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# CURRENT AND EMERGING AVENUES FOR ALZHEIMER'S DISEASE DRUG TARGETS

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## ABSTRACT

Alzheimer's disease (AD), the most frequent cause of dementia, is escalating as a global epidemic, and so far, there is neither cure nor treatment to alter its progression. The most important feature of the disease is neuronal death and loss of cognitive functions, caused probably from several pathological processes in the brain. The main neuropathological features of AD are widely described as amyloid beta (Aβ) plaques and neurofibrillary tangles of the aggregated protein tau, which contribute to the disease. Nevertheless, AD brains suffer from a variety of alterations in function, such as energy metabolism, inflammation and synaptic activity. The latest decades have seen an explosion of genes and molecules that can be employed as targets aiming to improve brain physiology, which can result in preventive strategies for AD. Moreover, therapeutics using these targets can help AD brains to sustain function during the development of AD pathology. Here, we review broadly recent information for potential targets that can modify AD through diverse pharmacological and nonpharmacological approaches including gene therapy. We propose that AD could be tackled not only using combination therapies including Aβ and tau, but also considering insulin and cholesterol metabolism, vascular function, synaptic plasticity, epigenetics, neurovascular junction and blood–brain barrier targets that have been studied recently. We also make a case for the role of gut microbiota in AD. Our hope is to promote the continuing research of diverse targets affecting AD and promote diverse targeting as a near-future strategy.

**KEYWORDS:** AD gene therapy, AD molecular targets, AD therapeutics, alzheimer´s disease, amyloid beta.

## INTRODUCTION

Alzheimer's disease is a steadily growing global epidemic. Estimates suggest more than 47 million people worldwide were affected in 2015 and a staggering 131 million is predicted 30 years from now.[1-4] AD is a neurodegenerative disorder characterized mainly by the loss of memory functions and accompanied by other symptoms in a wide range of classes from mood, verbalization to motor problems. The most striking outcome from this type of dementia is the incremental disability for performing everyday life routines and increasing dependence from others for care. Aging is the main risk factor for developing AD,[5,6] and the risk of developing AD dementia becomes even higher as life expectancy increases and the world population becomes older,[5,7] Other reviews have dealt extensively with the economic burden this disease represents for countries 1, 4, estimating it at 0.65% of the world gross domestic product, a cipher rarely seen for a single disease 8. Moreover, it is likely that the economic burden for AD is largely underestimated since it is difficult to account for the expenditure from family members paying for nursing or stop working to take care of their relatives.[7,9,10] Thus, solving the AD puzzle should be hand in hand with increasing the lifespan of humans, in order to reach for healthy aging, one of the main goals for the World Health Organization (WHO) and for many states worldwide.[11,12]

Currently, AD has no treatment available to modify its progression. Pioneering efforts from scientists and clinicians led to discovery and development of cholinesterase inhibitors for AD, capable of improving symptoms such as mood swings or dyskinesia, but these treatments do not halt AD progression nor improve memory performance in patients, as revised by Schneider et al.[13] and by Mangialasche et al.[14] Antibody therapies have been developed from the main pathological hallmarks of AD, Amyloid beta (Aβ) and Tau proteins, to normalize their levels in the brain. These therapies are based on the amyloid cascade hypothesis, proposing that Aβ and Tau accumulation in the brain mediates synapse loss and neuronal death, leading to diminished memory function.[15] Nevertheless, many clinical trials aimed at reducing amyloid levels have not reached significant improvement in memory performance, or caused secondary, often-adverse effects and have dropped out,[15] Moreover, some failed clinical trials also led to the scientific community to explore additional hypotheses for AD pathogenesis.[17,15]

Therefore, it has become more important to generate novel strategies and targets that will effectively alter in any form of the progression and the underlying causes of memory loss in AD. Novel evidence behind alternative mechanisms of the disease and improvements in technology from imaging to gene editing has opened new lines of research that could help to explain the origin and progression of AD,[22-25] In addition, the field is moving increasingly towards earlier and more accurate diagnostic of the pathology, where technology can help us to better classify and even redefine AD.[26] This review summarizes pioneering efforts in mechanisms of disease and novel drug targets for Alzheimer′s disease research. We would like to emphasize the importance of multidisciplinary research in finding new treatment avenues in what it is a complex disease with many challenges.

**Different treatment approaches based on pathogenesis**

**Synaptic plasticity and AD**

Neuroplasticity is a complex response of neurons to endogenous and exogenous stimuli; it is a continuous process that embraces learning and memory processes. Neuroplasticity comprises morphological and functional interchanges, including differences in synaptogenesis, remodeling of the synaptic, axon and dendritic structures, and generation of new neurons (neurogenesis). All brain tissues are associated to neuroplasticity but hippocampus, neocortical areas and cholinergic basal forebrain neurons, which are involved in the regulation of higher brain functions, such as learning, memory and cognition, maintain an elevated degree of plasticity during all life stages.

The adult central nervous system (CNS) has a limited, although effective, ability to restore synaptic circuitry and its impact on cognition remains controversial. Furthermore, mechanisms that regulate neuroplasticity seem to be involved in neurodegenerative diseases. It is of interest to note that brain regions with elevated neuronal plasticity develop more slowly during infancy and are the most vulnerable in the aging and in AD. A disproportion between synapse formation and elimination could be responsible for defective plasticity during ageing and disease. If defective mechanisms controlling developmental plasticity are reactivated in later life, they could contribute to inefficient plasticity processes.[27]

Memory deficits in AD could be related to early events that come before neurodegeneration, such as synaptic loss and dysfunction. Synapse degeneration is believed to begin with dendritic spines and with decreased quantity of molecules that regulate spine signaling.[28]

Insoluble Aβ fibrils are taken into consideration as the main responsible for spine pathology. On the other hand, in both transgenic mouse models of AD and human AD brain, synapse defects and memory loss correlate weakly with the presence of Aβ plaques and could take place before the formation of plaques. Indeed, small neurotoxins comprised of soluble Aβ oligomers (Aβ-derived diffusible ligands, ADDLs), present in the brain and cerebrospinal fluid of AD patients, are ligands able to compromise synaptic plasticity, even at nanomolar concentrations, by binding to dendritic spines or by the interference of transcription factor activation, mediated by N-methyl-D-aspartic acid (NMDA) receptors,[29-31] (See Fig. 1).

