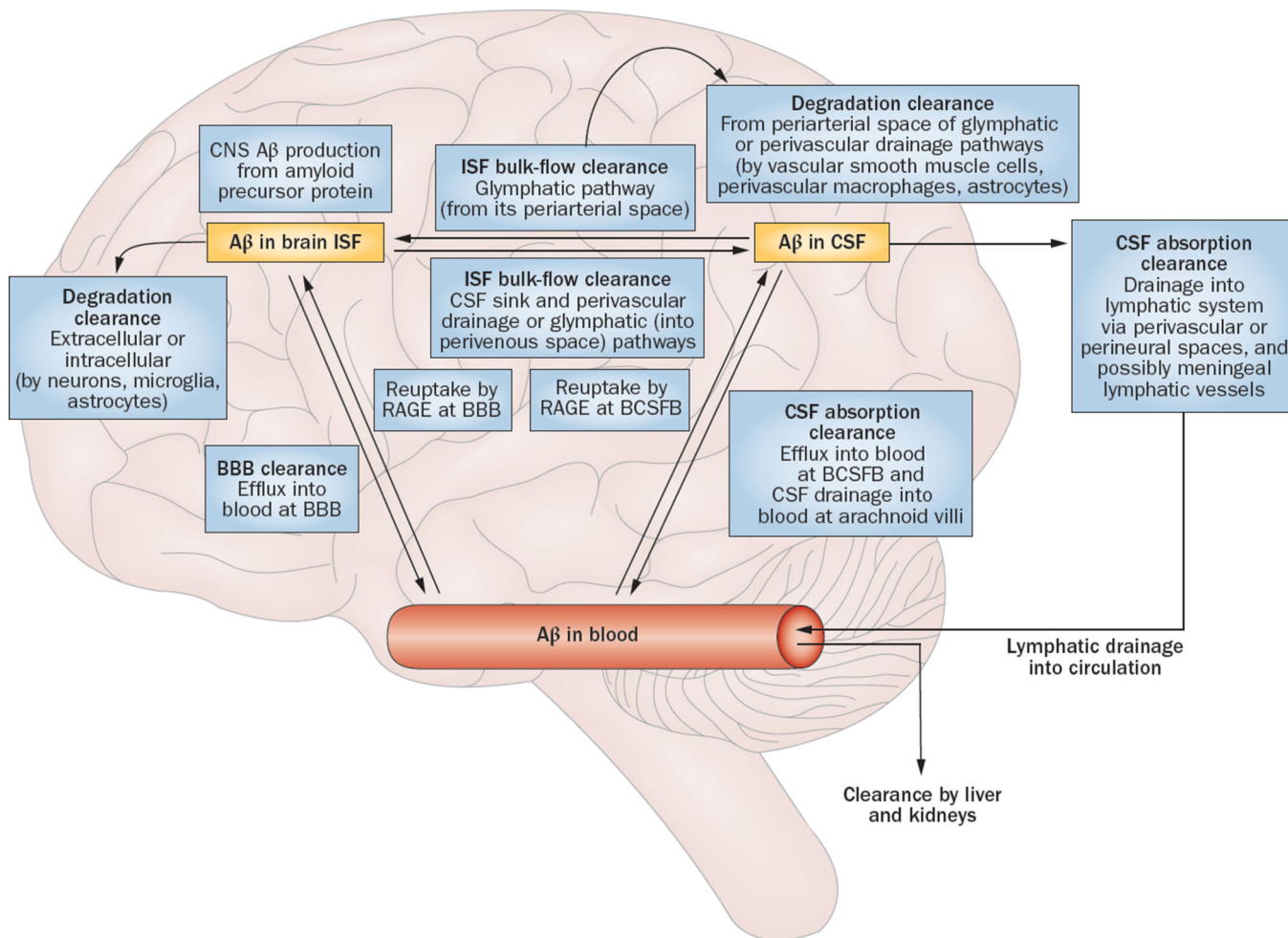
### Key points

- Accumulation of neurotoxic forms of amyloid- $\beta$  ( $A\beta$ ) and tau proteins is the pathological hallmark of Alzheimer disease (AD)
- Excess deposition of  $A\beta$  results from an imbalance between its production and clearance; in both early-onset and late-onset forms of AD,  $A\beta$  clearance seems already impaired at the prodromal stage
- $A\beta$  is removed from the brain by various overlapping and interacting clearance systems: degradation, blood-brain barrier (BBB) transport, interstitial fluid (ISF) bulk flow, and cerebrospinal fluid (CSF) absorption into the circulatory and peripheral lymphatic systems
- Although most extracellular  $A\beta$  undergoes BBB clearance, the recently discovered glymphatic pathway seems to be important for  $A\beta$  clearance
- Specific BBB transporters for tau have not been identified, suggesting that clearance of tau is less complex than that of  $A\beta$ , and mainly relies on degradation, ISF bulk flow, and CSF absorption
- Precise understanding of the mechanisms of clearance dysfunction in AD is paramount to develop strategies to reduce excess deposition of neurotoxic protein and to halt the related pathological changes

