Gene therapy-mediated enhancement of protective protein expression for the treatment of Alzheimer's disease

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Abbreviations

AAV, adeno-associated virus; AD, Alzheimer's disease; ADAM, a disintegrin and metalloprotease; AICD, amyloid precursor protein intracellular domain; ApoE, apolipoprotein E; APP, amyloid precursor protein; ARE, antioxidant response element; Aβ, amyloid beta; BACE1, β-site amyloid precursor protein cleaving enzyme 1; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; CBP, cyclic adenosine monophosphate-response element binding protein binding protein; CREB, cyclic adenosine monophosphate-response element binding protein; CTF, C-terminal fragment; ECE, endothelin converting enzyme; GLP-1, glucagon-like peptide 1; GPI, glycosylphosphatidylinositol; GSK-3β, glycogen synthase kinase 3β; HSP, heat-shock protein; HSV, herpes simplex virus; I_1PP2A, protein phosphatase 2A inhibitor 1; I_2PP2A, protein phosphatase 2A inhibitor 2; IL, interleukin; MAPK; mitogen-activated protein kinase; NEP, neprilysin; NFTs, neurofibrillary tangles; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NO, nitric oxide; Nrf2, nuclear factor E2-related factor 2; NSE, neuron-specific enolase; PHFs, paired helical fragments; PP2A, protein phosphatase 2A; PPARγ, peroxisome proliferator-activated receptor gamma; PrP, prion protein; PS, presenilin; RCAN1, Regulator of Calcineurin 1; ROS, reactive oxygen species; sAPPα, soluble amyloid precursor protein alpha; sAPPβ, soluble amyloid precursor protein beta; SeV, sendai virus; Tet, tetracycline; TFEB, transcription factor EB; TIIDM, type II diabetes mellitus; TNFα, tumour necrosis factor alpha; TREM2, triggering receptor expressed on myeloid cells 2.

Abstract

Alzheimer's disease (AD) is the leading form of dementia but lacks curative treatments. Current understanding of AD aetiology attributes the development of the disease to the misfolding of two proteins; amyloid-β (Aβ) and hyperphosphorylated tau, with their pathological accumulation leading to concomitant oxidative stress, neuroinflammation, and neuronal death. These processes are regulated at multiple levels to maintain homeostasis and avert disease. However, many of the relevant regulatory proteins appear to be downregulated in the AD-afflicted brain. Enhancement/restoration of these 'protective' proteins, therefore, represents an attractive therapeutic avenue. Gene therapy is a desirable means of achieving this because it is not associated with the side-effects linked to systemic protein administration, and sustained protein expression virtually eliminates compliance issues. The current article represents a focused and succinct review of the better established 'protective' protein targets for gene therapy enhancement/restoration rather than being designed as an exhaustive review incorporating less validated protein subjects. In addition, we will discuss how the risks associated with uncontrolled or irreversible gene expression might be mitigated through combining neuronal-specific promoters, inducible expression systems and localised injections. Whilst many of the gene therapy targets reviewed herein are yet to enter clinical trials, preclinical testing has thus far demonstrated encouraging potential for the gene therapy-based treatment of AD.

Keywords: Alzheimer's disease; gene therapy; protective proteins; amyloid beta; tau

1. Introduction

Alzheimer's disease (AD) is the predominant neurodegenerative disorder globally, with worldwide cases predicted to reach 75 million by 2030 (Prince *et al.* 2015). AD presents clinically with a progressive decline in memory and cognitive function, ultimately leading to death following complications including malnutrition or pneumonia. The pathological hallmarks of AD are the deposition of extracellular amyloid plaques (primarily in the cerebral neocortex) consisting of amyloid-β (Aβ) peptide (Thal *et al.* 2002), and neurofibrillary tangles (NFTs; originating in the entorhinal cortex) formed by abnormally phosphorylated aggregations of the microtubule-associated protein tau (Braak & Braak 1991). Plaques and tangles can develop in the brain three decades before clinical symptoms manifest (Perl 2010).

1.1. Abnormal protein aggregates are central to the aetiology of AD

Two well-accepted hypotheses tentatively explain the aetiology of AD. The 'amyloid hypothesis' (Hardy & Allsop 1991; Selkoe 1991) attributes disease development to the abnormal accumulation of 39-43 amino acid Aβ-peptides (Nunan & Small 2000). Aβ-peptides are formed by cleavage of the amyloid precursor protein (APP), an integral membrane protein concentrated at neuronal synapses (Priller *et al.* 2006). APP can be proteolytically cleaved via the amyloidogenic or the non-amyloidogenic pathways (Figure 1). In the latter, an α-secretase from the A Disintegrin And Metalloprotease (ADAM) family initiates juxta-membrane cleavage at the Lys16-Leu17 bond within the Aβ-peptide region (Anderson *et al.* 1991). This generates soluble APPα (sAPPα) and a membrane-bound C-terminal fragment (CTFα). Further cleavage of CTFα by the γ-secretase complex produces the APP intracellular domain (AICD) and the short P3 peptide (Chow *et al.* 2010).

In the amyloidogenic pathway, APP is cleaved N-terminally to Asp1 of the Aβ region by β-secretase (β-site APP cleaving enzyme 1, BACE1), yielding soluble APPβ (sAPPβ) and CTFβ (Vassar *et al.* 1999). Subsequent intramembrane cleavage of CTFβ by the γ-secretase complex produces AICD and Aβ-peptides, the latter of which exist in two predominant forms; an abundant 40 amino acid form ($A\beta_{(1-40)}$) and a more hydrophobic 42 amino acid form ($A\beta_{(1-42)}$) prone to aggregation (Mori *et al.* 1992; Jarrett *et al.* 1993). Aggregated Aβ initially forms oligomers before progressing through a protofibril intermediate to form mature fibrils (Verma *et al.* 2015). Mature fibrils were initially thought to cause the neurotoxicity observed in AD, but Aβ oligomers were subsequently identified as the primary neurotoxic species (He *et al.* 2012; Nimmrich *et al.* 2008).

The amyloid hypothesis is supported by the identification of mutations in *APP* and *PRESENILIN* (*PS1* and *PS2*) genes that pre-dispose to AD, and are associated with increased levels of Aβ-peptides or a higher ratio of $A\beta_{(1-42)}$ to $A\beta_{(1-40)}$ (Goate *et al.* 1991; Lanoiselée *et al.* 2017). In healthy individuals, Aβ-peptides are rapidly degraded by various proteases that collectively target monomeric, oligomeric and fibrillar forms (Saido & Leissring 2012). The ability to degrade Aβ-peptide decreases with age and following oxidative stress (Caccamo *et al.* 2005; Shinall *et al.* 2005; Wang *et al.* 2003) and is reduced in AD subjects (Mawuenyega *et al.* 2010). Additionally, clearance of Aβ-peptides from the brain into the circulation by low density lipoprotein receptor-related protein 1 may be defective in AD, although this remains

controversial [Reviewed in Shinohara *et al.* (2017)]. Nevertheless, failure to remove Aβ-peptides through degradation or clearance processes leads to their accumulation and aggregation in the brain.