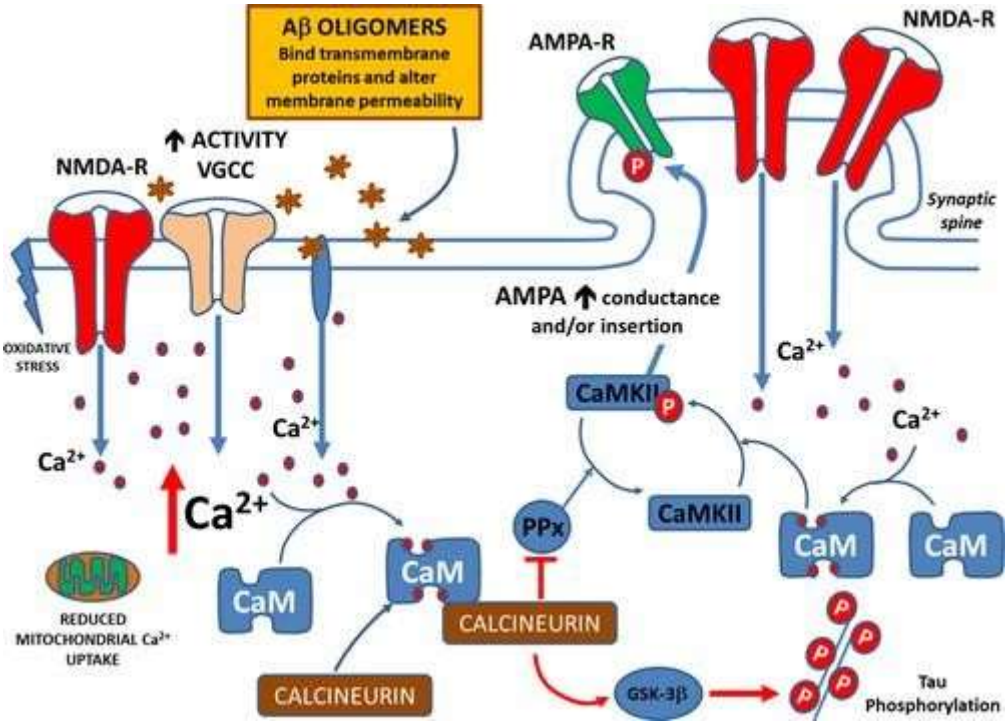



**Figure 1: Open in figure viewer Power Point.**

**Caption**

Bidirectional trafficking of proteins at postsynaptic level is a mechanism involved in synaptic plasticity. For example, synaptic activity and activation of AMPA/NMDA receptors control AMPA receptor sorting. Moreover, endocytosis and exocytosis are involved in long-term potentiation (LTP) and long-term depression (LTD) of hippocampal synapses. Induction of LTP and LTD are prevented by blocking exocytosis and endocytosis, respectively. Recycling endosomes located at the spine level regulates spine growth, suggesting that stimulation of endocytosis and dendritic spine could promote plasticity.[32]

Kinases play a critical role in synapse formation and plasticity. For example, the mitogen- activated protein kinase (MAPK)/extracellular signal-regulated kinase (Erk) pathway mediates the synaptogenic action of neurotrophic factors. This intracellular pathway could contribute to long-term synaptic plasticity by coordinating the activity of transcription factors and their subsequent nuclear translocation. MAPKs are located and active in synaptic terminals, suggesting a role in subcellular compartments during short- and long-term plasticity by phosphorylation of synaptic targets. Cyclin-dependent kinase-5 shows many roles in spine formation, expression of proteins in postsynaptic neurons, as well as in the phosphorylation of numerous molecules important for synaptic plasticity.[33]

The immunoglobulin and cadherin super families of cell adhesion molecules control cell migration, growth of axons and synapse formation. The neural cell adhesion molecule (NCAM), expressed in neuron and glia cell surface plasma membrane, regulates the consolidation of learning and memory processes. Enreptin, a peptide agonist of NCAM, enhances long-term memory and reduces neuronal death. Furthermore, the cell adhesion molecule N-cadherin regulates spine stability. Synaptic cell adhesion molecules interact with Aβ and also control its production by regulating the activity of enzymes involved in Aβ formation. Aβ-dependent reduction of synaptic adhesion alters function and integrity of synapses, indicating an important role of synaptic adhesion in the maintenance of neuronal integrity.[34]

The family of neurotrophins regulates synapse formation and synaptic plasticity. Nerve growth factor (NGF), a member of the neurotrophin family, promotes the synaptic function of cholinergic basal forebrain neurons, which contribute to memory process. Considering the regenerative effect of NGF on cholinergic neurons, its targeted delivery has emerged as a potential therapy for AD. Recently, a small clinical trial inserting encapsulated NGF-producing cells in AD patients has shown safety and tolerability increasing cholinergic markers in CSF 35. Long-term exposure of hippocampal neurons to brain-derived neurotrophic factor (BDNF, another neurotrophin with structural similarity to NGF) modulates synaptic transmission and plasticity and effects structural changes of dendrites, spines and presynaptic terminals.

Moreover, BDNF exposure has effects on the synaptic proteome, by affecting protein synthesis or degradation.[36]

Glial cells are involved in nervous system stability and synaptic plasticity. Glial processes ensheath synapses, support their development and functions and secrete

proteins (e.g. thrombospondins) that promote CNS synaptogenesis. Complement C1q and C3, upregulated in neurons exposed to astrocytes, participate in synapse elimination. Patients with frontotemporal dementia have low levels of progranulin (a protein antagonist of tumor necrosis factor-alpha (TNF-α)), which results in defects of lysosomal functions and excessive activation of complement, causing synaptic pruning by microglia and behavioural defects rescued by blocking complement activation.[37]

17β-estradiol (E2) supports dendrite growth, spine and synapse formation in both developing and adult CNS. In the hippocampus, E2 modulates synaptic plasticity slowly (genomically via classical nuclear receptors) and rapidly (nongenomically via extranuclear receptors).[38]

Nanomolar concentrations of E2 cause changes in hippocampal spine morphology. Activation of neuronal glutamate receptors, by glutamate released from astrocytes in response to PGE2, modulates dendritic spine density.[39]

The role of inflammation in AD is well recognized. Elevated levels of TNF-α, a pro-inflammatory cytokine responsible for the neuroinflammatory response, have been reported in brain and plasma of AD patients. TNF-α modifies synaptic transmission and strength. Synaptic scaling, which is a homeostatic mechanism that takes part in the synaptic dysfunction in AD may be the mechanism involved in these events. As TNF-α modulates synaptic scaling, alteration of synapsis mediated by elevated levels of TNF-α could contribute to cognitive and behavioural impairments in AD.[40]

In summary, in the mammalian CNS, dendritic spines are essential for synaptic function and plasticity. AD and other CNS disorders have a strong relation with aberrant dendritic spines. Synaptic plasticity and spine alteration can be influenced by many factors, including Aβ, impaired glucose and lipid metabolism, steroids, kinase pathways, cell adhesion molecules, neurotrophic factors, glial cells and inflammation. A better knowledge of cellular and molecular molecules able to control the age and/or cognitive abilities may lead to effective treatments for age-associated memory impairment and for other, more severe cognitive impairments, in particular AD.

**Epigenetics and AD**

Epigenetics involves heritable changes in gene function not caused by mutations in DNA sequence 41. Such changes may relate to chromosomal ones that affect gene activity and expression, as well as heritable phenotypic changes that do not derive from genome modification. These effects on cellular and physiological phenotypic traits could be driven by external or environmental factors, or be part of a normal developmental programme.

Numerous CNS physiological functions (neural stem cell fate determination, neural plasticity, and learning and memory) have significant epigenetic components. This is the case also for neurodegenerative diseases. For example, in Alzheimer disease (AD), both genetic and non genetic factors contribute to disease etiopathology. Whilst over 250 gene mutations have been related to familial AD, <5% of AD cases are gene-related.

At least three systems including DNA methylation, histone modification and noncoding RNA (ncRNA)-associated gene silencing can initiate and sustain epigenetic change. More than likely nongenetic factors, probably triggered by environmental factors, are causative factors of late- onset AD. Many CNS pathologies, including AD are associated with dysregulation of DNA methylation, histone modifications (deacetylation, phosphorylation, ubiquitylation and SUMOylation) and ncRNAs.[42] Histone phosphorylation, in particular, appears to be part of a complex interplay between other epigenetic markers, such as histone acetylation and methylation, and DNA methylation.

