

REVIEW ARTICLE

Reactive astrocytes as treatment targets in Alzheimer's disease—Systematic review of studies using the APPswePS1dE9 mouse model

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Abstract

Astrocytes regulate synaptic communication and are essential for proper brain functioning. In Alzheimer's disease (AD) astrocytes become reactive, which is characterized by an increased expression of intermediate filament proteins and cellular hypertrophy. Reactive astrocytes are found in close association with amyloid-beta (A β) deposits. Synaptic communication and neuronal network function could be directly modulated by reactive astrocytes, potentially contributing to cognitive decline in AD. In this review, we focus on reactive astrocytes as treatment targets in AD in the APPswePS1dE9 AD mouse model, a widely used model to study amyloidosis and gliosis. We first give an overview of the model; that is, how it was generated, which cells express the transgenes, and the effect of its genetic background on A β pathology. Subsequently, to determine whether modifying reactive astrocytes in AD could influence pathogenesis and cognition, we review studies using this mouse model in which interventions were directly targeted at reactive astrocytes or had an indirect effect on reactive astrocytes. Overall, studies specifically targeting astrocytes to reduce astrogliosis showed beneficial effects on cognition, which indicates that targeting astrocytes should be included in developing novel therapies for AD.

KEYWORDS

AD mouse model, Alzheimer's disease, amyloid-beta, APPswePS1dE9, reactive astrocytes

1 | INTRODUCTION

Alzheimer's disease (AD) is a prime example of a central nervous system (CNS) disease with a clear presence of reactive astrocytes. AD is the most common form of dementia and is neuropathologically characterized by

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BOX 1 The APPswePS1dE9 mouse model

APPswePS1dE9 mice overexpress two transgenes; humanized amyloid precursor protein (hAPP) harboring the Swedish mutation (K595N/M596L; Johnston, O'Neill, Lannfelt, Winblad, & Cowburn, 1994; Mullan et al., 1992) and human PS1 with a deletion of exon 9 (PS1dE9) (Perez-Tur et al., 1995). The APPswePS1dE9 mice were made by co-injection of two plasmids into a single cell embryo derived from F2 hybrids of C57BL/6J and C3H/HeJ mice (Figure 1d). Each plasmid contained one of the two transgenes, either humanized APP or human PS1dE9, under the control of a mouse prion protein (PrP) promoter element (the MoPrP.Xho vector). The two transgenes co-integrated and co-segregated as a single locus (Jankowsky et al., 2001). For coinjection of both transgenes, the MoPrP.Xho vector was used. Within this modified prion protein (MoPrP) vector, an Xho I restriction site replaced the open reading frame (ORF) of the third exon, removing the coding sequence of PrP. The transgene sequences, containing a start and stop codon, were cloned into the vector at this Xho I site. The main advantage of the use of the MoPrP.Xho vector was that the ORF is located within an exon, and can, therefore, be easily replaced by a recombinant ORF. Furthermore, as the MoPrP.Xho vector is small by itself, large cDNA fragments can be introduced (Borchelt, Davis, et al., 1996; Jankowsky et al., 2001). The expression of the transgenes is driven by the mouse PrP promoter, which was thought to lead to a predominant neuronal expression of the transgenes (Borchelt, Davis, et al., 1996; Jankowsky et al., 2001). However, it is known that in addition to the nervous system, the promoter also drives the expression of transgenes in other organs, such as heart and kidneys (Borchelt, Davis, et al., 1996; Esquerda-Canals, Montoliu-Gaya, Güell-Bosch, & Villegas, 2017; Jankowsky et al., 2007). Although transgene expression in heart and kidneys probably would not affect the pathological conditions in the brain, it might be associated with the premature death of APPswePS1dE9 mice (Section 3.5).

Multiple lines of mice were derived from embryos with the coinjected vectors (Jankowsky et al., 2001). The mice of line 85 were deposited at the Jackson Laboratories (Jankowsky et al., 2004). In this review, we focus on APPswePS1dE9 mice on a C57BL/6J × C3H/HeJ genetic background, MMRRC Stock No: 34829 (formerly Jackson Lab Stock No: 004462) and APPswePS1dE9 mice on a congenic C57BL/6J genetic background MMRRC Stock No: 34832 (formerly Jackson Lab Stock No: 005864).

The first increase in soluble A β ₄₂ levels has been observed in 3-month-old APPswePS1dE9 mice (Van Tijn et al., 2012) and amyloid plaques in 4-month-old mice (Garcia-Alloza et al., 2006; Ruan, Kang, Pei, & Le, 2009). Impaired synaptic plasticity has been detected as early as

3.5 months in the cortex (Shemer et al., 2006). A decrease in presynaptic and postsynaptic terminals has been observed by 4 months of age in the hippocampus (Hong et al., 2016). Cognitive impairments have been observed as early as 3 months of age, in a contextual fear memory task and 6-month-old APPswePS1dE9 mice in the Morris water maze spatial memory task (Végh et al., 2014). Astrogliosis develops around the age of 6 months, based on the increased expression of GFAP (Ruan et al., 2009; Van Tijn et al., 2012; Végh et al., 2014).

extracellular deposits of amyloid-beta (A β) and intracellular neurofibrillary tangles, consisting of hyperphosphorylated tau (Selkoe, 1991). Moreover, A β plaques are invaded and surrounded by activated microglia (Itagaki, McGeer, & Akiyama, 1989; Yin et al., 2017), and reactive astrocytes (Kamphuis et al., 2014; Kato et al., 1998; Simpson et al., 2010). Over the past decade, there has been an increasing insight into the effect of reactive astrogliosis on neural functioning. Therefore, it is opportune to review reactive astrocytes in relation to cognitive function in a widely used AD mouse model: the APPswePS1dE9 mouse (Box 1).

Astrocytes are essential for proper brain functioning (Box 2; Kettenmann & Verkhratsky, 2008; Verkhratsky & Nedergaard, 2018). They are actively involved in synaptic communication (Araque et al., 2014; Araque, Parpura, Sanzgiri, & Haydon, 1998) and play a key role in maintaining ion, neurotransmitter (Kirischuk, Parpura, & Verkhratsky, 2012), and energy homeostasis in the CNS (Allaman, Bélanger, & Magistretti, 2011; Verkhratsky & Nedergaard, 2018). Perisynaptic astrocyte protrusions closely interact with synapses and together they form the tripartite synapse (Araque, Parpura, Sanzgiri, & Haydon, 1999). In the reactive state, astrocytes show cellular hypertrophy, which is likely to affect these protrusions. Besides, there is a change in the intermediate filament (IF) cytoskeleton, due to an increase in the expression of the IF proteins glial fibrillary acidic protein (GFAP; the classical marker for reactive astrocytes; Box 3), vimentin, and nestin (Hol & Pekny, 2015; Pekny et al., 2016). The changes associated with the reactive state can directly impact synaptic transmission and neuronal circuit activity, thereby potentially contributing to the cognitive problems observed in AD (Chung, Welsh, Barres, & Stevens, 2015; Dossi, Vasile, & Rouach, 2018; Jo et al., 2014; Kuchibhotla et al., 2008; Ortinski et al., 2010; Osborn, Kamphuis, Wadman, & Hol, 2016; Peters et al., 2009; Wu, Guo, Gearing, & Chen, 2012; Xing, Yang, Cui, & Chen, 2019).

To understand how the changes in reactive astrocytes contribute to cognitive decline in AD, it is crucial to study the alterations in neuron–glia interactions during AD pathogenesis, as these can directly affect neuronal functioning. In this systematic review, we focus on the APPswePS1dE9 mouse. This is a widely used AD mouse model, which excellently mimics amyloidosis and gliosis. Of note, in the scientific literature, this model is often referred to as APP/PS1 mice. Since the abbreviation APP/PS1 is also used to refer to other AD mouse models with different APP and PS1 mutations and driven by different promoters, we will use the full name APPswePS1dE9 throughout the

review. We will first introduce the amyloid hypothesis, on which the model is based, and review the characteristics and the merit of the APPswePS1dE9 mouse model for AD research. We then systematically review studies that describe interventions that directly or indirectly affected reactive astrocytes in the APPswePS1dE9 AD mouse model, to determine whether modifying reactive astrocytes in AD could influence pathogenesis and cognition.

2 | THE AMYLOID HYPOTHESIS AND THE APPSWEPS1DE9 MOUSE MODEL

Several AD mouse models, including the APPswePS1dE9 model, are based on the amyloid-cascade hypothesis. Even though the debate on the exact etiology of AD is still strongly ongoing (De Strooper &

Karran, 2016; Selkoe & Hardy, 2016), the amyloid hypothesis (also known as the A β hypothesis or amyloid cascade hypothesis) has been a main hypothesis in the AD field for almost 30 years (Hardy & Allsop, 1991; Hardy & Selkoe, 2002; Selkoe, 1991; Selkoe & Hardy, 2016). The hypothesis places A β , a 39–43 amino acid long peptide proteolytically cleaved from the amyloid precursor protein (APP; Figure 1a), at the beginning of the disease process. The amyloid hypothesis states that the overproduction of A β and its aggregation in plaques are the first events in AD pathogenesis, eventually resulting in the development of neurofibrillary tangles, loss of synapses, and neuronal death (Hardy & Allsop, 1991; Hardy & Selkoe, 2002; Selkoe, 1991). In the 1990s, AD-causative mutations were observed in APP and in genes that were later found to be components of γ -secretase that cleaves APP, that is, presenilin 1 (PS1), and presenilin 2 (PS2; Chartier-Harlin et al., 1991; Goate et al., 1991; Levy-lahad et al., 1995;

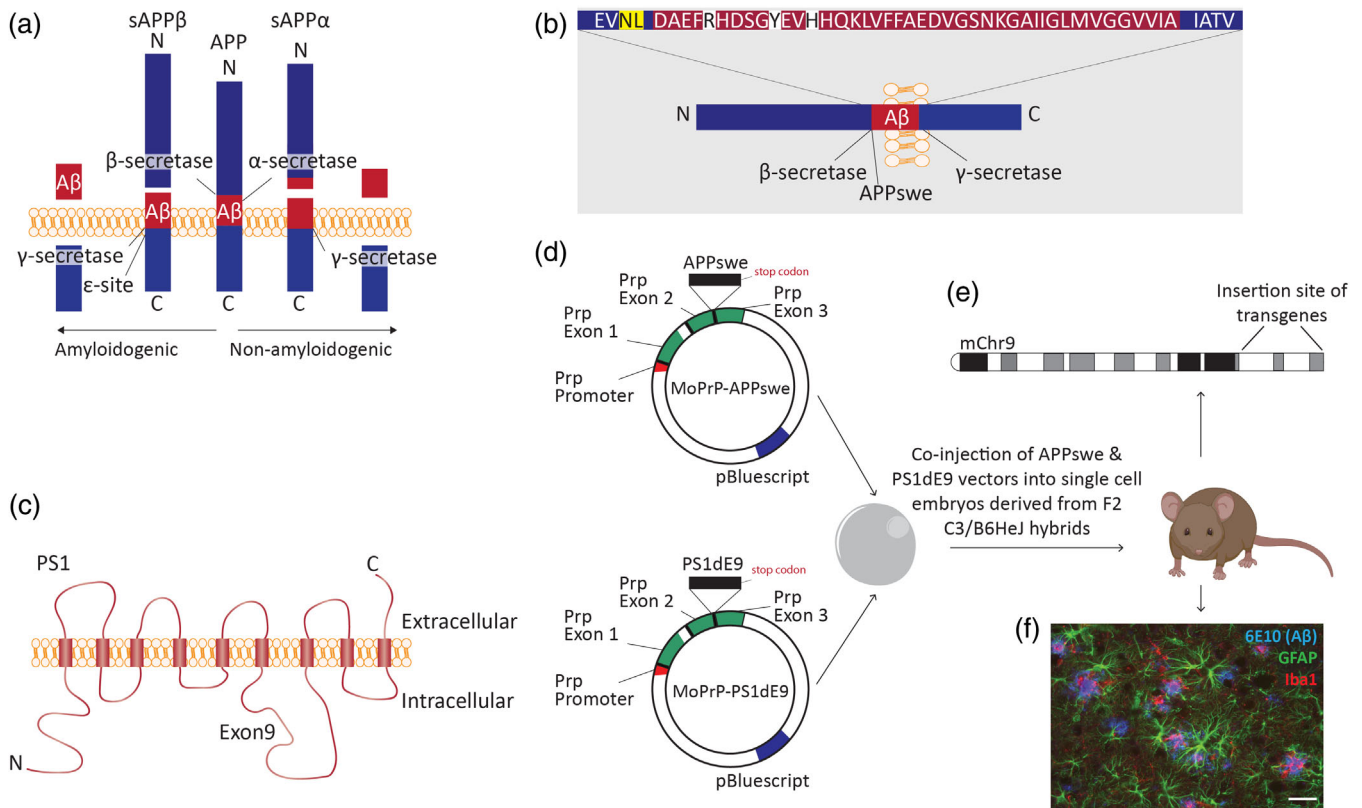


FIGURE 1 An overview of the amyloidogenic and non-amyloidogenic pathway, the generation of the APPswePS1dE9 mouse model, and gliosis. (a) Alternative processing of APP by α -, β -, and/or γ -secretases resulting in amyloidogenic and non-amyloidogenic cleavage. (b) Humanized mouse APP sequence (the white blocks indicate the human-specific amino acids that were introduced in the mouse sequence), containing the Swedish mutation (yellow block). (c) PS1 is a transmembrane protein and part of the γ -secretase complex. The PS1dE9 mutation results in the deletion of exon 9. (d) The APPswe and PS1dE9 transgenes were integrated in MoPrP vectors between exon 2 and 3; the coding sequence of PrP was completely removed, as described in Borchelt et al. (1996) and Jankowsky et al. (2001). The two vectors were co-injected into a single cell embryo derived from F2 hybrids of C57BL/6J and C3H/HeJ mice: C3/B6HeJ mice. This resulted in the generation of double transgenic APPswePS1dE9 mice, in which the expression of both the APP and the PS1 gene is driven by the mouse prion protein promoter. (e) Integration of the transgenes occurred at chromosome 9 between the Arpp21 and Pcd6ip genes (Jackson et al., 2015). (f) From the age of 6 months, reactive astrocytes (GFAP, green) and activated microglia (Iba1, red) surrounding A β plaques (6E10, blue) were detected, scale bar: 50 μ m, adjusted from original picture in Orre et al. (2014). A β , amyloid-beta; APP, amyloid precursor protein; ϵ -site, epsilon cleavage site; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adapter molecule 1; MoPrP, modified prion protein; PrP, prion protein; PS1, presenilin 1; sAPP α , soluble amyloid precursor protein- α ; sAPP β , soluble amyloid precursor protein- β . Based on Jackson et al. (2015) and Jankowsky et al. (2001, 2007). Scientific illustration toolkits from Motifolio were used to generate parts of this figure

Mullan et al., 1992; Rogaeve et al., 1995; Sherrington et al., 1995). These genetic findings point into the direction of an enhanced processing of APP and production of A β , and support the hypothesis of a primary involvement of A β in the disease process. A major concern with the amyloid hypothesis is the poor correlation between the development of cognitive impairment and A β plaque burden (Nelson et al., 2012). Still, mouse models based on mutations in APP, PS1, and PS2 are valuable tools to study specific parts of the AD pathogenesis.

Over the years, the amyloid hypothesis has been refined and now also includes the important role of A β oligomers, which is the most pathogenic and toxic form of A β (Selkoe & Hardy, 2016). Of the various forms of A β (monomers, oligomers, fibrils), it is oligomeric A β that disrupt synaptic function (Walsh et al., 2002). In several AD mouse models, based on genetic mutations in APP, PS1, and PS2, an impairment in synaptic transmission has been detected before fibrillar plaques become apparent (Larson, Lynch, Games, & Seubert, 1999; Moechars et al., 1999; Véghe et al., 2014).

In this review, we focus on APP^{swe}PS1dE9 mice (Box 1), which overexpress two transgenes: humanized amyloid precursor protein (hAPP) harboring the Swedish mutation (K595N/M596L, Figure 1b; Johnston et al., 1994; Mullan et al., 1992) and human PS1 with a deletion of exon 9 (PS1dE9, Figure 1c; Perez-Tur et al., 1995). Both mutations cause early-onset AD in humans (Mullan et al., 1992; Perez-Tur et al., 1995) and are known to change APP processing, resulting in an enhanced production of A β (Citron et al., 1992; Perez-Tur et al., 1995).