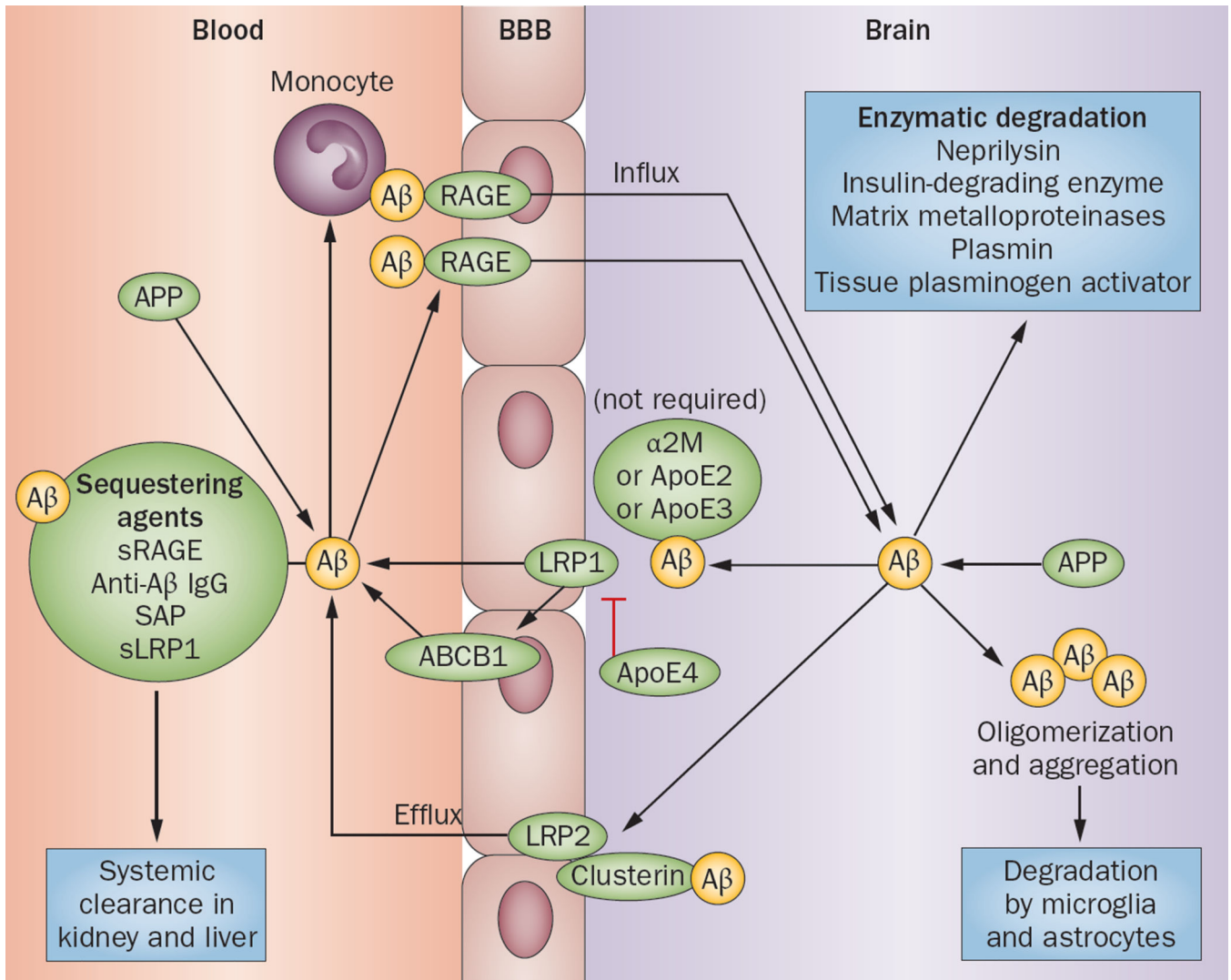




**Figure 1.** Perivascular clearance comprises perivascular drainage and glymphatic pathways. The perivascular drainage Nature Reviews | Neurology pathway (white arrows) moves waste into the periarterial space (located along smooth muscle cells and the capillary basement membrane) and towards the subarachnoid space in the direction opposite to blood flow. The glymphatic pathway (black arrows) clears waste from the ISF through the brain parenchyma, and comprises three functional components. (1) CSF influx, unidirectionally with blood flow, into the periarterial space (between the basement membrane of smooth muscle cells and pia mater), where the water component of CSF crosses astrocytic AQP4 channels to enter the brain parenchyma. CSF solutes can be cleared with astroglial transporters or channels, or can pass through the astrocytic endfeet clefts. (2) CSF–ISF exchange within the brain parenchyma. (3) CSF–ISF movement into the perivenous space of deep-draining veins. Effluxed waste can then recirculate with the CSF, or eventually be absorbed into the lymphatic system. Arrows indicate direction of flow. Abbreviations: AQP4, aquaporin-4; CSF, cerebrospinal fluid; ISF, interstitial fluid. Permission obtained from Cell Press © Nedergaard, M. *Science* **340**, 1529–1530 (2013).



**Figure 2.** Aβ clearance systems. Soluble Aβ can be removed from the brain by various clearance systems. Degradation clearance via extracellular and intracellular degradation pathways can involve either cellular uptake from the interstitium by neurons, microglia, and astrocytes, or uptake from the perivascular space by smooth muscle cells, perivascular macrophages, and astrocytes. BBB clearance involves Aβ efflux into the blood. ISF bulk flow