Indeed, histone phosphorylation increases pro-inflammatory gene activation 43. A number of proteins involved in AD pathology (amyloid precursor protein (APP), Tau, β-site amyloid precursor protein cleaving enzyme 1 (BACE1), glycogen synthase kinase-3β and c-Jun N- terminal kinase) are SUMO (small ubiquitin-like modifier) targets.[44] Furthermore, AD patients have altered levels of SUMOylation and SUMO-related protein expression.[45]

Amongst the classes of ncRNA, microRNAs (miRNAs) are highly expressed in CNS neurons, where they play a major role in neuron differentiation, synaptogenesis and plasticity.

MicroRNAs impact higher cognitive functions, as their functional impairment is involved in the aetiology of neurological diseases, including AD.[46] A growing body of evidence points to alterations in the miRNA network as active contributors to AD disease processes.[47] Alterations in the miRNA network contribute to AD disease pathogenesis by (i) regulating expression of APP and other enzymes involved in Aβ processing, in particular BACE1; (ii) Neurofibrillary tangles in AD brain are composed mainly of hyperphosphorylated Tau, whose state of phosphorylation represents a fine balance between kinases and phosphatases, processes that may be regulated by miRNAs; (iii) regulation of lipid metabolism 48; and (iv) neuroinflammation.[49]

Understanding epigenetic dysregulation in AD could contribute to our view of the origin and progression of AD and, possibly, the development of efficacious therapeutics. However, one caveat with epigenetic studies is the issue of causality. Yet, given the failure of AD clinical trials to date, focus is now shifting to diagnose AD at as early a stage as possible, even before

onset of cognitive decline. Despite the inherent difficulties, timely disease detection offers a multitude of benefits, not the least of which are opportunities for early intervention and better management of symptoms. miRNAs have emerged as potential candidates for reliable biomarkers of early-stage AD, being present in biofluids and displaying high stability in terms of storage/handling. Moreover, ncRNAs, miRNAs—and especially long ncRNAs—as therapeutic targets are only beginning to be considered. Even so, these transcripts represent potential targets for two reasons: (i) long ncRNA expression seems to be rather cell- and tissue-specific; (ii) the sequence-specific function of long ncRNA can be advantageous in designing specific therapies.

**Blood–brain barrier targets**

Blood–brain barrier (BBB) is a multicellular vascular structure that separates the central nervous system (CNS) from the peripheral blood circulation. The core anatomical element of the BBB is the cerebral blood vessel formed by endothelial cells (ECs). Mural cells represented by pericytes and astrocytes sit on the abluminal surface of the microvascular endothelial tube. Astrocytes interact with neurons and microglia. Both pericytes and astrocytes interact with ECs and maintain the sealing of interendothelial tight and adherent junctions, loss of leucocyte adhesion molecules and inhibition of transcytosis.[50-53] The vascular tube is surrounded by two basement membranes, the endothelial vascular basement membrane corresponded to an extracellular matrix secreted by the ECs and pericytes, and the parenchymal basement membrane primarily secreted by astrocytic processes that extend towards the vasculature. The molecular components of these basement membranes also contribute to the complexity of the barrier.[54]

Besides, these complex cellular interactions which correspond to the neurovascular unit instead of BBB, endothelial cells express different types of transporters and receptors amongst which some are involved in the efflux and influx of the amyloid peptide.[55,56] Beyond barrier function, influx and efflux are actively regulated at the blood–brain interface. Moreover, recent research has uncovered different transcription factors involved in phenotype change (zonation) along the vessels of the BBB.[57] The BBB maintains an environment that allows neurons to function properly by tightly controlling the passage of molecules and ions, instantaneously delivering nutrients and oxygen according to current neuronal needs, and by protecting the brain from toxins and pathogens. We now know that the cellular and molecular complexity of the BBB explains that the dysfunction of a cellular or molecular actor can disrupt its dynamics, although the precise process is unclear 58.

***Blood–brain barrier in AD***

Several impairments of the neurovascular unit have been described in Alzheimer's disease (AD), but the time-point at which they occur during disease pathogenesis remains unclear because they are too often seen in post-mortem brains. However, for the past 3 years, medical imaging has demonstrated the early BBB disruption in the hippocampus even before the onset of hippocampal atrophy.[59] In addition, many studies indicated cerebral microbleeds (micro haemorrhages) in AD,[60,61] Compared with controls, BBB P-glycoprotein activity was significantly lower in the parietotemporal, frontal and posterior cingulate cortices and hippocampus of mild AD subjects by PET-scan.[62] Besides, many morphological and functional changes in brain vasculature in AD were observed: thinning of microvessels, referred to as atrophic or string vessels; twisted or tortuous vessels and fragmented vessels.[63] thickening and vacuolization of the vascular basement membrane with increase of collagen IV.[64] leakage and accumulation of circulating plasma proteins with direct neurotoxic properties and erythrocyte- derived haemoglobin in brain.[56] Additional changes include also pericyte loss.[65] astromicrogliosis, many molecular changes directly impacting the clearance of the amyloid peptide (decrease of GLUT-1, LRP-1, P-gp and increase RAGE)[65] hypoperfusion and permeability failure.[66]

***Chemokines as critical targets for diagnosis or therapeutic strategies***

Amongst the peripheral molecular actors, we can target chemokines. Indeed, many articles have shown the involvement of chemokines in the pathophysiology of AD.[67] Of those that are deleterious, the pro-inflammatory chemokines CXCL10/CXCR3, CCL3, CCL4, CXCL8/CXCR8 and CX3CL1/CX3CR1 increase in AD, lead to inhibition of Aβ clearance, increased adhesion of PBMCs.[68,73] On the contrary, CCL5 is known as neuroprotective.[74,75] Besides the too high or too low levels of some chemokines including CCL2 are unfavourable in AD because the physiological activation of the CCL2/CCR2 signalling pathway is crucial to limit the progression of the disease in AD experimental models.[76,78]

In the light of these elements of the literature, we studied the impact of PBMCs issued from AD patients on the chemokines' signature at the level of a healthy BBB, given that the current data on chemokine levels are derived from isolated biological samples (Plasma, serum, brain and cell culture) whilst BBB displays a great cellular and molecular complexity, finely orchestrated to preserve the brain.

In a human BBB model comprising two cell lines, an endothelial cell line (hCMEC/D3) and U87 cell line (human glioblastoma), PBMCs from patients (control, mild and moderate AD patients) were added in the luminal medium. It should be noted that all analyses were also performed on isolated cultures of each cell type and on a BBB model without PBMCs. A previous work on a group of patients with AD at a moderate stage has already been published, and we also verified in this

study with the 3 groups of patients the interest to go to an integrated model to take into account the cellular and molecular interactions in the neurovascular unit.[79]

Results showed that PBMCs from moderate AD patients decreased CCL2 and CCL5 levels in luminal and abluminal compartments (2-3-fold) and CXCL10 only in the abluminal compartment (3-4-fold) compared to PBMCs from mild AD patients.

Levels of CCL2 and CCL5 also significantly decreased on PBMCs of moderate AD patients compared to PBMCs from mild AD patients. The CX3CL1 expression increased in luminal and abluminal compartments with PBMCs from mild AD patients compared to controls 80.

In both BBB models, the PBMCs come from patients or mice with advanced disease (moderate and 12 months) and the abluminal compartment is healthy. Even if the luminal compartment is AD in the mouse model, we observed:

A significant decrease in CCL2 in abluminal compartment with AD PBMCS (moderate stage or 12 months) An early increase in CX3CL1 (mild versus controls) in luminal and abluminal media and also an increase in abluminal compartment with mouse AD PBMCs (12 months versus 3 months).