2.1 | Amyloid precursor protein, start of the amyloidogenic pathway

AD-causative mutations were found in the APP gene, leading to an enhanced processing of the APP protein into A β . Even though its central place in the amyloid hypothesis, the normal function of APP and its cleaved fragments is largely unknown. APP is a ubiquitously expressed Type I membrane glycoprotein with only a small intracellular domain (Nalivaeva & Turner, 2013; Selkoe et al., 1996). In the brain, APP regulates the cell cycle progression of fetal neural stem cells (Joo et al., 2010) and stimulates neural cell migration, thereby playing an important role in brain development. In the adult brain, APP is involved in long-term potentiation (LTP; Weyer et al., 2011) and maintenance of calcium homeostasis (Octave, Pierrot, Ferrao Santos, Nalivaeva, & Turner, 2013).

APP can be processed by different proteolytic enzymes, that is, α -, β -, and γ -secretases, resulting in different products (Figure 1a). Via the non-amyloidogenic pathway, APP is sequentially cleaved by α - and γ -secretase, resulting in a soluble amyloid precursor protein- α (sAPP α), an extracellular P3, and an intracellular APP C-terminal domain (AICD) fragment. The amyloidogenic pathway yields sAPP β , A β , and AICD due to cleavage by β - and γ -secretases (Hall & Roberson, 2012; Salminen et al., 2013). The cleavage of APP by β -secretase results in the formation of sAPP β and an intramembrane

β -carboxyl-terminal fragment (β -CTF). The formation of A β_{40} or A β_{42} starts with ϵ -cleavage, followed by γ -cleavage of the β -CTF (Takami et al., 2009). Products of the primary ϵ -cleavage, A β_{49} , and AICD50-99 or A β_{48} and AICD49-99, are sequentially cleaved resulting in A β_{40} and A β_{42} , respectively. The two major pathways are A β_{49} \rightarrow A β_{46} \rightarrow A β_{43} \rightarrow A β_{40} and A β_{48} \rightarrow A β_{45} \rightarrow A β_{42} (tripeptide hypothesis; Kakuda et al., 2006; Szaruga et al., 2017; Takami et al., 2009). Over the years, also other proteolytic products of APP have been implicated in AD. In 2004, Cao and Südhof (2004) proposed a role for AICD in the regulation of gene transcription. Although others have shown that the effect on downstream targets is weak and might be indirect (Hébert et al., 2006). Thus, the role of AICD as a transcriptional regulator remains controversial (for a review, Bukhari et al., 2017). Also, the extracellular N-terminal fragment of APP (N-APP) produced after cleavage of APP by β -secretase, has been shown to mediate neurodegeneration. This outcome is distinct from effects mediated by A β peptides (Nikolaev, McLaughlin, O'Leary, & Tessier-Lavigne, 2009).

APP is differentially spliced, resulting in several isoforms. The most common and most stable isoforms are APP₆₉₅, APP₇₅₁, and APP₇₇₀, with the number referring to the number of amino acids (Johnston et al., 1994). In the brain, APP₆₉₅ is mainly expressed in neurons (Johnston et al., 1994; Nalivaeva & Turner, 2013). In neuronal cell lines, it has been shown that sAPP β , A β , and AICD are preferentially formed from APP₆₉₅ (Belyaev et al., 2010). The Kunitz-type protease inhibitor sequence in APP₇₇₀ and APP₇₅₁ promotes cleavage by α -secretase, not yielding A β . APP₆₉₅ lacks this sequence, making it the most amyloidogenic APP isoform (Nalivaeva & Turner, 2013).

2.2 | APP^{swe}PS1dE9 mouse: Swedish mutation in APP results in an increased A β production

For the generation of the APP^{swe}PS1dE9 model, humanized mouse APP₆₉₅ was used (Jankowsky et al., 2007). Human and mouse APP sequences differ in 17 amino acids, three of which are positioned within the A β -encoding part. In humanized mouse APP₆₉₅, the mouse-specific amino acids in the A β -encoding sequence were replaced by the human-specific amino acids (Figure 1b). Thus, glycine at position 5 in the A β domain was replaced by arginine, phenylalanine at position 10 by tyrosine, and arginine at position 13 by histidine. The last replacement causes A β to be more susceptible to zinc-mediated aggregation (Jankowsky et al., 2007; Nalivaeva & Turner, 2013). Importantly, the produced A β peptide in the mouse will be entirely human (Mellott et al., 2017), which is essential for testing therapies targeted at human A β .

The humanized APP sequence of the APP^{swe}PS1dE9 mice contains the Swedish mutation. Several mutations in APP have been linked to familial AD (FAD; <https://www.alzforum.org/mutations>). The humanized APP sequence of the APP^{swe}PS1dE9 mice contains the Swedish mutation. The Swedish mutation (APP^{swe}) was discovered in a Swedish family with early-onset AD. Two pedigrees showed a

double missense mutation in exon 16 of APP, which results in the replacement of the lysine residue at position 595 in APP₆₉₅ by asparagine (K595N) and the methionine residue at position 596 by leucine (M596L). The mutated codons are positioned just before the N-terminal side of the A β domain (Figure 1b). Due to the position of these mutations, β -secretase can cleave APP more efficiently. The level of A β in cells expressing APP_{swe} is 6–8 times higher than in cells expressing wild-type APP (Citron et al., 1992). Processing of wild-type APP by β -secretase occurs in early endosomes (Rajendran et al., 2006; Sannerud et al., 2011; Sannerud & Annaert, 2009), whereas APP_{swe} is likely processed by β -secretase in vesicles on their way to the cell surface (Haass et al., 1995). In short, the APP_{swe} mutation affects APP processing towards an increased A β production.

2.3 | Presenilin 1, function in the amyloidogenic pathway

Presenilin 1 (PS1) and 2 (PS2) are transmembrane proteins that undergo endoproteolytic processing. This yields large N-terminal and smaller C-terminal fragments (NTFs and CTFs) (Hardy, 1997), which accumulate equally in the brain (Lee et al., 1997). In contrast to the holoprotein, the NTFs and CTFs are stable protein fragments forming complexes that represent the active form of PSs (Xia, 2000).

PS1 is crucial for normal development as PS1^{-/-} mice die late in embryogenesis or at birth (Shen, 2014; Xia, 2000). PSs also play an essential role in synaptic function and neuronal survival in adult mice, since age-dependent synapse and neuron loss has been observed in a conditional double knockout mouse. These PS1^{-/-}/PS2^{-/-} mice showed memory and behavioral impairments (reviewed by Shen, 2014). In PS1-deficient neurons, accumulation of AICD fragments and loss of A β production have been observed (De Strooper et al., 1998). These studies revealed that PS1 is essential for the proteolytic function of γ -secretase (De Strooper et al., 1998; Wolfe et al., 1999). We now know that PS1 or PS2 form the catalytic active subunit of the γ -secretase intramembrane protease complexes, which also contain Nicastrin (Nct), anterior pharynx defective 1 (Aph-1), and presenilin-enhancer-2 (Pen-2; De Strooper, 2003; Francis et al., 2002; Goutte, Tsunozaki, Hale, & Priess, 2002; Yu et al., 2000).

Diverse molecular effects are induced by the different mutations in PSs. Mutations in PS1 and PS2 increase the ratio of A β ₄₂:A β ₄₀ (Borchelt et al., 1996). Consistent with this observation, A β deposition was observed earlier in transgenic mice in which APP_{swe} was co-expressed with mutant PS1 compared to mice only expressing APP_{swe} (Borchelt et al., 1997). The largest change in the A β ₄₂:A β ₄₀ ratio was observed when APP_{swe} was co-expressed with PS1 lacking exon 9, which shifted the ratio from 1:3 to 1:0.75 (Jankowsky et al., 2004). This change in the A β ₄₂:A β ₄₀ ratio could be caused by the subcellular distribution of PSs. PS1 and PS2 have distinct subcellular locations, contributing to substrate specificity. Whereas PS1 is broadly distributed in the cell, PS2 is directed to late endosomes/lysosomes. The strongest increase in the A β ₄₂:A β ₄₀ ratio was induced by

mutations in PS1 when it changed the localization of PS1 to late endosomes/lysosomes. FAD-associated mutations can affect the subcellular location of PS1 and overall increase the A β ₄₂:A β ₄₀ ratio, predominantly affecting the intracellular fraction and A β ₄₀ formation (Sannerud et al., 2016). Overall the mutations appear to decrease the C- to N-terminal cleavage of APP, resulting in longer, more hydrophobic, and self-aggregating A β peptides (Selkoe & Hardy, 2016).

When modulating PS1, it has to be noted that PSs/ γ -secretase not only cleave APP, but that they target numerous type I integral membrane proteins, such as Notch1, ErbB4, E-cadherin, CD44, and LDL-receptor related protein (LRP; De Strooper, 2003; Duggan & McCarthy, 2016; Wakabayashi & De Strooper, 2008). These changes could also contribute to AD pathogenesis.

2.4 | APP_{swe}PS1dE9 mouse: PS1 Exon 9 deletion results in an increased A β production

Mutations in PS1 and PS2, are the main cause of FAD (Jankowsky et al., 2004) and are linked to early-onset AD (Levy-lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). Together, they represent approximately 75% of all known FAD mutations (<https://www.alzforum.org/mutations>). For the generation of the APP_{swe}PS1dE9 model, human PS1 with a deletion of exon 9 (PS1dE9) was used (Jankowsky et al., 2007). The deletion of exon 9 causes early-onset FAD (Lee et al., 1997; Sherrington et al., 1995). The PS1dE9 mutation is a point mutation (G to T) in the splice site for exon 9, which breaks up the consensus sequence for the splice acceptor site, resulting in the (in-frame) deletion of exon 9 (Hardy, 1997; Steiner et al., 1999).

As this is the domain in which PS processing normally occurs, PS1dE9 ultimately results in the accumulation of the uncleavable PS holoprotein. However, the pathological function of PS1dE9 is independent of its defect to undergo proteolytic processing, but rather due to a missense translation that is the result of the aberrant exon 8/10 splice junction leading to S290C and an increase in A β ₄₂. Correcting the S290C change prevented the threefold increase in A β ₄₂ production (Steiner et al., 1999). Thus, the PS1dE9 variant affects APP processing towards an increased A β ₄₂ production. How the PS1dE9 mutation results in an increased production of A β ₄₂ remains unclear. A hypothesis is that this mutation results in disturbed transport of PS1 from the endoplasmic reticulum (Kim et al., 2007) and that this change in the subcellular location of PS1 may affect the ratio of A β ₄₂:A β ₄₀ formation (Section 2.3).

The PS1dE9 mutation is associated with atypical A β pathology and motor symptoms. In the cortex of several AD patients with this mutation large, loose, “cotton wool” A β deposits and few dense core plaques were detected (Crook et al., 1998; Mann et al., 2001; Smith et al., 2001). However, not all patients with the PS1dE9 mutation display this atypical pathology (Hiltunen et al., 2000; Ishii et al., 1997). In PS1dE9 transgenic mice, this atypical pathology is not observed and APP_{swe}PS1dE9 mice display characteristic neuritic plaques (Jankowsky et al., 2004).

BOX 2 Physiological functions of astrocytes

Astrocytes are involved in numerous physiological functions, including, but not limited to, maintaining molecular homeostasis of the CNS, recycling of neurotransmitters, supplying energy substrates to neurons, and maintaining the blood-brain barrier (BBB) (for a comprehensive review see Verkhratsky & Nedergaard, 2018).

Astrocyte processes are in close contact with neurons, forming the so-called tripartite synapse (Araque et al., 1999), and with the vasculature, forming the gliovascular unit (Giaume, Koulakoff, Roux, Holcman, & Rouach, 2010). Astrocytes interact with synapses, respond to signals of surrounding neurons, and can, in turn, modulate synaptic function. They can regulate synaptic transmission by buffering extracellular potassium, releasing gliotransmitters, for example, ATP, glutamate, or GABA, and taking up neurotransmitters from the extracellular environment, establishing tight and dynamic neuron–glia interactions (Shrivastava et al., 2013; Steinhäuser, Seifert, & Bedner, 2012). As part of the gliovascular unit, astrocytes form a link between the vasculature and neurons. Astroglial endfeet are involved in the regulation of BBB function, supplying nutrients, removal of toxic waste products, and maintenance of ionic balance around synapses (De Bock, Leybaert, & Giaume, 2017).

Even though astrocytes occupy individual domains (Bushong, Martone, Jones, & Ellisman, 2002), they are coupled via gap junctions (Giaume et al., 2010). Connexins (Cxs), specifically Cx30 and Cx43, are the proteins that form transmembrane hemichannels (HCs) or gap junctions between astrocytes. The intercellular gap junctions enable the diffusion of small molecules through the astrocyte network and play an important role in the metabolic support of neurons (Rouach, Koulakoff, Abudara, Willecke, & Giaume, 2008) and in potassium homeostasis (Bazzigaluppi, Weisspapir, Stefanovic, Leybaert, & Carlen, 2017; Wallraff et al., 2006). As a hemichannel, connexins also form a pore between the cytoplasm and the extracellular space (Yi et al., 2016).

Astrocytes have an active role in CNS physiology, and astrocyte dysfunction has been implicated in various CNS disorders. In AD, astrocytes undergo morphological, molecular, and functional changes, a process known as astrogliosis.

models (Elder, Gama Sosa, & De Gasperi, 2010). Around 100 models are now available to study AD pathogenesis (<https://www.alzforum.org/research-models>). Several are based on the amyloid-cascade hypothesis (APPswePS1dE9, PDAPP, J20, and 5xFAD), but other models are based on mutations in tau, Trem2, or ApoE (3xTG, Trem2 R47H knock-in, ApoE4 knock-in). The APPswePS1dE9 mouse model, which is the focus of this review, was generated in 2001 (Jankowsky et al., 2001) and has since been widely used.

3.1 | Cell-type-specific expression of the transgenes

In APPswePS1dE9 mice, the expression of the transgenes is driven by the mouse PrP promoter (Box 1, Figure 1d), which was thought to lead to a predominant neuronal expression of the transgenes (Borchelt, Davis, et al., 1996; Jankowsky et al., 2001).

However, by comparing expression levels of PrP in different cell types in the brain, the highest expression is found in astrocytes and endothelial cells, followed by neurons, oligodendrocyte progenitor cells (OPCs), and oligodendrocytes. The expression of the Prp gene in microglia is low (Zhang et al., 2014). Although it is mentioned in the original manuscript that there is MoPrP.Xho-driven transgene expression in astrocytes, it is not further discussed (Borchelt, Davis, et al., 1996). APP and PS1 are endogenously expressed by astrocytes and microglia of wild-type mice (Figure 2a; Orre, Kamphuis, Osborn, Jansen, et al., 2014; Tasic et al., 2018; Zhang et al., 2014). We observed expression of the APP and PS1 transgenes in cortical astrocytes (Figure 2b,d) and microglia (Figure 2c,e) isolated from 15- to 18-month-old APPswePS1dE9 mice (unpublished data, Willem Kamphuis and Tamar Smit). This shows that the transgenes are actively transcribed in glia, and therefore might also directly change glia function in the APPswePS1dE9 mice.

3.2 | Influence of genetic background on AD pathogenesis

The genetic background of mice can affect behavior and the timing of the formation of plaques (Hyman & Tanzi, 2019; Jackson et al., 2015; Neuner, Heuer, Huentelman, O'Connell, & Kaczorowski, 2019), and therefore should always be mentioned in manuscripts and taken into account when designing experiments. The original APPswePS1dE9 mouse was generated in F2 hybrids of C57BL/6J and C3H/HeJ (B6C3) mice. This mouse line has also been backcrossed to C57BL/6J, as this background is favorable for behavioral experiments to test memory and cognition.

