



# Geroprotective interventions in the 3xTg mouse model of Alzheimer's disease

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**Abstract** Alzheimer's disease (AD) is an age-associated neurodegenerative disease. As the population ages, the increasing prevalence of AD threatens massive healthcare costs in the coming decades. Unfortunately, traditional drug development efforts for AD have proven largely unsuccessful. A geroscience approach to AD suggests that since aging is the main driver of AD, targeting aging itself may be an effective way to prevent or treat AD. Here, we discuss the effectiveness of geroprotective interventions on AD pathology and cognition in the widely utilized triple-transgenic mouse model of AD (3xTg-AD) which develops both  $\beta$ -amyloid and tau pathologies characteristic of human AD, as well as cognitive deficits. We discuss the beneficial impacts of calorie restriction (CR), the gold standard for geroprotective interventions, and the effects of other dietary interventions including protein restriction. We also discuss the

promising preclinical results of geroprotective pharmaceuticals, including rapamycin and medications for type 2 diabetes. Though these interventions and treatments have beneficial effects in the 3xTg-AD model, there is no guarantee that they will be as effective in humans, and we discuss the need to examine these interventions in additional animal models as well as the urgent need to test if some of these approaches can be translated from the lab to the bedside for the treatment of humans with AD.

**Keywords** Alzheimer's disease · Mice · 3xTg · Tau ·  $\beta$ -amyloid · Geroprotectors

## Introduction

With improvements in medicine and public health, the number of aged individuals has increased dramatically [1]. The number of individuals 65 years or older in the USA is expected to reach 88 million by 2050; this population already numbered 55 million in 2019 [2]. As age increases, so does susceptibility to age-related diseases such as Alzheimer's disease (AD). AD is a neurodegenerative disease in which patients exhibit impaired memory, motor function, and language due to neuronal damage [2]. In 2019, approximately 5.8 million people were living with AD in the USA, resulting in a total healthcare cost of \$290 billion [2]. By 2050, those healthcare costs are expected to be over \$1 trillion [3]. Due to the

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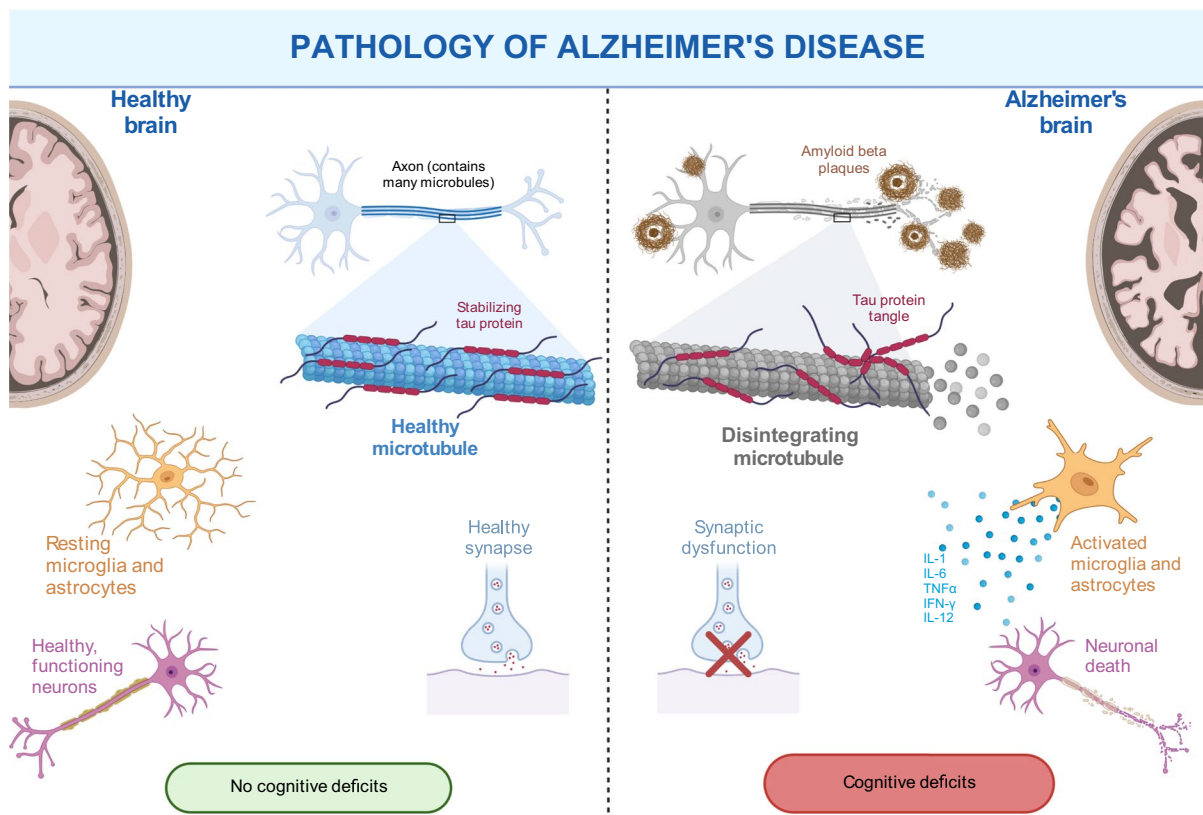
high prevalence and cost of this disease, it is imperative to determine effective ways to prevent, delay, or treat AD.

There are two different types of AD. The most common is “late-onset”, also known as “sporadic”, in which AD develops late in life. Of the 5.8 million Americans living with Alzheimer’s disease in 2019, approximately 95% were late-onset [4]. The cause of late-onset AD is not known, but aging and possessing an apolipoprotein E4 allele (apoE4) are both risk factors [4]. In contrast, the other 5% of cases of AD were in younger adults and are characterized as “early-onset” or “familial” AD (FAD). FAD is primarily characterized by inherited amyloid precursor protein (APP), presenilin-1 (PSEN1), or presenilin-2 (PSEN2) mutations [4, 5]. Though FAD occurs due to one or more specific genetic mutations, and specific

allelic variants increase the risk of late-onset AD, age is the greatest risk factor for all forms of AD [6].

### Pathology of Alzheimer’s disease

The progression of both types of AD are similar and are characterized by several different physiological changes (summarized in Figure 1). In healthy brains, tau is associated with microtubules and acts to stabilize them [7]. Binding of tau to microtubules is regulated by post-translational modifications, primarily phosphorylation [7, 8]. Phosphorylation of tau decreases its affinity toward microtubules, so varying amounts of phosphorylation allow for intra-axonal sorting, with phosphorylated tau aggregating closer to the soma and dephosphorylated tau located near



**Fig. 1** Characteristics of AD pathology in the central nervous system. During the progression of AD, many changes occur in the brain. Microtubules disintegration occurs due to the hyperphosphorylation of tau and formation of NFTs. Meanwhile, A $\beta$  clearance is disrupted leading to aggregation into plaques.

Astrocytes and microglia are activated, and pro-inflammatory cytokines are released. Each of these factors then contribute to synaptic dysfunction and neuronal death, which ultimately result in cognitive deficits. Created with [BioRender.com](https://www.biorender.com)

the growth cone [7]. When hyperphosphorylated, tau completely dissociates from the microtubules and aggregates together to form neurofibrillary tangles (NFTs) [8]. NFT aggregation is strongly associated with neuronal death [9, 10]. Another contributing factor to AD development is microRNA (miRNA) dysregulation [11]. Dicer is an miRNA-processing enzyme which is upregulated during exercise, and ablation of this enzyme lowers miRNA abundance, leading to neuronal loss, tau hyperphosphorylation, and changes in behavior—similar phenotypes as seen in AD pathology, leading researchers to conclude that normal miRNA abundance and Dicer processing are important in overall brain health [12–14].

Another aspect of AD pathology is the accumulation of  $\beta$ -amyloid ( $A\beta$ ) plaques. In the healthy brain, APP is cleaved into  $A\beta$ , which acts to decrease synaptic activity and regulate potassium and calcium channel production to control excitability and cell survival [15]. However, problems arise when too much  $A\beta$  is produced or clearance mechanisms are no longer sufficient to keep  $A\beta$  at a healthy level (reviewed in [16]). One potential mechanism for excessive  $A\beta$  production is beta-site APP cleaving enzyme 1 (BACE1), which cleaves APP to produce  $A\beta$  [17, 18]. Patients with AD have higher BACE1 levels, and deletion of BACE1 in mice prevents  $A\beta$  accumulation and ameliorates AD pathology [17–19]. Decreased autophagy [20, 21] and a disruption of insulin signaling [22–24] are both thought to contribute to the accumulation of  $A\beta$  to form extracellular plaques. During aging, two specific enzymes that are responsible for the majority of  $A\beta$  degradation are downregulated: neprilysin and insulin-degrading enzyme [25]. The downregulation of these two enzymes may contribute greatly to the accumulation of  $A\beta$ .

$A\beta$  pathogenicity differs somewhat between FAD and late-onset AD, with FAD exhibiting altered  $A\beta$  ratios while late-onset AD does not [26]. There are a variety of different  $A\beta$  isoforms, with  $A\beta_{40}$  levels being significantly more abundant than  $A\beta_{42}$ .  $A\beta_{42}$  is the more hydrophobic and fibrillogenic of the  $A\beta$  isoforms, and is greatly increased in the brain during FAD, leading to the formation of amyloid plaques (reviewed in [25]). Transgenic mouse models have shown that amyloid deposition is primarily due to  $A\beta_{42}$ , and that  $A\beta$  increases NFT production. Aggregated  $A\beta$  also contributes to immune (microglia) activation which leads to high levels of

neuroinflammation and may exacerbate neuronal stress.

There is considerable interplay between the different pathological features of AD, some of which are still not well known [27]. Current findings suggest that  $A\beta$  contributes to tau pathology and tau pathology in return may promote  $A\beta$  pathology. In addition, NFTs may increase  $A\beta$  toxicity, suggesting that tau pathology may indeed may be a major driver of AD pathology [28].

During the preclinical stage, there are no clinical systems evident, but there are changes in biomarkers [2, 27]. Evident biomarkers in the preclinical stage may include reduced  $A\beta_{1-42}$  in the cerebrospinal fluid and increased amyloid tracers during PET scans, increased phosphorylated tau (p-tau) levels in the cerebrospinal fluid, and changes in MRI scans [27]. As preclinical AD progresses, clinical symptoms eventually appear, though this occurs in humans at least 15 years after the beginning of  $A\beta$  accumulation [27]. Clinical symptoms start as mild cognitive impairment, such as memory lapses and difficulty thinking, and then progress to AD dementia [2, 27]. AD dementia patients may have trouble communicating and performing routine tasks as well as deficits in motor control and swallowing. They may also exhibit personality and behavior changes, suspiciousness, and agitation [2]. AD has dramatic negative impacts not only on the quality of life of the patient, but also greatly affects those who must care for the individual.

Another compounding factor in the use and cost of caregiving for AD patients is the prevalence of comorbidity with a number of other age-related diseases, including cardiovascular disease, diabetes, kidney disease, and cancer [2]. Coexisting conditions such as these increase caregiving time and cost and may exacerbate the symptoms and progression of AD.

### Diabetes and Alzheimer's disease

In humans, the development of AD is often closely tied to diabetes and obesity. In 2014, 37% of Medicare beneficiaries who were 65 or older with dementia also had diabetes [2]. The high prevalence of comorbidity between these two diseases has led to the suggestion that AD development may be linked to dysfunctional insulin signaling and glucose homeostasis [2, 29]. While the exact relationship

between glucose metabolism and AD is unclear, the risk of AD is increased by type 2 diabetes, and glucose metabolism is impaired in individuals with AD (reviewed in [30]). Glucose metabolism is disrupted in the brains and neurons of AD patients, with defects in glucose uptake (reviewed in [30]) and mitochondrial dysfunction, which may stem from disruption of the TCA cycle, electron transport chain, or fission-fusion [31–33]. It has been well established that there is impaired insulin signaling in the brain during AD, so much so that AD is often referred to as type III diabetes [34–40]. In many tissues, insulin acts to stimulate glucose uptake via translocation of glucose transporters [39]. This allows tissues such as muscle to take up blood glucose, thereby lowering post-prandial blood glucose and allowing the tissue to utilize the glucose for energy.