It is known that the variations of these two chemokines are deleterious in AD, and it has been demonstrated that they are induced by PBMCs from AD patients or mice with advanced AD. Thus, the results join other publications highlighting an origin of peripheral blood in AD. The modulation of the blood–brain interface by targeting CCL2 and CX3CL1 could be a new therapeutic pathway.

Neurovascular junction damage and therapeutic targets: insights from preclinical research on vascular dementia and microbleeds.

Vascular dementia and its most prominent subtype, subcortical atherosclerotic encephalopathy (M. Binswanger), represent the second most frequent and important form of dementia in the elderly after Alzheimer's disease. It represents about 15% of all dementia cases, whilst another 15% of cases are mixed forms occurring together with Alzheimer's disease. Vascular dementia has therefore rapidly gained attention as a growing medical and socioeconomic burden. Vascular dementia and, in particular, M. Binswanger are characterized by progressive white matter lesions that are strongly related to cognitive decline and believed to be an important pathophysiological hallmark of almost half of all dementias in the elderly (and even beyond ‘pure’ forms of vascular dementia.[81] Symptoms of vascular dementia are also observed in cases of disseminated cerebral microbleeds.

Despite its significant impact, relatively little is known about central pathogenic mechanisms, and no casual treatments are available so far. It is known, however, that hypertension plays an important role in vascular dementia and rigorously controlling blood pressure may slow down its progress. In turn, increased systolic BP progressively disrupts white matter integrity already in young adults and increases the risk for late-life dementia.[82]

In human vascular dementia patients, microbleeds and lacunar infarcts typically occur in the basal ganglia whilst white matter hyperintensities preferentially develop in the centrum semiovale. Anatomical factors might explain these differing predilection sites: arterioles entering the deep white matter from the superficial cortex are coated by a single leptomeningeal layer rendering them more susceptible to hypertension-related vascular damage,[83,84] Microbleeds preferably appear in the basal ganglia.

In this section, current findings will be outlined from preclinical research that may indicate such novel therapeutic targets for vascular dementia, which, at least in part, may also be relevant for Alzheimer's disease. Potential therapeutic approaches will also briefly be presented

***Preclinical research in vascular dementia: state of the art***

Preclinical research in vascular dementia relies on a number of animal models, most of which separately mimic a selected aspect of human disease, predominantly lacunar infarcts, white matter damage and vessel dysfunction. An important animal model is stroke-prone spontaneously hypertensive rats (SHR-SP). They feature most of the cardinal histopathological signs of cerebral small vessel disease (cSVD)[85] likely as a consequence of chronically increased arterial blood pressure that causes vascular dysfunction.[86] However, the SHR-SP model is biased towards the bleeding facet of cSVD,[87] which might be due to genetically fixed alterations of the endothelial tight junctions,[88] and a massively increased blood pressure by far exceeding that observed in human patients. Recent research on SHR, which present high, but not extremely increased systolic blood pressure, revealed very similar behavioural and histological findings as seen in human vascular dementia patients,[89] (Fig. 2). Moreover, a number of disease-driving alterations such as focal BBB breakdown, macro- and microglial activation, and immune alterations may also provide promising targets for early-stage AD (Fig. 2).

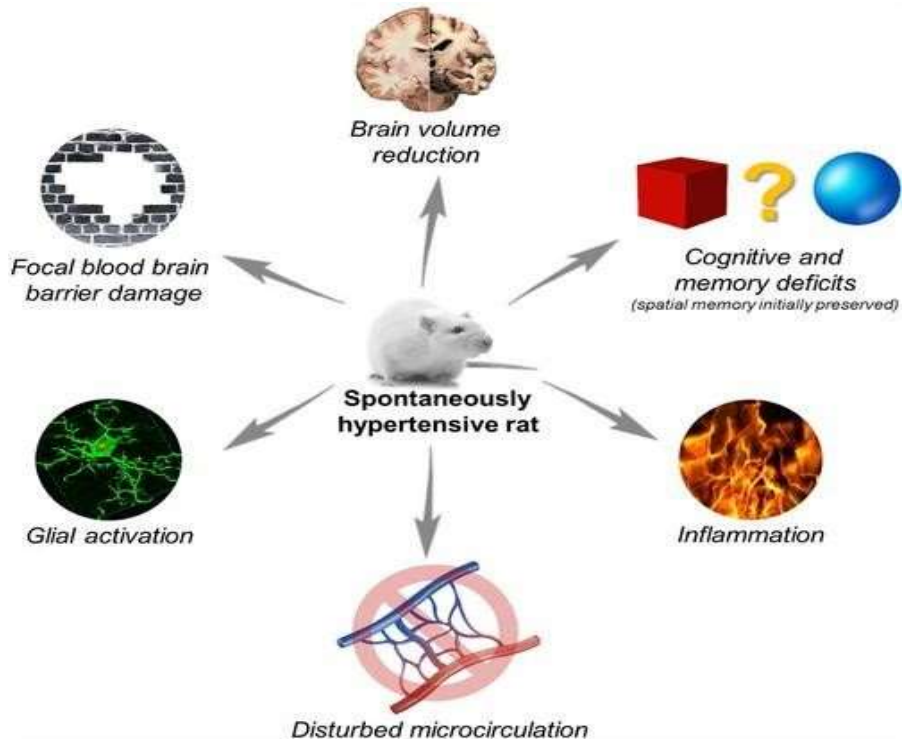



**Figure 2**

***Behavioural changes in SHR, white matter and BBB breakdown***

Middle-aged SHR showed a reduced discrimination capability between known and unknown objects, indicating a decline of the nonspatial working memory, primarily related to frontal- subcortical circuits.[90] Spatial memory is initially not affected. However, spatial memory deficits being typical in human vascular dementia patients,[91] may develop over time since time-dependent loss of cornu ammonis 1 pyramidal neurons occurs in SHR.[91]

***Macro- and microglial activation***

A sustained macro- and microglial activation in deep cortical regions can be observed in SHR. Although the number of Iba1-positive microglia in DCR is comparable results between SHR and normotensive Wistar Kyoto rats (WKY), single-cell morphological analysis increased cellular volumes being indicative of microglial hypertrophy. Microglial activation is further indicated by increased CD11b expression.[89]

***Immunological mechanisms potentially contributing to vascular dementia***

There is increasing evidence that the immune system significantly contributes to the development and progression of vascular dementia. For instance, serum levels of soluble adhesion molecules were increased in patients with white matter lesions,[93] and c-reactive protein (CRP) levels correlate with the existence and progression of white matter damage.[91] The association of inflammation and vascular dementia is not surprising since chronic inflammation also plays an important role in the pathophysiology of its primary risk factor hypertension,[94,95] However, whether such inflammatory processes initiate vascular and tissue damage, promote its propagation or simply constitute a response to ongoing reorganization remains unclear. Similar relationships have been described for Alzheimer's disease.

Distribution of blood-borne leucocytes differs between SHR and WKY strains. In WKY, T cells were mostly localized within the meninges and the choroid plexus (CP), whilst the majority of T cells populated microvessels within the SHR brain parenchyma. The different T cell distribution patterns may be explained, for instance, by an upregulation of VCAM-1 in brain endothelial cells, which occurs as a consequence of an activated renin-angiotensin system during arterial hypertension in hypertensive rats,[96] and vascular dementia patients.[97] The increased presence of T cells adhering to the luminal side of cerebral microvessels might indicate slowed vascular transit time of leucocytes due to pseudopod formation,[98] or may be part of a systemic adaptive immune response against vascular neoantigens. Importantly, T cells directly promote endothelial dysfunction.