The partial C3H/HeJ background is especially relevant concerning neuroinflammation and reactive gliosis research in this AD mouse model, as the C3H/HeJ mice have a mutation in the Toll-like receptor-4 gene (Tlr4). Normally, peripheral administration of the bacterial endotoxin lipopolysaccharide (LPS) induces a microglia response and reactive astrocytes. However, a spontaneous mutation in the Lps

3 | CHARACTERIZATION OF THE APPSWEPS1DE9 MOUSE MODEL

The discovery of AD-causative mutations in the APP, PS1, and PS2 genes contributed to the generation of various transgenic AD mouse

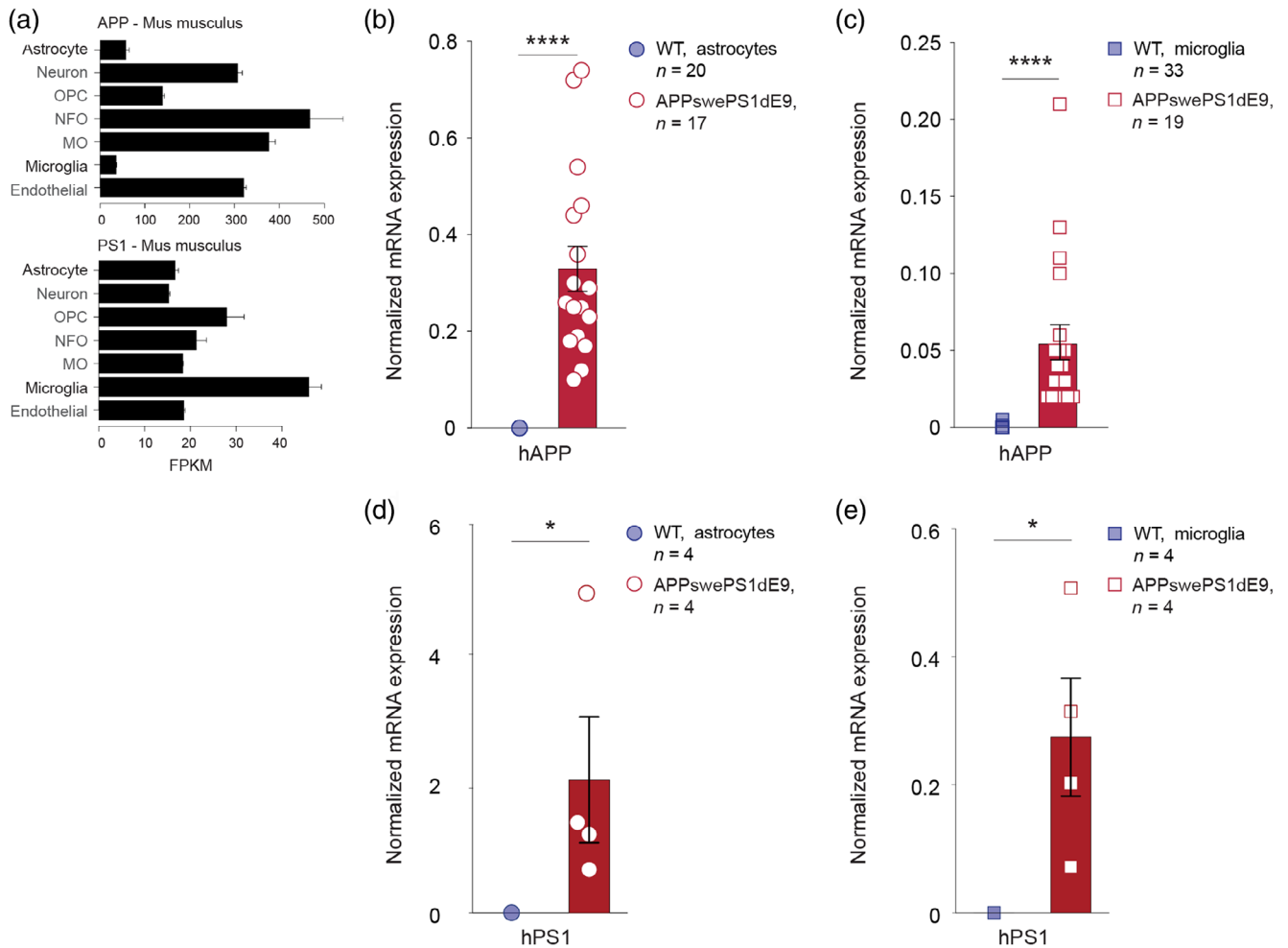


FIGURE 2 Endogenous and transgenic APP and PS1 expression in astrocytes and microglia. (a). Expression levels of endogenous APP and PS1, modified from https://web.stanford.edu/group/barres_lab/brain_rnaseq.html. Cells were isolated from the cortices of P7 mice (Zhang et al., 2014). (b,c) Cortical astrocytes and microglia were isolated from 15- to 18-month-old WT (blue) and APP^{swe}PS1^{dE9} mice (red) by FACS procedures, described in detail by Orre et al. (2014). RNA was isolated using TRIre and cDNA was generated following the manufacturer's instructions (Quantitect – Qiagen). Resulting cDNA served as a template in real-time qPCR assays (SYBR Green PCR Master Mix; Applied Biosystems), as described by Kamphuis et al. (2015). To determine expression levels of the humanized APP (hAPP) transgene the following primers were used: FW: TGAACCATTTCAACCGAGCTG and REV: GTGGGTACTCCAGAGCC. Transcript levels were normalized to HPRT and GAPDH levels. (b) Normalized mRNA expression of hAPP is higher in astrocytes of APP^{swe}PS1^{dE9} mice ($n = 17$ mice) compared to WT ($n = 20$ mice, the level of hAPP was below the limit of detection in 17 samples). (c) Normalized mRNA expression of hAPP is higher in microglia of APP^{swe}PS1^{dE9} mice ($n = 19$ mice) compared to WT ($n = 33$ mice, the level of hAPP was below the limit of detection in 29 samples). (d,e) Cortical astrocytes and microglia were isolated from 4-month-old wild-type (blue) and APP^{swe}PS1^{dE9} mice (red) by MACS procedures, adapted from protocol by Orre, Kamphuis, Osborn, Melief, et al. (2014). To determine expression levels of the hPS1 transgene the following primers were used: FW: GAGGACAACCACCTGAGCAA and REV: ATCTTGCTCCACCACCTGC. Transcript levels were normalized to HPRT and GAPDH levels. (d) Normalized mRNA expression of hPS1 is higher in astrocytes of APP^{swe}PS1^{dE9} mice ($n = 4$ mice) compared to WT ($n = 4$ mice, the level of hPS1 was below the limit of detection in three samples). (e) Normalized mRNA expression of hPS1 is higher in microglia of APP^{swe}PS1^{dE9} mice ($n = 4$ mice) compared to WT ($n = 4$ mice, the level of hPS1 was below the limit of detection in three samples). FACS, fluorescence-activated cell sorting; FPKM, fragments per kilobase million; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hAPP, humanized amyloid precursor protein; HPRT, hypoxanthine phosphoribosyltransferase; hPS1, human presenilin 1; MACS, magnetic-activated cell sorting; MO, myelinating oligodendrocyte; NFO, newly formed oligodendrocyte; OPC, oligodendrocyte progenitor cell; PS1, presenilin 1; WT, wild-type. * $p < .05$, **** $p < .0001$, Mann-Whitney test

locus (defective LPS response: Lps^{d/d}) makes C3H/HeJ mice resistant to LPS (Barber, Perera, & Vogel, 1995; Poltorak et al., 1998). C57BL/6J mice do not have this mutation (Lps^{n/n} allele) and therefore have a normal LPS response. This Lps gene was later identified as the Tlr4 gene, and a single mutation in the third exon of the Tlr4 gene is

responsible for the LPS resistance. Aside from their inability to respond to LPS, C3H/HeJ mice are developmentally and immunologically normal (Poltorak et al., 1998). Maintaining APP^{swe}PS1^{dE9} mice on a B6C3 background will result in a mixed litter with different Tlr4 genotypes. Mice can be homozygous (Lps^{d/d}), heterozygous (Lps^{d/n}),

or wild-type ($Lps^{n/n}$) for the Tlr4 mutation (Tahara et al., 2006). This is of importance for AD research, as TLR4 ($lps^{d/d}$) mediates the A β -induced activation of microglia. Indeed microglia cultures of C3H/HeJ mice exposed to A β show a reduced production of nitrite and IL-6 in comparison to microglia from wild-type C3H/HeN mice (which do not have a mutation in the TLR4 gene) (Walter et al., 2007). TLR4 signaling is involved in the clearance and uptake of A β (Tahara et al., 2006) and studies indicate that this Tlr4 mutation increases the A β load in APPswePS1dE9 mice, without affecting APP and PS1 levels (Song et al., 2011; Tahara et al., 2006). Moreover, the $Lps^{d/d}$ -APPswePS1dE9 mice are more vulnerable to cognitive deficits compared to $Lps^{n/n}$ -APPswePS1dE9 of the same age, as assessed by the Morris water maze (Song et al., 2011). Therefore, it is important to indicate the specific strain and for B6C3 mice the specific Lps genotype, when reporting results, as the background strain of APPswePS1dE9 mice affects amyloidosis, microglia activation, and cognition (Song et al., 2011; Tahara et al., 2006). Unfortunately, this information is not always specified in the scientific literature.

The Jackson Laboratory provides two strains of the APPswePS1dE9 mice, one on a mixed B6C3 background (B6C3-Tg (APPswe, PSEN1dE9)85Dbo/Mmjax; Stock No: 34829) and one on a C57BL/6J congenic background (B6.Cg-Tg(APPswe, PSEN1dE9)85Dbo/Mmjax; Stock No: 34832). For the studies selected in part 4 of this review, we indicate the background used when specified.

3.3 | Neuropathological, neurobiological, and behavioral characteristics

Transgenic mouse models of AD with a mutation only in APP are characterized by a relatively slow accumulation of A β , whereas mice with mutations in both APP and PS1 accumulate A β faster, develop plaques earlier in life, and show behavioral impairments at younger ages (Jankowsky & Zheng, 2017). APPswePS1dE9 mice develop the main pathological hallmarks observed in AD patients, including aggregation of A β in plaques, reactive astrocytes, neuroinflammation, and cognitive impairments (Malm, Koistinaho, & Kanninen, 2011; Xiong et al., 2011). In the hippocampus of 12-month-old APPswePS1dE9 mice, even tau hyperphosphorylation is increased compared to wild-type controls (Sui, Zhang, Dong, Xu, & Sun, 2019; Zhu, Xu, Sun, Zhu, & Sui, 2017). However, it is important to highlight that tau pathology in the form of neurofibrillary tangles is not observed in APPswePS1dE9 mice and other AD mouse models, except when the animals also express human mutant tau (Duyckaerts, Potier, & Delatour, 2008; Moreno-Gonzalez, Estrada, Sanchez-Mejias, & Soto, 2013).

In 3-month-old APPswePS1dE9 mice, an increase in soluble A β_{42} is detected in the posterior cerebral cortex (Van Tijn et al., 2012). Plaques are detected in 4-month-old mice in the (frontal) cortex and hippocampus (Garcia-Alloza et al., 2006; Ruan et al., 2009) or in the cortex and hippocampus of 6-month-old mice (Jankowsky et al., 2004). In general, the A β pathology and glial activation progress during aging (Garcia-Alloza et al., 2006; Kamphuis, Orre, Kooijman,

Dahmen, & Hol, 2012; Orre, Kamphuis, Osborn, Jansen, et al., 2014; Ruan et al., 2009; Wirz et al., 2013). The age at which A β plaques are detected is depending on the method used, the sex of the animals, and the genetic background. For instance, the studies by Tahara et al. (2006) and Song et al. (2011) showed that in APPswePS1dE9 mice on a mixed B6C3 background, the A β burden is increased in $LPS^{d/d}$ and $LPS^{d/n}$ animals compared to $LPS^{n/n}$ mice (Song et al., 2011; Tahara et al., 2006). Also, sex influences the A β pathology in APPswePS1dE9 mice (Jiao et al., 2016). The study by Jiao et al. (2016) shows that there are sex differences in other AD pathology markers as well. In 12-month-old female APPswePS1dE9 mice higher levels of the astroglial protein GFAP, the microglial marker CD45, and proinflammatory cytokines are found together with an increased A β burden and a lower level of synaptic markers compared to male mice (Jiao et al., 2016). These studies indicate that the genetic background and sex are important factors that should be considered when designing studies.

Plaque-associated reactive astrocytes and activated microglia are detected in APPswePS1dE9 mice from the age of 6 months (Ruan et al., 2009; Van Tijn et al., 2012; Végh et al., 2014), making it a good model to study the development of both amyloidosis and gliosis.

Electrophysiologically, the first changes are observed at the onset of A β pathology. Impaired synaptic plasticity is detected as early as 3.5 months in the cortex of APPswePS1dE9 mice (Shemer et al., 2006). In the hippocampus, no major defects in glutamatergic transmission or plasticity are found in 3-month-old APPswePS1dE9 mice. Only the induction of transient LTP is affected, in an age-independent manner (Volianskis et al., 2010). Loss of synapses or neurons could mediate impairment in synaptic plasticity. While some studies indicate a decrease in the number of presynaptic and postsynaptic terminals in the hippocampus as early as 4 months (Hong et al., 2016) or 7 months (Ding et al., 2008; Woo et al., 2015), others found no changes in synaptic density in the cortex at 3 or 6–7 months (Shemer et al., 2006; Van Tijn et al., 2012) or even an increase in the number of synaptic contacts in the CA1 region of the hippocampus of 12-month-old APPswePS1dE9 mice (West, Bach, Söderman, & Jensen, 2009).

Cognitive impairments are first observed in 3-month-old APPswePS1dE9 mice in a contextual fear memory task and in 6-month-old mice in the Morris water maze spatial memory task (Végh et al., 2014). In other studies, impairments have been found in a spatial working memory task in 4-month-old APPswePS1dE9 mice (Park et al., 2006), and 6-month-old APPswePS1dE9 mice made more mistakes and showed longer latencies in the radial arm water maze (RAWM) test compared to age-matched wild-type mice (Xiong et al., 2011). Whereas another study found no differences in spatial learning between wild-type and APPswePS1dE9 mice at the age of 7 months, the performance of APPswePS1dE9 mice in the RAWM was impaired at the age of 12–13 months (Volianskis et al., 2010). The first changes in cognition are detected by hippocampal-dependent cognitive tests, including the contextual fear memory task, of which the performance inversely correlates with regions in which A β pathology was found.



BOX 3 GFAP

Glial fibrillary acidic protein (GFAP) is the characteristic intermediate filament (IF) protein in astrocytes. Astrocytes express 10 different isoforms of GFAP, which form together with vimentin and nestin the cytoskeleton of astrocytes (Hol & Pekny, 2015; Pekny et al., 2016). GFAP expression is highly upregulated in disease. Reactive astrocytes are observed in various brain pathologies, for example, epilepsy, neurodegenerative diseases, ischemia, infection, and brain trauma. An increased GFAP expression and changed morphology are the most used markers for reactive astrocytes (Escartin, Guillemaud, & Carrillo-de Sauvage, 2019; Hol & Pekny, 2015; Pekny et al., 2016), also evident from the studies listed in Tables 1 and 2 of this review. Although some functional changes after the loss of GFAP have been described (Hughes, Maguire, McMinn, Scholz, & Sutherland, 2004; Liedtke et al., 1996), the link between increased GFAP expression and functional changes in reactive astrocytes remain largely unstudied. Even though GFAP is a valid and valuable marker for reactive astrocytes, there are some remaining concerns and limitations. GFAP-immunostainings only show the IF cytoskeleton, which is about 10% of the astrocyte surface, and therefore cannot be used to determine cellular hypertrophy. Cortical astrocytes have lower expression levels of GFAP in comparison to hippocampal astrocytes, and it remains to be determined whether these astrocytes respond with a similar GFAP increase in response to AD pathology. In conclusion, by only studying GFAP, early changes and subpopulation specific changes of reactive astrocytes are missed in AD. Alternative and AD specific reactive astrocyte markers should be determined to fully understand the pathological changes in AD astrocytes.