In the CNS, insulin has a number of different functions such as signaling food intake and energy regulation in the hypothalamus and effecting synaptic plasticity in the hippocampus [34, 39]. Insulin also greatly influences learning and memory, neurotransmitter signaling, and neuronal development [38, 39]. Insulin promotes neuronal glucose uptake as well [39]. In insulin-resistant patients, the inability of tissues to respond to insulin and initiate glucose uptake leads to hyperinsulinemia [38]. Peripheral hyperinsulinemia in turn leads to downregulation of insulin receptors in the blood brain barrier, leading to decreased uptake of insulin into the brain [34, 38]. Decreased insulin function in the brain is detrimental to learning and memory but also leads to decreased glucose uptake into both the brain and neurons themselves [39, 40]. Decreased glucose metabolism has been found in AD patients and correlates with a reduction in cognitive performance [40].

In 3xTg mice, a number of studies have examined how the progression of AD pathology correlates with disruptions in insulin signaling and glucose homeostasis [29, 41–43]. Older 3xTg mice exhibit impaired glucose tolerance as well as elevated fasting blood glucose levels [42, 43], and diet-induced obesity accelerates the cognitive decline of 3xTg mice [44]. According to Vandal et al., impaired glucose tolerance is evident by 10 months of age, but not at 6 months of age [42]. In contrast, Velazquez et al. did not find differences in glucose tolerance at 10 months of age but did by 16 months of age [43]. Inconsistencies in timing between these two studies

may be due in part to differences in the glucose tolerance test protocol [42, 43]; but in both studies, 3xTg mice do exhibit an age-dependent increase in glucose intolerance. However, 3xTg mice do not show impaired performance on an insulin tolerance test [42]. In the central nervous system, 3xTg-AD mice exhibit an age-dependent increase in insulin resistance, occurring prior to peripheral insulin resistance [43]. These results suggest that as in humans, insulin signaling and glucose homeostasis are likely linked to AD, although how these are linked remains to be determined. To accelerate the process of determining these links between AD and metabolic health, as well as developing treatments for AD, scientists have developed animal models of AD to aid research into the development, progression, and treatment of the disease.

### The 3xTg-AD mouse model

There are over 180 mouse models for AD, many of which have only a single gene mutation; as a result, these models exhibit only either A $\beta$  or tau pathology, and do not encompass the full scope of neurodegenerative issues that occur in the brain of an AD patient [4, 45]. The 3xTg-AD mouse model, developed by Oddo and colleagues in 2003, was made by comicro-injection of two independent transgenes containing APP<sub>Swe</sub> and tau<sub>P301L</sub> into the embryos of homozygous mutant PS1<sub>M146V</sub> knock-in mice [46]. The 3xTg-AD model is useful for studying AD treatments as they exhibit a full range of AD pathologies.

These mice exhibit robust A $\beta$  pathology, with an age-dependent increase in A $\beta$ <sub>40</sub> as well as the longer, more amyloidogenic A $\beta$ <sub>42</sub> form [42, 46, 47]. By 3–4 months of age, 3xTg-AD mice exhibit intracellular A $\beta$  immunoreactivity in parts of the hippocampus and cortex, with extracellular A $\beta$  plaque accumulation seen starting at 12–15 months of age [46–49]. Tau pathology develops later, with the first signs of human tau immunoreactivity appearing at 12 months of age; more than 6 months after A $\beta$  pathology begins [46, 49]. Once tau pathology begins to develop, it progresses in a fashion very similar to what is observed in humans. First, tau hyperphosphorylation is evident in the hippocampus, particularly in the pyramidal neurons of the CA1 subfield, and developing in the cortex at later stages of the pathology [46]. In humans, tau pathology in the cerebrospinal

fluid correlates much more strongly with neuronal death and cognitive defects than A $\beta$ , but A $\beta$  is thought to be a primary driver for the overall pathology of AD [4, 49]. Thus, the appearance of A $\beta$  pathology prior to tau pathology follows a similar trend to human AD.

In humans, synaptic loss in the frontal cortex is primarily responsible for cognitive defects and occurs early in the onset of AD [50–52]. 3xTg mice also display synaptic dysfunction and impaired long-term potentiation by 6 months of age, likely due to tau pathology [46]. Inflammation, another hallmark of AD pathogenesis, is also present in 3xTg-AD mice, with microglia activation beginning by 6–12 months of age, and astrogliosis present by 12 months [47, 53]. There is some inconsistencies in the performance of 3xTg-AD mice in motor coordination tests, but most reports agree that the mice appear to exhibit impaired motor function starting at about 6 months of age, which is consistent with motor function impairments seen in some AD patients [2, 54].

Although in humans, increased A $\beta$  levels in the brain may have been seen up to 22 years prior to exhibiting symptoms [2], the accumulation of A $\beta$  and cognitive deficits are more concurrent in 3xTg mice, which exhibit mild cognitive defects in spatial learning and memory by 4–6 months of age [47, 48, 55]. Deficits have also been found in contextual fear memory [48]. Overall, the 3xTg mouse model displays a progression of A $\beta$  and tau pathology, along with changes in neuronal function and inflammatory state, that closely mimics the progression of AD in humans, though the timing in some of these aspects are skewed, likely by the much shorter lifespan of mice.

One avenue of interest in potential AD treatments is repurposing dietary and pharmaceutical interventions that are effective in other age-related diseases in the treatment of AD. Aging is the primary risk factor for both late-onset and FAD, so geroprotective interventions may be highly beneficial in the regulation of AD. The 3xTg model has been widely used in examining the effects of these interventions in AD, which is the topic of this review and summarized in Table 1.

### mTOR signaling and Rapamycin

One potential link between insulin signaling and the progression of AD is the protein kinase mechanistic Target Of Rapamycin Complex 1 (mTORC1) (Table 1, Figure 2). mTORC1 acts as a central hub

for nutrient signaling, integrating nutritional information as well as hormonal cues to regulate the activation of anabolic processes essential for cell and organismal growth, including protein translation, ribosomal biogenesis, lipogenesis and nucleotide biogenesis; mTORC1 also negatively regulates autophagy [56, 57]. mTORC1 is a key downstream effector of insulin signaling and provides feedback inhibition of insulin signaling through its substrates S6K1 and Grb10, thus promoting the degradation of insulin receptor substrate [58–60]. As such, sustained activation of mTORC1 by nutrient overload, obesity, and aging has been suggested as an underlying molecular mechanism that drives the development of insulin resistance.

mTORC1 signaling has been linked to age-related diseases and lifespan in diverse species including worms, flies, and mammals [56, 61]. Genetically reducing mTORC1 signaling extends the lifespan of yeast, worms, flies, and mice [62–68]. Studies by multiple independent laboratories and groups have shown that the mTOR inhibitor rapamycin extends lifespan in worms, flies, and multiple strains of wild-type mice, even when treatment is intermittent or conducted for only a relatively short period of time [69–78]. Rapamycin improves multiple parameters of healthspan in mice [79]. mTORC1 signaling has been implicated in cognitive decline with normal, non-pathogenic aging, and treatment of wild-type mice with rapamycin ameliorates deficits in learning and memory, perhaps in part by preserving cerebral blood flow, and blunts age-related declines in synaptic density [80, 81].

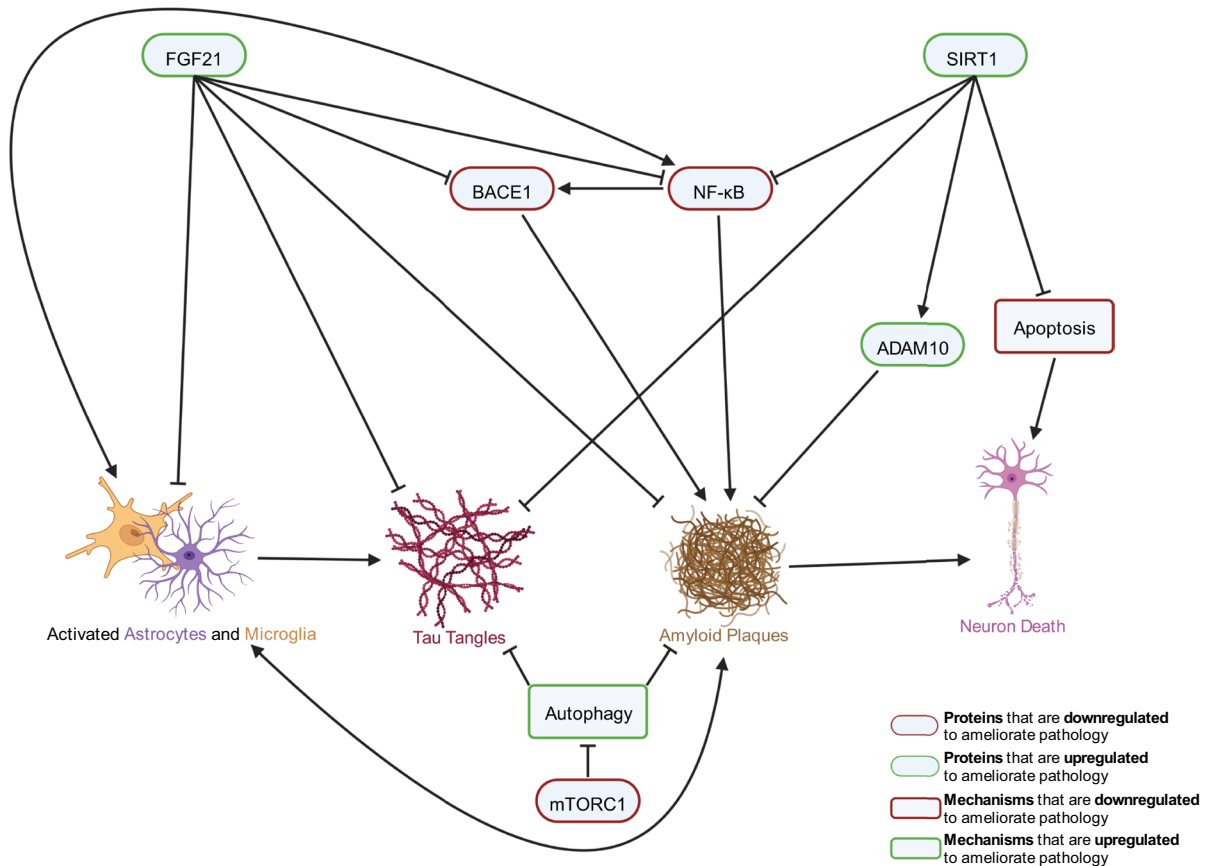
mTORC1 signaling is elevated in the brains of human AD patients [82–85], and genetic depletion of *S6K1*, a substrate and effector of mTORC1, is sufficient to improve memory and reduce AD pathology in 3xTg mice [86]. Similarly, treatment with the mTOR inhibitor rapamycin prevents the progression of AD pathology and prevents cognitive deficits in 3xTg mice, perhaps in part due to increased autophagy [85] (Table 1). In 3xTg-AD mice, autophagy improvement was seen following both short and long-term rapamycin treatment, with increases in the autophagy proteins ATG5 and ATG7, which are both necessary for autophagy induction, as well as increased LC3-II, a marker of autophagy that is incorporated into the autophagosome membrane [85].