An interesting finding was the considerable decrease of T cells in the meningeal space and the choroid plexus of SHR. Meningeal T cells have a significant impact on learning behaviour, memory function and mood stabilization.[99] Moreover, higher amounts of natural killer (NK) cells were present in the SHR brain.

***Preclinical research on post haemorrhage neuronal damage: novel insights***

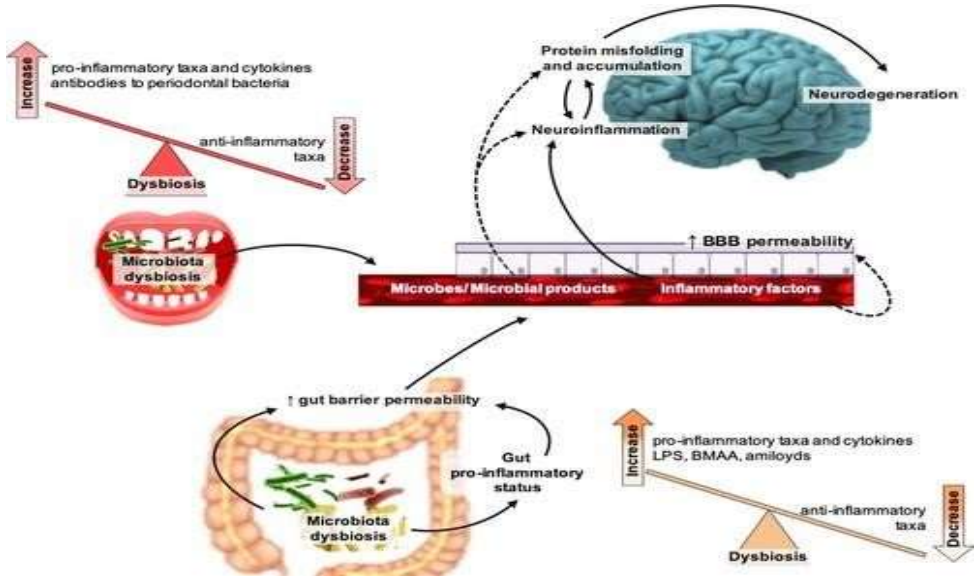
Recent clinical evidence suggests that the occurrence of microbleeds leads to a greater cognitive decline in vascular dementia and AD,[102,104] In the Rotterdam Study, higher levels of plasma Aβ were associated with increasing lacunar and microbleed counts 105. However, microbleeds are often functionally asymptomatic in patients 106, 107 and are therefore difficult to detect clinically, except using modern imaging technology.

Furthermore, cognitive decline is particularly worsened when microbleeds occur in deep brain regions or simultaneously in lobar and deep structures.[103] Blood breakdown products may lead to axonal and white matter injury of fibres trespassing the lesion site resulting in delayed, distal cell death. There is evidence from larger brain haemorrhages in the basal ganglia that axonal degeneration occurs in the internal capsule due to its close proximity. For example, Wallerian degeneration is common in intracerebral haemorrhage (ICH) patients and occurs particularly in the corticospinal tract in deep ICH.[108]

The underlying molecular mechanisms of how microbleeds promote cognitive decline and axonal degeneration/white matter damage remain incompletely understood. Blood breakdown products released from the bleed can cause neuronal cell death engaging nonapoptotic forms of regulated cell death 109, 110. In addition, it is known that degeneration of axons, in general, occurs actively, but autonomously from neuronal cell body death, and via different molecular mechanisms 111. Whilst neuronal cell bodies may die via the canonical caspase-3-dependent apoptotic pathway, blockade of this pathway does not prevent axonal degeneration 112. Axon degeneration depends on the proapoptotic family member bax and requires caspase-6 113.

**Gut microbiota and AD**

In AD subjects, higher levels of pro-inflammatory cytokines have been found, together with reactive microglial cells co-localizing with amyloid plaques. It has been proposed that high levels of inflammation are a consequence of Aβ signalling 114, 115. However, recently this hypothesis has been revised since the induction of the pro-inflammatory state can promote the amyloid cascade. It is in this context that we look at the role of the microbiota.




The gut microbiota has been named our other brain for the functional connections between the two. The microbiota weighs as much as the brain itself (up to 1.5 kg 116) and is made of bacteria, viruses and fungi. The number of bacteria in the gut exceeds the number of somatic cells by 10-fold and the number of microbial genes (the microbiome) exceeds the number of human genes by 100-fold 116, 117. The human gut has bacteria with pro-inflammatory and others with anti-inflammatory properties, in dynamic homeostatic balance. Different stressors can lead to dysbiosis, that is an imbalance between pro- and anti-inflammatory bacteria that has been invoked to explain observations in patients with rheumatoid arthritis, atherosclerosis, obesity and other diseases (Fig. 3) 118.

***The Gut microbiota and the immune system in AD***

Differences in the gut microbiota composition have been described, suggesting a specific microbial signature typical of AD 120. The question arises of the causality behind these intestinal changes and brain pathology. The immune system seems to play a crucial role in the gut–brain communication.

Higher levels of peripheral and central pro-inflammatory cytokines have been found in AD patients as compared to controls; reactive microglial cells co-localize with amyloid plaques, indicating that the pathology is accompanied by peripheral and neuroinflammation 114, 115. Importantly, gut microbiota communicates with the immune system, for example by inducing T regulatory

cells to turn off inflammatory processes 121, but alterations in its composition have been seen to be related to inflammatory pathologies. A dysbiotic flora, indeed, produces metabolites or release molecules, such as lipopolysaccharides, that can induce a peripheral inflammatory response, which, in turn, could reach the brain. In case of gut microbial dysregulation, both the intestinal barrier and the blood–brain barriers become more leaky, leading to an augmented passage of these molecules, from the gut into the circulation 122, 123.

In AD, inflammation has always been considered one of the downstream phenomena of amyloid deposition. However, recent findings showed that immune system activation and a pro-inflammatory state could promote amyloid deposition 114. In this regard, preclinical studies indicated that Aβ exerts antimicrobial properties: temporal lobe homogenates from AD patients inhibit Candida albicans growth, in a dose-dependent manner, as compared to non- AD temporal lobe homogenates or to cerebellum homogenates from AD patients 124. In a mouse and a nematode model of AD the presence of Aβ protected from Salmonella or C.albicans infection by creating a net that entrapped microbes and prevented their adhesion to the host 125. As the immune system regularly produces amyloid nets to entrap uninvited guests 126, Aβ could represent a first immune response against a microbial invader in the brain. Interestingly, fungi and the bacterial component lipopolysaccharide have been found in post-mortem brains of AD patients, especially in the area where Aβ plaques are present124, 125, 127, 128, raising new hypothesis of AD pathogenesis.

**Restoring insulin action & glucose metabolism in AD: our short-term perspectives**

Besides the known effects of amyloid-β (Aβ) and hyperphosphorylated Tau protein in the central nervous system (CNS) in AD, they may be also important at the periphery131. For instance, Aβ may compete with insulin and bind to its receptors at the periphery, impairing pancreatic β-cells and leading to insulin resistance and glucose dysmetabolism 132, 133. This may in turn exacerbate Aβ deposition 134, 135, creating a vicious cycle of dysfunctional CNS insulin signalling, oxidative stress and neuroinflammation, culminating in cognitive deficits 136.