The second hypothesis of AD pathogenesis proposes that the hyperphosphorylation of tau is the causative event. Physiologically, tau phosphorylation is responsible for the polymerisation and stabilisation of microtubules, key components of the cellular cytoskeleton (Weingarten *et al.* 1975). In the human brain, alternate splicing of the *MAPT* gene produces six different tau isoforms (Goedert *et al.* 1989). In AD all isoforms of tau are hyperphosphorylated, resulting in dissociation from microtubules and aggregation into paired helical filaments (PHFs) that comprise the NFTs characteristic of AD (Grundke-Iqbal *et al.* 1986).

NFT hyperphosphorylated tau cannot bind tubulin or promote microtubule formation (Alonso *et al.* 2006). In its non-fibrillised hyperphosphorylated form, tau also prevents binding of lesser phosphorylated tau to tubulin. This destabilises existing microtubules and promotes the formation of degradation-resistant insoluble PHFs (Alonso *et al.* 1994; Yamada *et al.* 2015). This accumulation of insoluble tau impairs axonal transport (Cabrales Fontela *et al.* 2017), compromises mitochondrial integrity (DuBoff *et al.* 2012) and impairs synaptic function (Zhou *et al.* 2017), eventually leading to neuronal loss.

The amyloid and tau hypotheses are not mutually exclusive. For example, Aβ-peptides can induce tau phosphorylation through mitogen-activated protein kinase (MAPK) p38 or glycogen synthase kinase 3β (GSK-3β) signalling (Terwel *et al.* 2008; Zheng *et al.* 2002; Zhu *et al.* 2000). Indeed, Aβ-induced oxidative stress increases Regulator of Calcineurin 1 (RCAN1) expression, which inhibits calcineurin and promotes GSK-3β expression (Lloret *et al.* 2011). This increases GSK-3β content and promotes Aβ-peptide neurotoxicity (Koh *et al.* 2008).

Calcineurin inhibition and GSK-3β upregulation can both increase hyperphosphorylated tau levels, by inhibiting tau dephosphorylation and promoting tau hyperphosphorylation, respectively (Wei *et al.* 2002). Similarly, Aβ-induced oxidative stress increases MAPK p38 activation in APP/PS1 transgenic mice, supporting a multi-mechanistic action of Aβ-peptide in tau hyperphosphorylation (Giraldo *et al.* 2014).

1.2. AD-associated protein aggregates promote oxidative stress and neuroinflammation

Oxidative stress has been implicated in the pathogenesis of AD, possibly through Cu^{2+} binding to Aβ-peptides (Jiang *et al.* 2007) generating neurotoxic reactive oxygen species (ROS) such as $HO^{\bullet}$ (Guilloreau *et al.* 2007). ROS oxidise lipids, proteins, and DNA (Dorfman & Adams 1973), leading to cell death through membrane lipid peroxidation, the impairment of cellular metabolism, or mitochondrial DNA damage (Markesbery 1997; Tramutola *et al.* 2017).

Neuroinflammation is also critically involved in AD (Rogers *et al.* 1988). Aβ oligomers and fibrils bind to immune cell receptors such as CD14, CD36, scavenger receptor A, and toll-like receptors 4 and 6 (Hickman *et al.* 2008; Liu *et al.* 2005b; Stewart *et al.* 2010), inducing microglial cells to release pro-inflammatory cytokines and migrate into plaques to phagocytose Aβ-peptides in an attempt to clear them (Stalder *et al.* 1999). Astrocytes also

release cytokines and nitric oxide (NO), while accumulating around Aβ-peptide brain deposits (Beach & McGeer 1988). As the disease progresses, chronic activation of brain immune cells results in the sustained production of pro-inflammatory molecules and reduced Aβ clearance (Hickman *et al.* 2008). More recently, tau has also been implicated in neuroinflammation, following its colocalization with astrocytes and microglia (Nilson *et al.* 2017). In glial cell cultures, truncated tau increases NO levels, and activates the MAPK pathway, leading to tau hyperphosphorylation (Zhu *et al.* 2000), and increases proinflammatory cytokines including interleukin-1β (IL-1β), IL-6, and tumour necrosis factor α (TNFα) (Kovac *et al.* 2011).

Hence, Aβ deposition and tau hyperphosphorylation contribute to oxidative stress and neuroinflammation, which further exacerbate the disease.

1.3. Current drug treatments for AD are limited to symptomatic relief

Four drugs are currently licensed for AD treatment. The acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine) enhance cholinergic neurotransmission and compensate for cholinergic neurodegeneration (Mehta *et al.* 2012), while the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist memantine limits excitotoxicity-induced neurodegeneration (Schmitt 2005; van Marum 2009). Neither treatment is curative, only temporarily managing AD symptoms (Areosa *et al.* 2005; Farlow 2002). Treatments that target the causative factors of AD are, therefore, required.

Whilst many small molecule drugs and protein/peptide-based treatments have reached clinical trials, few afford great benefit in AD due to lack of efficacy, side-effects and compliance issues (Mehta *et al.* 2017). AD is a long-term disease which makes the inducible upregulation of therapeutic proteins (e.g. by gene therapy) an attractive proposition (Nikol & Huehns 2001). Gene therapy could involve: (1) replacing a known deleterious allele with a functional copy, or (2) boosting the expression of beneficial proteins (Somia & Verma 2000). As AD is largely idiopathic, the former applies to a minority with familial inheritance, while the latter potentially applies to a broader range of patients and is thus the focus of the current gene therapy development, reviewed herein.

2. Enhancing protective protein expression by gene therapy for AD

2.1. Gene therapies that modulate pathogenic protein aggregation as potential treatments for AD

2.1.1. Reducing the generation of Aβ-peptides

Although a myriad of drugs have been developed that, mechanistically, impair β- or γ-secretase function (reviewed in Zhao *et al*. 2020), with a view to reducing Aβ-peptide generation few, if any, have resulted in an appreciable reduction in the rate of cognitive decline in patients (reviewed in Zhao *et al*. 2020; Kumar *et al*. 2018; Selkoe 2019). Such failures are exemplified no more effectively than by the recent late stage clinical trial failures of BACE1 inhibitors such as MK8931, AZD-3293, JNJ-54861911, E2609 and CNP520 which have not only shown little clinically relevant improvement in cognitive performance but, in some cases, have even worsened cognitive function (Moussa-Pacha *et al*. 2020; Wessels *et al*. 2020). There

is, therefore, a clear requirement for alternative strategies geared towards reducing Aβ-peptide generation.