**Table 1** Dietary interventions, mimetics, and dietary supplements examined in the 3xTg mouse model

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>Rapamycin</b>				
Caccamo et al., 2010	6 months	10 weeks	8–9 months	Improved MWM performance Decreased p-tau and A $\beta$ Decreased mTOR signaling Increased autophagy
Majumder et al., 2011	2 months	16 months	18 months	Improved NOR and MWM performance Decreased p-tau and A $\beta$ Decreased microglia activation/inflammation Increased autophagy
	15 months	3 months	18 months	No change in NOR or MWM performance No change in p-tau or A $\beta$ No change in microglia activation/inflammation Increased autophagy
<b>Caloric restriction</b>				
Halagappa et al., 2017	3 months	7 months	10 months	Improved MWM performance No change in open field test
	3 months	14 months	17 months	Improved MWM performance Increased Open Field activity Decreased p-tau and A $\beta$
Rangan et al., 2022	3.5 months	15 months	18.5 months	Improved Y-maze, NOR, and BM performance Decreased p-tau and A $\beta$ Decreased microglia levels Increased neurogenesis
	6.5 months	2 months	8.5 months	No change in Y-maze performance Improved NOR performance Decreased p-tau and A $\beta$ in males but no change in females Decreased microglial activation in females
<b>Intermittent fasting</b>				
Halagappa et al., 2017	3 months	7 months	10 months	Improved MWM performance No change in open field test
	3 months	14 months	17 months	Improved MWM performance Increased Open Field activity No change in p-tau or A $\beta$
<b>Protein restriction</b>				
Parrella et al., 2013	8–9 months	18 weeks	12.5–13.5 months	Improved NOR and Y-maze performance No change in Plus Maze performance No change in p-tau Decreased A $\beta$ No change in microglia activation/inflammation No change in glucose metabolism
<b>BCAA restriction</b>				
Torunissac et al., 2018	16 months	2 months	18 months	Improved NOR performance Decreased p-tau No change in A $\beta$
<b>Carnosine</b>				
Corona et al., 2011	1 month	11–13 months	12–14 months	No change in MWM performance No change in p-tau Decreased A $\beta$
<b>Curcumin + Fish Oil</b>				
Ma et al., 2019	5 months	4 months	9 months	Improved Y-maze performance

**Table 1** (continued)

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>Vitamin A</b>				
Biyoung et al., 2021	8 months	6 months	14 months	Improved Y maze performance No change in body weight or food intake Decreased Aβ in males but not females No change in ADAM10, BACE1, IDE, GFAP, or IBA-1
Lerner et al., 2012	7 months	8 weeks	9 months	Improved Morris radial arm water maze performance Decreased Aβ levels Increased total tau levels
Takamura et al., 2016	12 months	4 weeks	13 months	Improved cell proliferation levels Decreased microglia levels No change in astrocyte levels
Watamura et al., 2015	22 months	4 weeks	23 months	Decreased tau phosphorylation Decreased microglia levels No change in astrocyte levels



**Fig. 2** Potential pathways to target in the treatment or prevention of AD. FGF21, mTORC1, and SIRT1 are three of the most promising pathways for influencing AD pathology. Downregulation of mTORC1 may improve AD pathology through increased autophagy, leading to improved clearance of Aβ and p-tau. Upregulation of FGF21 may improve a variety of AD characteristics directly, as well as through inhibition of

both BACE1 and NF-κB. SIRT1 upregulation may improve AD pathology via inhibition of both apoptosis and NF-κB as well as promotion of ADAM10 to reduce Aβ plaque formation and neuron death. Due to the interplay between the different aspects of AD pathology, even treatments that only target one aspect may have long-reaching effects on overall AD pathology. Created with [BioRender.com](https://BioRender.com)

Rapamycin-treated 3xTg-AD mice performed better than untreated 3xTg-AD mice as well as wild-type control mice in Morris Water Maze (MWM), a hippocampal-dependent task [85, 87]. Novel Object Recognition (NOR) also showed improvements in 3xTg-AD mice treated with rapamycin [87]. Overall A $\beta$  pathology was improved in treated 3xTg-AD mice, as shown by decreased soluble and insoluble A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> levels and decreased A $\beta$  deposition [85, 87]. Tau pathology was likewise improved, and tau phosphorylation was decreased at multiple residues [85, 87]. Finally, rapamycin-treated 3xTg-AD mice showed lower levels of activated microglia, and as such, lower levels of brain inflammation [87]. Although autophagy is increased in both short and long-term rapamycin treatment, beneficial effects of rapamycin treatment on A $\beta$  and tau pathology is only seen when treatment begins before the formation of plaques and tangles, suggesting a preventative effect rather than curative [87].

As a result of the potent effects of rapamycin in the 3xTg mouse model of AD, as well as similar beneficial effects of rapamycin in other early onset AD mouse models [88–90], rapamycin has been proposed as a potential prophylactic therapy for AD [91]. However, there are potential safety concerns associated with the long-term use of rapamycin, which is FDA-approved as an immunosuppressant agent, and which also causes a variety of metabolic side effects when rodents or people are treated for long periods of time, particularly if high doses are used (reviewed by [92]). Additionally, it has been suggested that administration of rapamycin in late-stage AD may actually be detrimental due to impairment of the lysosomal system [93]. Though there is not yet any data on how AD patients may respond to rapamycin treatments, there are currently two clinical trials in progress at the University of Texas Health Science Center at San Antonio to determine the safety, tolerability, and cognitive effects of rapamycin administration in older adults with mild cognitive impairment or AD [94, 95].

### Dietary interventions in the 3xTg mouse model of AD

Caloric restriction (CR) has represented the gold standard for geroprotective dietary interventions for almost a century, robustly extending lifespan in

numerous species ranging from yeast, worms, and flies to mice, rats, dogs, and even non-human primates (reviewed in [96]). CR is an attractive option when considering AD prevention due to the beneficial effects of CR on many age-related diseases and its ability to decrease age-related disease-associated mortality [96, 97]. However, while dietary interventions such as CR may be efficacious in the laboratory, the ability of people to adhere to such an abstemious diet in the real world is limited. As such, research has begun to focus more strongly on other dietary interventions that may be easier to maintain long-term. The three dietary interventions that have been studied in the 3xTg-AD mouse model that we will discuss here are CR, protein restriction (PR), and branched-chain amino acid restriction (BCAA-R) (summarized in Table 1).

#### Calorie restriction

In addition to extending lifespan, CR also extends healthspan, with benefits including decreased adiposity, increased insulin sensitivity, decreased inflammation, decreased reactive oxidative species (ROS), and decreased lipids in the bloodstream, as well as reduced rates of diabetes and beneficial effects on brain function [96, 98–101]. Due to both the metabolic and geroprotective benefits of CR, there has been substantial interest in the use of CR as a way to treat or prevent AD. It is important to note, however, that fasting is an important aspect of many CR models. Most laboratory CR models involve feeding a reduced amount of food once a day, which results in an unintentional prolonged fasting period after the food is quickly consumed. Pak et al. examined whether fasting is required for the beneficial effects of CR [102]. They found that daily prolonged fasting without the restriction of calories recapitulated many of the benefits of a typical laboratory CR diet. In contrast, when mice were fed a diet that modeled CR without prolonged fasting, there was a large reduction in the beneficial effects of the CR diet.

Notably, mTORC1 is thought to be a key effector of the response to CR, and in yeast, deletion of *TOR1* is epistatic with CR [62]. However, studies of CR and mTOR in worms and flies have not clearly demonstrated an epistatic relationship; in flies, lifespan is extended by rapamycin at every level of calorie intake [103–107]. Extensive mammalian “omics” studies



suggest that rapamycin and CR have distinct, largely non-overlapping effects [108–113].

In various models of AD, CR decreases both A $\beta$  and tau pathology, decreases neuroinflammation, improves neurogenesis and neuroplasticity, and improves cognition [100, 114–119]. Some mechanisms by which CR may induce these beneficial effects include attenuated immune and hormone changes, increased damage repair, changes in gene expression, increased autophagy and apoptosis, altered insulin/insulin-like growth factor-1 (IGF-1) signaling, altered mTORC1 signaling, activation of sirtuins, decreased metabolic rate, and attenuated ROS generation [96, 100, 117]. In the 3xTg mouse model of AD, CR ameliorates age-related deficits in Morris Water Maze (MWM) performance and open-field activity [120]. CR also decreases A $\beta$  and tau pathology in the hippocampus, as evidenced by decreases in both A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> levels and decreased tau phosphorylation at Ser202. Halagappa et al. also looked at intermittent fasting alone and found that there were similar improvements in MWM and open-field, but observed no change in A $\beta$  or p-tau levels as a result of intermittent fasting [120]. This suggests that CR may be a useful tool in controlling the pathology of AD, but unlike with the metabolic benefits of CR, fasting alone may not recapitulate all neurological improvements.

Rangan et al. utilized a fasting-mimicking diet (FMD) composed of low calories, low protein, and high unsaturated fats which produces similar longevity-related markers as water-only fasting but provides various nutrients to lessen the burden of prolonged fasting [121]. In the first experiment, FMD was administered starting at 3.5 months of age in 4–5 day cycles, depending on sex. FMD mice had improvements in both Y-maze and NOR performance, particularly in males, at 10.5 months of age. At 18 months of age, FMD female 3xTg mice performed better in the Barnes Maze (BM). A $\beta$  load, p-tau, and microglia levels were decreased and neurogenesis was increased in FMD mice. In the second experiment, FMD was administered to 6.5-month-old mice, which are old enough that AD pathology can be observed. After 2 months of FMD, there was no change in Y-maze performance, but NOR recognition index was improved in males. A $\beta$  and p-tau load was decreased in males, but unaltered in females. However, FMD female 3xTg mice had diminished microglial activation. Overall,

FMD reduced inflammation, improved short-term memory, and ameliorated A $\beta$  and tau pathology.

Emerging data suggests that CR may have beneficial effects for non-human primates as well as for humans. CR attenuates the progression of AD-type amyloidosis in Squirrel monkeys [122]. In humans, both clinical trials and observational studies have shown that caloric restriction, particularly when paired with exercise, ameliorates mild cognitive impairment and reduces risk of AD (reviewed in [123, 124]). Given this human data and supporting data from animal models, CR seems to be a promising avenue for AD patients, despite the difficulties in adherence to a CR diet.

### Protein restriction

Ever since the very first studies of CR, scientists have wondered if the effects of CR on aging were due to reduced intake of calories generally, or due to a reduction in specific nutrients. Recently, dietary protein has been identified as a key macronutrient for aging, with studies in flies and rodents finding that animals consuming diets with less protein have better metabolic health and live longer [125–129]. While protein restriction (PR) has many of the same beneficial effects as CR, diets based on alterations in dietary macronutrients are thought to be easier to maintain than an abstemious, low-calorie CR regimen [96].

While PR has not yet been fully explored in the context of AD, 3xTg mice cycled on and off of a protein-free (PF) diet had improved working memory in the Y-maze and spatial memory in NOR [130]. Interestingly, while a CR diet reduces both A $\beta$  and tau pathology, a PF diet specifically reduced tau pathology and phosphorylation, without altering A $\beta$  immunoreactivity or A $\beta$  plaque size in the hippocampus [120, 130]. Finally, a PF diet did not affect microglial activation, suggesting that PF does not influence inflammation in the brain [130]. PF did not lead to chronic low body weight in 3xTg mice, and did not significantly decrease blood glucose levels, but does reduce circulating levels of IGF-1 and IGF binding protein-3 (IGFBP-3) levels [130]. Administration of a cyclic 7-day 4% PR diet from 3.5 months to 18 months of age resulted in improved Y-maze and NOR performance, decreased A $\beta$  and p-tau loads, and increased neurogenesis [121].

One potential mechanism by which reductions in dietary protein may prevent or treat AD is through

reduced activity of mTORC1 signaling. As amino acids are potent agonists of mTORC1, it is logical that a reduction in dietary protein might limit mTORC1 signaling and have similar effects; PR has been shown to reduce mTORC1 signaling in vivo in multiple other tissues [128, 131], and preliminary work suggests that PR may be able to reduce mTORC1 signaling in the brain [132]. Though there is some contradicting evidence from clinical and observational studies in humans, many studies suggest that high protein intake is correlated with reduced risk of AD and improved cognition [133–135]. It is unclear if the differences between animal models and human studies are due to between-species effects or age-related differences. Many AD animal models, particularly in 3xTg mice, are utilized when they are still relatively young. It is possible that PR helps to delay the onset of AD pathology but is not sufficient to decrease AD prevalence. Conversely, it is likely that differing levels of protein intake are beneficial at different life stages. In this instance, PR may be more beneficial for the young, while increased protein intake may be beneficial late in life.

#### Restriction of branched-chain amino acids

The branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) were identified as metabolically interesting in the late 1960s, when the blood levels of the BCAAs were found to be elevated in humans with obesity [136]. Since that time, blood levels of the BCAAs have been associated with cancer, cardiovascular disease and diabetes [137, 138]. As essential amino acids, blood levels of BCAAs are highly correlated with dietary levels, and increased consumption of BCAAs is associated with increases in both liver and blood levels of the BCAAs as well as the incidence of diabetes [128, 139, 140]. The BCAAs are potent agonists of mTORC1, and activation of mTORC1 has been suggested to be linked to their effects on insulin sensitivity as well as cancer [141, 142]. Dietary BCAA levels correlate negatively with lifespan in mice, with high BCAA diets shortening lifespan and BCAA restricted diets extending lifespan [127, 143].