clearance can occur into the CSF sink (ventricles and subarachnoid space), via perivascular drainage pathway, or via glymphatic pathway. CSF absorption clearance involves absorption either into the circulatory system from the arachnoid villi and BCSFB, or into the lymphatic system from the perivascular and perineural spaces—and possibly through meningeal lymphatic vessels. Abbreviations: Aβ, amyloid-β; BBB, blood–brain barrier; BCSFB, blood–CSF barrier; CSF, cerebrospinal fluid; ISF, interstitial fluid; RAGE, advanced glycosylation end productspecific receptor. Adapted with permission from Nature Publishing Group © Erickson, M. A. & Banks, W. A. *J. Cerebr. Blood Flow & Metabol.* **33**, 1500–1513 (2013).



**Figure 3.** Aβ efflux and influx through the BBB. Aβ can enter the brain via RAGE as a free plasma-derived peptide, or can be transported by monocytes. Sequestering agents (soluble transporters that chaperone Aβ for systemic degradation) can prevent Aβ entry from the circulation into the brain. Aβ is eliminated from the brain enzymatically or by transportation through the BBB. LRP1 mediates efflux of unbound Aβ and Aβ bound to ApoE2, ApoE3 or α2M from the brain parenchyma into the blood with the help of ABCB1; ApoE4 inhibits this transport process. Aβ bound to clusterin is transported through the BBB by LRP2. Abbreviations: α2M, α2-macroglobulin; Aβ, amyloid-β; ABCB1, multidrug resistance protein 1 (also known as P-glycoprotein 1); ApoE, apolipoprotein E; BBB, blood–brain barrier; LRP, LDL receptor-related protein; RAGE, advanced glycosylation end product-specific receptor; SAP, serum amyloid P; sLRP1, soluble LRP1; sRAGE, soluble form of RAGE. Permission obtained from Nature Publishing Group © Zlokovic, B. V. *et al Nat. Rev. Neurosci.* **12**, 723–738 (2011).