Surprisingly few therapeutic opportunities for enhancing protective protein expression to impair the generation of Aβ-peptides have been identified. The regulation of APP proteolysis has been closely linked to cholesterol metabolism and the lateral segregation of the protein and its secretases between lipid raft and non-raft regions of the cell membrane (reviewed in Arbor *et al.* 2016). The clearance of cholesterol from the brain is dependent on the conversion of the lipid into 24S-hydroxycholesterol (24S-HC) which, unlike cholesterol, can freely cross the blood-brain-barrier. 24S-HC, generated from cholesterol by the action of cholesterol 24-hydroxylase (endoded by the *CYP46A1* gene), has been shown to enhance α-secretase activity and increase the α- to β-secretase activity ratio (Famer *et al*. 2007). Hudry *et al*. (2010) subsequently showed that adeno-associated virus gene therapy with *CYP46A1* reduced amyloid pathology both before and after the onset of plaque deposition in APP23 transgenic mice. The authors further suggested that, mechanistically, the phenomenon might involve a reduced recruitment of APP and presenilin 1 to lipid rafts. Similarly, the over-expression, in APP/PS1 transgenic mice, of the transcription factor Forkhead box O1 (FoxO1), involved in the regulation of cell growth, differentiation and metabolism, has been shown to significantly decrease Aβ-peptide production through an attenuation of amyloidogenic APP processing by the β- and γ–secretases (Zhang *et al*. 2020).

Changes in endosomal morphology and function are early events in the AD-afflicted brain (reviewed in Nixon *et al*. 2000) and the lysosomal compartment has been linked to disease progression through reduced autophagic clearance and the accumulation of lysosomal cathepsins in amyloid plaques (Nixon *et al*. 2006). Therefore, it is possible that enhancing the expression of proteins that promote lysosomal function might represent a viable therapy for AD although such global cellular changes may have unintended and undesirable consequences. However, some precedent does exist in this respect. Annunziata *et al.* (2013) showed that mice deficient in the lysosomal sialidase neuramindase 1 (NEU1) spontaneously developed amyloid plaques. The authors proposed that this resulted from the lysosomal accumulation of an 'oversialylated' form of APP (resulting in enhanced amyloidogenic processing of the protein) together with enhanced extracellular release of Aβ-peptides due to excessive lysosomal exocytosis. Most importantly, in the context of the current review, adenoviral-mediated restoration of NEU1 expression in 5XFAD mice through hippocampal stereotactic injection resulted in a dramatic decrease in plaque pathology leading the authors to conclude that enhancing NEU1 expression might represent a novel therapy for the treatment of AD.

However, as far as enhancing protective protein expression with a view to reducing Aβ-peptide generation is concerned, by far the most research in this respect has been conducted on the non-amyloidogenic secretase ADAM family of enzymes. Several ADAM family members have been implicated as potential α-secretases, including ADAMs 8, 9, 10, 17, 19 and 33 (reviewed in Gough *et al*. 2011), however, only in the case of ADAM10 has the over-expression of the protein *in vivo* been shown to result in an appreciable lowering of Aβ-peptide plaque burden in an animal model (Postina *et al*. 2004). Interestingly, ADAM9 was

first shown to enhance sAPPα production in cell cultures by Koike *et al*. (1999) but was subsequently shown to require the presence of ADAM10 for its ability to cleave both APP and PrP (Cissé *et al*. 2005). Our group was the first to show that ADAM9 was capable of proteolytically shedding ADAM10 from the cell surface (Parkin & Harris 2009) which presented somewhat of a paradox given that the former enzyme had previously been shown to enhance sAPPα generation (Koike *et al*. 1999). This was further compounded by the subsequent confirmation that inhibiting ADAM9 using a recombinant form of its prodomain increased membrane-associated levels of ADAM10 resulting in enhanced α-secretase processing of APP (Moss *et al*. 2011). Whilst this latter observation might not explain the apparent paradox, it does open up the possibility of enhancing the expression of the ADAM9 prodomain as a potential gene therapy for AD. Furthermore, a more recent publication by Scharfenberg *et al*. (2019) showed that soluble ADAM10 was able to degrade sAPPα but not full-length APP which implies that ADAM9-mediated shedding of ADAM10 would not only promote amyloidogenic APP processing but also result in decreased levels of the protective sAPPα fragment making the afore mentioned strategy employing the ADAM9 prodomain all the more attractive.

Despite other members of the ADAM family being implicated to varying degrees in α-secretase activity, ADAM10 remains the major target for treatments aimed at enhancing protein expression to reduce Aβ-peptide production (Peron *et al.* 2018), with loss-of-function mutations in *ADAM10* being associated with late-onset AD (Kim *et al.* 2009). The potential validity of this approach is supported by data from $APP_{[V717I]}$ transgenic mice overexpressing ADAM10 that show a reduced Aβ plaque load and improved learning and memory as compared to controls (Postina *et al.* 2004).

However, enhanced ADAM10 levels have been circumstantially associated with increased cancer progression, possibly due to the role of the enzyme in Notch signalling (Gavert *et al.* 2007; Guo *et al.* 2012; Yuan *et al.* 2013). This might partly explain why ADAM10 gene transfer has not been progressed further as a viable AD therapy in humans.

Interestingly, prescribed drugs and small molecular weight molecules that enhance ADAM10 expression [Reviewed in Wetzel *et al.* (2017)], including fibrates and retinoids, do not increase cancer incidence (Bonovas *et al.* 2012). In fact, retinoids have shown therapeutic benefits in breast, melanoma and prostate cancers (Tang & Gudas 2011), and two large studies on fibrates reported an inverse association with liver cancer incidence (Li *et al.* 2019; Iakobishvili *et al.* 2019). Collectively, these studies suggest that ADAM10 enhancement would not promote cancer *in vivo.*

Moreover, undesirable effects of enhanced ADAM10 on Notch signalling might be mitigated by specifically targeting the enzyme to lipid rafts (the proposed site of Aβ-peptide generation) using a synthetic glycosylphosphatidylinositol (GPI)-anchored form. Indeed, GPI-anchored ADAM10 targeted to rafts reduces amyloidogenic APP processing in SH-SY5Y cells (Harris *et al.* 2009).

While ADAM10 has yet to be tested as a gene therapy, lentiviral-mediated gene transfer of peroxisome proliferator-activated receptor γ (PPARγ)-coactivator-1α has been

shown to increase ADAM17 mRNA levels in APP23 transgenic mice (Katsouri *et al.* 2016). Interestingly, these ADAM17-overexpressing mice showed fewer Aβ plaques, decreased microglial activation, and improved spatial recognition memory, supporting the potential therapeutic value of this approach.

2.1.2. Inhibiting Aβ-peptide aggregation

The prion protein (PrP) is a cell surface glycoprotein with functions in neuronal differentiation, myelin maintenance, and stress handling (Castle & Gill 2017). Although controversial (Calella *et al.* 2010; Cissé *et al.* 2011; Kessels *et al.* 2010), PrP may partially mediate the toxic effects of Aβ-peptides (Barry *et al.* 2011; Gimbel *et al.* 2010; Kudo *et al.* 2012; Laurén *et al.* 2009), by acting as a cell surface receptor [Reviewed in (Salazar & Strittmatter 2017)]. ADAM10 is partly responsible for PrP shedding from the cell membrane, producing a soluble form (Taylor *et al.* 2009) which, in addition to preventing the toxicity mediated by membrane-bound PrP, also interferes with Aβ-peptide aggregation and neurotoxicity (Altmeppen *et al.* 2013; Nieznanski *et al.* 2012). Furthermore, PrP cleavage by ADAM10/ADAM17 yields an N-terminal fragment (N1) (Vincent *et al.* 2001), that binds Aβ oligomers to inhibit fibrillisation (*in vitro*) and attenuate memory deficits in mice (Fluharty *et al.* 2013). Gene transfer of either soluble PrP or N1 has yet to be examined but may provide a therapeutic option for AD by preventing PrP-mediated neurotoxicity of Aβ-peptides and reducing Aβ-peptide accumulation. Furthermore, upregulating ADAM10/ADAM17 (section 2.1.1), might also alleviate AD pathology through both the shedding of membrane bound PrP and the generation of the neuroprotective N1 fragment, independent of any effects on Aβ-peptide production.