3.4 | Reactive astrocytes

Due to their active and crucial function in the brain, changes in astrocytes in pathological conditions are no longer considered as a non-specific or passive secondary reaction (Escartin et al., 2019). A variety of pathological stimuli can induce morphological, molecular, and functional changes in astrocytes (Escartin et al., 2019; Hol & Pekny, 2015; Pekny et al., 2016; Escartin et al., 2021). Different terms, including (astro)gliosis, astrocyte activation, reactive astrocytes, and astrocyte reactivity are used in literature to describe these astroglial changes (for a comprehensive review, Escartin et al., 2019, 2021). Detrimental effects on brain function can be mediated by the loss of homeostatic functions and/or gain of functions related to the reactive state of astrocytes (Osborn et al., 2016). Nevertheless, beneficial functions of reactive astrocytes have also been found. Reactive astrocytes form a

glial scar to restrict damage, repair the blood–brain barrier, and provide energy substrates (Buffo, Rolando, & Ceruti, 2010; Sofroniew, 2015). Preventing the reactive astrocytes-associated increase in GFAP (sometimes together with vimentin) can exacerbate inflammation and pathology associated with autoimmune encephalomyelitis (Liedtke, Edelmann, Chiu, Kucherlapati, & Raine, 1998), stroke (Li et al., 2008; Liu et al., 2014), and even A β accumulation (Kraft et al., 2013), although the latter could not be repeated by us in a similar experiment (Kamphuis et al., 2015).

Plaque-associated reactive astrocytes are detected in APPswePS1dE9 mice at the age of 6 months, based on the increased expression of GFAP (Ruan et al., 2009; Van Tijn et al., 2012; Végh et al., 2014). Despite the widespread presence of reactive astrocytes in APPswePS1dE9 mice at late stages of AD pathology, no evidence has been found for proliferation of astrocytes (Kamphuis et al., 2012). At the age of 15–18 months, isolated cortical astrocytes revealed a proinflammatory phenotype and a reduced expression of genes involved in neuronal support and communication (Orre, Kamphuis, Osborn, Jansen, et al., 2014). The expression of immunoproteasome subunits is increased in astrocytes during ageing, starting in 9- to 12-month-old APPswePS1dE9 mice (Orre et al., 2013). Elevated resting intracellular calcium levels and more frequent calcium transients were found in cortical astrocytes after the development of senile plaques (6–8 months). These astrocytic calcium transients originated near plaques, occurred synchronously over longer distances, and were uncoupled from neuronal activity (Kuchibhotla, Lattarulo, Hyman, & Bacskai, 2009). When astrocytes become reactive they enhance the release of various gliotransmitters, including GABA, glutamate, and ATP (Jo et al., 2014; Yi et al., 2016). The astroglial GABA immunoreactivity is inversely correlated with the distance from amyloid plaques (Jo et al., 2014). These findings indicate that there are clear morphological, molecular, and functional changes in reactive astrocytes in APPswePS1dE9 mice.

3.5 | Advantages and limitations

Although no animal model fully mimics the pathology and cognitive impairments seen in human AD patients, AD mouse models have been invaluable in AD research. Each AD (mouse) model has its strengths and limitations (Sasaguri et al., 2017). AD mouse models enable investigating astrocyte function in an intact system and studying behavioral changes. However, it should be taken into account that there are species differences between mouse and human astrocytes at a molecular and functional level (Oberheim et al., 2009; Zhou et al., 2020). The characterization of astrocytes acutely isolated from human brain tissue (Zhang et al., 2016) and induced pluripotent stem cell (iPSC)-derived astrocytes help to address these species differences (Mungenast, Siegert, & Tsai, 2016; Sullivan & Young-Pearse, 2017). Validation of these findings in a complete organism, to assess its effect on AD pathology and on memory and cognition, remains an essential step in therapy development for AD. This makes AD mouse models extremely valuable.

A major advantage of the APPswePS1dE9 mouse model is that it enables efficient crossing with other mice, as the transgenes were incorporated at the same chromosomal location and co-segregate as a single locus (Jankowsky et al., 2001). APPswePS1dE9 mice develop the main pathological hallmarks as observed in AD patients; age-related aggregation of A β in plaques, neuroinflammation, reactive gliosis, and cognitive impairments (Malm et al., 2011; Wirz et al., 2013; Xiong et al., 2011). Moreover, due to the expression of both APPswe and PS1dE9, the mice develop A β pathology at an early age (Borchelt et al., 1997). However, as the APPswePS1dE9 mice overexpress mutant transgenes from an early age (embryonic), it might affect brain development and function. If and how overexpression of humanized APPswe and human PS1dE9 affects endogenous APP- and/or PS1-processes beyond A β production (Sections 2.1 and 2.3) and influence AD pathogenesis remains largely unstudied. Finally, it must be noted that there are no AD patients with mutations in both APP and PS1. While the APP and PS1 mutations used in the APPswePS1dE9 mouse model are linked to FAD (Mullan et al., 1992; Perez-Tur et al., 1995), FAD accounts for <5% of all AD patients (Malm et al., 2011).

In AD patients, the earliest pathological changes are observed in the medial temporal lobe (entorhinal cortex and hippocampus; Braak & Braak, 1991). This spatial and temporal expression is not mimicked by the transgene expression that is driven by the PrP promoter. However, also in the APPswePS1dE9 mice, A β pathology is detected from an early age in the cortex and hippocampus (Garcia-Alloza et al., 2006; Ruan et al., 2009; Van Tijn et al., 2012). In AD knock-in models, in which the endogenous mouse APP gene is humanized, the target gene is under the control of its native promoter. While this leaves the temporal, spatial, and gene expression level of APP undisturbed (Guo et al., 2013; Saito et al., 2014), these models also do not exhibit tau pathology. Another potential disadvantage of these knock-in models is the less severe pathology development and minor behavioral changes (Sasaguri et al., 2017).

Variable premature death rates, between 10% and 35%, have been reported for APPswePS1dE9 mice. Although cardiomyocyte contractile dysfunction was found in APPswePS1dE9 mice, this was not linked to the mortality rate (Turdi et al., 2009). Overall, gross pathological abnormalities were not observed in these mice (Xiong et al., 2011). One study using female mice indicates that before the age of 6 months, about 35% of the APPswePS1dE9 mice kept as a hemizygote on a C57BL/6J congenic background died. Seizures might be a factor accounting for the premature death of APPswePS1dE9 mice (Minkeviciene et al., 2009; Xiong et al., 2011). Indeed, patch-clamp electrophysiology identified that a depolarized resting membrane potential contributed to the increased neuronal network excitability observed in this transgenic line (Minkeviciene et al., 2009). The mortality rate seems to be highly variable, as in our group it is around 10% for APPswePS1dE9 mice at 6 months of age (personal communication Willem Kamphuis). Whether the variable mortality rate is due to the specific background used of the

APPswePS1dE9 mice, the environment in which the mice are housed including the type of caging, noise levels, food, or social housing is not clear.

Despite its limitations, the APPswePS1dE9 mouse model is overall a good model to study amyloidosis and the development of reactive astrocytes and its consequences, which forms the focus of this review.

4 | SYSTEMATIC REVIEW: REACTIVE ASTROCYTES AS A TREATMENT TARGET IN THE APPSWEPS1DE9 MOUSE MODEL

Astrocytes display a reactive phenotype in the CNS of AD patients and APPswePS1dE9 mice (Section 3.4); meaning hypertrophic soma and a pronounced upregulation of the intermediate filament cytoskeleton. How this affects astrocyte normal function (Box 2) and astrocyte–neuron interactions is just beginning to be unraveled.

To determine how intervening with reactive astrocytes could affect AD pathogenesis and cognition, we have systematically reviewed studies on the APPswePS1dE9 mouse model in which astrocytes were targeted by either direct or indirect interventions. Besides the effect on astrocytes, we also included the effect on A β burden, microglia activation, synaptic density, and memory. When indicated in the study, we included the specific APPswePS1dE9 strain purchased, including genetic background, and which sex was used. The review is based on a PubMed search, updated last on April 2, 2021, with the following search terms: (((Alzheimer*) AND mouse model*) NOT (5xFAD OR 2xKI OR 3xTg OR Tg2576 OR TgCRND8 OR TASTPM OR ARTE10 OR EAE OR NL-F OR v7171 OR J20 OR JNPL3 OR P301L OR P301S OR PS19 OR APP23 OR PDAPP OR TAPP)) AND (reactive astrocyt* OR astrocytosis OR astrogliosis OR gliosis)) AND ("2001"[Date - Publication]: "3000"[Date - Publication]). We only included articles written in English. This search term yielded 314 research articles (Figure 3). We screened the title and abstract of these research articles and selected studies referring to an APP/PS1 model or an unspecified AD mouse model. Selecting the studies that found an effect of astrocytes and/or gliosis or used an astrocyte-specific intervention resulted in the inclusion of 101 articles. After screening the full text, 55 articles were selected that used the APPswePS1dE9 mouse model. In nine studies, astrocytes were directly targeted in APPswePS1dE9 mice either by blocking the negative functional changes of reactive astrocytes, by inducing gene expression in astrocytes, or by preventing astrocytes to become reactive (Table 1). We found 46 studies in which astrocytes were indirectly affected by various interventions. We divided these studies into five categories based on the main target of the intervention: A β , inflammation, transmitter release, neuroprotection, or environmental risk factors (Table 2). Altogether the results from the studies indicate that targeting astrocytes in AD should be included in developing novel therapies.

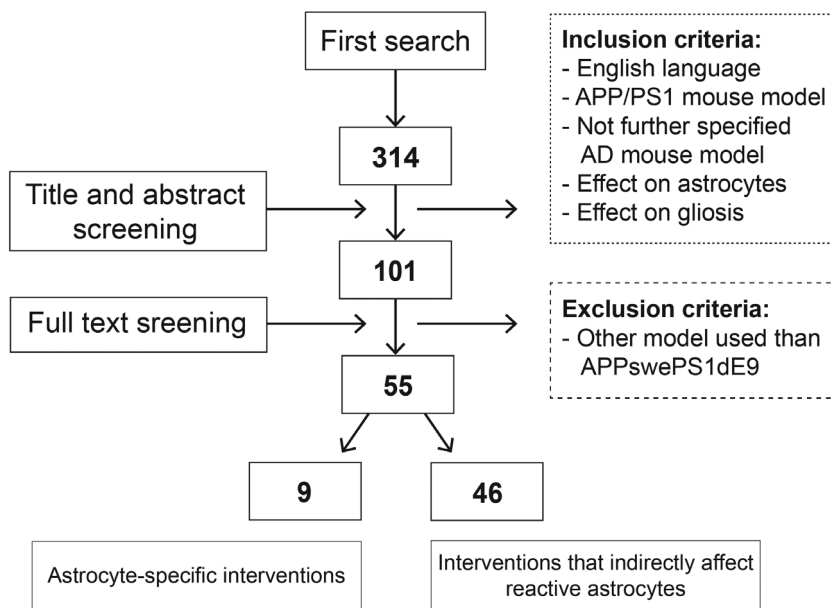


FIGURE 3 Flowchart of study selection. Refer to Section 4 for the search terms used in the first search

4.1 | Interventions directly affecting reactive astrocytes

The following studies applied various astrocyte-specific interventions in APPswePS1dE9 mice to unravel the contribution of reactive astrocytes to AD pathogenesis (Table 1). Studies with a similar approach to target reactive astrocytes are discussed together in the following sections.

4.1.1 | Intervening with connexins in reactive astrocytes

Connexin30 (Cx30) and Cx43 are the proteins that form gap junctions between astrocytes or form transmembrane hemichannels (HCs), which are pores between the cytoplasm and the extracellular milieu of astrocytes. Reactive astrocytes, that surround A β plaques, upregulate the expression of Cx30 and Cx43 (Yi et al., 2016). Regardless of their distance to plaques, the gap junction communication between APPswePS1dE9 astrocytes, however, was similar to that in wild-type astrocytes. In the hippocampus of APPswePS1dE9 mice, the HCs composed of Cx43 were chronically open, indicating that specifically the hemichannel function is affected in AD (Yi et al., 2016). Opening of the Cx43 HCs can be triggered by the presence of A β (A β -induced) inflammation, or an increased concentration of intracellular calcium ([Ca²⁺]_i) in astrocytes, inducing the release of gliotransmitters such as ATP and glutamate from reactive astrocytes (Yi et al., 2016). ATP activates P2X7 or P2Y1 receptors on microglia and astrocytes, leading to increased calcium transients and calcium waves in astrocytes, as has been shown in another AD mouse model: the APPPS1 model driven by the Thy1 promoter (Delekate et al., 2014). HC-dependent glutamate and ATP release amplify a vicious circle in astrocytes by inducing high [Ca²⁺]_i levels that trigger Cx43 HC opening contributing to a

maintenance of elevated [Ca²⁺]_i. The effect of the resulting chronic release of glutamate and ATP mediated by activated HCs can be detrimental and/or protective for neurons (Yi et al., 2016). This is shown by cell culture experiments that indicate that glutamate release can contribute to synaptic loss by activation of extrasynaptic NMDA receptors (Talanta, Sanz-Blasco, & Zhang, 2013), or mediate neuronal survival by activation of the astroglial mGluR3 (Durand et al., 2014). And, excessive ATP release can mediate a neurotoxic effect by stimulation of IL-1 β secretion by microglia (Pelegriin & Surprenant, 2009) or a neuroprotective effect by increasing the release of IL-6 (Fujita, Tozaki-Saitoh, & Inoue, 2009).

Astrocyte-specific Cx43 deficiency was obtained by crossing a GFAP-cre mouse line with a floxed-Cx43 mouse line (Ren et al., 2018; Yi et al., 2016). Astroglial Cx43 deficiency in 9-month-old APPswePS1dE9 mice resulted in a reduced release of the gliotransmitters glutamate and ATP. The release of GABA was not affected in these mice (Yi et al., 2016). This latter finding is in line with the study by Jo et al. (2014), which shows that the release of GABA is mediated by Best1 channels in astrocytes (Jo et al., 2014), and thus not by HCs.

Besides the reduced gliotransmitter release, there was a significant decrease in GFAP levels in the cortical and hippocampal astrocytes of 12-month-old Cx43-deficient APPswePS1dE9 mice, as compared to APPswePS1dE9 astrocytes. The decrease in GFAP was not caused by a decrease in A β plaque load, as this was similar in both mouse strains (Ren et al., 2018). The Cx43 deficiency also led to a reduction in neuronal damage, as shown by a decrease in oxidative stress and neuritic dystrophy in the hippocampus (Yi et al., 2016), and an increase in dendritic spine density and postsynaptic density 95 (PSD95) protein level (Ren et al., 2018). The Cx43 deficient APPswePS1dE9 mice had a better spatial and nonspatial memory compared to APPswePS1dE9 mice. Re-expression of Cx43 by a viral vector in 7-month-old mice resulted in reactive astrocytes and aggravated synaptic function assessed 2 months later (Ren et al., 2018).