**Table 1**

## Clearance systems in the brain

Clearance system	Source	Destination	Factors affecting clearance system	Clearance pathways
Blood–brain barrier clearance <sup>61</sup>	ISF	Blood	Transporter expression and activity Ligand affinity and competition Vascular integrity	Efflux transporters and mediators Influx transporters and mediators
Degradation clearance <sup>56</sup>				
Intracellular	ICS	Degradation	Enzyme expression and activity Ligand affinity and competition Initiation of intracellular degradation pathways	Ubiquitin–proteasome pathway Autophagy–lysosome pathway Endosome–lysosome pathway Proteases
Extracellular	ISF	Degradation or cellular uptake	Enzyme expression and activity Ligand affinity and competition Activation of cellular uptake	Proteases Glial phagocytes
ISF bulk flow clearance <sup>86</sup>				
CSF sink	ISF	CSF sink (subarachnoid space, ventricles)	Intrinsic ISF flow rate	ISF efflux into CSF sink
Perivascular drainage	ISF	Periarterial space to peripheral lymph	<i>APOE*ε4</i> Immune complex deposition Arterial age Arterial pulsation (hypothetical)	ISF efflux into basement membrane of capillary and arterial walls
Perivascular glymphatic	ISF	Perivenous space to peripheral lymph or ventricles	Molecular size Arterial pulsation <i>AQP4</i> expression and localization Sleep	CSF influx into periarterial space CSF–ISF exchange within interstitium CSF–ISF efflux along perivenous space
CSF absorption clearance <sup>18,98</sup>				
Circulatory	CSF	Blood	CSF production BCSFB transporters Arachnoid villi resistance	Arachnoid villi integrity and BCSFB efflux and influx transporters and mediators
Lymphatic	CSF	Peripheral lymph	Lymphatic absorption of CSF	Perivascular space Perineural space
Meningeal lymphatic vessels	CSF	Lymph	Unknown	Subarachnoid CSF into meningeal lymphatic vessels

Abbreviations: *APOE\*ε4*, apolipoprotein E ε4 allele; *AQP4*, aquaporin-4; BCSFB, blood–CSF barrier; CSF, cerebrospinal fluid; ICS, intracellular space; ISF, interstitial fluid.

**Table 2**Clearance of A $\beta$  and tau from the brain

Clearance system	A $\beta$ <sup>9,16,44,109,125</sup>	Tau <sup>135,210</sup>
Blood–brain barrier clearance	Majority of eA $\beta$ clearance LRP1 efflux ABCB1 efflux ApoE-mediated efflux $\alpha$ 2M-mediated efflux LRP2-mediated efflux RAGE influx	Unknown
Degradation clearance		
Intracellular	Ubiquitin–proteasome pathway Autophagy–lysosome pathway Endosome–lysosome pathway Proteases	Ubiquitin–proteasome pathway Autophagy–lysosome pathway Endosome–lysosome pathway Proteases
Extracellular	Proteases Glial phagocytosis	Unknown
ISF bulk flow clearance		
CSF sink	Contributes to eA $\beta$ clearance	Unknown
Perivascular drainage	Contribution % to eA $\beta$ clearance unknown	Unknown
Perivascular glymphatic	Contributes to eA $\beta$ clearance (55–65%) Likely to facilitate blood–brain barrier clearance	Might contribute to clearance of non-endocytosed tau
CSF absorption clearance		
Circulatory	Arachnoid villi Blood–CSF barrier transporters (e.g. LRP1 efflux)	Arachnoid villi
Lymphatic	CSF lymphatic absorption	Unknown

Abbreviations:  $\alpha$ 2M,  $\alpha$ 2-macroglobulin; A $\beta$ , amyloid- $\beta$ ; ABCB1, multidrug resistance protein 1 (also known as P-glycoprotein 1); ApoE, apolipoprotein E; CSF, cerebrospinal fluid; e-tau, extracellular tau; eA $\beta$ , extracellular A $\beta$ ; ISF, interstitial fluid; LRP, LDL receptor-related protein; RAGE, advanced glycosylation end product-specific receptor.