Finally, there is some limited circumstantial evidence to suggest that the over-expression of various heat shock proteins (HSPs) might be beneficial in the treatment of Alzheimer's disease. HSPs can interact with Aβ-peptides to prevent their aggregation, and can also inhibit tau aggregation (Dou *et al.* 2003; Wilhelmus *et al.* 2007). HSP70 and HSP90 are the most studied HSPs in AD, and HSP70 levels are elevated in AD (Koren *et al.* 2009; Son *et al.* 2015). However, AD studies have predominantly researched HSP inhibitors [Reviewed in Campanella *et al.* (2018)]. This may be due to the observed upregulation of HSP70 in AD brains and the possibility of defective chaperones promoting disease progression rather than attenuating it (Marino Gammazza *et al.* 2016). While gene therapy for overexpression of specific HSPs may provide a means of preventing Aβ-peptide accumulation, we first require a deeper understanding of HSP individual and collective functions in AD pathogenesis.

2.1.3. Enhancing Aβ-peptide degradation and clearance

Just as the failure of BACE1 inhibitor clinical trials has exemplified the need for alternative strategies to reduce Aβ-peptide generation, the need for alternative strategies for the post-generation elimination of these peptides is exemplified by the failure of multiple anti-amyloid antibody clinical trials (Reviewed in Aisen *et al*. 2020). In 2019, Biogen announced the termination of two clinical trials of the anti-Aβ peptide monoclonal antibody aducanumab based on a lack of benefit or 'futility'. Biogen is currently working with the U.S. Food and Drug Administration (FDA) for regulatory approval of the drug based on subsequent extended analyses that purported to show a slowing of cognitive decline in participants who

were given a higher dose over a longer period of time. However, clinical trial failures in recent years of multiple other anti-amyloid antibodies such as gantenerumab and crenezumab (both Roche), solanezumab and donanemab (both Eli Lilly) and BAN 2401 (Eisai) does not bode well for this therapeutic approach despite the very recent attempts to resurrect some of these drugs based on marginal effects in participant sub-cohorts. There is, therefore, an immediate need for alternative approaches for the elimination of Aβ-peptides from the brain. This need may well be met through the gene therapy-mediated enhancement of proteins that either degrade or facilitate the clearance of Aβ-peptides.

Aβ-peptide levels are tightly regulated by an array of proteases (Saido & Leissring 2012), amongst which the zinc metalloendopeptidase, neprilysin (NEP), is responsible for most Aβ-peptide degradation (Howell *et al.* 1995). Consistent with NEP being a rate-limiting enzyme in Aβ-catabolism (Takaki *et al.* 2000), reduced NEP expression is associated with increased Aβ plaque burden in *post-mortem* AD brains (Grimm *et al.* 2013). Conversely, herpes simplex virus (HSV), lentiviral and adeno-associated virus (AAV)-mediated NEP gene transfer into APP transgenic mouse models has been shown to reduce Aβ-peptide deposition, IL-6 levels, astrocyte activation and improves memory function (El-Amouri *et al.* 2008; Hong *et al.* 2006; Iwata *et al.* 2013; Marr *et al.* 2003; Spencer *et al.* 2008). Collectively, these studies provide convincing preclinical evidence for direct overexpression of NEP in AD treatment.

In parallel to NEP, zinc metalloendopeptidases endothelin converting enzymes (ECE)-1 and -2 are also involved in Aβ catabolism (Eckman *et al.* 2001). Individuals homozygous for the C338A polymorphism, that enhances *ECE1* promoter activity, have a lower risk of AD (Funalot *et al.* 2004). Accordingly, upregulation of ECE1 via intracranial administration using a recombinant AAV reduced total Aβ-peptide levels in the brains of APP/PS1 mice by 50% (Carty *et al.* 2008). The potential effects of ECE1 gene transfer on neuroinflammation or memory impairment were not examined, and these certainly warrant further investigation.

Aβ-peptide clearance across the blood-brain barrier (BBB) into the bloodstream is mediated by apolipoprotein E (ApoE) (Kline 2012). In the brain, ApoE is primarily expressed by astrocytes (Grehan *et al.* 2001). There are three common ApoE isoforms; ApoE2 (Cys112, Cys158), ApoE3 (Cys112, Arg158), and ApoE4 (Arg112, Arg158) (Rall *et al.* 1982). ApoE4 increases the risk of AD in heterozygous carriers 2-3-fold, and 14-fold in homozygous individuals, while the ApoE2 allele reduces risk, with *APOE* genotype being a strong risk factor for AD (Farrer *et al.* 1997). Lin *et al.* (2018) used CRISPR/Cas9 gene editing to create isogenic induced pluripotent stem cell (iPSC) lines homozygous for *APOE4* from unaffected parental *APOE3* cells. Neurons derived from the former iPSCs exhibited enhanced Aβ-peptide secretion relative to the parental cells whilst similarly derived astrocytes exhibited a compromised uptake of Aβ-peptides. Microglial cells derived from the edited iPSCs also exhibited a relative decrease in Aβ-peptide uptake and increased inflammatory gene activations. Conversely, the authors also observed that converting *APOE4* to *APOE3* in brain cell types derived from sporadic AD iPSCs attenuated multiple AD-related pathologies. Additional studies using similar CRISPR/Cas9 editing in iPSC-derived neurons seem to indicate that a toxic gain of function of *APOE4* is responsible for AD-related pathology as such changes can largely be ameliorated following editing to *APOE3* (Wang *et al.* 2018 and Wadhwani *et al*. 2019).

Whilst gene editing to ameliorate the toxic effects of ApoE4 holds promise and is currently progressing to preclinical models, the fact that ApoE2 appears to be protective against the development of AD and is most effective at clearing Aβ-peptides from the brain (Castellano *et al.* 2011) has already led to the investigation of ApoE2 gene transfer in preclinical models of AD. In a first study, lentivirus-driven expression of ApoE2 in PDAPP transgenic mice reduced total Aβ-peptide burden when compared to GFP and ApoE4 controls (Dodart et al. 2005). More recently, AAV-driven expression of ApoE2 was shown to maintain synaptic density in APP/PS1 mice (Hudry *et al.* 2013) and AAV-mediated enhancement of ApoE2 in PDAPP mice also reduced ApoE4-associated Aβ-peptide pathology, dependent on ApoE2 expression and pre-existing amyloid pathology (Zhao *et al.* 2016). As intracisternal administration is the optimal method for AAV-mediated gene delivery in non-human primates (Rosenberg *et al.* 2018), a phase I clinical trial was initiated in 2019, with an ApoE2-expression AAV vector administered intracisternally to 15 ApoE4 homozygous individuals (NCT03634007).