TABLE 1 Astrocyte-specific interventions in APPswePS1dE9 mice

Intervention	Target	Age	Sex	Background	Amyloid-beta	Astrocytes	Microglia	Synaptic density	Memory	References
Gfap-Cx43 ^{-/-} - APPswePS1dE9 ^a	Cx43	9 mo	F/M	-	-	ATP release↓ (ATPite) Glutamate release↓ (CE-LIF) GABA release→ (CE-LIF)	-	Hip: RTN-3 dystrophic neurites↓ (IHC)	-	Yi et al. (2016)
Gfap-Cx43 ^{-/-} - APPswePS1dE9 ^b	Cx43	12 mo	-	BALB/c × C57BL/6J × 129SvJ SVEvBrd-Hprt ^{b-m2}	Cx and Hip: Aβ number→ (IHC, 6E10)	Cx and Hip: GFAP number↓ (IHC) GFAP, GLAST and MEGF10↓ (qPCR and WB)	-	Hip: Syn→, PSD95↓ (WB)	MWM↓ NOR↑	Ren, Zhang, and Wang (2018)
GFAP-IE3-APPswePS1dE9 ^c Induced from birth	ApoE	6 mo ¹ or 9 mo ²	F/M	C57BL/6J × C3H/HeJ	Cx: (in)soluble Aβ ₄₀ , Aβ ₄₂ → (ELISA) Cx: Aβ→ (IHC)	Cx and Hip: GFAP↑ ² (IHC)	Cx and Hip: Iba1↑ ² (IHC)	Cx: Syn→ ² , PSD95↓ ² (WB)	-	Liu et al. (2017)
GFAP-IE4-APPswePS1dE9 ^c Induced from birth ^{1,2} or 6 mo ³	ApoE	6 mo ¹ , 9 mo ² or 9 mo ³	F/M	C57BL/6J × C3H/HeJ	Cx: (in)soluble Aβ ₄₀ , Aβ ₄₂ ↑ ^{1,2} (ELISA) Cx: (in)soluble Aβ ₄₀ , Aβ ₄₂ → ³ (ELISA) Cx and Hip: Aβ↑ ^{1,2} (IHC) Cx and Hip: Aβ→ ³ (IHC)	Cx and Hip: GFAP↑ ^{1,2} Cx and Hip: GFAP→ ³ (IHC)	Cx and Hip: Iba1↑ ² (IHC)	Cx: Syn→ ² , PSD95→ (WB)	-	Liu et al. (2017)
AAV-GFAP104-DTR injection in hippocampus at 10–12 mo	Oxidative stress	11–13 mo	F/M	C57BL/6J	-	Hip: GFAP intensity↓ (IHC)	Hip: Iba1 area↓ (IHC)	NeuN number↓ (IHC)	NPR↓	Chun et al. (2020)
Intracerebroventricular injection of AAV-GFAP-CLU at 2 d	Clusterin	8 mo	F/M	C57BL/6J × C3H/HeJ	Cx: (in)soluble Aβ ₄₀ , Aβ ₄₂ ↓ (ELISA) Cx and Hip: Aβ area↓ (IHC, X- 34)	Cx: GFAP intensity↓ (IHC) Cx: GFAP↓ (qPCR)	Cx: Iba1 intensity↓ (IHC)	Cx and Hip: Dystrophic neurites↓ (IHC)	-	Wojtas et al. (2020)
AAV-gfaABC1D-SOCS3 injection in hippocampus at 3–4 mo ¹ or injection at 15 mo ²	STAT3	9–10 mo ¹ or 16 mo ²	M	C57BL/6J × C3H/HeJ	Hip: Aβ number↑ ¹ (IHC, BAM10) Hip: Aβ ₄₀ , Aβ ₄₂ → ¹ (ELISA)	Hip: GFAP intensity↓ (IHC and WB) Vim intensity↑ ¹ (IHC)	Hip: Iba1 number per plaque→ ¹ (IHC)	-	MWM↑ ¹	Ceyzériat et al. (2018)
Cx43-STAT3 ^{-/-} - APPswePS1dE9 ^b Induced from 6 wk	STAT3	8 mo	F/M	C57BL/6N	Aβ area↓ (IHC, IC16, and ThS) Soluble Aβ ₄₀ , Aβ ₄₂ ↓ (ELISA)	Cx and Hip: GFAP area→ (IHC) Peri-plaque astroglial volume↓	Cx and Hip: Iba1 area→ (IHC) Number of microglia branches and junctions↑	-	MWM↓	Reichenbach et al. (2019)
APPswePS1dE9 ^c - GFAP ^{-/-} -vimentin ^{-/-}	Intermediate filament proteins	4 mo ¹ , 8 mo ² and 12 mo ³	-	B6C3 × C57BL × 129SV × 129Ola	Cx and Hip: Aβ area↑ ^{2,3} (IHC, HJ3.4 and X-34) Cx: insoluble Aβ ₄₀ , Aβ ₄₂ → ¹ Cx: insoluble Aβ ₄₀ ↑ ³ , Aβ ₄₂ ↓ ² (ELISA)	Plaque-astrocyte overlap↑ ³ GFAP number→ ³ (IHC)	Plaque-microglia overlap↑ Iba1 number↑ ² (IHC) Iba1↑ ³ (qPCR)	ECx: RTN-3↑ ³ (IHC)	-	Kraft et al. (2013)
APPswePS1dE9 ^c - GFAP ^{-/-} -vimentin ^{-/-} and APPswePS1dE9 ^b -GFAP ^{-/-}	Intermediate filament protein(s)	6 mo, 9 mo, and 15 mo	-	C57BL/6 × 129SV × 129Ola	Aβ area→ (IHC, 6E10)	Plaque-astrocyte overlap↓ Vim area↑ ² (IHC)	Iba1 number→ (IHC)	-	-	Kamphuis et al. (2015)

Abbreviations: General—Specified interventions or ages indicated with ^{1–3}. When not indicated: same effect for all interventions or ages. When a specific group is not indicated: read-out was not determined for that group; ↓, Increased or improved (Memory) compared to control of same age; ↑, decreased or impaired (Memory); →, no (significant) difference; -, not studied; d, day(s); mo, month(s); wk, week(s). Intervention and Target—Different APPswePS1dE9 strains indicated with ^{a–c}. APPswePS1dE9 mice, specific strain not indicated; ^bMMRRC Stock No: 34832-JAX-C57BL/6J; ^cMMRRC Stock No: 34829-JAX-C57BL/6J × C3H/HeJ. AAV, adeno-associated virus-based vectors; ApoE, apolipoprotein E; CLU, clusterin; Cx43, Connexin43; DTR, diphtheria toxin receptor; gfaABC1D, promoter to mediate astrocyte-specific expression; GFAP, glial fibrillary acidic protein; IE3, inducible human ApoE3 expression; IE4, inducible human ApoE4 expression; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3. Age—at read-out. Sex—Female (F), Male (M), or both (F/M). -, not indicated. Background: Genetic background of mice used in the study. X, Crossed with *Brain Regions*—Cx, cortex; ECx, entorhinal cortex; Hip, hippocampus. Techniques—ATPite, Luciferin-luciferase bioluminescence assay; CE-LIF, capillary electrophoresis with laser-induced fluorescence; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; MWM, Morris water maze; NOR, novel object recognition; NPR, novel place recognition; qPCR, quantitative polymerase chain reaction; WB, western blot. Genes, proteins, and transmitters—6E10, Aβ_{1–16} antibody; Aβ, amyloid-beta; ATP, adenosine triphosphate; BAM10, Aβ_{1–40} antibody; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; GLAST, glutamate-aspartate transporter; HJ3.4, Aβ_{1–13} antibody; Iba1, ionized calcium-binding adapter molecule 1; IC16, Aβ_{1–16} antibody; MEGF10, multiple EGF-like domains 10; PSD95, postsynaptic density protein 95; RTN-3, reticulon-3, marker of neurotrophic dystrophy; Syn, synaptophysin; ThS, thioflavin-S; Vim, vimentin; X-34 dye, fluorescent amyloid-specific dye.



Thus, both studies support that astroglial connexins, that is, Cx43 in particular, are potential therapeutic molecular targets to prevent reactive astrocytes and synaptic degeneration in AD (Ren et al., 2018; Yi et al., 2016).

4.1.2 | Inducible expression of human Apolipoprotein E isoforms in astrocytes

The lipid/cholesterol carrier apolipoprotein E (ApoE) is coded by the polymorphic ApoE gene. The gene has three major alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. Genetically, the ApoE gene is a well-established AD risk locus. The presence of 1 or 2 $\epsilon 4$ alleles strongly increases the risk to develop late-onset AD (Esquerda-Canals et al., 2017). ApoE is mainly expressed in glia, with the highest expression levels in astrocytes and microglia, followed by oligodendrocyte precursors. The expression in neurons is low (Zhang et al., 2014).

To determine at which age ApoE has the most impact on A β pathology, Liu et al. (2017) developed APPswePS1dE9 mice with inducible expression of human ApoE3 or ApoE4 in astrocytes. They generated a mouse line with cre-dependent and doxycycline regulated ApoE3 or ApoE4 expression. Crossing with a GFAP-cre mouse line resulted in astrocyte-specific, inducible ApoE expression. ApoE3 expression in APPswePS1dE9 mice at any stage of A β pathology had no effect on insoluble A β levels and the A β plaque development, but led to decreased levels of cortical and hippocampal GFAP, implying that ApoE3 reduces A β -induced reactive astrocytes. APPswePS1dE9-ApoE3 mice also had elevated levels of PSD95, suggesting that ApoE3 can prevent synaptic impairment. In contrast, ApoE4 expressed at 0–6 months revealed a critical role of ApoE4 before and during the onset of A β pathology. During this stage, astroglial expression of ApoE4 significantly increased insoluble A β levels and A β plaque burden, impaired hippocampal A β clearance, and induced reactive gliosis in the cortex and hippocampus (i.e., increased GFAP and microglial Iba1 levels). Notably, inducing ApoE4 expression later in life, when A β plaques are present (6–9 months), barely affected amyloidosis (Liu et al., 2017). Surprisingly, crossing the original version of the APPswePS1dE9 mice, resulting from the crossing of the APPswe line C3-3 and the PSdE9 line S-9, with a mouse line expressing human ApoE4 driven by the mouse prion protein promoter did not enhance A β pathology at 6 months (Lesuisse et al., 2001).

These findings show that the astrocyte-specific expression of ApoE3 or ApoE4 impact A β pathology and astrogliosis differently and indicate that the expression of ApoE isoforms in astrocytes is a potential therapeutic target to modulate reactive astrogliosis and synaptic degeneration in AD.

4.1.3 | Increasing reactive astrocytes by oxidative stress

Oxidative stress plays an important role in the pathogenesis of AD (Bhatia & Sharma, 2021). Chun et al. (2020) studied whether the

severity of reactive astrocytes could affect other hallmarks of AD. In this study, the diphtheria toxin receptor (DTR) was expressed in astrocytes by injecting AAVs with DTR driven by the GFAP104 promoter in the CA1 region of the hippocampus of 10- to 12-month-old APPswePS1dE9 mice. Administration of diphtheria toxin (DT) in these mice induced astroglial hydrogen peroxide (H₂O₂) production and resulted in an increase of reactive astrocytes, based on GFAP expression. Furthermore, it reduced the expression of NeuN, and increased expression of cleaved caspase-3, suggesting the induction of neurodegeneration. Moreover, the severe reactive astrocytes in astrocyte-specific DTR-expressing APPswePS1dE9 mice impaired spike probability compared to APPswePS1dE9 mice (Chun et al., 2020). Whether this was due to synaptic degeneration and/or increased tonic inhibition by the severe reactive astrocytes, as indicated by a previous study (Jo et al., 2014; Section 4.2), was not determined. The DTR-expressing APPswePS1dE9 mice showed impaired spatial memory assessed by the novel place recognition test, known as a hippocampal memory task. The effect on A β burden was not assessed in this study (Chun et al., 2020).

Taken together, the administration of DT induced severe reactive astrocytes, triggered neurodegeneration, and impaired spatial memory in DTR-expressing APPswePS1dE9 mice. This study indicates that reducing oxidative stress could reduce reactive astrocytes, presenting a therapeutic strategy for AD.

4.1.4 | Reducing reactive astrocytes by overexpression of clusterin

Clusterin (CLU), also known as apolipoprotein J (APOJ), is a multifunctional glycoprotein, involved in heterogeneous processes, including lipid homeostasis, complement inhibition, and apoptosis (Foster, Dangla-Valls, Lovestone, Ribe, & Buckley, 2019). Large-scale genome-wide association studies have identified an association of common polymorphisms within the CLU gene with the risk of developing AD (Lambert et al., 2013). CLU could form complexes with A β , thereby influencing its solubility and preventing amyloid fibril formation.

Astrocytes are a major source of CLU expression in the brain (Zhang et al., 2016). Wojtas et al., 2020 investigated the impact of overexpression of astroglial CLU on AD pathology. An AAV vector expressing CLU under the control of the GFAP promoter was used to induce a ~30% overexpression of CLU in astrocytes in 8-month-old APPswePS1dE9 mice. This CLU overexpression ameliorates amyloid pathology and reduced the number of dystrophic neurites in the cortex and hippocampus of APPswePS1dE9 mice. Also, gliosis, assessed by GFAP and Iba1 immunostaining, was reduced in 8-month-old AAV-CLU-APPswePS1dE9 mice compared to APPswePS1dE9 mice injected with control AAV-GFP vectors. These results indicate that the overexpression of CLU in astrocytes impacts amyloid burden and associated pathologies and offers a novel therapeutic molecular target to reduce reactive astrocytes and synaptic degeneration in AD (Wojtas et al., 2020).

4.1.5 | Reducing reactive astrocytes by disrupting or inhibiting the JAK2/STAT3 pathway

Reactive astrocytes can be directly induced by activating the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway (Ceyzériat et al., 2018). The JAK/STAT3 pathway is activated in astrocytes of adult APPswePS1dE9 mice (8 months) (Haim et al., 2015). Binding of several cytokines and growth factors to their receptors activates JAK2, results in the phosphorylation and nuclear translocation of STAT3. Nuclear STAT3 induces the transcription of multiple target genes, including GFAP (Ceyzériat et al., 2018), the classical marker for reactive astrocytes.

Inhibition of the JAK2/STAT3 pathway via adeno-associated virus-based vectors (AAV)-induced overexpression of Suppressor of Cytokine Signaling 3 (SOCS3) in the hippocampus of 3-month-old APPswePS1dE9 mice prevented the upregulation of GFAP and A β plaque load assessed 6 months later (Ceyzériat et al., 2018). In this experiment, SOCS3 was driven by the gfaABC₁D promoter, which makes the expression astrocyte-specific. The SOCS3-mediated inhibition of JAK2/STAT3 normalized the expression of multiple reactive astrocyte markers, including the expression of cytokines and complement factors in 9-month-old APPswePS1dE9 mice. Moreover, inhibition of STAT3 improved spatial learning in APPswePS1dE9 mice, as assessed by the Morris water maze test. Vice versa, stimulation of the JAK2/STAT3 pathway induced reactive astrocytes in wild-type mice and enhanced reactive astrogliosis in APPswePS1dE9 mice. More A β plaques were present in APPswePS1dE9 mice with an enhanced astrocyte reactivity (Ceyzériat et al., 2018). However, this study is in contrast with previous studies in which astrocyte reactivity was reduced by GFAP and vimentin deficiency (see next section).

Astrocyte-specific STAT3 deficiency can be obtained by crossing a tamoxifen-inducible Cx43-Cre mouse line with a floxed-STAT3 mouse line. Deletion of STAT3 in astrocytes from an early age, that is, 6 weeks, resulted in a reduced A β burden at the age of 8 months and an increase in internalization of A β in microglia (Reichenbach et al., 2019). While Ceyzériat et al., 2018 observed reduced GFAP immunoreactivity, this conditional STAT3 deficiency in astrocytes did not change the overall coverage of astrocytes and microglia (Reichenbach et al., 2019). Around plaques, even an increase in astrocyte volume was found, which is a characteristic of reactive astrocytes. The morphology of microglia near plaques was affected, displaying a higher number of branches and longer processes. It was suggested that STAT3 deletion triggers a switch in astrocytes from a neurotoxic to a protective astrocyte phenotype, based on the expression of specific markers (including Amigo2, C3, and Tm4sf1). Moreover, STAT3 deletion in astrocytes reduced the astrocyte calcium hyperactivity, and induced an improvement in spatial learning and memory. The application of a preclinical systemic STAT3 inhibitor for 6 weeks also protected 8-month-old APPswePS1dE9 mice from cognitive decline (Reichenbach et al., 2019). These results show that reactive astrocytes contribute to AD pathogenesis and that blocking the JAK/STAT3 pathway in astrocytes, therefore, could offer a novel

therapeutic strategy for AD (Ceyzériat et al., 2018; Reichenbach et al., 2019).