Despite controversial, this may also involve the hyperphosphorylated Tau-induced destabilization of microtubules in β-cells, blunting insulin secretion 137 and insulin- mediated trafficking of glucose transporter-4 (GLUT4)-containing vesicles to the plasma membrane. Hence, glucose uptake into skeletal muscle and adipocytes is inhibited and type 2 diabetes (T2D) may arise 138-140.

Insulin and its downstream signalling cascades play a crucial role against CNS damage and disease. Besides the known regulation of brain glucose/bioenergetic homeostasis 141-143, insulin signalling protects against oxidative stress, (neuro)inflammation and dysfunctional intracellular quality control mechanisms 144-147, rescuing synaptic/neuronal function 148, 149 and cognition150. This downregulation of bioenergetic metabolism in insulin-resistant brain may arise years before the onset of clinical symptoms (possibly during midlife), affecting Aβ or Tau homeostasis and rendering people (especially women) more prone to dementia and AD 151-155. Thus, AD has been increasingly considered a metabolic disorder, also termed ‘type 3 diabetes’ 156.

Evidence for AD-related brain glucose hypometabolism includes the slowdown in cerebral blood flow due, for example, to brain vascular atrophy 157. This, together with the lower levels of GLUT-3 and -4 in AD brain, may attenuate the glucose uptake across the blood–brain barrier (BBB) and its use by CNS 158, 159. AD also inhibits brain enzymes from glycolysis and Krebs cycle (e.g. lactate dehydrogenase (LDH), aconitase, glutamine synthetase, creatine kinase, pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (α-KGDH)) 160, 161, depending on disease progression 162. Besides the possible direct impact of PDH inhibition in lowering the levels of acetyl-coenzyme A, acetylcholine, cholesterol and neurosteroidal hormones (e.g. oestrogen) upon AD 163, these metabolic changes further associate with mitochondrial alterations along disease progression 164. In this perspective, Aβ is widely known to deregulate mitochondrial proteins, blunting mitochondrial cytochrome c oxidase (or complex IV) activity and oxygen respiration rate, either centrally and/or peripherally (e.g. in platelets) 165-168. This may be also due to a reduction in the neuronal expression of nuclear genes that code for mitochondrial electron transport chain subunits 169, or to a decrease in the number of neuronal mitochondria 155. Importantly, the disruption between mitochondrial respiration and energy metabolism in AD was also associated with oxidative stress 156, 170, possibly due to activation of p38MAPK signalling and subsequent hippocampal glutamatergic synaptotoxicity/death, culminating in the AD cognitive deficits 170, 171. Alternatively, disrupted mitochondrial dynamics (fission and fusion) and trafficking upon AD may hamper the development and maturation of synapses 164, 172, 173. Moreover, the correlation between early deficits in synaptic mitochondria and synaptic loss in AD 162, 174 reinforce the notion that brain glucose (energy) hypometabolism may constitute an early event in disease pathogenesis, starting decades before its diagnosis (probably during midlife) 175, 176. This may impair neuronal insulin signalling, creating a vicious cycle of Aβ-mediated and hyperphosphorylated Tau-mediated injury 177, 178.

Although this is not the aim herein, there are extensive differences (even at the level of gene expression) between male and female brain (metabolism) upon ageing and/or AD 153, 179 that may further condition the whole discovery/development of successful

preventive and therapeutic strategies against the disease.

***Opportunities in drug development in AD***

The 'charm' of repurposing efficient anti-T2D drugs to recover brain insulin signalling and glucose metabolism in AD

The failures described above point to the urgent need to unveil the precise aetiology and pathophysiological mechanisms of AD, as these will be also crucial to discover more accurate diagnostic and efficient therapeutic tools 157, 180. They also emphasize the need of supporting Phase III clinical trials on strong and accurate preclinical data and to tackle multiple therapeutic targets 157. Moreover, the refocus on preventive strategies and/or drugs targeting the prodromal or very early stages of AD (before the onset of dementia) will hopefully maintain a longer quality of life 157.

Amongst such promising therapeutic (and preventive?) strategies in AD, one tempting target is the rescue of brain insulin signalling and glucose metabolism 157. Accordingly, an increasing attention has been given to the potential benefits of repositioning efficient, commercialized anti- T2D drugs to treat AD 181-184. This hypothesis is supported by the molecular mechanisms shared by T2D and AD 183, 184. This is also tempting due to the potential targeting of preclinical/prodromal AD, mild cognitive impairment (MCI), or at-risk conditions (prevention), rather than just its later stages 157, 183, 184.

**The temptation of using biguanides (metformin) against AD: a friend or foe?**

Metformin is the most efficient anti-T2D biguanide 183-185. It is relatively inexpensive and with a low risk of hypoglycaemia 183. Metformin inhibits insulin-mediated hepatic glucose production and promotes peripheral glucose disposal by activating liver and skeletal muscle AMPK signalling 183, 184. Given its good tolerability, metformin can be used as mono- or multi-therapy at all stages of T2D 183. Amongst its adverse effects are gastrointestinal distress, hepatic dysfunction, congestive heart failure, dehydration and alcoholism 183, 184. Therefore, metformin must be used with caution in elderly patients.

Preclinical data suggest that metformin may be neuroprotective, probably by recovering brain insulin action and energy metabolism 183-185. Metformin also increased markers for mitochondrial biogenesis and fusion (e.g. Mfn2 and OPA1), attenuated mitochondrial transition pore opening and oxidative stress, protecting against apoptosis and cognitive deficits 183-185. It also modulated lipid and protein synthesis, fatty acid oxidation and promoted neurogenesis 183. However, the rescue in hippocampal JNK signalling and synaptic markers achieved by metformin did not improve cognitive function in obese T2D mice 186 and even promoted hepatic mitochondrial dysfunction and cell death 187.

Concerning its role in ageing and AD, metformin decreased the risk for dementia in aged individuals and improved cognition in AD patients 188, 189. This may involve the attenuation in neuronal insulin resistance and AD-like neuropathology, most likely via AMPK-related regulation of APP amyloidogenic processing; inhibition of mTOR and subsequent autophagic/lysosomal removal of Aβ; and/or the stimulation of PP2A activity and decreased Tau hyperphosphorylation 190, 191. Given such promising data, according to ClinicalTrials.gov, two Phase II clinical trials on the effects of metformin administration in middle-aged and aged obese patients with amnestic MCI (NCT00620191), or in MCI and early AD patients (NCT01965756) were recently completed and results are awaited soon.

**The potential of thiazolidinediones to tackle AD**

The main thiazolidinediones (TZDs) used in T2D are Rosiglitazone, Pioglitazone and troglitazone 183. Though TZDs are relatively expensive, they are very efficient in the long-term management of T2D 183, 184. These drugs act as PPARγ agonists to promote the transcription of genes related to lipid and glucose metabolism 192, 193. More specifically, TZDs increase insulin-induced glucose uptake (most likely via GLUT-1 and GLUT-4) and decrease lipid accumulation by skeletal muscle, stimulate triglyceride storage in adipocytes, hepatic fatty acid oxidation and inhibit hepatic gluconeogenesis 184. Amongst their adverse effects are a possible weight gain and increased risk of myocardial infarction 184.