2.1.4. Decreasing tau phosphorylation

A complementary approach to reducing Aβ-peptide accumulation is the promotion of tau dephosphorylation by serine/threonine phosphatases. The potential importance of such an approach is underlined by several recent publications identifying tau phosphorylated at threonine-217 (p-tau217) as a potential biomarker for AD. Indeed both p-tau217 and p-tau181 levels have been reported to increase as early as two decades before the occurrence of aggregated tau pathology (Barthélemy *et al.* 2020) but the former, when quantified in cerebrospinal fluid, performs better as an AD biomarker (Janelizde *et al*. 2020). Furthermore, plasma p-tau217 has recently been shown to increase in early AD (Mattsson-Carlgren *et al.* 2020) and to effectively discriminate the disease from other neurodegenerative diseases (Palmqvist *et al*. 2020).

Protein phosphatase 2A (PP2A) accounts for ~70% of tau dephosphorylation activity (Liu *et al.* 2005a), which is decreased by ~30% in AD brains (Gong *et al.* 1995), consistent with reduced PP2A mRNA levels in the hippocampus of AD patients (Vogelsberg-Ragaglia *et al.* 2001). Mouse studies have demonstrated that starvation-induced PP2A inhibition contributes to tau hyperphosphorylation even when tau kinases such as GSK-3β are inhibited (Planel *et al.* 2001). Therefore, upregulation of PP2A, rather than inhibition of tau kinases, could offer treatment options for AD.

PP2A activity is modulated by heat-stable inhibitors 1 (I_1PP2A) and 2 (I_2PP2A) with differential efficacy at distinct tau phosphorylation sites (Li *et al.* 1995; Tsujio *et al.* 2005). Lentiviral vectors expressing I_2PP2A siRNA were designed to enhance PP2A activity in AD patients. Hippocampal injection of I_2PP2A siRNA reduced tau hyperphosphorylation at multiple residues, decreased GSK-3β activation, improved neuronal spine density, and rescued memory deficits in human tau-transgenic mice (Zhang *et al.* 2014).

However, PP2A has diverse substrates, impacting on cellular functions such as β-catenin signalling, p53 tumour suppressor activity, and c-Myc accumulation [Reviewed in Virshup and Shenolikar (2009)]. Thus, while the side-effects of unregulated PP2A expression

are unknown, they could be extensive. Further research into PP2A and other phosphatase isoforms specifically involved in tau dephosphorylation is required (Sontag & Sontag 2014).

2.1.5. Enhancing tau clearance

Reducing hyperphosphorylated tau may also be achieved through increasing clearance, through proteolytic and degradative mechanisms. Studies *in vitro* have demonstrated monomeric tau cleavage by the proteases thrombin, calpain, and puromycin-sensitive aminopeptidase (Khlistunova *et al.* 2006; Liu *et al.* 2011; Sengupta *et al.* 2006). However, several tau proteolysis products are themselves toxic (Garg *et al.* 2011; Khlistunova *et al.* 2006; Wang *et al.* 2007), requiring downstream degradation by proteasomal and autophagic machineries to be neutralised.

Tau can also be cleared by autophagy (Wang & Mandelkow 2012). Notably, pre-lysosomal autophagic vacuoles are abundant in AD brains, suggesting that, whilst autophagy is induced in AD, lysosomal maturation is impaired, thereby potentially preventing the neuroprotective actions of autophagy in respect of tau clearance (Nixon *et al.* 2005). Inducing autophagy thus appears to be a potential treatment option that warrants further investigation. Using gene therapy, overexpression of autophagy modulators has been successfully achieved in rodent models [Reviewed in Levine *et al.* (2015)]. For example, direct overexpression of transcription factor EB (TFEB), by injection of an AAV-TFEB vector in the substantia nigra of PD rats, led to TFEB expression and increased mRNA levels of lysosomal markers in dopaminergic neurons, suggesting increased activity of the autophagy-lysosomal pathway (Decressac *et al.* 2013). Importantly, the expression of lysosomal markers suggests progression of autophagy through to the degradation stage, implying that TFEB gene transfer might promote tau degradation in AD.

Finally, gene therapy mediated-overexpression of parkin, a protein responsible for trafficking of mitochondria to the perinuclear region for autophagy (Vives-Bauza *et al.* 2010), has been trialled preclinically for AD. 3xTg-AD transgenic mice administered with a lentiviral vector for parkin overexpression exhibited increases in autophagy-related protein levels three months after injection, and reduced Aβ-peptide levels (Khandelwal *et al.* 2011).

Numerous studies in other diseases demonstrate that promoting autophagy is both possible and efficacious (Levine *et al.* 2015; Byun *et al.* 2017). However, since autophagy contributes to cell death further research is required to prove the safety of autophagy inducers in AD (Levine *et al.* 2015).

2.2. Gene therapies that modulate neuroinflammation as potential treatments for AD

2.2.1. Anti-inflammatory cytokines

A critical component of neuroinflammation in the AD-afflicted brain is the production of pro-inflammatory cytokines by activated microglia and astrocytes. However, the role of anti-inflammatory cytokines in AD pathology is more ambiguous (Domingues *et al.* 2017). For example, short-term AAV-mediated expression of the anti-inflammatory cytokine IL-4 in the TgCRND8 transgenic mouse model exacerbated Aβ-peptide deposition (Chakrabarty *et al.* 2012), but long-term expression in APP/PS1 mice significantly reduced microglial

accumulation, astrogliosis, and Aβ-peptide load (Kiyota *et al.* 2010). Similarly, AAV-mediated gene delivery of murine IL-10 in APP/PS1 mice suppressed astrogliosis, enhanced neurogenesis, and rescued spatial learning deficits (Kiyota *et al.* 2012). However, a complex relationship between IL-10 levels and AD-related pathology may exist, as deficiency of the protein in APP/PS1 mice was shown to enhance microglial phagocytosis of Aβ-peptide and rescued behavioural impairment (Guillot-Sestier *et al.* 2015). This suggests a complicated relationship between anti-inflammatory cytokine levels and AD relevant pathology, which may be independent of their effects on Aβ-peptide. Further research is required to characterise the mechanisms underlying cytokines as a potential therapy in AD.

2.2.2. TREM2

Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane receptor expressed on myeloid cells including microglia (Colonna & Wang 2016; Schmid *et al.* 2002). TREM2 stimulation in mice primary microglial cultures increases phagocytosis while reducing the expression of the proinflammatory cytokines TNFα and IL-1β (Takahashi *et al.* 2005). The R47H mutation in TREM2, which may cause a decrease in mRNA levels (Cheng-Hathaway *et al.* 2018), increases the risk of late-onset AD 2- to 3-fold (Carmona *et al.* 2018). Interestingly, overexpression of TREM2 via lentiviral gene transfer in APP/PS1 mice significantly reduced Aβ plaque density, lowered levels of proinflammatory cytokines, and improved spatial memory (Jiang *et al.* 2014). This study highlights the potential of TREM2 gene therapy in AD, but further research is required.