It has been hypothesized that defects in BCAA catabolism, which elevate brain levels of the BCAAs, may contribute to the pathogenesis of AD by activating mTORC1 [137]. In the 3xTg-AD mouse model,

increased BCAA intake is closely associated with diabetes and AD, possibly due to down-regulation of BCAA transaminase (BCAT) in the brain, which in turn leads to leucine accumulation [137]. Leucine accumulation is positively associated with risk for developing AD [137, 138]. Supplementation of BCAAs leads to increased cognitive deficits, increased tau phosphorylation, and, when paired with HFD, premature death of 3xTg mice [137, 138]. Conversely, restriction of BCAA intake improves cognitive performance of 3xTg mice in NOR, although it did not decrease tau phosphorylation [138].

While the BCAAs have most often been considered as a group, an emerging realization is that the individual BCAAs have distinct effects on metabolism and signaling. The valine catabolite 3-hydroxyisobutyrate (3-HIB) has been shown to promote insulin resistance in mice by stimulating fatty acid and glucose uptake into skeletal muscle [144, 145]. Isoleucine restriction improves glucose tolerance and reduces adiposity in mice, while in humans higher dietary levels of isoleucine are associated with greater body mass index, and higher blood levels of isoleucine are associated with increased mortality [146, 147].

When considering BCAA levels in humans, Tynkynen et al. found that decreased BCAA levels are associated with increased risk of dementia and AD [148]. There have not been any studies examining the effect of individual BCAAs on cognition and AD pathology, however one study has found that a genetic predisposition to increased plasma isoleucine is positively associated with AD, suggesting that pharmacological or dietary interventions that reduce isoleucine levels may improve AD pathology [149]. Future research in animal models of AD may help to determine if the individual BCAAs have specific roles in driving the progression of AD.

#### Fgf21

All three of the dietary interventions discussed above — CR, PR, and BCAA restriction — have been shown to regulate Fibroblast growth factor 21 (FGF21), an endocrine hormone produced primarily by the liver but also by other tissues including adipose and skeletal muscle that was discovered over 20 years ago [150, 151]. FGF21 is strongly induced by protein restriction in both humans and rodents [152, 153], and is required for some of the effects

of PR on lifespan and metabolic health [154–156], at least in C57BL/6J male mice [157]. While the effects of CR on FGF21 are not as clear, there are at least some reports that CR upregulates FGF21 signaling [158]. In mice, BCAA restriction upregulates FGF21, although the effect may depend upon the exact diet and length of restriction [127, 147, 152, 159, 160]. Restriction of isoleucine, which is the most critical BCAA for metabolic responses, strongly induces FGF21 in C57BL/6J males [147].

After release from the liver, FGF21 crosses the blood brain barrier to stimulate sympathetic nerve activity via  $\beta$ -klotho which results in an overall increase in energy expenditure and insulin sensitivity and decreased food intake and body weight [151, 153, 155, 156, 161–163]. Due to the ability of FGF21 to cross the blood brain barrier, its effects on cognition have been well studied. In humans, AD patients have lower FGF21 levels [164] and increased FGF21 expression correlates with improved cognition, learning ability, and both short- and long-term memory [165]. In Wistar rats, administration of FGF21 led to decreased systemic and neural inflammation, improved hippocampal synaptic plasticity, increased dendritic spine density, improved brain mitochondrial function, decreased oxidative stress, improved neurogenesis, and decreased tau pathology [166–169]. Interestingly, intraperitoneal FGF21 administration did not increase brain insulin sensitivity in Wistar rats [167]. Other studies have also found neuroprotective effects of FGF21. FGF21 is able to decrease A $\beta$  toxicity and protects against oxidative stress and apoptosis in cultured neuroblastomas [170] and protected cultured neurons against excitotoxicity [171]. Given these many cognitive and neuroprotective improvements associated with increased FGF21 signaling, any interventions or pharmaceutical treatments that upregulate FGF21 are good candidates for improving AD pathology (Figure 2).

To summarize, dietary interventions have great potential for treatment of AD, but determining which components are most beneficial to increase or decrease in the daily diet is important, as well as identifying the molecular mediators. If increased FGF21 contributes to the beneficial effects of these diets on AD, other interventions which stimulate FGF21 — including diets low in methionine or chronic cold exposure [172–175] — may also be able

to help preserve cognition and slow the development of AD pathology. In fact, Tournissac et al. examined the ability of repeated short cold exposure to protect against cold-induced tau phosphorylation [176]. Exposing 15-month-old 3xTg mice to short-term cold over a period of 4 weeks increased brown adipose tissue thermogenesis and plasma FGF21 levels, improved glucose tolerance, and decreased tau hyperphosphorylation. They found that plasma FGF21 levels were negatively correlated with p-tau levels in the hippocampus. These results suggest that increased FGF21 production via any mechanism may be beneficial for AD pathology. It is also possible that FGF21 may interact with other mechanisms to ameliorate AD pathology, as it has been reported that FGF21 may repress hepatic mTORC1 activity [177], while diminished mTORC1 activity in muscle may promote expression of FGF21 [178].

## Dietary supplements

### Carnosine

Carnosine ( $\beta$ -alanyl-L-histidine) is synthesized in the body from  $\beta$ -alanine and L-histidine, and increased ingestion of  $\beta$ -alanine leads to increased levels of carnosine [179].  $\beta$ -alanine is found in high concentrations in meat, but due to the benefits of low-protein diets, consuming sufficient levels of  $\beta$ -alanine to significantly raise serum carnosine levels is impractical. This suggests that supplementation of  $\beta$ -alanine or carnosine may be more advantageous for the various metabolic and neurological benefits of carnosine. Carnosine, which is found in high concentrations in glial and neuronal cells throughout the brain, has been found in other models to function as a chelator for zinc as well as an antioxidant and free-radical scavenger; carnosine exerts anti-aging activity by attenuating the toxicity of byproducts of lipid peroxidation, and inhibits A $\beta$  toxicity [179–185]. Mitochondrial dysfunction, which is prevalent as an early marker in AD, tends toward enhanced ROS generation, which in turn increases free zinc levels in neurons [180, 186–188]. This increase in zinc levels then leads to increased zinc-dependent ROS formation and A $\beta$  oligomerization, all of which contribute individually to the development of AD pathology and each other [180, 187, 189–191]. Thus, carnosine's activity

as a chelator and antioxidant may be highly beneficial in AD. Additionally, carnosine acts as a rapamycin mimetic, and as such may construe beneficial effects in AD via mTOR inhibition [192].

Treatment of 3xTg male mice with carnosine for 11–13 months starting at 1 month of age did not significantly improve MWM performance or affect tau phosphorylation at the Thr231/Ser235 site, but did reduce intraneuronal A $\beta$  in the hippocampus [180]. Carnosine supplementation also reversed age-dependent mitochondria deficits commonly associated with the 3xTg-AD mouse model [180, 188].

Human AD patients have reduced plasma carnosine levels [193]. In addition, several studies have found that carnosine supplementation is associated with improved cognition [194]. Though these data are limited, the potential for carnosine to reduce toxicity and improve cognition may result in long-term benefits for AD.

### Curcumin

Curcumin is a component of the spice turmeric. Curcumin has been found to have anti-inflammatory effects, and is often used for arthritis, asthma, atherosclerosis, cancer, and diabetes [195, 196]. In vitro results have found that curcumin prevents neuronal damage, attenuates neuroinflammation, reduces oxidative damage, and ameliorates A $\beta$  accumulation in the brain [196–201]. In the 3xTg-AD mouse model, curcumin has been studied in conjunction with DHA-rich fish oil as well as by itself [199]. Both curcumin and DHA reduced phosphorylation of c-Jun N-terminal kinase (JNK), insulin receptor substrate 1 (IRS-1), and tau (at Ser422), but did not affect performance on the Y-maze [199]. The combination treatment (curcumin + fish oil) had more significant effects on each of these parameters and also showed improvements in Y-maze performance [199].

In humans, consumption in diets high in curcumin are associated with lower incidence of AD [202, 203]. Several clinical studies have shown that curcumin has a number of health benefits in multiple organ systems. Specific to AD pathology, curcumin supplementation may prevent cognitive decline, induce A $\beta$  clearance, and prevent some aspects of AD pathology (reviewed in [202, 203]). Potential mechanisms by which curcumin may induce beneficial effects on AD include

the previously mentioned anti-inflammatory effects as well as stimulating FGF21 production [204], downregulating of mTOR signaling [205], increasing SIRT1 levels [206], and inhibiting Nuclear factor kappa B (NF- $\kappa$ B) [200].

### Vitamin A

Vitamin A is a nutrient obtained through the food which is critical for proper brain function in adults, particularly in learning and memory processes—two aspects of cognition that are heavily deficient in AD [207–210]. Retinoids, including retinol, are vitamin A derivatives and are important in modulating the inflammatory response, including neuroinflammation, and contribute to normal brain function [211, 212]. Interestingly, circulating levels of vitamin A are decreased in elderly individuals concurrently with the risk of developing AD [207, 208, 213, 214], and retinol levels are also lower in AD patients [215–217]. This suggests that supplementation of vitamin A may help ameliorate AD pathology through the retinoid signaling pathway.

Male and female 3xTg mice show some improvements in AD pathology when vitamin is supplemented in diet from 8 to 14 months of age [207]. Specifically, vitamin A-supplementation increased discrimination between the familiar and novel arms of the Y-maze during the short-term memory test, without a change in visual acuity. Vitamin A supplementation decreased both A $\beta$  and p-tau in males but not females, suggesting a sex-dependent effect in some aspects of retinoid signaling. Vitamin signaling did not affect A Disintegrin and Metalloproteinase 10 (ADAM10) or BACE1 signaling, nor did it alter the inflammatory environment in the brain.

All-trans retinoic acid (ATRA) has been FDA-approved for the treatment of acute promyelocytic leukemia and acne vulgaris. Recently, it has gained interest as a potential treatment for AD. It is thought that ATRA can modulate APP cleavage regulation via promotion of ADAM10 transcription [212]. Alternative possible mechanisms for the benefits of ATRA and other retinoids is through stimulating phospholipase activity, which plays an important part in neuronal membranes [212]. Several studies have been performed in the 3xTg-AD mouse model to determine the beneficial effects of ATRA on AD pathology [212, 218, 219]. Treatment with ATRA

for 8 weeks starting at 7 months of age results in improved performance in the Morris radial arm water maze test as well as decreased A $\beta$  levels [212]. Total tau levels were higher in ATRA-treated 3xTg-AD mice, but levels of phosphorylated tau were not measured [212]. Treatment of 3xTg mice with ATRA for 4 weeks starting at either 12 or 22 months had beneficial effects, including increased cell proliferation, decreased p-tau levels, and reduced microgliosis [218, 219].

Though there do not appear to be any clinical trials on the effects of vitamin A in AD patients, the decreased levels of vitamin A during AD and improved AD pathology in 3xTg mice suggest that vitamin A supplementation may be beneficial for AD patients. There are a number of ongoing clinical trials utilizing various retinoids or retinoid X receptor agonists [220–222]. One study on isotretinoin that has already been published in cognitively healthy subjects found that isotretinoin may have dose-dependent improvements in hippocampal-based learning [223]. Furthermore, a 1 year treatment of beta carotene (a provitamin A carotenoid) in individuals older than 65 years resulted in cognitive benefits, but short-term administration of beta carotene had no impact [224]. One potential hurdle in utilizing vitamin A or retinoids for AD is the potential negative side effect. Retinoids are potentially teratogenic, and as such would not be able to be used in pregnant or breastfeeding mothers [212]. In addition, prolonged usage of retinoids may lead to neurotoxicity or gastrointestinal hemorrhage in addition to arrhythmia, nausea, abnormal liver function, skin irritation, and depression [212]. As such, treatment dosage and period will both be important considerations when utilizing retinoids to combat AD.

## Exercise

Physical exercise is well known for its beneficial effects on obesity, insulin resistance, and type 2 diabetes [225, 226], but importantly exercise also has a number of beneficial effects on the brain, promoting synaptic plasticity, neurotransmission, and neurogenesis, and inhibiting AD progression in mice [226–229]. In humans, physical exercise is correlated with decreased risk of developing MCI and improved

cognition in both AD and MCI [227, 230]. Several studies on exercise have been performed in the 3xTg-AD mouse model, utilizing both aerobic and resistance exercise (summarized in Table 2).