4.1.6 | Reducing reactive astrocytes by deleting intermediate filament proteins

Upregulation of the IF proteins GFAP and vimentin is a well-known characteristic of reactive astrogliosis, the functional alterations associated with it, however, remain up to now largely unknown. The response of astrocytes to A β seems dependent on the presence of GFAP, as astrocytes of GFAP-deficient ($^{-/-}$) mice failed to form a barrier-like structure around an A β plaque in the hippocampus, as shown in slice cultures (Xu, Malouf, Messing, & Silver, 1999). By comparing the transcriptome of cortical astrocytes of 15- to 18-month-old APPswePS1dE9-GFAP $^{-/-}$, APPswePS1dE9-GFAP $^{-/-}$ -vimentin $^{-/-}$, and control APPswePS1dE9 mice, Kamphuis et al. (2015) discovered that the transcriptional response of reactive astrocytes was different in astrocytes with and without GFAP and vimentin. Astrocytes of GFAP- and GFAP-vimentin-deficient APPswePS1dE9 mice displayed an aggravated inflammatory response and the decrease of neuronal support genes, as shown earlier (Orre, Kamphuis, Osborn, Jansen, et al., 2014), was absent. Whether this indicates a deteriorated or improved inflammatory status is still unclear, as the changed genes include pro-inflammatory and anti-inflammatory genes. No differences in A β plaque load were found between both IF-deficient and APPswePS1dE9 mice with IFs in mice of 6, 9, and 15 months of age, indicating that the IFs themselves do not play a prominent role in amyloidosis (Kamphuis et al., 2015). These results are in contrast with earlier findings of Kraft et al., 2013, which showed an increase in plaque load and insoluble A β in 8- and 12-month-old APPswePS1dE9-GFAP $^{-/-}$ -vimentin $^{-/-}$ mice, without an effect on the expression or processing of APP (Kraft et al., 2013). They found that the number of astrocytes was unaffected, but the overlap between astrocyte processes and amyloid plaques was reduced after deletion of IF proteins (which is also seen in the study of Kamphuis et al.), whereas microglia number and overlap with plaques were increased. These results suggest that reactive astrocytes are needed to limit plaque growth (Kraft et al., 2013). Discrepancies between the studies can be caused by the different age of animals used in the studies, the mixed backgrounds of the IF-deficient animals, or different staining techniques used to visualize A β plaques. Kamphuis et al., 2015 kept the animals at a genetic background of predominantly C57BL/6 with a variable contribution of 129Sv and 129Ola while Kraft et al., 2013 kept them on a mixed B6C3, C57BL/6, 129Sv, and 129Ola background (Kamphuis et al., 2015; Kraft et al., 2013). Discrepancies with the study by Ceyzériat et al., 2018, can be explained by the different interventions used. In contrast to the inhibition of the JAK2/STAT3 pathway to prevent the upregulation of GFAP (Ceyzériat et al., 2018), the crossings by Kraft et al., 2013 and Kamphuis et al., 2015 resulted in the complete removal of GFAP and vimentin. Additional factors that might contribute to the differences are the genetic background of the mouse lines and the timing of the intervention, as disruption of



the JAK/STAT3 pathway was only induced from the age of 3–4 months (Ceyzeriat et al., 2018), in contrast to a complete deficiency of GFAP (Kamphuis et al., 2015; Kraft et al., 2013).

In summary, although IF-deficiencies affect the transcriptional response of reactive astrocytes (Kamphuis et al., 2015), contradicting findings have been reported on the effect on the A β burden (Kamphuis et al., 2015; Kraft et al., 2013). How background strain and pathology stage exactly affect this outcome is still unknown. Moreover, the effect of IF-deficiency on cognitive decline in the APPswePS1dE9 still needs to be established.

4.2 | Interventions indirectly affecting reactive astrocytes

Reactive astrocytes were also indirectly affected by various intervention studies, which we categorized on the main target of their intervention, A β , inflammation, transmitter release, neuroprotection, or environmental risk factors. In the following sections, we give a short overview of the findings of these studies, which are listed in Table 2.

4.2.1 | Amyloid-beta

To reduce the A β load or prevent the formation of A β in APPswePS1dE9 mice, many different strategies have been used over the years, such as A β immunization (Qu et al., 2007; Wang et al., 2016), stimulation of A β clearance (Bernstein et al., 2014; Gao et al., 2020; Koronyo-Hamaoui et al., 2020; Luo et al., 2020), prevention of A β oligomerization (Tao et al., 2020), and modulation of APP cleavage enzymes (Mori et al., 2012, 2020; Prikhodko et al., 2020). Besides reducing the A β load, almost all of these interventions also reduced reactive astrogliosis based on GFAP expression and, when studied, showed improved memory of the APPswePS1dE9 mice (Table 2). In one study, however, the intervention to stimulate A β clearance resulted in an upregulation of GFAP around A β plaques. Also, LC3, an autophagy-related protein, was increased in these astrocytes. These findings suggest that the intervention recruited astrocytes to plaques and enhanced A β clearance via autophagy by astrocytes (Luo et al., 2020).

4.2.2 | Inflammation

Expectedly, many interventions that also affected reactive astrocytes were aimed at the modulation of inflammation, for example through inhibiting NF- κ B (Malm et al., 2007; Zhang et al., 2009), interfering with chemokine binding (Sui et al., 2019; Zhu et al., 2017), or cross-breeding the APPswePS1dE9 mice with mice that were deficient in genes that led to an altered inflammatory response (Lee et al., 2016; Manocha et al., 2017). In the majority of studies, reducing inflammation also decreased the expression of GFAP, and in some studies also led to a decrease in the A β load. In the studies in which also the

cognitive function was studied, an improvement of memory was found in the studies that report on reduced reactive astrogliosis.

In three studies, however, the inflammation-targeted interventions led to an upregulation of GFAP (Button et al., 2019; Jin et al., 2008; Lee et al., 2016). For two of these studies, this was expected considering the genotypes of the mice that were generated by cross-breeding with APPswePS1dE9 mice, the LPS^{d/d}-APPswePS1dE9 mice (Jin et al., 2008) and the ApoA-I deficient APPswePS1dE9 mice (Button et al., 2019). The LPS^{d/d} genotype is known to enhance AD pathology in APPswePS1dE9 mice (see 3.2, Tahara et al., 2006; Song et al., 2011) and ApoA-I, a primary component of high-density lipoprotein (HDL) complex, is proposed to have a protective role in AD pathogenesis (Button et al., 2019). In the third study, the transient receptor potential cation channel, subfamily A1 (TRPA1)-deficient APPswePS1dE9 mice were investigated. In TRPA1 deficient APPswePS1dE9 mice a reduction of A β load, inflammatory cytokine secretion, and improved cognition were found. Even though GFAP was increased in TRPA1-deficient mice, a reduced expression of IL-1 β , IL-6, and IL-4 in astrocytes was detected. These findings indicate that despite an increased GFAP expression, astroglial inflammation was reduced. However, as TRPA1 is expressed in endothelial cells, neurons, and astrocytes, it remains unclear whether it was the TRPA1 deficiency in astrocytes in APPswePS1dE9 mice that contributed to the reduced inflammatory response and improvement in learning and memory.

Astrocytes are known to closely interact with microglia; both cells are involved in reactive gliosis in AD. The studies by Zhu et al. (2017) and Sui et al. (2019) highlight the role of microglia in AD pathogenesis. They used a C-C chemokine receptor type 3 (CCR3) antagonist and CCR3-deficient APPswePS1dE9 mice to target microglia, which led to a reduced GFAP expression and A β load and an improved memory (Sui et al., 2019; Zhu et al., 2017).

4.2.3 | Transmitter release

The study by Yi et al. (2017) emphasizes the important role of hemichannel Cx43 function (Section 4.1; Yi et al., 2016; Ren et al., 2018). They used Boldine, which has been shown in *in vitro* experiments to block HCs and not gap junctions. Boldine administration to 6-month-old APPswePS1dE9 mice for 3 months, via drinking water, reduced the release of ATP and glutamate, without affecting the release of D-serine or GABA. Although not specifically mentioned, these transmitters can either be released from astrocytes (Araque et al., 2014; Volterra & Meldolesi, 2005) or neurons. A β load was unaffected and the number of dystrophic neurites was reduced (Yi et al., 2017). The studies by Jo et al. (2014) and Park et al. (2019) also focus on the role of transmitter release by astrocytes, specifically GABA. Both studies used MAO-B inhibitors and showed a reduction in GABA production and release by astrocytes. Neuronal plasticity was increased and the inhibitors prevented memory impairment in APPswePS1dE9 mice (Jo et al., 2014; Park et al., 2019). It is important to note, that the drugs used in these three studies are not astrocyte-



TABLE 2 Interventions in APPswE9 mice indirectly affecting reactive astrocytes

Intervention	Target	Age	Sex	Background	Amyloid-beta	Astrocytes	Microglia	Synaptic density	Memory	Reference
Amyloid-beta										
Monthly immunization targeting Aβ ₄₂ from 3 mo	Aβ ₄₂	15-16 mo	-	C57BL/6J × C3H/HeJ	Cx and Hip: Aβ ₄₂ area _↓ (IHC) Aβ ₄₂ (ELISA)	Cx and Hip: GFAP _↑ (IHC)	-	-	-	Qu et al. (2007)
Daily oral administration of Tannic acid from 6 mo	β-secretase	12 mo	F/M	C57BL/6J × C3H/HeJ [†]	Cx, ECx, and Hip: Aβ number and size _↓ (IHC, 4G8) Wb: Aβ ₄₀ , Aβ ₄₂ (ELISA)	Cx, ECx, and Hip: GFAP area _↓ (IHC)	CC, ECx, and Hip: Iba1 area _↓ (IHC)	-	NOR [†] MWM [†]	Mori et al. (2012)
c-fms-ACE ^{10/10} , APPswE9 ^{1dE9c}	Aβ ₄₂	5 mo ¹ , 7 mo ² , 8.5 mo ³ , 11-12 mo ⁴ or 13 mo ⁵	F/M	C57BL/6	Cx and Hip: Aβ ³ (IHC, 6E10 and ThS) Soluble Aβ ₄₀ ² Soluble Aβ ₄₂ ^{1,2,5} (ELISA)	GFAP area and number ^{2,5} (IHC)	-	-	BM ^{†4}	Bernstein et al. (2014)
Weekly injection with low (L) or high (H) doses of IVIG ¹ , Oil-Nabs ² or Blue-Nabs ³	Aβ ₄₂ oligomers	8-9 mo	M	C57BL/6J	Aβ ^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42} (IHC, 6E10) Insoluble Aβ ₄₀ ^{3H, 4, 1, 2, 3A, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42} (IHC) Soluble Aβ ₄₀ ^{1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42} (ELISA)	Cx: GFAP ^{2,3H, 4, 1, 3L} Hip: GFAP area ^{2, 4, 1, 3L} (IHC)	Cx and Hip: Iba1 ^{1, 2, 3H, 4, 1, 3L} (IHC)	Syn ^{1, 2H, 3H, 4, 1, 2L, 3L} (WB)	MWM ^{1, 2L, 3H, 4, 1, 2H, 3L} NOR [†]	Wang et al. (2016)
Bone marrow transplantation of WT ¹ or c-fms-ACE ^{10/10.2} mice at 2 mo	Aβ ₄₂	9 mo	F/M	C57BL/6J	Cx: Aβ area ² (IHC, 6E10) Cx: Soluble Aβ ₄₂ ² (ELISA)	Cx: GFAP area ² , number _↓ (IHC)	-	-	-	Koronyo-Hamaoui et al. (2020)
Oral administration of BPN-15606 from 3 mo ¹ or 6 mo ²	γ-secretase	6 mo ¹ or 9 mo ²	F	C57BL/6J × C3H/HeJ	Cx: Aβ number and size ¹ (IHC, 82E1) Hip: Aβ size ¹ Cx and Hip: Aβ ² (IHC) Insoluble Aβ ₃₈ , Aβ ₄₀ and Aβ ₄₂ ² Soluble Aβ ₃₈ , Aβ ₄₀ ² and Aβ ₄₂ ^{1, 2} (ELISA)	Cx and Hip: GFAP area and size ² Cx and Hip: Iba1 area and number ^{1, 2} (IHC)	Cx and Hip: Iba1 area and number ^{1, 2} (IHC)	-	MWM ^{1, 2}	Prikhodko et al. (2020)
Injection of IDOL ASO at 3 mo and 6 mo	Aβ	8-9 mo	M	C57BL/6J	Aβ area _↓ (IHC, 82E1 and X-34) (Insoluble Aβ ₄₀ , Aβ ₄₂ (ELISA)	GFAP area _↓ (IHC)	Iba1 intensity ² Iba1 soma size _↓ (IHC)	-	MWM [†] Contextual and cued FC ²	Gao et al. (2020)
Ad libitum access to Gemfibrozil ¹ or Wy14643 ² via drinking water	Aβ	8-10 mo	F/M	-	Aβ _↓ (IHC, 4G8) Cx and Hip: (insoluble) Aβ ₄₀ , Aβ ₄₂ (ELISA)	Cx and Hip: GFAP [†] (IHC and WB)	Cx and Hip: Iba1 [†] (IHC and WB)	Cx and Hip: Syn and PSD95 [†] (WB)	MWM [†] Open Field test [†]	Luo et al. (2020)
Gal-3 ^{+/+} → APPswE9 ^{1dE9c}	Aβ	7 mo	F/M	C57BL/6J × 129/Sv × SJL	Hip: Aβ _↓ (IHC) Aβ oligomers _↓ (WB)	Hip: GFAP _↓ (IHC)	Hip: Iba1 _↓ (IHC)	-	MWM [†]	Tao et al. (2020)
Daily oral intake of Gallic Acid from 12 mo	α/β-secretase	18 mo	M	C57BL/6J	Cx, ECx, and Hip: Aβ area and number _↓ (IHC, 4G8)	Cx, ECx, and Hip: GFAP area _↓ (IHC)	Cx, ECx, and Hip: Iba1 area _↓ (IHC)	-	NOR [†] RAWM [†] YM [†]	Mori et al. (2020)
Inflammation										
Daily oral intake of PDC from 9 mo	NF-κB	16 mo	-	C57BL/6J × C3H/HeJ [†]	Cx: Aβ area ² (IHC, pan-Aβ, and ThS) Hip: (insoluble) Aβ ₄₀ , Aβ ₄₂ ² (ELISA)	Cx: GFAP intensity ² (IHC) Cx: GLT-1 [†] (WB)	Cx: CD11b ² Cx: CD45 intensity ² (IHC)	-	MWM [†]	Malm et al. (2007)

(Continues)



TABLE 2 (Continued)