Some neuroprotective effects were described for TZDs, including a decrease in stroke-related damage and neurological deficits in T2D mice 194. Others suggested that TZDs-mediated reduction in brain oxidative stress and rescue in STAT3/Wnt signalling pathways may promote neuronal progenitor cells proliferation and differentiation upon T2D 195. This, together with a protection against amyloidogenic processing of APP, Tau hyperphosphorylation, neuroinflammation and Aβ-induced neuronal insulin resistance may account for the recovery in memory and cognitive performance in patients and rodent models 183, 184. In line with this, in a randomized clinical trial, Rosiglitazone improved cognitive function in mild to moderate AD patients 196, whereas in a Phase III study the drug did not show beneficial effects in AD patients, and the long-term use of thiazolidinediones did not attenuate the risk for AD 197.

According to ClinicalTrials.gov, a Phase III clinical trial is currently analysing the potential of Pioglitazone as a β-secretase inhibitor (TOMMORROW; NCT01931566) in people aged 65-83 years, at risk of MCI due to AD. A masked extension of this study (NCT02284906; phase III) is planned with 316 individuals with an MCI diagnosis due to AD that complete the TOMMORROW

study.

Is it still worthy to evaluate (intranasal) insulin for AD treatment? The pros and cons.

Insulin has been increasingly used in T2D, not only for blood glucose management but also to prevent its chronic microvascular complications and death 198.

**However, some controversy persists on its efficacy, which may be lost upon T2D progression.**

Physiologically, brain insulin signalling promotes synaptic remodelling and memory formation 199, 200. We also found that restoring insulin and IGF-1 signalling recovered both peripheral and brain glucose metabolism, and motor function in vitro and in vivo in Huntington's disease models 201-203. Moreover, insulin decreased synaptic Aβ accumulation, oxidative damage and mitochondrial dysfunction 143, 204. This was accompanied by a protection against Aβ-induced neuronal insulin resistance 205, 206. However, associated with insulin administration is the high risk of recurrent hypoglycaemia, which has been increasingly related to neuronal dysfunction/death and cognitive deficits 207, 208. But since restoring brain insulin signalling constitutes a promising approach against AD, an alternative could be the potential use of intranasal insulin herein.

Intranasal insulin promoted brain insulin signalling in AD, without affecting blood insulin or glucose levels 209. Clinical trials involving MCI or early AD patients showed that intranasal insulin improved brain glucose metabolism and stabilized or even rescued their memory and cognitive deficits 199, 210, 211. According to ClinicalTrials.gov, results are awaited from two recently completed Phase II/III and II clinical trials on insulin (SNIFF; NCT01767909) and glulisine (a rapid-action insulin analog that regulates glucose metabolism and counteracts Aβ) (NCT02503501), involving middle-aged and aged MCI or mild AD individuals. Possible limitations to the use of intranasal insulin for AD treatment could be the generalized increase in brain insulin levels and its possible adverse consequences on brain regions (like hypothalamus) that control, for example, water and food intake 212.

**The increasing therapeutic potential of incretin drugs in AD**

**Dipeptidyl peptidase-IV inhibitors**

Sitagliptin, Saxagliptin, Linagliptin, Vildagliptin, Alogliptin, Tenegliptin, Dutogliptin and Gemigliptin are the main dipeptidyl peptidase-IV (DPP-IV) inhibitors used to treat T2D 183, 184, 213. DPP-IV inhibitors are oral small molecules that blunt the degradation of native GLP-1 by the aminopeptidase DPP-IV, increasing its half-time and circulating levels, together with the attenuation of glucagon effects 213-215. DPP-IV inhibitors are well tolerated and can be used either as mono- or multi-therapy 184, 213. Apparently, these drugs do not affect gastric emptying, body weight or cardiovascular function and present a low risk of hypoglycaemia 184, 213. However, their efficacy may be lost upon T2D progression 216.

Sitagliptin attenuated mouse hippocampal AD neuropathological hallmarks, improving also acetylcholine and adiponectin receptor levels in T2D rat brains 217, 218. Sitagliptin and Vildagliptin also decreased peripheral T2D and oxidative stress markers and rescued learning and memory deficits in insulin-resistant and T2D rats 217, 219, 220. Vildagliptin also decreased the levels of Aβ, hyperphosphorylated Tau and neuroinflammatory markers, and rescued memory deficits upon AD 221. However, it is still debated whether DPP-IV inhibitors can cross the blood–brain barrier and exert direct effects in the brain or if their effects are mostly peripheral 184, 213. Further research is needed before including DPP-IV inhibitors into clinical trials in AD.

**GLP-1 receptor agonists**

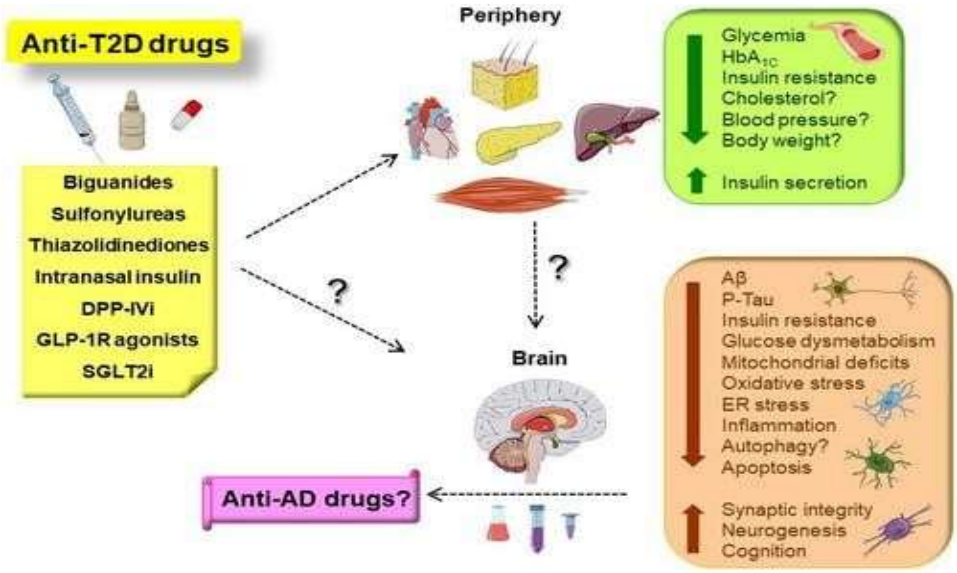
Exendin-4, liraglutide and lixisenatide are the most used GLP-1 receptor (GLP-1R) agonists in T2D 183, 184, 213, 222. They act as incretin mimetics, promoting insulin secretion in a glucose- dependent manner to overcome insulin resistance 183, 213, 222. Besides their minimum risk of hypoglycaemia, GLP-1R agonists have also potent, long-lasting anti-obesogenic effects, possibly via a hypothalamic-regulated decrease in appetite and food intake 213. They also showed benefits in blood pressure, cholesterol and triglycerides levels, as well as in cardiac function upon T2D 213. Though the mechanisms involved herein remain poorly known, they may rely on a decrement in markers for cardiovascular risk (as IL-6, TNFα), endothelial dysfunction, oxidative/endoplasmic reticulum (ER) stress and inflammatory pathways 213. Interestingly, liraglutide promoted GLUT4 translocation in mouse skeletal muscle via cAMP signalling and may thus affect glucose uptake and metabolism 223.