Resistance exercise via weighted ladder climbs resulted in improved grip strength, increased hippocampal IGF-1, and decreased A $\beta$  in females from 3 to 5 months of age [231]. In 9–10-month-old males, resistance exercise increased recognition index in NOR, improved Y-maze latency and errors, increased synaptic protein levels, reduced A $\beta$  deposits, decreased tau hyperphosphorylation, reduced microglia activation, and decreased pro-inflammatory and increased anti-inflammatory cytokine levels [232]. When 3xTg mice are given free access to running wheels — a form of aerobic exercise — from 6 until 12 months of age, they exhibit decreased neophobia, decreased anxiety, improved MWM performance, decreased A $\beta$  levels, decreased brain oxidative stress markers, and decreased mitochondrial DNA deficits [233]. Treadmill exercise 5 days a week for 9 weeks (beginning at 30 minutes per day and slowly increasing to 90 minutes per day) in 3-month-old females led to improved rotarod performance and increased hippocampal IGF-1 levels [231]. Forced running exercise on a motorized wheel bed for 12 weeks in 2.5 to 5.5 month old 3xTg-AD mice leads to reduced foot slips in the beam test, reduced removal time in adhesive test, and improved spatial recall in MWM [234]. Kim et al. found that aerobic exercise alleviates high fat diet-induced defects in metabolic function and insulin signaling while also improving cognitive function, decreasing A $\beta$  plaque accumulation and A $\beta$ <sub>42</sub> levels, and reducing tau phosphorylation [226].

Dungan et al. used a combination resistance/aerobic exercise program called PoWeR (progressive weighted wheel running) where a running wheel is weighted with 2 g of resistance for the first 2 weeks then slowly increased to 5 g of resistance [19]. They began exercising female 3xTg-AD mice at 2 months of age and exercised them for 20 weeks. Though the authors did not look at hallmarks of AD pathology, they did look into potential mechanisms for how exercise may improve AD pathology, specifically BACE1, Dicer, and miRNA abundance. Endurance exercise has been found to downregulate BACE1, making it a possible mechanism for exercise-derived improvements in AD [17, 18]. PoWeR-exercised mice have lowered BACE1, increased Dicer expression, and

**Table 2** Exercise and exercised plasma in the 3xTg mouse model

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>Exercise</b>				
Bareiss et al., 2022	2.5 months	12 weeks	5.5 months	No change in body weight Improved challenging beam test performance Improved adhesive removal test performance No change in open field test Improved MWM performance
Dungan et al., 2020	2 months	20 weeks	7.5–8 months	Increased Dicer gene expression Decreased BACE1 Changed overall mRNA abundance
García-Mesa et al., 2012	6 months	6 months	12 months	No change in body weight Improved reflex test, wooden rod test, and wire rod test Ameliorated neophobia/improved open field performance Decreased latency to lit area in dark/light box Decreased A $\beta$ Decreased p-tau (when used in conjunction with melatonin) Improved MWM performance Decreased brain oxidative stress Ameliorated mitochondrial DNA deficits
Kim et al., 2017	4 months	20 weeks	9–10 months	Improved NOR and MWM performance Decreased p-tau and A $\beta$ Decreased microglia activation/inflammation Increased synaptic plasticity Decreased neurodegeneration Decreased HOMA-IR and GTT AUC
Liu et al., 2020	9 months	4 weeks	10 months	No change in open field performance Decreased body weight Improved NOR and Y-maze performance Increased synaptic protein levels Decreased A $\beta$ deposits and tau hyperphosphorylation Decreased microglia activation and pro-inflammatory cytokines Increased anti-inflammatory cytokines
Pena et al., 2020	3 months	9 weeks	5 months	Resistance training improved grip strength and decreased A $\beta$ Aerobic training improved rotarod performance but did not change A $\beta$ levels Both trainings increased hippocampal IGF-1 levels No change to p-IGF-1 R, p-AKT, p-MAPK, p-GSK3 $\beta$ , cathepsin B, p-CREB, or BDNF No change to hippocampal respiration
<b>Exercised plasma</b>				
Kim et al., 2017	12 months	1 month	13 months	Increased BDNF Improved MWM performance No changes in p-tau or p-GSK3 $\beta$ Improved mitochondria function in hippocampus Inhibited apoptosis and cell death in hippocampus Increased levels of synaptic proteins in hippocampus Increased neurogenesis

*HOMA-IR*, Homeostatic Model Assessment for Insulin Resistance; *GTT*, glucose tolerance test; *MAPK*, mitogen-activated protein kinase; *GSK3 $\beta$* , glycogen synthase kinase-3 beta; *CREB*, cAMP response element binding protein; *BDNF*, brain-derived neurotropic factor

overall beneficial changes in miRNA abundance, suggesting that these may be some of the mechanisms by which exercise improves AD [19].

Unfortunately for AD patients, late-stage AD pathology may lead to decreased motility and overall inability to exercise, making any benefits that exercise may have on AD pathology null. However, plasma from young, exercised individuals may be used to construe the same beneficial effects. Treating 12-month-old male 3xTg mice either young plasma or plasma from young, exercised mice for 1 month increased levels of synaptic proteins in the hippocampus, and mice treated with plasma from young, exercised mice had increased brain-derived neurotrophic factor (BDNF), learning ability and both short- and long-term memory in MWM, increased neurogenesis. Young, exercised plasma reduced ROS, inhibited apoptosis and cell death in the hippocampus, and maintained mitochondria homeostasis [235].

Though more studies are needed to parse out the exact mechanisms by which exercise may ameliorate AD pathology, there is evidence that exercise promotes FGF21 expression and may activate the SIRT1 pathway [232]. These studies show that exercise alone may have a significant effect on AD. If the patient is unable to exercise, exercised plasma transfusions from another individual may be able to substitute for exercise, or small molecules which mimic the beneficial effects of exercise on AD may be developed. It is also possible that exercise may be combined with dietary interventions or pharmaceutical treatment to have even greater beneficial effects on cognition.

In humans, exercise has diverse metabolic and anti-aging benefits. It also has limited side effects compared to other geroprotective interventions and as such is well studied in human AD patients [225, 227, 230, 236–250]. Though there is a lot of inter-individual heterogeneity, the sheer number of clinical trials that have been performed allow meta-analyses to be performed and to determine that there are overall benefits to cognition, activities of daily living (ADL), and even AD risk (reviewed in [230, 239, 240, 244]). Of special note, the planned ExPlas study is unique from other exercise clinical trials as it will utilize plasma from exercise-trained donors [245]. If effective, plasma from exercised donors will be especially beneficial in AD treatment as AD is associated with motor impairments and AD patients may not be able to exercise effectively.

In addition to physical limitations of AD patients, exercise as a geroprotective intervention also faces a strong barrier in motivation to exercise. In an effort to encourage exercise in elderly adults, particularly those with AD, a number of programs have been developed, including telerehabilitation and community-based exercise programs [237, 241, 246]. Though it is hard to enforce participation in programs such as these, these programs should be readily available to patients as the benefits of exercise in old age and AD are strongly supported by both animal models and human clinical trials.

## Type 2 diabetes mellitus drugs

Due to the high prevalence of AD and diabetes co-occurrence, the use of diabetes drugs to treat AD has been of considerable interest (diabetes drugs tested in 3xTg mice summarized in Table 3). Linagliptin is a Dipeptidyl peptidase 4 (DPP-4) inhibitor, and often one of the first drugs used to treat diabetes in patients due to its strong inhibitory effects [251, 252]. DPP-4 inactivates glucagon-like peptide (GLP) and glucose-dependent insulinotropic peptide (GIP), which regulate postprandial plasma glucose concentrations and sensitize pancreatic  $\beta$ -cells to glucose stimulation, respectively [252]. The GLP-1 receptor is highly expressed in the central nervous system, particularly in the hypothalamus and hippocampus [253, 254]. In addition to its direct effects on DPP-4, linagliptin may also ameliorate AD pathology via mTOR, SIRT1 [251, 255]. In the 3xTg-AD mouse model, females treated with linagliptin exhibited improved cognition and pathology, including reduced  $A\beta_{42}$  levels and reduced tau phosphorylation [256]. Linagliptin-treated 3xTg-AD mice had improved performance on both the MWM and Y-Maze, which suggests improvements in spatial learning and working memory compared to non-treated mice. Finally, linagliptin-treated 3xTg-AD female mice had decreased levels of glial fibrillary acidic protein (GFAP), a marker for inflammation [256].

Instead of blocking the action of DPP-4, an alternative way to activate GLP-1 signaling is with synthetic GLP-1 receptor agonists such as liraglutide and exenatide. Additional potential mechanisms for improvements by GLP-1 receptor agonists in AD may be due to increased FGF21 and SIRT1 activity,

**Table 3** Type II diabetes mellitus drugs tested in the 3xTg mouse model

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>Linagliptin</b>				
Kosaraju et al., 2017	9–10 months	8 weeks	11–12 months	Improved MWM and Y-maze performance Decreased p-tau and A $\beta$ Decreased microglia activation/inflammation No change in plasma glucose levels
<b>Liraglutide</b>				
Chen et al., 2017	7 months	8 weeks	9 months	Improved MWM performance Decreased neurodegeneration No change in plasma glucose levels
<b>Exenatide</b>				
Bomba et al., 2013	3 months	9 months	12 months	No change in MWM performance No change in LDH activity or anaerobic glucose catabolism
<b>Pioglitazone</b>				
Masciopinto et al., 2012	3 months	9 months	12 months	No change in NOR or MWM performance No changes in plasma glucose levels, GTT, or serum insulin levels
Searcy et al., 2012	10 months	14 weeks	14–15 months	Improved avoidance in AASB Decreased p-tau and A $\beta$ Increased synaptic plasticity
Yu et al., 2014	10 months	4 months	14 months	No change in spontaneous exploratory activity, locomotion, or anxiety Improved MWM performance Decreased p-tau Decreased astrocyte levels
<b>Rosiglitazone</b>				
Yu et al., 2014	10 months	4 months	14 months	No change in spontaneous exploratory activity, locomotion, or anxiety No change in MWM performance Decreased p-tau Decreased astrocyte levels
<b>GLP-1/GIP/Glucagon triagonist</b>				
Li et al., 2018	7 months	1 month	8 months	Improved MWM and Y-maze performance Increased Open Field activity Decreased p-tau and A $\beta$ Increased synaptic plasticity No changes in plasma glucose levels
Li et al., 2020	7 months	45 days	8 months	Improved radial arm maze performance Increased synaptophysin and PSD-95 in the hippocampus Regulated altered neuron activity in the hippocampus
<b>Insulin</b>				
Chen et al., 2014	9 months	7 days	9 months	Increased levels of synaptic proteins Decreased microglia activation Decreased A $\beta$ levels No change in p-tau levels



**Table 3** (continued)

Source	Starting age	Treatment period	Age at analysis	Treatment effects
Sanguinetti et al., 2019	2 months	6 months	8 months	Improved Y-maze test performance Normalized cytokine levels No change in brain mass Normalized thickness of brain layers
	2 months	12 months	14 months	Improved Y-maze test performance Decreased pro-inflammatory cytokine levels No change in brain mass Normalized thickness of brain layers
Vandal et al., 2014	6 months	9 months	15 months	Improved NOR performance Decreased A $\beta$ levels

*LDH*, lactate dehydrogenase; *GTT*, glucose tolerance test; *AASB*, active avoidance shuttle box

decreased inflammation, and increased insulin sensitivity [257, 258]. The effects of both liraglutide and exenatide have been examined in the 3xTg-AD mouse model [253, 259]. Liraglutide improved performance in MWM and attenuated tau phosphorylation at Thr231, Ser396, Ser214, and Ser199/202, but not Thr217 [253]. Liraglutide also protected the 3xTg-AD mice from neurodegeneration and promoted JNK and extracellular signal-regulated kinase (ERK) signaling [253]. Exenatide did not improve cognition or pathology in 3xTg mice, though it did improve cognition in a different mouse model of AD [259].