2.3. Gene therapies modulating neurotrophic and neuroprotective factors as potential treatments for AD

2.3.1. sAPPα

sAPPα, generated via the non-amyloidogenic proteolysis of APP, has been implicated in neurogenesis, brain development and plasticity [Reviewed in Dar and Glazner (2020)]. The neuroprotective actions of sAPPα *in vivo* include enhancement of neuronal survival, protection from ROS and decreased glutamate-mediated excitotoxicity (Araki *et al.* 1991; Goodman & Mattson 1994; Mattson *et al.* 1993). Furthermore, evidence suggests that sAPPα inhibits BACE1, theoretically reducing amyloidogenic APP processing (Obregon *et al.* 2012). Gene transfer of sAPPα, using an AAV vector, has been examined in APP/PS1 transgenic mice. Direct overexpression of sAPPα in these mice partially reduced Aβ burden, improved synaptic plasticity and rescued spatial reference memory deficits. An increase in microglia recruitment to amyloid plaques was also observed, indicating the possibility of increased Aβ-peptide phagocytosis (Fol *et al.* 2016). No further studies utilising gene transfer for overexpression of sAPPα have been reported.

2.3.2. Neurotrophins

Neurotrophins are growth factors important for the proliferation, survival, differentiation and migration of neurons (Kerschensteiner *et al.* 2003). Additionally, they play a role in development and synaptic plasticity (Ledda & Paratcha 2016). The first of these to be discovered, nerve growth factor (NGF) (Levi-Montalcini & Angeletti 1963), promotes the survival of cholinergic neurons following injury (Aloe *et al.* 2015). This is particularly

interesting given the degeneration of cholinergic neurons in AD, the role of this neuronal subtype in the cognitive symptoms of the disorder and the fact that drugs targeting this system are effective in treating symptoms (Whitehouse *et al.* 1981; Whitehouse *et al.* 1982). Therefore, NGF has been implicated as a therapy for AD as a means of preserving cholinergic neurotransmission. Gene transfer of NGF is a widely studied topic, with many preclinical studies (Bishop *et al.* 2008; Blesch *et al.* 2005; Fischer *et al.* 1987; Kordower *et al.* 1994)[Reviewed in Tuszynski (2007)] and several clinical trials being undertaken. The results of the first clinical trial for NGF gene therapy were reported in 2005 (NCT00017940), following *ex vivo* delivery of NGF to eight patients with early-stage AD; the rate of cognitive decline in patients was slowed with no adverse effects (Tuszynski *et al.* 2005). A second phase I clinical trial (NCT00087789) demonstrated the safety of AAV-mediated NGF delivery, in addition to a possible dose-dependent reduction in cognitive decline (Rafii *et al.* 2014). *Post-mortem* examination of brains from patients in both studies identified cholinergic axonal sprouting in sites of NGF delivery, demonstrating that degenerating neurons can still respond to growth factors (Tuszynski *et al.* 2015). A phase II trial (NCT00876863) failed to demonstrate significant improvements in primary clinical outcomes (Rafii *et al.* 2018). However, pathological analysis revealed that, although targeting of the injection was accurate in most cases, the limited spread of AAV-NGF from the injection site prevented NGF from reaching cholinergic neurons (Castle *et al.* 2020).

A second neurotrophin, brain-derived neurotrophic factor (BDNF), partly elicits its effects through the modulation of neuroinflammatory processes. BDNF overexpression decreases levels of the proinflammatory cytokine TNF-α whilst increasing levels of the anti-inflammatory cytokines IL-4, IL-10, and IL-11 (Makar *et al.* 2009). BDNF is synthesised in many brain regions, including the entorhinal cortex, where it is anterogradely trafficked to the hippocampus (Leal *et al.* 2017). Protein levels of BDNF are lowered in the entorhinal cortex and hippocampus of the AD-afflicted brain (Hock *et al.* 2000; Narisawa-Saito *et al.* 1996). Several studies have investigated BDNF gene therapy in models of AD. Nagahara *et al*. demonstrated that lentiviral-mediated gene transfer of BDNF to J20 transgenic mice reversed synapse loss and memory impairment independent of effects on Aβ-peptide load, with similar effects being observed in rat and primate models (Nagahara *et al.* 2009). Hippocampal administration, mediated by a Sendai viral (SeV) vector, to Tg2576 transgenic mice confirmed these findings (Iwasaki *et al.* 2012). The former study was replicated in younger mice with earlier administration of BDNF shown to prevent neuronal loss, with effects being independent of Aβ-peptide load (Nagahara *et al.* 2013). More recently, magnetic resonance imaging has been used to guide AAV-BDNF delivery to the entorhinal cortex of non-human primates to maximise targeted expression. Such delivery elevated BDNF labelling in the hippocampal dentate gyrus, but functional and behavioural consequences were not reported (Nagahara *et al.* 2018). In addition to these targeted and selective BDNF manipulations, the indirect enhancement of BDNF expression by gene therapy has also been investigated; increasing levels of CREB (cyclic adenosine monophosphate-response element binding protein) binding protein (CBP) via lentiviral delivery induced BDNF expression and rescued the learning and memory impairment in 3xTg-AD mice (Caccamo *et al.* 2010).

Collectively, these results implicate direct or indirect BDNF overexpression as a valid candidate for AD. Importantly, MRI-guided injection could prevent inaccurate administration and improve the limited spread that caused the phase II clinical trial failure of NGF.

2.3.3. Progranulin

Progranulin is a trophic factor that promotes neuronal development, survival and neurite outgrowth (Van Damme *et al.* 2008), in addition to modulating neuroinflammation (Yin *et al.* 2010). Mutations in the *GRN* gene encoding progranulin are strongly associated with frontotemporal dementia (Mackenzie *et al.* 2006; Yu *et al.* 2010) and are also a risk factor for AD (Perry *et al.* 2013). P301L tau transgenic mice hemizygous for *GRN* exhibited enhanced tau phosphorylation (Hosokawa *et al.* 2015), although introduction of the *GRN* hemizygote into APP transgenic mice actually decreased Aβ-peptide accumulation (Hosokawa *et al.* 2018).

Administration of *GRN* using AAV-mediated gene therapy reduced microgliosis and improved lysosomal abnormalities in transgenic mouse models of frontotemporal dementia (Arrant *et al.* 2018). AAV-GRN has recently received approval to enter clinical trials for frontotemporal dementia (Prevail Therapeutics 2020). Extending this gene therapy for AD may, therefore, prove beneficial through decreasing both tau hyperphosphorylation and neuroinflammation.

2.3.4. Glucagon-like peptide 1 (GLP1) signalling

GLP-1 is an incretin hormone primarily synthesised in the gut and released into the bloodstream after food intake (Yildirim Simsir *et al.* 2018). In the brain, GLP-1 is a growth factor that promotes survival, proliferation, and repair whilst inhibiting apoptosis (Perry & Greig 2004; Sharma *et al.* 2014). On binding to its receptor, activation of the downstream signalling pathway facilitates insulin signalling and culminates in inhibition of GSK-3β (Mussmann *et al.* 2007).