Intervention	Target	Age	Sex	Background	Amyloid-beta	Astrocytes	Microglia	Synaptic density	Memory	Reference
<i>LPS</i> ^{d/d} -APPswPS1dE9	Innate immune response	13–15 mo	-	C57BL/6J × C3H/HeJ	-	Cx: GFAP area [↑] (IHC) GFAP [↑] (WB)	Cx: CD11b area [↑] (IHC) CD11b [→] (WB) CD45 [→] (IHC and WB)	-	-	Jin, Kim, Maxwell, Li, and Fukuchi (2008)
Daily injection of PDTC	NF-κB	7–12 mo	-	C57BL/6J × C3H/HeJ	Cx and Hip: Aβ ₄₀ [→] , Aβ ₄₂ [↑] (ELISA)	Cx and Hip: GFAP [↓] (IHC and WB)	-	-	-	Zhang et al. (2009)
Daily injection of Valproic acid from 7 mo	Inflammation/neuroprotection	8 mo	-	C57BL/6J × C3H/HeJ	Cx and Hip: Aβ area and number [↓] (IHC, 4G8) Soluble Aβ ₄₀ , Aβ ₄₂ [↓] (ELISA)	Hip: GFAP number [↓] (IHC)	Hip: Iba1 number [↓] (IHC)	-	MWM [↑]	Xuan et al. (2015)
Immunization with GA ¹ , Mo ^{BM2} or GA and Mo ^{BM3} from 10 mo	Myelin-derived antigens/inflammation	12 mo	M	C57BL/6J	Cx and Hip: Aβ area [↓] (IHC, 4G8, and Th5) Aβ ₄₀ , Aβ ₄₂ [↓] (ELISA)	Cx: GFAP area and number [↓] (IHC)	-	Hip: VGLUT1 [↑] (IHC)	BM [↓]	Koronyo et al. (2015)
TRPA1 ^{-/-} -APPswPS1dE9 ^c	Inflammation/Calcium signaling	8 mo	-	C57BL/6J	Aβ oligomers, monomers [↓] (WB)	GFAP [↑] (WB)	-	-	MWM [↑] YM [↑]	Lee et al. (2016)
NFATc2 ^{-/-} -APPswPS1dE9 ^c	Cytokines	8 mo	M	C57BL/6J × 129X1/SvJ	Cx: Aβ intensity [→] (IHC, 4G8) and Campbell-Switzer staining	Cx: GFAP intensity [↓] (IHC)	Cx: Iba1 [→] and CD68 intensity [↓] (IHC)	Hip: PSD95 [↓] and Syn [→] (WB)	-	Manocha et al. (2017)
Early postnatal antibiotic treatment for 1 wk from P14	Inflammation	6.5 mo	M	C57BL/6J × C3H/HeJ	Aβ area [↓] (IHC, 3D6) (Insoluble Aβ ₄₀ , Aβ ₄₂ [→] (ELISA)	GFAP number [↓] (IHC)	Iba1 number [↓] (IHC)	-	-	Minter et al. (2017)
Daily administration of <i>Humululus Japonicus</i> from 5 mo	Oxidative stress/inflammation	7.5 mo	F/M	C57BL/6J × C3H/HeJ	Cx: Aβ area [↓] (IHC, BAM10)	Cx: GFAP area [↓] (IHC)	Cx: Iba1 number [↓] (IHC)	-	NOR [↑] YM [↑]	Park et al. (2017)
CCR3 ^{-/-} -APPswPS1dE9 ^b	CCR3	12 mo	M	C57BL/6J × -	Cx and Hip: Aβ number [↓] (IHC, 6E10)	Cx and Hip: GFAP number [↓] (IHC)	Cx and Hip: Iba1 number [↓] (IHC)	Hip: Drebrin and PSD95 [↓] (WB) Hip: Synapse number [↓] (EM)	MWM [↑]	Zhu et al. (2017)
Weekly immunization with anti-CD49d ¹ or IgG control ² from 9 mo	Proinflammatory microglia	10 mo	F	C57BL/6J	Cx: (Insoluble Aβ ₄₀ , Aβ ₄₂ [→] (WB) Wb: Aβ [→] (IHC, 4G8)	Cx: GFAP ^{1, →2} (WB) Wb: GFAP ^{1, →2} (IHC)	Wb: CD68 and Iba1 [↓] (IHC)	Cx: Syn [→] and PSD95 [↓] (WB)	-	Manocha, Ghatak, Puig, and Combs (2018)
ApoA-1 ^{-/-} -APPswPS1dE9 ^c KO was compared to hemizygous group	ApoA-1	12 mo	F/M	C57BL/6J	Cx: Aβ [↓] (IHC) Hip: Aβ [→] (IHC) Hemisphere: (Insoluble Aβ ₄₀ , Aβ ₄₂ [→] (ELISA)	Cx and Hip: GFAP area [↓] (IHC)	-	-	Contextual and cued FC [→]	Button et al. (2019)
Daily oral administration of UA for 13 d from 7 mo	Inflammation	7.5 mo	F	C57BL/6J × C3H/HeJ	Cx and Hip: Aβ ₄₀ , Aβ ₄₂ number and area [↓] (IHC)	Cx and Hip: GFAP number [↓] (IHC)	Cx and Hip: Iba1 number [↓] (IHC)	-	MWM [↑]	Gong et al. (2019)
Two daily injections of YM344031 from 10 mo	CCR3	12 mo	M	C57BL/6J × C3H/HeJ	Hip and NCx: Aβ number [↓] (IHC)	Cx: GFAP number [↓] (IHC)	Cx: Iba1 number [↓] (IHC)	Hip: Syn, drebrin and PSD95 [↓] (WB)	MWM [↑]	Sui et al. (2019)
pMCAO ¹ or sham-operated ² daily injection of ONO-8713 for 14 d from 4.5 mo	EP1 receptor	5 mo	M	C57BL/6J × C3H/HeJ	Cx: Aβ ₄₀ , Aβ ₄₂ [→] (ELISA)	Cx: GFAP [→] Hip: GFAP ^{↓2, →1} (IHC)	Cx: Iba1 ^{↓1, →2} Hip: Iba1 [→] (IHC)	-	PAT ^{1, →2}	Mendes et al. (2020)

TABLE 2 (Continued)

Intervention	Target	Age	Sex	Background	Amyloid-beta	Astrocytes	Microglia	Synaptic density	Memory	Reference
Weekly injection of GA from 20 mo	Myelin-derived antigens/Inflammation	22 mo	F/M	C57BL/6J	Cx, ECx, and Hip: Aβ area _↓ (IHC, 6E10)	Cx, ECx, and Hip: GFAP area _↓ (IHC)	Cx, ECx, and Hip: Iba1 area _↓ (IHC)	ECx: PSD95 _↑ Hip and Wb: PSD95 _→ (IHC) Syn _↑ (MS)	BM _↑	Doustar et al. (2020)
<i>Transmitter release</i>										
Daily oral intake of selegiline for 3 d ¹ , 1 wk ² or 3 wk ³ from 10 mo	MAO-B	10–12 mo	F/M	C57BL/6J and C3H/HeJ	Aβ _→ ² (WB)	GABA within GFAP positive area _↓ ¹ (IHC)	-	-	MWM _→ ³ PAT _↑ ²	Jo et al. (2014)
Boldine in drinking water from 6 mo	Hemichannel	9 mo	F/M	C57BL/6J	Cx and Hip: Aβ area _→ (IHC)	GFAP _→ (WB) D-serine and GABA release _→ ATP and glutamate release _↓ (CE-LIF)	Iba1 _→ (WB)	Hip: RTN-3 dystrophic neurites _↓ (IHC)	-	Yi et al. (2017)
Daily oral intake of selegiline for 3 d ¹ , 4 wk ² or KDS2010 from 8 mo ³	MAO-B	8 mo ¹ , 9 mo ² , or 13 mo ³	F/M	C57BL/6J X C3H/HeJ	-	GABA within GFAP positive area _↓ ^{1,3} , _→ ² GFAP _↓ ³ (IHC)	-	-	PAT _↑ ^{1,3} , _→ ² MWM _↑ ³	Park et al. (2019)
<i>Neuroprotection</i>										
Daily injection of Tetrahydroperforin (IDN5706) from 12 mo	Inflammation, oxidative activity, and monoamines/neurotransmitter uptake	13 mo	M	C57BL/6J X C3H/HeJ	Hip: Aβ area _→ (IHC, Aβ ₁₋₁₇) Aβ area _↓ (IHC, ThS)	Hip: GFAP number _→ Hip: GFAP intensity soma and soma size _↓ (IHC)	-	-	MWM _↑	Cerpa et al. (2010)
Daily administration of peroxisome proliferator WY ¹ or 4-PB ² via drinking water from 7 mo	Peroxisomes	9 mo	F/M	C57BL/6J X C3H/HeJ	Aβ area _↓ (IHC, 4G8 and ThS)	Cx: GFAP area _→ ¹ , _↓ ² Hip: GFAP area _↓ (IHC)	Cx: CD11b area _→ ¹ , _↓ ² Hip: CD11b area _↓ (IHC)	Hip: Syn, VAMP1/2 and VGlut1 _→ GluR2, PSD95 and NR2B _↑ (WB)	MWM _↑ NOR _→	Inestrosa et al. (2013)
Biweekly injection of recombinant NOIs vaccine from 2 to 3 mo	Neurite outgrowth	5 mo	F/M	C57BL/6J	Cx and Hip: Aβ area and number _↓ (IHC, 6E10) Hemisphere: Aβ _↓ (WB, 6E10)	Cx and Hip: GFAP area _↓ (IHC)	-	-	MWM _↑	Zhang et al. (2013)
Endo-B1 ^{-/-} -APPsweP1dE9 ^c	Apoptosis, autophagy, and mitochondrial function	6 mo ¹ or 12 mo ²	M	C57BL/6J	Cx and DG: Aβ area and number _↑ ¹ (IHC, 6E10) Soluble Aβ ₄₀ , Aβ ₄₂ ¹ (Luminex assay)	Cx and Hip: GFAP area _↑ ¹ (IHC)	-	CA3: Syn _↓ (IHC)	MWM _↓	Wang et al. (2015)
Coflin ^{+/-} -APPsweP51dE9 ^a	Filamentous actin	7 mo	F/M	- X 129 5/SvEv ^{Brd} X C57BL/6J	-	Hip: GFAP intensity _↓ (IHC)	-	Hip: PSD95 and Syn intensity _↑ (IHC)	Contextual FC _↓ Cued FC _→	Woo et al. (2015)
Perinatal choline-supplemented diet	Choline	6 mo ¹ , 9 mo ² or 12 mo ³	F/M	C57BL/6J X C3H/HeJ	Hip: Aβ40, Aβ42 area _↓ ^{2,3} Hip: Aβ40, Aβ42 number _↓ ^{2,3} (IHC) Soluble Aβ _{1-2F,3M} , _→ ^{3F,2M} (WB, 6E10) Soluble Aβ ₄₀ , Aβ ₄₂ _→ ^{1,2M,3F} , _↓ ^{2F,3M} (ELISA)	GFAP _↓ ^{2F} , _→ ^{3F,2M,3M} (WB)	-	-	-	Mellott et al. (2017)

(Continues)



TABLE 2 (Continued)

Intervention	Target	Age	Sex	Background	Amyloid-beta	Astrocytes	Microglia	Synaptic density	Memory	Reference
Daily injection of ASS234 from 10 wk	AChE/MAO	4.5 mo	M	-	Cx: A β Hip: A β \leftrightarrow (IHC, ThS)	Cx: GFAP number \downarrow (IHC)	Cx: Iba1 number \downarrow (IHC)	-	-	Serrano et al. (2017)
Daily injection of liraglutide ¹ or palm ¹¹ -PrRP31 ² from 7 to 8 mo	GLP-1	9–10 mo	M	C57BL/6J	Cx: A β area \leftrightarrow Hip: A β area \rightarrow , \downarrow ² (IHC)	Cx: GFAP area \downarrow Hip: GFAP area \rightarrow , \downarrow ² (IHC)	Cx: Iba1 area \leftrightarrow Hip: Iba1 area \rightarrow , \downarrow ² (IHC)	Hip: Syn ^{1,2} (WB)	-	Holubová et al. (2019)
Pyk2 ^{-/-} -APPswePS1dE9 ^c	Pyk2	12 mo	F/M	C57BL/6J	Hip: A β area \leftrightarrow (IHC)	Hip: GFAP area \downarrow (IHC)	Hip: Iba1 area \leftrightarrow (IHC)	DG: PSD95 and SV2A area \uparrow (IHC)	MWM \uparrow NOR \uparrow PAT \uparrow	Salazar et al. (2019)
Oral administration of WBQ5187 from 6 mo	Multiple targets	9 mo	M	C57BL/6J	A β area \downarrow (IHC) Soluble A β ₄₀ , A β ₄₂ \downarrow (ELISA)	GFAP \downarrow (IHC)	Iba1 \downarrow (IHC)	-	MWM \uparrow	Wang et al. (2019)
Daily injections of liraglutide ¹ or DA-JC1 ² from 10 to 12 mo	GLP-1	11–13 mo	F	C57BL/6J x C3H/HeJ	CA1, Cx, and DG: A β area \downarrow (IHC)	Hip: GFAP area \downarrow , \rightarrow ¹ (IHC)	Hip: Iba1 area \downarrow (IHC)	-	-	Salles et al. (2020)
Daily leptin injection for 1 wk from 3 mo ¹ or 12 mo ²	Neurogenesis	3 mo or 12 mo	M	C57BL/6J	Hip: A β number \downarrow (IHC, 6E10)	Hip: Gfap \rightarrow , \downarrow ² (qPCR) SGZ: BrdU*GFAP \rightarrow , \downarrow ² SVZ: BrdU*GFAP \rightarrow (IHC)	Hip: Iba1 number \downarrow (IHC)	-	-	Cañó et al. (2021)
Environmental risk factors										
Restraint stress for 21 d, 6 hrs a day from 4 mo	Stress	5 mo	M	C57BL/6J	(In)soluble A β ₄₀ , A β ₄₂ \leftrightarrow (ELISA)	Cx and Hip: GFAP area \uparrow (IHC)	-	-	-	Perez-Nievas et al. (2011)
Five days a week: Low ¹ or high ² dose cigarette smoke exposure from 3 mo	Smoking	7 mo	F/M	C57BL/6J x C3H/HeJ	Cx: A β \rightarrow , \downarrow ² Hip: A β area \downarrow Cx and Hip: A β number \rightarrow , \downarrow ² (IHC, 4G8) Cx and Hip: A β area \rightarrow , \downarrow ² Subiculum: A β area \leftrightarrow (IHC, ThS)	Cx and Hip: GFAP area \rightarrow , \downarrow ² (IHC) \downarrow ² (IHC)	Cx and Hip: Iba1 area \rightarrow , \downarrow ² (IHC)	-	-	Moreno-Gonzalez et al. (2013)
Voluntary wheel running from 5 mo	Exercise	7.5 mo	-	C57BL/6J x C3H/HeJ	Cx: A β area \leftrightarrow (IHC, 4G8, and ThS) Cx: A β number \downarrow (IHC, ThS) Hip: A β area \downarrow (IHC, A11, 4G8 and ThS) Hip: A β number \downarrow (IHC, ThS)	Cx and Hip: GFAP intensity \downarrow (IHC)	-	MWM \uparrow	-	Tapia-Rojas, Aranguiz, Varela-Nallar, and Inestrosa (2016)
Social housing: Social isolation ¹ Social contact with one ² or social contact with five ³ from 6 mo	Social interaction	12 mo	M	C57BL/6J	Hip: A β area and number \leftrightarrow (IHC, ThS and 6E10) A β ₄₀ , A β ₄₂ \leftrightarrow (ELISA)	Hip: GFAP number \uparrow ³ (IHC)	-	Syn, SNAP-25, PSD95 and GluN2B \uparrow , ³ GluA2 and GluN2A \leftrightarrow (WB) Hip: Dendritic spine density \rightarrow , \downarrow ³ (IHC)	MWM \rightarrow , \downarrow ³	Liang, Yang, Zhang, and Hao (2019)

TABLE 2 (Continued)

Intervention	Target	Age	Sex	Background	Amyloid-beta	Astrocytes	Microglia	Synaptic density	Memory	Reference
Early life stress by limited nesting and bedding from P2-9	Early life stress	4 mo ¹ or 10 mo ²	M	C57BL/6J	-	ECx and Hip: GFAP area \rightarrow (IHC) Hip: <i>Aldh1l1</i> , <i>Aqp4</i> , <i>GFAP</i> , <i>GLAST</i> , <i>GLT1</i> , <i>GluS</i> , <i>Vim</i> \rightarrow , <i>Fosn1</i> ¹ (qPCR)	-	-	-	Abbink et al. (2020)