Pioglitazone is an insulin sensitizer used in T2DM treatment [251]. In addition to improving insulin sensitivity and as a result glycemic control, pioglitazone also downregulates NF $\kappa$ B transcription and mTOR signaling [260, 261]. Masciopinto et al. studied the effect of pioglitazone administered to 3xTg mice for 9 months starting at 3 months of age [262]. Pioglitazone did not improve memory performance on either MWM nor NOR; the mice also did not show any improvements in glucose metabolism or brain mitochondrial function [262]. This is at odds with other studies, which found that treatment with pioglitazone for 4 months starting at 10 months of age had a variety of benefits for 3xTg mice [260, 263]. These 14-month-old mice showed reduced soluble intracellular A $\beta$  levels in the CA1 region of the hippocampus [260] along with decreased tau phosphorylation [260, 263]. While they did not show improvements in light/dark preference, spontaneous exploratory activity, locomotion, or anxiety, they did have improved avoidance learning and MWM performance [260, 263]. These mice also had improved long-term plasticity

and increased membrane hyperpolarization, suggesting a younger neuronal phenotype [260]. Finally, pioglitazone-treated mice exhibited decreased astrocyte levels [263].

Rosiglitazone is another insulin sensitizer commonly used in T2DM patients with similar mechanisms as pioglitazone. When 10-month-old 3xTg-AD mice were treated with rosiglitazone for 4 months, they exhibited similar improvements as pioglitazone [263]. Pioglitazone-treated mice had improved MWM performance, decreased p-tau levels, and decreased astrocyte activation, but showed no changes in spontaneous exploratory activity, locomotion, or anxiety [263].

A novel GLP-1/GIP/Glucagon receptor (Gcg) triagonist developed by Finan et al. in 2015 may also have potential as an AD treatment [264]. It has been found to ameliorate obesity and diabetes in rodents through improvements in glucose homeostasis and increased FGF21 levels [264]. When tested in 7-month-old 3xTg mice, this novel triagonist improved long-term spatial memory in the MWM as well as working memory in the Y-maze and radial arm maze [265, 266]. Long-term potentiation and synaptic plasticity in the CA1 region of the hippocampus was also improved, along with decreased A $\beta$  and tau aggregation in the hippocampus [265]. Synaptic markers synaptophysin and postsynaptic density protein 95 (PSD-95) were increased in the hippocampus [266]. Finally, the triagonist normalized spontaneous excitatory synaptic activities and hyperexcitability of hippocampal neurons [266].

Finally, something as simple as insulin may be beneficial in the treatment of AD. Insulin injections

are commonly used in T2DM, and due to the prevalence of AD and T2DM occurring comorbidly, insulin injections may not only help with treating T2DM but AD as well, by improving insulin signaling in the brain. After placing 3xTg-AD mice on a HFD, they develop impaired glucose tolerance as well as greatly worsened A $\beta$  pathology and cognitive function [267]. Injecting these much with a single injection of insulin improves memory in the novel object recognition tests, reduced soluble A $\beta_{42}$  levels, and even decreased A $\beta$  production and increased A $\beta$  clearance [267]. Intranasal administration of insulin is an effective way of bypassing the blood-brain barrier and delivering insulin directly to the brain [268–271]. An important question to consider when examining the potential benefits of intranasal insulin administration is whether chronic administration is necessary or if acute treatment is sufficient. Chen et al. examined short-term intranasal insulin administration in 3xTg-AD mice and found that a 7-day treatment period was sufficient to increase levels of synaptic proteins and decrease bot microglia and A $\beta$  levels, but was not able to decrease tau phosphorylation [268]. In HFD-fed 3xTg-AD mice, weekly intranasal insulin therapy for either 6 or 12 months ameliorated some aspects of HFD-exacerbated pathology [29]. HFD resulted in impaired spatial memory, which ameliorated by intranasal insulin in both 8 and 14-month-old mice [29]. In addition, intranasal insulin altered cytokine levels and was able to normalize some aspects of brain physiology [29]. These results suggest that insulin injections, both peripherally and intranasal, may be beneficial in AD, likely via improvements in insulin signaling.

These studies suggest that there is strong potential for already available T2D drugs to have benefits on AD pathology. Clinical trials in AD patients are needed to determine how translatable these benefits are as well as whether the benefits are due solely to improvements in glucose homeostasis or occur independently in metabolically healthy individuals. So far, some clinical trials utilizing T2D drugs in patients with AD and MCI have already been performed. Of the drugs discussed here, liraglutide treatment had neuroprotective effects, and both pioglitazone and rosiglitazone induced cognitive improvements in a number of studies (reviewed in [272]). Intranasal insulin has been widely studied in patients with AD and MCI (reviewed in [272]). As a whole, the

findings of clinical trials on intranasal insulin have been largely positive. Intranasal insulin improves memory and cognition, and functional ability, and has additional neuroprotective effects [269–273]. Further research is needed to determine if benefits are solely due to improvements in glycemic control (and thus may only benefit patients with T2D) or if there are additional off-target effects of these drugs such via mechanisms like mTOR, SIRT1, and FGF21.

### Anti-cancer therapeutics

As another age-related disease, therapies that are protective against various cancers may also be beneficial in the treatment of AD. Many of the aspects of AD pathology are shared with cancer such as microtubule disassembly and signaling pathway abnormalities. There have been preliminary studies on the efficacy of anti-cancer therapeutics in the 3xTg model that show promising results (Table 4).

Paclitaxel is a commonly used microtubule-stabilizing drug in cancer patients [274–277]. In cancer, microtubule-stabilizing drugs work by preventing microtubule disassembly to inhibit cellular mitosis and thus tumor growth [277, 278]. In AD, microtubule-stabilizing drugs may prevent neurodegeneration and preserve already damaged neurons [277]. Many microtubule-stabilizing drugs including paclitaxel are limited in their ability to cross the BBB, making delivery method an important consideration [277]. Cross et al. treated 3xTg mice intranasally as a non-invasive method of bypassing the BBB [277]. Two cohorts of mice were treated; a young cohort treated from 2 to 6.5 months to determine efficacy of preventing AD pathology and an old cohort treated from 11 to 17 months to determine the ability of paclitaxel to improve cognitive performance and reduce anxiety. In the young cohort, paclitaxel reduced p-tau levels, reactive gliosis, and astrocyte activation while also increasing axonal transport. In older mice with already developed AD pathology, paclitaxel improved performance in the radial water tread maze and decreased anxiety in the elevated plus maze.

Polyphillin I (PPI) is a drug isolated from plants that may inhibit the proliferation of cancer cells via downregulation of the expression of cancerous inhibitor of protein phosphatase 2A (CIP2A) which is upregulated in the brains of AD patients [279, 280].

**Table 4** Anti-cancer therapies examined in 3xTg mice

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>Paclitaxel</b>				
Cross et al., 2021	2 months	4.5 months	6.5 months	Decreased p-tau Decreased Iba-1 and GFAP Increased axonal transport
	11 months	6 months	17 months	Improved radial water tread maze performance Decreased anxiety in elevated plus maze
<b>Polyphillin I</b>				
Zhou et al., 2020	10 months	1 month	11 months	Improved objection recognition and object location memory Improved MWM performance Improved fear conditioning memory Increased neuron and spine numbers Restored PP2A activity Decreased A $\beta$ and p-tau in hippocampus
<b>Radiotherapy</b>				
Wilson et al., 2020	16 months	5 days	18 months	Decreased A $\beta$ plaques Decreased NFTs

Treatment of 10-month-old male 3xTg mice for 1 month results in improved object recognition performance and object location memory, improved spatial learning and memory in MWM, and rescued fear conditioning memory impairment [280]. PPI increased neuron and spine numbers while reducing A $\beta$  loads and tau phosphorylation in the hippocampus. Zhou et al. confirmed that the reduction in tau phosphorylation was due directly to restored protein phosphatase 2A (PP2A) activity via in vitro experiments.

Radiotherapy is a well-known anti-cancer therapy. In adult patients with small cell lung cancer and pediatric leukemia, the standard of care frequently includes low dose prophylactic cranial radiation to decrease central nervous system relapse [281, 282]. Radiotherapy is successful in treating extracranial amyloidosis [282–284]. In 16 month old female 3xTg mice low dose irradiation results in decreases in both A $\beta$  plaques and neurofibrillary tangles [282].

Interest in low dose irradiation as an AD treatment began with a single case study in 2015 in which a patient with advanced AD showed improvements in cognition, memory, and speech after consecutive CT scans [285]. Based on the results of that case, Cutler et al. treated four patients with three consecutive treatments of low-dose irradiation and witnessed improvements in cognition and behavior for three out of the four patients [286]. There are a large number of additional ongoing clinical studies on the efficacy of

low-dose radiotherapy in AD listed on [clinicaltrials.gov](https://clinicaltrials.gov). Neither paclitaxel nor PPI have been studied in humans with AD or MCI, however a number of other anti-cancer drugs have had AD or MCI-related clinical trials, the results of which have been extremely varied but promising (reviewed in [287]).

### Histone deacetylase inhibitors

Histone deacetylases (HDACs) are enzymes that remove acetyl groups from lysine residues on histones [288]. HDAC enzymes are categorized into four classes, the Class I Rpd3-like proteins, Class II Hda1-like proteins, Class III Sir2-like proteins, and the Class IV protein [288]. In addition to deacetylation of histones, HDACs also deacetylate a number of non-histone substrates, primarily those involved in apoptosis, autophagy, and cell cycle regulation [289]. HDAC inhibitors increase histone acetylation and enhance memory and plasticity, leading them to be a potential modulator of AD pathology [290–293].

RGFP-966 is a selective HDAC3 (a class I HDAC) inhibitor that has been found to cross the blood-brain barrier [294]. HDAC3 is highest in brain regions involved in memory and learning, such as the hippocampus and cortex, where AD pathology is the highest [294–296]. Treatment with RGFP-966 in 9-month-old 3xTg-AD mice for 3 months improves

spatial and recognition memory in the Y-maze, NOR, and BM tests [294]. RGFP-966 also improved motor function, as indicated by rotarod, treadmill and balance beam [294]. A $\beta$  pathology was also ameliorated; A $\beta$ <sub>42</sub> levels were decreased in the brain and plasma, and there was increased activity of neprilysin, an A $\beta$  degrading enzyme, suggesting improved A $\beta$  degradation and clearance [294]. Finally, treatment with RGFP-966 reduced tau phosphorylation at Thr181 in the entorhinal cortex and phosphorylation at Ser396 in the entorhinal cortex and hippocampus [294].

Nicotinamide is released from NAD<sup>+</sup> during deacetylation by sirtuins, a class III NAD<sup>+</sup>-dependent HDAC. It acts as a non-competitive inhibitor to sirtuins, as well as a Poly [ADP-Ribose] Polymerase I (PARP-1) inhibitor [290, 297]. PARP-1 typically functions as a DNA repair enzyme, but under intense DNA damage can lead to non-apoptotic cell death, which has been associated with a variety of neurodegenerative diseases [297]. Nicotinamide also plays a role in regulation of mTOR [298]. Administration of nicotinamide for 4 months to 4-month-old 3xTg mice improved both short- and long-term spatial learning in the MWM and improved contextual learning in the fear-conditioning assay [290]. No changes in A $\beta$  pathology were found but tau pathology was ameliorated. In addition to ameliorating tau pathology and cognitive deficits, Nicotinamide treatment had a number of other beneficial effects on overall neuronal function. Interestingly, Green et al. also treated 20-month-old mice with nicotinamide for 4 months and found that once tau pathology has progressed to such an advanced stage, nicotinamide treatment does not induce any changes in cognitive function or tau phosphorylation, but did significantly reduce soluble A $\beta$ <sub>42</sub> levels. Sirtuin activity was reduced in the nicotinamide-treated mice.

The response of 3xTg mice to HDAC inhibitors were extremely positive, which means that HDAC inhibitors are a strong possible candidate in the treatment of AD. To our knowledge, there have not yet been any clinical studies utilizing RGFP-966 in AD but there are two clinical trials for the administration of nicotinamide to patients with AD or MCI have been completed but the results of which are not yet available [299]. One possible problem with utilizing HDAC inhibitors to treat AD is that HDAC I reduction is seen in AD patients, and decreased HDAC I levels are associated with neurodegeneration and

cognitive decline [300]. In addition, SIRT1 is a class III HDAC of special interest in AD as its levels are decreased in AD patients [288, 301, 302]. Therefore, inhibition of HDACs may actually be detrimental in AD, which suggests the benefits of nicotinamide administration may be due primarily to its inhibition of PARP-1 or regulation of mTOR.