Many studies have demonstrated the potential neuroprotective roles of GLP-1 in AD [Reviewed in Yildirim Simsir *et al.* (2018)]. For example, inhibition of GSK-3β by GLP-1 reduces age-dependent tau hyperphosphorylation in a diabetic transgenic mouse model (Ma *et al.* 2015). GLP-1 treatment reduces intracellular Aβ-peptide in PC12 cells and protects primary cultured neurons from Aβ-induced damage (Perry *et al.* 2003). In APP/PS1 mice, Liraglutide, a GLP-1 agonist, reduced Aβ plaque load and rescued learning and memory impairment (McClean *et al.* 2011). Similarly, another GLP-1 agonist, Lixisenatide, reduced Aβ-peptide load, NFTs and neuroinflammation in APP/PS1/tau mice (Cai *et al.* 2018). This reduction in neuroinflammation may not solely be Aβ- and tau-dependent, as GLP-1 reduces IL-1β mRNA expression, microglial activation, and oxidative damage *in vitro* and *in vivo* (Iwai *et al.* 2006; Spielman & Klegeris 2014; Teramoto *et al.* 2011).

While safe and efficacious GLP-1 drugs are currently available for the treatment of type II diabetes mellitus (TIIDM), disadvantages include long-term compliance issues and systemic side effects. Selective overexpression of GLP-1 in the CNS may, therefore, present a novel multi-modal therapy for AD. Gene transfer of GLP-1 has yet to be reported in *in vivo* models of AD, although it has been examined in relation to TIIDM (Tasyurek *et al.* 2014). Whilst these studies focused on systemic expression of GLP-1, as opposed to selective

neuronal expression, they validate the possibility of sustained GLP-1 expression via gene therapy. By modifying the promoter and/or route of administration, gene transfer of GLP-1 selectively to neurons may exhibit neuroprotective effects in AD transgenic mouse models. Although this remains to be adequately tested.

2.4. Enhancing proteins that regulate oxidative stress

Antioxidants previously studied in relation to AD include glutathione, carotenoids, vitamin C, vitamin E, and α-lipoic acid (Mirończuk-Chodakowska *et al.* 2018). The effect of orally administered vitamin E on AD has been examined in many clinical trials over 20 years (Sano *et al.* 1997), although results remain inconclusive (Browne *et al.* 2019).

Nuclear factor E2-related factor 2 (Nrf2) binds to the antioxidant response element (ARE) to promote transcription of anti-oxidative genes (Liu *et al.* 2017) and several preclinical studies support the therapeutic potential of modulating Nrf2. For example, administration of sulforaphane, an Nrf2 activator, in PS1V97L-Tg mice decreased amyloid pathology and rescued cognitive deficits (Tian *et al.* 2019). Moreover, lentiviral-mediated gene transfer of Nrf2 to the hippocampus of APP/PS1 mice reduced astrocytosis and improved spatial learning (Kanninen *et al.* 2009). Nrf2 has also been implicated as a negative regulator BACE1 transcription in mouse embryonic fibroblasts (Bahn *et al.* 2019), suggesting that upregulation could provide a multi-mechanistic treatment for AD involving both reduced oxidation and modified amyloid processing. Further upstream, α-lipoic acid may act through several mechanisms to activate Nrf2 (Brandes & Gray 2020). Gene transfer of α-lipoic acid synthetase, an enzyme involved in α-lipoic acid biosynthesis, may also be a potential treatment option. However, the bioavailability of many antioxidants makes antioxidant gene therapy in AD an understudied area.

3. Gene transfer technologies can allow specific and highly regulated protein expression

A range of preclinical and, to a lesser extent, clinical studies have already examined gene therapy strategies in the treatment of AD. However, there are clear safety considerations to be made before such strategies reach the clinical stage. One of the key concerns in this respect is host immune response to the therapy both in terms of an immediate adverse reaction and in terms of an adaptive immune response resulting in the production of therapy neutralizing antibodies (reviewed in Shirley *et al*. 2020). In the case of Alzheimer's disease, such reactions may, to some extent, be mitigated by stereotactic therapy injection although, for obvious reasons, this is far from ideal in the clinic. Gene therapy may also cause severe toxicity if the expression level of the transgene is not sufficiently regulated or expressed in off-target cells/tissue. Related toxic consequences in non-human primates have previously included ataxia, impaired ambulation, proprioceptive deficits and damaged dorsal root ganglia (Hinderer *et al*. 2018). In addition, insertional mutagenesis and genotoxicity are likely to be concerns when certain transgenes are injected with high-dose vectors (Chandler *et al.* 2017). Nonetheless, gene therapy for the treatment of AD remains an attractive and topical proposition and, to some extent, some of these problems can already be mitigated as discussed below.

3.1. Specificity

Most potential gene therapies for AD use direct injection into the brain to ensure region specificity of expression. Neuronal-specific promoters can also be used for targeted gene expression. For example, the neuron-specific enolase (NSE) promoter and the platelet-derived growth factor promoter drive gene expression exclusively in neurons, with expression lasting for over two months in the adult rat (Peel *et al.* 1997). Similarly, the neuronal-specificity of the human synapsin-1, α-tubulin and calcium/calmodulin-dependent protein kinase IIα (CaMKIIα) promoters have previously been described (Kügler *et al.* 2003; Mayford *et al.* 1996; Gloster *et al.* 1994). Additionally, all five of these promoters have been successfully fused to the cytomegalovirus enhancer to create hybrid promoters with 2- to 4-fold increased expression levels relative to the native neuronal-specific promoters (Hioki *et al.* 2007).

3.2. Inducible systems

Conventional gene therapy cannot regulate, or reverse, gene expression once initiated. Uncontrolled systemic gene expression carries a plethora of adverse effects (Liu & Kirn 2007). In an attempt to resolve such issues, inducible gene expression systems have been developed [for review see Kallunki *et al.* (2019)].

The most widely used is the tetracycline (Tet)-inducible expression system, which has the advantage that tetracycline derivatives are prevalent in clinical practice (Naidoo & Young 2012). The system has three possible configurations: (1) repression-based, (2) Tet-off, and (3) Tet-on (Kallunki *et al.* 2019). In the latter of these, presence of the drug activates transcription of the target gene. This configuration is advantageous in gene therapy as the tetracycline derivative can be discontinued when gene overexpression is no longer required or adverse effects occur. The system has been further optimised to ensure negligible "leakiness" in the absence of the drug (Shaikh & Nicholson 2006; Zhou *et al.* 2006).

The use of inducible expression systems for disease treatment has been validated in cancer therapy where systemic administration of IL-12 to advanced renal cell carcinoma patients had previously resulted in severe toxicity and two deaths (Leonard *et al.* 1997). Utilising the RheoSwitch Therapeutic System®, which uses veledimex as the activator ligand, results in tumour-specific inducible IL-12 expression that is well tolerated in both preclinical studies and Phase I clinical trials for glioblastoma and advanced melanoma (Barrett *et al.* 2018; Linette *et al.* 2013; Schwartzentruber *et al.* 2011).