Abbreviations: **General**—Specified interventions or ages indicated with ¹⁻⁵. When not indicated: the same effect for all interventions or ages. When a specific group is not indicated: read-out was not determined for that group; \uparrow , increased or improved (Memory) compared to the control group of the same age; \downarrow , decreased or impaired (Memory); \rightarrow , no (significant) difference; \rightarrow , not studied; d, day(s); mo, month(s); wk, week(s). **Intervention and target**—Different APPswPS1dE9 strains indicated with ^{a-c}. **c**—APPswPS1dE9 mice, specific strain not indicated; ¹MMRRC Stock No: 34829-JAX-C57BL/6J \times C3H/HeJ; ²MMRRC Stock No: 34832-JAX-C57BL/6J \times C57BL/6J. **4-PB**, 4-phenylbutyrate: activates peroxisome proliferation; AChE, acetylcholinesterase; ApoA-1, apolipoprotein A1; primary component of high-density lipoproteins; ASS234, multipotent acetyl and butyrylcholinesterase/monoamine oxidase A-B inhibitor; Blue-Nabs, naturally occurring autoantibodies against A β oligomers purified by Cibacron Blue; Boidine, inhibitor of hemichannel activity; BPN-15606, γ -secretase modulator; CCR3, C-C chemokine receptor 3; CD49d, antibody against α 4-integrin; c-fms-ACE^{10/10}, mouse line overexpressing angiotensin-converting enzyme (ACE) under the control of the c-fms promoter; resulting in myelomonocytic lineage cells; Cofilin, filamentous-actin-severing protein; DA-JC1, Glucagon-like peptide-1 (GLP-1)/glucagon-dependent insulinotropic polypeptide dual agonist; Endo-B1, endophilin-B1 (Bif-1; Bax-interacting factor 1); GA, Glatiramer acetate: weak agonist of myelin-derived antigens. Gal-3, Galactin-3; A β oligomerization and A β toxicity; Gemfibrozil, peroxisome proliferator-activated receptor agonist; GLP-1, glucagon-like peptide-1; IDOL ASO, inducible degrader of low-density lipoprotein receptor (IDOL) antisense oligonucleotide (ASO); IVIG, intravenous immunoglobulin; KDS2020, reversible monoamine oxidase-B inhibitor; Liraglutide, glucagon-like peptide-1 (GLP-1) receptor analogue; LPS^{d/d}, mouse line expressing a spontaneous mutation in the lipopolysaccharide (LPS) locus: resulting in a defective LPS response; MAO-A-B, monoamine oxidase-A and B; Mo^{shw}, bone marrow-derived CD115⁺ monocytes; NFATc2, nuclear factor of activated T cells, isoform c2; NO1, neurite outgrowth inhibitor; Oli-Nabs, naturally occurring autoantibodies against A β oligomers purified by A β ₄₂ oligomers; ONO-8713, Selective prostaglandin receptor 1 (EP1) antagonist; Palm-PrRP, prolactin-release peptide (PrRP) palmitoylated at the N-terminus; pdMCAO, permanent middle cerebral artery occlusion; PDTC, pyrrolidine dithiocarbamate; NF- κ B, nuclear factor- κ B inhibitor; Pyk2, (PTK2B) Protein tyrosine kinase 2; Selegiline, monoamine oxidase-B inhibitor; Tannic acid, anti-amyloidogenic polyphenol (flavonoid); TRPA1, transient receptor potential ankyrin 1; cation channel; UA, urolithin A; gut-microbial metabolite of ellagic acid; WBQ5187, Quinolone-benzofuran derivative; WT, wild-type; WY, WY-14643 peroxisome proliferator-activated receptor agonist; activators of peroxisome proliferators receptors agonist; YM344031, C-C chemokine receptor 3 antagonist. Age — at read-out. Sex — female (F), male (M), or both (F/M). —, not indicated. **Background**: Genetic background of mice used in the study. **x**, Crossed with ¹MMRRC Stock No: 34829-JAX-C57BL/6J \times C3H/HeJ crossed with C57BL/6J. **Brain regions**—CA, cornu ammonis; CC, cingulate cortex; Cx, cortex; DG, dentate gyrus; ECx, entorhinal cortex; Hip, Hippocampus; NCx, neocortex; SGZ, subgranular zone of the hippocampal dentate gyrus; SVZ, subventricular zone; Wb, Whole brain. **Techniques**—BM, Barnes Maze; BrdU, 5-bromo-2'-deoxyuridine; CE-LIF, capillary electrophoresis with laser-induced fluorescence; ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy; FC, fear conditioning; IHC, immunohistochemistry; MS, mass spectrometry; MWMM, Morris water maze; NOR, novel object recognition; PAT, passive avoidance test; RAWMI, radial arm water maze; qPCR, quantitative polymerase chain reaction; WB, western blot; YM, Y-Maze test. **Genes, proteins, and transmitters**—3D6, A β ₁₋₅ antibody; 4G8, A β ₁₇₋₂₄ antibody; 6E10, A β ₁₋₁₆ antibody; 82E1, Soluble and fibrillar A β ; A11, oligomer antibody; A β , amyloid-beta; Aldh1l1, 10-formyltetrahydrofolate dehydrogenase; Aqp4, aquaporin-4; ATP, adenosine triphosphate; BAM10, A β ₁₋₄₀ antibody; CD, cluster of differentiation molecule; Fasn, fatty acid synthase; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; GLAST, glutamate-aspartate transporter; GLT-1, glutamate transporter 1; GluA2, glutamate receptor, ionotropic, AMPA2 (alpha 2); GluN2A, glutamate receptor, ionotropic, NMDA2A (epsilon 1); GluN2B, glutamate receptor, ionotropic, NMDA2B (epsilon 2); GluR2, glutamate receptor 2; GluS, glutamate synthetase; Iba1, ionized calcium-binding adapter molecule 1; NR2B, N-methyl D-aspartate receptor subtype 2B; PSD95, postsynaptic density protein 95; RTN-3, reticulon-3, marker of neuritic dystrophy; SNAP-25, synaptosomal nerve-associated protein 25; SV2A, synaptic vesicle glycoprotein 2A; Syn, synaptophysin; ThS, thioflavin-S; VAMP1, vesicle-associated membrane protein 1; VGlut, vesicular glutamate transporter; Vim, Vimentin; X-34 dye, fluorescent amyloid-specific dye.



specific and thus a contribution of neurons or microglia has to be considered (Jo et al., 2014; Park et al., 2019; Yi et al., 2017).

4.2.4 | Neuroprotection

Because AD is a progressive neurodegenerative disease, various interventions have been targeted at neuroprotection in APPswePS1dE9 mice. These interventions included choline supplementation (Mellott et al., 2017), drugs targeting peroxisomes (Abbott, Toledo, Aranguiz, Inestrosa, & Varela-Nallar, 2013), and crossing APPswePS1dE9 mice with mice that are lacking a neuroprotective protein (Wang et al., 2015) or late-onset AD risk factor Pyk2 (Salazar et al., 2019). All studies categorized in the neuroprotective group, except one, showed a reduction of GFAP expression and A β burden, and some also showed an improvement in memory function (when examined), in response to the neuroprotective intervention.

Crossbreeding of Endophilin-B1 (Endo-B1) deficient mice with APPswePS1dE9 mice resulted in the upregulation of GFAP, an increase in A β burden, aggravation of synaptic degeneration, and behavioral impairment compared to APPswePS1dE9 mice (Wang et al., 2015). Endo-B1 (also known as Bax-interacting factor 1) has multiple functions, including the regulation of apoptosis, autophagy, and mitochondrial function. Endo-B1 is neuroprotective, as over-expression of Endo-B1 in neuronal cultures protected against A β -induced apoptosis and mitochondrial dysfunction (Wang et al., 2014). In cortical tissue of AD patients and 10-month-old APPswePS1dE9 mice, Endo-B1 protein levels are decreased compared to controls (Wang et al., 2015).

Other neuroprotective interventions were aimed at the cholinergic network, modulated oxidative stress, or targeted the AD risk factor Pyk2. The basal forebrain cholinergic system is vulnerable to degeneration in AD patients (Hampel et al., 2018; Whitehouse et al., 1982). Even though the number of cholinergic neurons in the forebrain remained unchanged, dystrophic cholinergic neurites were found in the cortex, hippocampus, and striatum of APPswePS1dE9 mice (Perez, Dar, Ikonovic, DeKosky, & Mufson, 2007). The use of perinatal choline supplementation prevented the reduction of the cholinergic marker choline acetyltransferase in 9- and 12-month-old APPswePS1dE9 mice. It also reduced amyloidosis and the GFAP levels specifically in 9-month-old female APPswePS1dE9 mice (Mellott et al., 2017).

One of the mechanisms by which A β induces neurotoxicity is oxidative stress (Inestrosa et al., 2013). Reducing oxidative stress by applying a semi-synthetic derivative of the St. John's Wort (Cerpa et al., 2010) or a drug that enhances peroxisomal proliferation (Inestrosa et al., 2013) both resulted in an increased synaptic density and improved cognitive function in APPswePS1dE9 mice. These interventions also reduced the A β burden and GFAP levels (Cerpa et al., 2010; Inestrosa et al., 2013). Vice versa, increasing reactive astrocytes by oxidative stress in astrocyte-specific DTR expressing APPswePS1dE9 mice resulted in neurodegeneration

and impaired spatial memory compared to APPswePS1dE9 mice (Chun et al., 2020).

The kinase Pyk2 (PTK2B) has been implicated as a late-onset AD risk factor, and is primarily expressed by neurons. Pyk2-deficiency in APPswePS1dE9 prevented synapse loss and improved cognitive functioning compared to 12-month-old APPswePS1dE9 mice. Also, the A β burden and GFAP levels were reduced in Pyk2-deficient mice (Salazar et al., 2019).

4.2.5 | Environmental risk factors

Environmental factors can either be protective, such as exercise and social interaction, or a risk factor for developing AD, such as smoking, stress, and social isolation. Voluntary wheel running reduced GFAP and A β levels (Tapia-Rojas et al., 2016), whereas exposure to cigarette smoke or restraint stress increased GFAP expression in the APPswePS1dE9 mice (Moreno-Gonzalez et al., 2013; Perez-Nievas et al., 2011). Liang et al., 2019 performed a social housing experiment with APPswePS1dE9 mice, which showed that enhancing social interaction with one or multiple cage mates, improved cognitive function, but also increased the expression of GFAP without affecting A β burden (Liang et al., 2019).

To conclude, the majority of interventions reduced both A β burden and GFAP expression, although some studies showed that these could be independently affected. When the studies also included cognitive testing, an improvement in cognitive function was detected in most studies. As most interventions summarized in Table 2 were aimed to reduce A β load, inflammation, or neuronal damage, the expression of GFAP could be affected in various ways. It is important to note that these studies impact reactive astrocytes indirectly and they were not designed to determine causality between reactive astrogliosis and AD pathogenesis. Therefore, conclusions should be made with caution.

Although GFAP expression is a valuable, but limited readout for reactive astrocytes, in almost all intervention studies (listed in Table 2) except five (Abbink et al., 2020; Jo et al., 2014; Malm et al., 2007; Park et al., 2019; Yi et al., 2017), GFAP expression was used to determine the effect on reactive astrocytes. Jo et al. (2014) and Park et al. (2019) showed a reduced GABA release as a readout to show the effect of their intervention on reactive astrocytes, and Yi et al. (2017) showed a reduction in the release of the gliotransmitters glutamate and ATP. Malm et al. (2007) found an increase in the level of astroglial GLT-1 after inhibiting NF- κ B, although GFAP levels did not change. Abbink et al., 2020 found an increase in Fasn expression, without changes in other astrocyte-related genes after early life stress exposure in APPswePS1dE9 mice (Abbink et al., 2020). Even though studies using alternative markers for reactive astrocytes are limited, this indicates that (functional) changes can be induced in reactive astrocytes by interventions, in some cases without affecting the expression of GFAP (Abbink et al., 2020; Malm et al., 2007; Yi et al., 2017). The causality and underlying mechanisms that mediate a positive effect on A β burden, reactive astrocytes, and cognitive

performance require further investigation. An astrocyte-specific approach to unravel the contribution of astrocytes in the interventions listed in Table 2 is needed to further explore astrocytes as a therapeutic target to treat AD patients.

5 | DISCUSSION

In this review, we have listed direct and indirect interventions that attenuate the occurrence of reactive astrocytes in the APPswePS1dE9 AD mouse model. Considering their crucial role in proper brain functioning, the functional changes associated with the reactive state of astrocytes can have a direct impact on synapse function and neuronal circuit activity, potentially contributing to cognitive problems in AD (Chung et al., 2015; Kuchibhotla et al., 2008; Ortinski et al., 2010; Osborn et al., 2016). Classically, most studies investigating the changes in reactive astrocytes focus on GFAP expression, rather than on functional changes.

An increased GFAP expression and changed morphology are the most often used markers for reactive astrocytes (Escartin et al., 2019; Liddelov et al., 2017), also evident from the studies listed in Tables 1 and 2. However, it remains unclear whether an increase in GFAP expression is an appropriate marker for all reactive astrocytes. Moreover, this focus on GFAP expression and morphological changes in astrocytes have so far not been sufficient to reveal what happens in the early stages of AD pathology. While an increase in soluble A β ₄₂ is detected in 3-month-old APPswePS1dE9 mice (Van Tijn et al., 2012) and plaques in 4-month-old animals (Garcia-Alloza et al., 2006; Ruan et al., 2009), reactive GFAP positive astrocytes around amyloid deposits are first detected at 6 months of age in APPswePS1dE9 mice (Ruan et al., 2009; Van Tijn et al., 2012; Végh et al., 2014). Whether the early increase in A β oligomers is sufficient to induce reactive astrocytes, and whether GFAP upregulation is a sensitive marker for the early and subtle functional changes in astrocytes is unknown.

To determine the contribution of reactive astrocytes to AD pathogenesis, it is necessary to unravel the effect of their functional changes to synaptic dysfunction and/or loss and eventually dementia. The consequences of disturbances in astroglial function, including but not limited to the release of gliotransmitters, uptake of neurotransmitters, ion buffering, and BBB maintenance (Osborn et al., 2016; Verkhratsky & Nedergaard, 2018) are largely unstudied in AD. There is an emerging interest in astrocyte function and more tools are becoming available to study this using *in vivo*, *ex vivo*, and *in vitro* systems (Almad & Maragakis, 2018). Astrocyte-specific techniques are being developed to manipulate astrocyte function and to study neuron–glia interactions. Optogenetics and chemogenetics enable the manipulation of astrocytes (Aguilhon et al., 2013; Mederos et al., 2018; Xie, Yang, Song, Quan, & Qing, 2019). Genetically encoded calcium indicators and sensors for transmitters allow for the functional measurement in astrocytes (Almad & Maragakis, 2018). The field is relatively young, and in recent years, an increase in studies focusing on astrocyte heterogeneity, function, and astrocyte–neuron interactions (Escartin et al., 2019) is observed. This will undoubtedly

lead to more insight in astrocyte function in health and disease, which is essential to understand the full spectrum of AD pathogenesis.

To develop astrocyte-specific and well-timed interventions, changes in astroglial function at different stages of the disease need to be determined. This information will enable astrocyte-directed therapies to be effective in reversing the astrocyte-induced damage or allow for repair and/or recovery. When the damage is too severe, it might be irreversible. As more therapeutics will be targeted towards the earlier stages of AD, identifying pathological changes in astrocyte function in the early stages of pathology is important. This should coincide with the development of accurate biomarkers for specific disease stages to make the treatment of AD patients possible.

Transcriptomics and proteomics are important tools to find changes in astrocytes at the gene or protein level (Pestana, Edwards-Faret, Belgard, Martirosyan, & Holt, 2020; Westergard & Rothstein, 2020). The A1 and A2 profiles identifying neuroinflammation and ischemia-induced subtypes of reactive astrocytes, respectively (Liddelov et al., 2017), are a good start of this endeavor. However, limitations of this classification have also been revealed, as aged astrocytes take on a A1-like neuroinflammatory phenotype (Clarke et al., 2018) and reactive astrocytes co-expressing A1 and A2 genes are observed in APPswePS1dE9 mice, the MCAO model, and the aging mouse brain (Clarke et al., 2018; Orre, Kamphuis, Osborn, Jansen, et al., 2014). These findings indicate that it may be difficult to define classes of reactive astrocytes with only a few genes. Recent single-cell RNAseq approaches are likely to reveal more and more disease and cell-type-specific profiles. A recent single-cell RNA sequencing study identified five transcriptomically distinct types of astrocytes in the hippocampus and cortex of adult mice (Batiuk et al., 2020). Still little is known about the effect of the molecular and functional heterogeneous subtypes of astrocytes and reactive astrocytes and their contribution to AD pathogenesis. Using hippocampal astrocytes of 7-month-old 5xFAD AD mice, Habib et al., 2020 identified a disease-associated astrocyte (DAA) population (Habib et al., 2020). Not all genes in the DAA profile are unique for AD-associated astrocytes as the profile shared a significant number of upregulated genes compared with the Gfap-high population, a population also present in wild-type mice. It remains to be determined whether these markers for reactive astrocytes are universal for different AD mouse models, pathology stages, and brain regions studied.

Overall, state-of-the-art sequencing approaches will discover novel disease- and subtype-specific profiles, which also need to be validated in AD patient material. These profiles will help to identify specific pathways that can be targeted with the use of cell-specific tools and enable the development of astrocyte-specific therapies. Connecting gene and protein changes to functional changes will be the first step in the development of astrocyte- and pathway-specific AD therapies.

6 | CONCLUSION

Reactive astrocytes are present in the APPswePS1dE9 mice when plaques become apparent, coinciding with cognitive decline (Ruan



et al., 2009; Van Tijn et al., 2012; Végh et al., 2014). Still little is known about the effect of the functional changes associated with their reactive state and their contribution to AD pathogenesis. Specific treatment windows and astrocyte-specific interventions need to be further developed. The few astrocyte-specific interventions highlighted in this review produced encouraging results (Ceyzériat et al., 2018; Reichenbach et al., 2019; Ren et al., 2018; Yi et al., 2016), indicating that targeting reactive astrocytes could be a promising therapeutic area for AD.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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