### Sirtuin activators

Nicotinamide riboside (NR) is a NAD<sup>+</sup> precursor. NAD<sup>+</sup> depletion is an important aspect of mitochondria dysfunction, which is a hallmark of many neurodegenerative diseases including AD [303, 304]. NAD<sup>+</sup> is a metabolite that is necessary for mitochondrial function, stem cell self-renewal, and neuronal stress resistance, and as such, is especially important in the brain [303, 305]. Neurons in the brain are thought to be especially sensitive to a depletion of NAD<sup>+</sup> due to their high energy demands, so supplementation of a NAD<sup>+</sup> precursor such as NR may improve neuronal function [303, 304]. As sirtuins are NAD<sup>+</sup>-dependent HDACs, increased NAD<sup>+</sup> levels also lead to increased sirtuin activity. In addition, NR alleviates FGF21 resistance to improve FGF21 signaling [306]. Administration of NR for 6 months to 16–18-month-old 3xTg-AD mice increased the NAD<sup>+</sup>/NADH ratio in the cortex of the brain, which is typically lower in this model compared to wild-type controls; this suggests that orally administered NR crosses the blood-brain barrier to boost NAD<sup>+</sup> levels in the brain [303]. NR treatment at this age also improved spatial learning and working memory on both the MWM and Y-maze as well as motor function in both rotarod and grip strength analysis. No changes in A $\beta$  pathology were seen with NR treatment, but tau hyperphosphorylation was ameliorated. In addition to ameliorating tau pathology and cognitive deficits, NR treatment had a number of other beneficial effects on overall neuronal function, suppressed inflammatory responses, upregulated insulin-signaling pathways, improved long-term potentiation, decreased mitochondrial ROS levels, decreased DNA damage and decreased apoptosis.

Resveratrol is a natural compound found in grapes, red wine, peanuts, soy, and tea [307–309]. It has been found in animal models to have antioxidant, anti-inflammatory, and neuroprotective with longevity-promoting properties, making it a potential candidate

for AD treatment [307, 309–311]. Resveratrol has been proposed to mimic the health benefits of caloric restriction through activation of SIRT1 [312–314].

Resveratrol treatment of 3xTg mice for 10 months starting at 2 months of age improved motor function as well as spatial and recognition memory in MWM and NOR assays [309]. Resveratrol also protected against both A $\beta$  and tau pathology, as indicated by increased levels of the amyloid-degrading enzyme neprilysin, decreased intracellular APP and A $\beta$  levels, and decreased tau phosphorylation at Ser202 [309]. Resveratrol did not improve neuroplasticity, unlike many of the other potential AD treatments, but did enhance the ubiquitin-proteasome system activity, which is important for A $\beta$  and p-tau clearance [309]. When resveratrol was administered for only 5 months, treated mice exhibited decreased A $\beta$  levels, inflammation, apoptosis, autophagy-related protein expression, BACE1 levels and NF- $\kappa$ B levels [315]. Resveratrol treatment also decreased  $\alpha$ -synuclein, which can form amyloid-like fibrils under oxidative stress [315]. Resveratrol also increased neurotrophin and synaptic marker levels such as BDNF, NGF, synaptophysin, and PSD-95 levels. SIRT1 activation was increased, supporting the mechanism by which resveratrol may enact its beneficial effects [315]. These results suggest that even short-term resveratrol treatment can have beneficial effects on AD pathology, though it is still unknown how long lasting these benefits may be.

Recently, a novel formulation of resveratrol that has increased pharmacokinetic properties has been developed [316]. Dennison et al. performed both a sub-chronic reversal as well as a prophylactic study utilizing 3xTg mice to examine the potential benefits this drug may have on AD pathology [316]. In the sub-chronic reversal study, 15-month-old male 3xTg mice were treated for 36 days. At the end of the treatment, they exhibited increased ADAM10 in the hippocampus and decreased inflammatory cytokines, tumor necrosis factor alpha (TNF $\alpha$ ), and interleukin 6 (IL-6) in the liver and spleen. In the prophylactic study, 4-month-old male and female 3xTg mice were treated for 9 months. The treated mice showed improvements in object location memory and NOR but had no improvements in spatial memory or sensorimotor behavior. Tau levels in the hippocampus and p-tau in the prefrontal cortex were decreased but A $\beta$ <sub>42</sub> levels were decreased only in the entorhinal cortex. AD-related gene expression as a whole was improved; particularly increased SIRT1

activity, increased *Adam10* and *Bdnf* expression, and decreased *Bace1* and *Psen1*. Inflammatory markers *Tnf* and *Il6* were also decreased.

In summary, sirtuin activators are a promising avenue to explore in the treatment of AD pathology (Figure 2). Of the sirtuin activators studied in the 3xTg mouse model, there have been many beneficial effects and they may mimic many of the benefits of caloric restriction without the difficulties of a strict dietary regimen (Table 5). In addition to activating sirtuin, many of the sirtuin activators studied also inhibit NF- $\kappa$ B production, such as resveratrol [310, 311, 315]. In a clinical trial, nicotinamide riboside administration did not improve cognition, though the experimenters did see positive changes in brain function via fMRI [317]. In contrast, resveratrol showed much more promising results. Resveratrol was found to improve A $\beta$  clearance, as shown by stabilized levels of A $\beta$  in CSF and plasma, and modulated the inflammatory response [318]. Finally, there was a small amelioration of functional decline as quantified by the Alzheimer's Disease Cooperative Study-Activities of Daily Living measure [318]. As such, both preclinical and clinical studies have shown sirtuin activators to be effective at ameliorating AD pathology.

## Fingolimod

Sphingosine-1-phosphate (S1P) is a phospholipid that has been implicated in metabolic dysfunction such as in diabetes and obesity [319, 320]. S1P regulates many cellular processes through its paracrine and autocrine signaling. S1P is positively associated with obesity, but is thought to actually ameliorate obesity through energy homeostasis regulation [319]. In diabetes, S1P levels are increased, but it appears to have differing effects depending on which receptor it is bound to [319]. S1P1R activation ameliorates diabetes, whereas S1P2R activation exacerbates it. Furthermore, ceramide, a precursor of S1P, has important effects on cell growth, proliferation, cell migration, apoptosis, and differentiation [319, 320]. Ceramides have been found to inhibit insulin signaling and stimulate pancreatic  $\beta$  cell death [320]. S1P, however, appears to prevent cell death, suggesting that increased conversion of ceramide (and potentially other S1P precursors) may be beneficial in diabetes [320]. Further study on S1P signaling in obesity

**Table 5** Miscellaneous geroprotective therapies tested in the 3xTg mouse

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>RGFP-966</b>				
Janczura et al., 2018	9 months	3 months	12 months	Improved NOR, BM, and Y-maze performance Improved motor function Decreased p-tau and A $\beta$
<b>Nicotinamide</b>				
Green et al., 2008	4 months	4 months	8 months	No change in NOR performance Improved MWM performance Increased contextual freezing Decreased p-tau No change in A $\beta$
<b>Nicotinamide Riboside</b>				
Hou et al., 2018	16–18 months	3–6 months	19–24 months	Improved NOR, MWM, and Y-maze performance Improved motor function Increased cued freezing, no change in context freezing Decreased p-tau No change in A $\beta$ Decreased microglia activation/inflammation Increased synaptic plasticity No change in neurodegeneration
<b>Resveratrol</b>				
Broderick et al., 2020	2 months	5 months	7 months	Decreased inflammation Decreased A $\beta$ Decreased $\alpha$ -synuclein, BACE1, NF- $\kappa$ B, GFAP, PARP Increased BDNF, NGF, synaptophysin, and PSD-95 Decreased apoptosis and autophagy protein expression, increased p62 levels
Corpas et al., 2019	2 months	10 months	12 months	Improved NOR and MWM performance Increased light preference in AASB Increased Open Field Activity Decreased p-tau and A $\beta$
Dennison et al., 2022	15 months	36 days	16 months	Increased ADAM10 in hippocampus Decreased TNF $\alpha$ and IL-6 in liver and spleen
	4 months	9 months	13 months	Improvement in object location memory and NOR performance No change in BM, open field or rotarod Decreased A $\beta$ , tau, and p-tau Increased SIRT1 activity Increased <i>Adam10</i> and <i>Bdnf</i> levels Decreased <i>Bace1</i> , <i>Psen1</i> , <i>Tnf</i> and <i>Il6</i> levels
<b>Fingolimod</b>				
Fagan et al., 2022	6 months	6 months	12 months	Improved novel object location memory and MWM performance Reduced microglial activation Reduced circulating and infiltrating lymphocytes Decreased IL-6 and increased IL-10 Decreased p-tau and APP in cortex
<b><math>\alpha</math>7 nAChR agonist</b>				
Medeiros et al., 2014	15–18 months	3 months	18–21 months	Improved NOR and MWM performance Increased contextual freezing No change in p-tau or A $\beta$ No change in microglia activation/inflammation

NGF, nerve growth factor; AASB, active avoidance shuttle box

and diabetes is needed as there is controversy over whether increased S1P exacerbates metabolic syndrome or S1P is increased as a compensatory mechanism to combat metabolic syndrome [319, 320]. In AD, the S1P system is altered, suggesting that pharmaceuticals that normalize S1P system function, like fingolimod, may be beneficial in the treatment of AD [321, 322]. In AD, S1P kinase-1 and -2 levels are decreased and sphingosine lyase is increased, leading to a decrease in S1P [323]. S1P in AD models inhibits neuronal death, protects against A $\beta$  toxicity, and reduces neuroinflammation [321, 323]. Ceramide levels are increased in AD, and may contribute to axonal degeneration and neuronal death via increased ROS production [323].

Fingolimod is an oral neuroimmunomodulatory drug used to treat patients with multiple sclerosis [322, 324]. It readily passes through the BBB to bind to the S1P receptors in the brain, except for S1P<sub>2</sub> [322]. Fingolimod has been found to target S1P receptors on neuronal and glial cells, leading to regulation of neurogenesis, myelination, inflammation, and astrogliosis. Male 3xTg mice treated with fingolimod from 6 to 12 months of age had improved AD pathology and improved memory as assessed by the NOR and MWM tests [322]. Fingolimod also reduced microglial activation and the presence of both circulating and infiltrating CD3+ T cells. The inflammatory profile in the brain was improved, as shown by decreased IL-6 and increased interleukin 10 (IL-10) levels. The cortex had decreased p-tau and APP levels, suggesting an overall diminished AD phenotype. Although fingolimod has extremely promising results in 3xTg mice and other animal models, the number and severity of possible side effects introduce an inherent roadblock in utilizing fingolimod in AD patients [325, 326].

### $\alpha_7$ Nicotinic acetylcholine receptor agonist

Cholinergic dysfunction is prevalent during neurodegenerative diseases such as AD [327, 328]. Of the cholinergic receptors, the  $\alpha_7$  nicotinic acetylcholine receptors ( $\alpha_7$  nAChRs) have been found to be closely linked with neurodegenerative and inflammatory diseases, particularly in the brain, as they are primarily expressed on neurons, macrophages, microglia, and astrocytes [196, 327, 329–331].  $\alpha_7$  nAChRs are

important in AD because their levels are decreased in the cortex of AD patients and A $\beta$ <sub>42</sub> has been found to bind to  $\alpha_7$  nAChRs, allowing it to accumulate in neurons [327, 332–334].

Both blocking and activating  $\alpha_7$  nAChRs have been proposed as potential treatments for AD [327]. In 3xTg-AD mice, administration of A-582941, an  $\alpha_7$  nAChR, restores learning and memory in the MWM, NOR, and contextual fear conditioning [327]. A-582941 treatment did increase the levels of memory-associated proteins in the brain, such as c-Fos levels and CREB, TrkB, and BDNF phosphorylation, but did not affect A $\beta$  or tau pathology [327]. Finally, A-582941 treatment did not ameliorate neuroinflammation in the brains of 3xTg-AD mice [327]. These results suggest that while A-582941 treatment does improve some of the downstream cognition effects of AD pathology, it does not target the main causes, namely A $\beta$  and tau accumulation.  $\alpha_7$  nAChRs may be useful in treating symptoms of AD in later stage AD patients but may not have a beneficial effect on the disease progression itself.