In combination with a cell or tissue-specific promoter and, possibly, local injection, inducible systems might mitigate adverse events from more widespread unregulated overexpression of the therapeutic protein. For example, the forebrain-specific CaMKIIα promoter used within a Tet-inducible system permits inducible and reversible expression specifically within neurons in mice (Michalon *et al.* 2005). Similarly, tamoxifen-inducible expression driven by the NSE promoter resulted in exclusive cerebellar granule cell expression in mice (Pohlkamp *et al.* 2014). Therefore, integration of inducible systems and neuronal-

specific promoters into future research should permit controlled expression of therapeutic proteins by gene therapy in AD, which may improve success in clinical trials.

4. Concluding remarks

Despite the large number of candidate treatments previously developed for AD, many drugs have failed in clinical testing. While gene therapy itself is not a new concept, our expanding knowledge of AD aetiology and recent developments in gene therapy systems provides new promise for this currently incurable disease. Here, we have discussed several therapeutic proteins that remain to be validated using gene therapy. The range of putative therapeutic target proteins will likely increase as our understanding of AD further develops. Whilst further preclinical validation of gene therapy-mediated protein overexpression is needed before testing in clinical trials, the *in vitro* and *in vivo* studies described herein demonstrate the feasibility of gene therapy as a future treatment strategy for AD.

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Figure 1: Inter-relationships between pathogenic events culminating in neuronal injury and disease exacerbation in AD. APP can be cleaved by ADAM10, forming neuroprotective sAPPα and, following γ-secretase cleavage, P3 and AICD. Alternatively, BACE1 cleavage results in Aβ-peptide formation, which can misfold and accumulate into oligomers and fibrils, leading to oxidative stress, neuroinflammation and tau hyperphosphorylation. Tau hyperphosphorylation can occur independently of Aβ-peptide accumulation, resulting in its dissociation from microtubules and accumulation in PHFs and NFTs. Pink boxes denote the detrimental effects of these pathways whilst green boxes denote mechanisms leading to reduced Aβ-peptide or tau accumulation. Yellow bolts denote putative therapeutic targets for gene therapy-mediated overexpression.

Table 1: Summary of potential gene therapies for AD discussed in this review.

Gene	Phase of trial	Vector	Outcomes	References
sAPPα	Preclinical	AAV	Reduced Aβ-peptide accumulation and improved synaptic plasticity, spine density, and spatial memory in PS1ΔE9 mice.	(Fol *et al.* 2016)
PGC-1α	Preclinical	Lentivirus	Reduced BACE1 and Aβ plaques, improved spatial memory, and attenuation of neuroinflammation in APP23 mice.	(Katsouri *et al.* 2016)
NEP	Preclinical	Lentivirus	Reduced Aβ-peptide deposits in two transgenic human APP mouse models.	(Marr *et al.* 2003)
		Lentivirus	Reduced Aβ-peptide, IL-6 levels, and oxidative stress in APPswe/PS1ΔE9 mice.	(El-Amouri *et al.* 2008)
		AAV	Reduced Aβ-peptide accumulation and improved memory in APP23 mice.	(Iwata *et al.* 2013)
		Lentivirus	Reduced intracellular Aβ-peptide and improved spatial memory in J9M mice.	(Spencer *et al.* 2008)
		HSV	Reduced Aβ-peptide production in C57BL/6 mice transfected with lentiviral APPswe.	(Hong *et al.* 2006)
ECE1	Preclinical	AAV	Reduction in total Aβ-peptide load and fibrillar Aβ in APP+PS1 mice.	(Carty *et al.* 2008)
ApoE2	Preclinical	Lentivirus	Reduction in total Aβ-peptide burden in PDAPP mice.	(Dodart *et al.* 2005)
		AAV	Reduced soluble Aβ-peptide and synapse loss in APPswe/PS1ΔE9 mice.	(Hudry *et al.* 2013)
		AAV	Reduction in ApoE4-dependent amyloid pathology in several transgenic mice models.	(Zhao *et al.* 2016)
		AAV	Intracisternal administration optimal method in non-human primates.	(Rosenberg *et al.* 2018)
	Phase I	AAV	Ongoing in 15 ApoeE4 homozygotes.	NCT03634007
***PP2A* (via *I_2PP2A*)**	Preclinical	Lentivirus	Inhibition of GSK-3β and tau hyperphosphorylation, improved memory and spine density in human tau mice.	(Zhang *et al.* 2014)
TFEB	Preclinical	AAV	Protected neurons from toxicity of α-synuclein in a PD mouse model. Could be used to induce autophagy in AD.	(Decressac *et al.* 2013)
Parkin	Preclinical	Lentivirus	Increased levels of autophagic markers, and a reduction in Aβ-peptide levels and oxidative stress in 3xTg-AD mice.	(Khandelwal *et al.* 2011)
IL-4	Preclinical	AAV	Attenuation of neuroinflammation and reduced Aβ-peptide load in APP+PS1 mice.	(Kiyota *et al.* 2010)
IL-10	Preclinical	AAV	No effect on Aβ-peptide deposition but reduced neuroinflammation in APP+PS1 mice.	(Kiyota *et al.* 2012)
NGF	Preclinical	Various	Prevention of cholinergic neurodegeneration and improvement in memory function in various models.	(Tuszynski 2007)
	Phase I	Fibroblasts	Slowed cognitive decline and showed no long-term adverse effects in 8 mild AD patients.	(Tuszynski *et al.* 2005)
		AAV	Long-term NGF expression was well-tolerated in 10 AD patients.	(Rafii *et al.* 2014)
		Fibroblasts and AAV	*Post-mortem* evaluation of both phase I trials revealed neuronal sprouting towards side of injection.	(Tuszynski *et al.* 2015)
	Phase II	AAV	No difference in primary outcomes to those administered sham injection.	(Rafii *et al.* 2018)
BDNF	Preclinical	Lentivirus	Reversal of synapse loss and improved learning and memory in J20 mice.	(Nagahara *et al.* 2009)
		SeV	Improved memory and reduced synaptic degeneration in Tg2576 mice.	(Iwasaki *et al.* 2012)
		Lentivirus	Prevention of neuronal loss following early administration in J20 mice.	(Nagahara *et al.* 2013)
		AAV	MRI-guided delivery allowed accurate targeting of the entorhinal cortex.	(Nagahara *et al.* 2018)
***BDNF* (via *CBP*)**	Preclinical	Lentivirus	Increased BDNF levels leading to rescued memory impairment in 3xTg-AD mice.	(Caccamo *et al.* 2010)
GRN	Preclinical	AAV	Reduced microgliosis and improved lysosomal function in a frontotemporal dementia mouse model.	(Arrant *et al.* 2018)
GLP-1	Preclinical	Various	Successful overexpression of GLP-1 leading to therapeutic effects in animal models of type 2 diabetes mellitus.	(Tasyurek *et al.* 2014)
TREM2	Preclinical	Lentivirus	Decreased Aβ-peptide burden, protection against synapse loss, and improved memory in APPswe/PS1ΔE9 mice.	(Jiang *et al.* 2014)
Nrf2	Preclinical	Lentivirus	Reduced Aβ-peptide burden and improvement in learning in APP/PS1 mice.	(Kanninen *et al.* 2009)

Figure 1.