In humans, some clinical trials of  $\alpha_7$  nAChR modulators have shown promising results for use in the treatment of AD pathology [335, 336]. Deardoff et al. used EVP-6124 (also known as encenicline), an  $\alpha_7$  nAChR partial agonist, and found improvements in both cognitive and functional measures [335]. Galantamine modulates nicotinic receptors and inhibits acetylcholinesterase to regulate nicotinic neurotransmission [336]. In AD patients, galantamine improves cognitive function and ADL scores but does not alter behavioral symptoms [336]. There is also an ongoing clinical trial to determine if daily transdermal nicotine impacts cognitive function in patients with MCI [337]. It is important to note that the majority of currently FDA approved therapeutics for AD target the cholinergic system, including galantamine, donepezil, rivastigmine, and tacrine, with mostly favorable results, which suggests that the cholinergic system and nAChR modulation may be promising targets for AD patients [338].

### Immunotherapy and immunizations

Immunotherapy treatments targeting both A $\beta$  and p-tau have been examined in the 3xTg-AD mouse model [339–346]. Oddo et al. looked at A $\beta$

immunotherapy in the 3xTg mouse model by injecting anti-A $\beta$  antibodies directly into the hippocampus [339]. By 7 days post-injection, there was a dramatic decrease in extracellular A $\beta$  plaques, as well as a decrease in intracellular A $\beta$  [339, 340]. Interestingly, anti-A $\beta$  antibody treatment also reversed tau pathology, as shown by a complete reduction in intracellular tau immunoreactivity [339]. In older mice with already well-established A $\beta$  and tau pathology, A $\beta$  immunotherapy still cleared A $\beta$  accumulation, but did not diminish tau aggregation. On the other hand, Walls et al. examined the effect of intrahippocampal phospho(Ser202/Thr205)-tau immunotherapy in the 3xTg-AD mouse model [340]. Within 7 days post-injection, 3xTg-AD mice showed decreased total tau levels [340]. p-Tau immunotherapy reduces pathological tau levels in both young and old mice but did not affect A $\beta$  levels at any age [340].

St-Amour et al. examined the effects of a similar immune-based therapy: intravenous immunoglobulin (IVIg) [346]. IVIg is relatively safe and well tolerated, is being used in an increasing number of diseases, and is relatively easy to administer long-term, especially when compared to intrahippocampal immunotherapy injections [346, 347]. It has been found to improve cognition and reduce A $\beta$  in cerebrospinal fluid of AD patients due to the naturally occurring anti-A $\beta$  antibodies present in the blood of healthy individuals and thus IVIg preparations [346, 348–350]. 3xTg mice injected with IVIg for 1–3 months exhibited improved recognition memory in the NOR test, but spatial memory on the BM was not changed [346]. Soluble A $\beta_{42}$  levels were decreased in 16-month-old mice, but not in 12-month-old mice, and no change was seen in insoluble A $\beta$  levels or extracellular A $\beta$  plaques [346]. Similarly, no changes were seen in tau phosphorylation at either the Thr181 or Ser396/Ser404 sites [346]. IVIg injections also modified the immune state of the 3xTg-AD mice, resulting in an overall decrease in inflammatory state as indicated by decreases in pro-inflammatory cytokines in the cortex [346]. Vaccines for AD have also been heavily studied in the 3xTg mouse model [341–345]. Most studies focus on utilizing the A $\beta$ -specific scFv-h3D6 vaccine, with promising results such as reduced A $\beta$  plaques, decreased tau levels, ameliorated pro-inflammatory state as shown by decreased GFAP, ionized calcium binding adaptor molecule 1 (Iba1), and IL-6 levels, and improvements in spatial memory in the

MWM [341, 344, 345]. Another study used a DNA epitope vaccine to produce anti-A $\beta$  reactivity without the negative side effects that have been seen in some studies on A $\beta$  vaccines or immunotherapy such as a T-cell mediated pro-inflammatory immune response or microhemorrhages in the brain [342]. The DNA epitope-vaccinated mice had decreased A $\beta$  loads, improved spatial memory, decreased inflammation, and overall strong anti-A $\beta$ -specific antibody production with an anti-inflammatory immune response [342]. In contrast, Rajamohamedsait et al. utilized a tau vaccine and found decreased tau pathology and A $\beta$  plaque burden as well as decreased microgliosis [343]. The tau-vaccinated mice exhibited no change in astrocyte activation or neuronal density.

Though there is a lot more research that needs to be done on utilizing the immune response to combat the buildup of A $\beta$  plaques and hyperphosphorylated tau during AD, the preliminary findings in the 3xTg model are promising (Table 6). Additionally, a number of clinical trials have already been performed using various immunotherapy strategies, with mixed results (reviewed in [351, 352]). Of the strategies discussed here, the IVIg studied by St-Amour et al. did not exhibit cognitive improvements as a whole, but subgroup analyses revealed that cognition was improved specifically in individuals with APOE4 [346]. Due to the increasing interest in immunotherapy approaches to AD, there are now two FDA-approved immunotherapy treatments [338]. The first immunotherapy to receive FDA approval was aducanumab (aduhelm), which was approved under the FDA's accelerated approval process in June of 2021 [338]. Since its approval, aducanumab has received much controversy, with many patients developing amyloid-related imaging abnormalities, particularly individuals with APOE4. In January of 2023, a second anti-AD immunotherapy, lecanemab (leqembi), was approved by the FDA [338]. Although many clinical trials of lecanemab are still ongoing, preliminary results show a slowing of cognitive decline after lecanemab administration as well as ameliorated tau and amyloid pathology. Lecanemab has exhibited fewer negative side effects than aducanumab thus far. Further research is needed to determine the most effective immunotherapy approach that will produce a robust anti-A $\beta$  or tau response in all individuals without increasing neuroinflammation.



**Table 6** Immunotherapy and immunizations tested in the 3xTg mouse model

Source	Starting age	Treatment period	Age at analysis	Treatment effects
<b>A<math>\beta</math> immunotherapy</b>				
Oddo et al., 2004	12 months	Single injection	12–13 months	Decreased p-tau and A $\beta$
<b>p-tau immunotherapy</b>				
Walls et al., 2014	15–18 months	Single injection	15–19 months	Decreased p-tau No change in A $\beta$
<b>Intravenous Immunoglobulin</b>				
St-Amour et al., 2014	9 months	3 months	12 months	No change in NOR performance Increased light preference in AASB No change in p-tau or A $\beta$
	12–14 months	1 or 3 months	16 months	Improved NOR performance No change in BM or Open Field performance No change in p-tau Decreased A $\beta$ Decreased microglia activation/inflammation
<b>Vaccines</b>				
Güell-Bosch et al., 2020	5 months	7 months	12 months	Decreased A $\beta$ plaques Decreased IL-6 levels Decreased loss of brain volume
Movsesyan et al., 2008	3 months	1 month	4 months	Improved MWM performance Decreased A $\beta$ load No change in tau levels Decreased microglia activation and astrogliosis
Rajamohamedsait et al., 2017	3 months	3 months	22 months	No change in cognition (no deficit seen) Decreased tau pathology Decreased A $\beta$ plaques No change in neuronal density Decreased microgliosis No change in astrocytes
Roda et al. 2020	5 months	4 months	9 months	No change in anxiety Improved MWM at 5 months of age Slightly decreased A $\beta$ load
Roda et al., 2020	22 months	15 days	22 months	Decreased A $\beta$ load Decreased tau levels Decreased GFAP and Iba1 levels No change in TNF $\alpha$ , IL-33, IL-1b, or IL-6

AASB, active avoidance shuttle box

## Conclusion

There are many different mouse models of AD available, all with different strengths and weaknesses. Here, we have discussed studies performed using the 3xTg mouse model of AD. A major advantage of this strain is that they develop both A $\beta$  and tau pathology. The main drawback to the 3xTg-AD model is that it is a model for early-onset AD, which is not nearly as common as late onset AD. While most AD rodent models have been developed via autosomal-dominant AD transgenes that have non-physiological expression

patterns [353], there has recently been a push for developing mouse models with knocked in humanized A $\beta$  or tau, though these knock-ins aren't always sufficient to cause full AD pathology with severe cognition impairment as seen in human patients [353–355]. Another option for a more translatable model of late-onset AD is non-human primates, which closely resemble humans in the progression of cognitive decline and neuropathy during aging [356]. Non-human primates also display age-related A $\beta$  plaque accumulation and tau pathology, making them a good option for modeling late-onset AD [356].

A number of different potential treatments — many of which have been demonstrated to function as geroprotectors, extending the lifespan of wild-type mice — have been discussed above. Many of these interventions, including rapamycin, are now being tested in humans to examine the potential efficacy against this deadly disease. Dietary and lifestyle interventions also have a lot of potential and are low-cost as compared to specialized pharmaceuticals; exercise in particular is extremely beneficial in the 3xTg model. However, a limitation of both dietary changes and exercise is that these may be particularly difficult for many aged individuals to follow.

The NIA Interventions Testing Program as well as other researchers have identified and continue to identify many different interventions that robustly extend the lifespan of mice. We have discussed some of these above, including rapamycin. Other interventions that have shown promise on their own or in combination with rapamycin include drugs for type 2 diabetes because there is such a close connection between AD and other metabolic disorders. These include acarbose, canagliflozin and metformin [357–361]. It will be important to test these and additional geroprotectors in mouse models of AD to determine if they might also have potential for the treatment or prevention of AD.

Regardless of the model species used, an important consideration in any animal model is genetic diversity. The human population is extremely genetically diverse, and in order for a model to be more representative of the human condition, sex and strain differences must be considered when drawing conclusions and attempting to translate results into humans. One great example of a genetically diverse AD model is the AD-BXD model proposed by Neuner et al. [362]. In this model, 5XFAD mice are bred to the well-studied BXD strains, allowing researchers to determine which sequence variants may contribute to exacerbated AD phenotype or protect against the development of AD. Though the 5XFAD is a model of early-onset AD, the AD-BXD model highlights the importance of genetic diversity in AD studies. Future genetically diverse mouse models of late-onset AD will be extremely useful in bringing a personalized medicine approach to the treatment of AD.

It is important to determine what the mechanisms are for the effectiveness of various dietary interventions, to make them as easy to stick to as possible.

Diets based on altered levels of macronutrients are likely to be more sustainable than diets based on reduced energy intake; likewise, time restricted feeding is believed to be more sustainable for many than diets that involve calorie counting. While PR has performed well in short-term studies, the long-term adherence to such diets is unknown; there are also potential concerns as the elderly may have increased need for dietary protein to fight sarcopenia. Thus, diets reducing just once amino acid — for instance, isoleucine [147] — or drugs which mimic the effect of these restrictions, for example by altering BCAA uptake or catabolism — could allow many more individuals to benefit from these type of interventions. Likewise, exercise mimetics may be of particular interest for individuals who are not physically able to exercise.

Examining mechanisms by which dietary interventions may protect against AD pathology is of equal importance. Many of the dietary interventions discussed here, including CR, PR, and restriction of BCAAs, reduces activity of mTORC1; and as rapamycin delays or prevents AD in mouse models including 3xTg, it is logical to assume that these diets work at least in part by restricting mTORC1 signaling. Ketogenic diets also have been reported to reduce mTORC1 signaling but have not been tested in the 3xTg mouse model. However, several of the dietary interventions discussed promote SIRT1 and FGF21 activity, which may also be mechanisms by which they improve AD pathology. Further study of how these diets work, as well as the precise downstream effectors of their beneficial effects, may be critical to developing new pharmaceuticals to target AD.

In conclusion, due to advances in medical science and public health, the average person is now living much longer than in the past. This increase in lifespan has not been associated with an equal increase in healthspan, and as a result, we have witnessed an enormous increase in the prevalence of age-related diseases, including AD. Thus, there is now more than ever an urgent need to identify ways to effectively treat or prevent AD. Targeting the aging process itself may provide new and effective ways to target AD, and as such, there has been substantial research on the ability of geroprotective interventions to slow or prevent AD. Here, we have summarized research into the effect of many interventions on the development and progression of AD in the 3xTg mouse model.

This model has provided important insights and will likely continue to be an important tool in the future due to its ability to model multiple aspects of AD; yet continued progress requires the development and use of mouse models that recapitulate late-onset AD in a genetically heterogeneous population.

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## Declarations

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