**The novel and still unexplored anti-AD therapeutic potential of SGLT2 inhibitors**

The main SGLT2 inhibitors used to treat T2D are empagliflozin and dapagliflozin. Although they exert their glucose-lowering effects mainly through a novel, insulin- independent mechanism (via increased renal glycosuria), one cannot exclude the increase in peripheral insulin sensitivity, GLP-1 levels and/or β-cell function 227- 232. This may be accompanied by decreased leptin levels, endothelial dysfunction, oxidative stress and inflammation markers, ultimately reducing blood pressure and body weight 233. Thus, SGLT2 inhibitors may optimally reduce the long-term complications associated with T2D, with a low risk of hypoglycaemia and hypotension 230, 234. Although little is known on its neuroprotective role, empagliflozin may protect obese T2D mice against brain oxidative stress and DNA damage probably via the recovery in

serum insulin levels and vascular function, ultimately rescuing their learning and memory function 235, 236. Additionally, dapagliflozin-mediated attenuation of retinal capillary hyperperfusion, arteriole wall thickening and microvascular remodelling in T2D were followed by a decrease in brain markers for oxidative stress, inflammation and apoptosis 235, 237, 238. This was further accompanied by an improved insulin action, mitochondrial function, synaptic density/plasticity, neurogenesis and in learning and memory in T2D patients and animal models 235-238.

To our knowledge, there are no current clinical trials on the use of SGLT2 inhibitors to tackle AD. In conclusion, one can hypothesize that either by ameliorating peripheral insulin action and glucose homeostasis and/or by crossing the blood– brain barrier and exerting similar effects in the central nervous system, anti-T2D drugs from the different classes may represent promising therapeutic approaches to tackle AD (Fig. 4).




**Restoring brain cholesterol metabolism by CYP46A1 gene therapy**

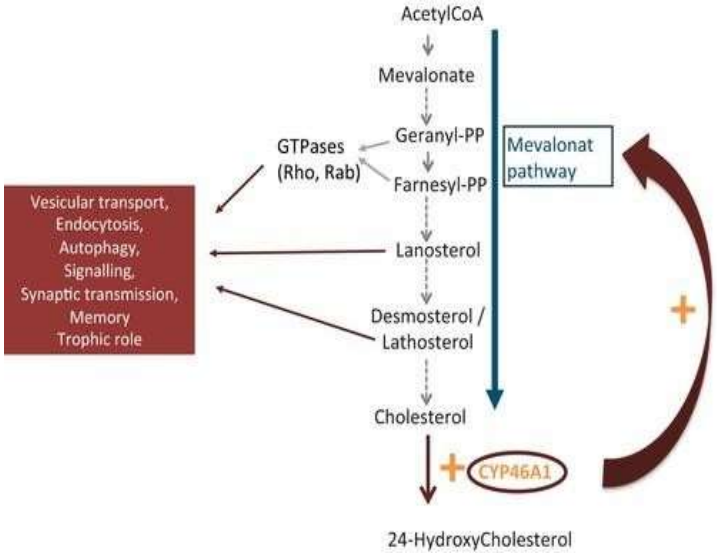
Increasing evidence demonstrates the role of brain cholesterol in the physiopathology of neurodegenerative disease particularly in Alzheimer's disease and Huntington's disease239, 240. Brain contains a particularly high portion of total body cholesterol, since our brain represents 2% of our body weight, but contains 25% of total cholesterol. Besides the important (70%) myelin fraction, brain cholesterol is a major constituent of neuronal membranes and plays crucial role in synaptic function and neuronal survival.

Increasing arguments link brain cholesterol metabolism and AD. Tangles of Tau are observed In Niemann–Pick-C, a genetic disease of cholesterol metabolism, confirming the direct connection between dysfunction of cholesterol in the brain and the tangles of Tau. The role of ApoE, the main cholesterol transporter in the brain, and of the ApoE4 allele has been long recognized as the main risk factor (after age) for Alzheimer's disease 6. More recently, GWAS analysis has identified several genes of lipid metabolism, like SORL, ABCA7 and CLU in association with AD 241. Cholesterol increased concentration has been evidenced in the brain of AD patients. The role of statins (inhibitors of HMGCoA reductase key enzyme of cholesterol synthesis) is still debated. However, a recent retrospective study on 400 000 patient receiving long-term treatments by statins evidenced a link between a decreased frequency of Alzheimer's disease to chronic administration of statins, a link varying upon sex, ethnicity and molecules 242. Yet, the discussion on statins and cognitive decline in AD remains open 243.

Cholesterol is directly associated with plaques and tangles. In vitro and in vivo studies have shown that increased cholesterol content in membranes is associated with increased A-beta production. Conversely, decreased cholesterol in the membranes favours the nonamyloidogenic pathway of APP processing 244.

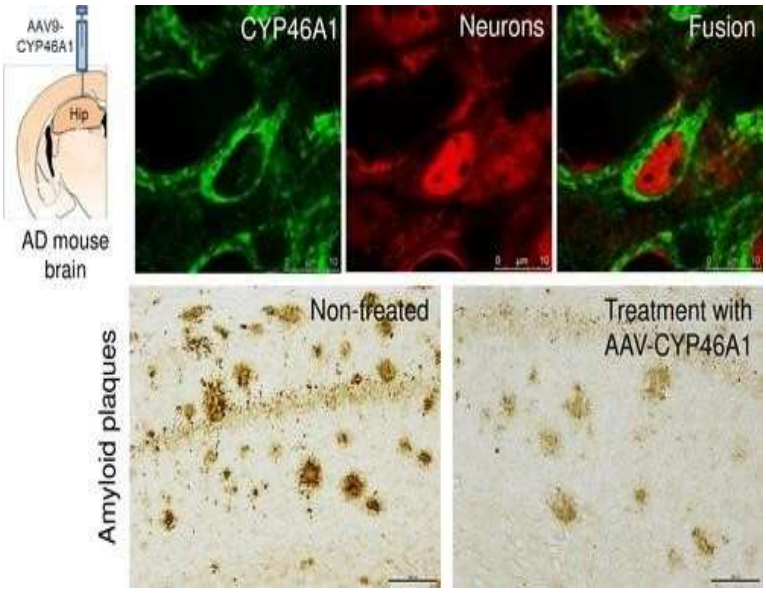
Cholesterol cannot cross the blood–brain barrier (BBB), and brain cholesterol is produced in situ, mostly by astrocytes in adults. It is then transported to neurons by APOE, which are the major consumers of the generated cholesterol. To some extent, cholesterol is also produced by synthesis in neurons and this is an important part of the brain cholesterol homeostasis. Cholesterol is excreted from the brain mostly after transformation into 24-hydroxycholesterol (24-OH), that can freely cross the BBB and is metabolized in the liver. 24-OH is produced by CYP46A1, a cytochrome enzyme specifically expressed in the brain 245, 246. CYP46A1 is a key enzyme of brain cholesterol metabolism. Not only CYP46A1 allows most cholesterol efflux from the brain, it also activates the whole pathway of cholesterol metabolism, the so-called mevalonate pathway (Fig. 5) and represents an important stress response factor to noxious stimuli like ageing, toxic protein aggregates,

disease conditions like AD 247, 248. CYP46A1 was shown in response to stress, to induce the relocation of Trkb in plasma membranes, leading to its activation and to postsynaptic stress response signalling, a pathway that could be associated with improved cognition and synaptic plasticity.




The decrease in CYP46A1 function in normal mouse hippocampus, using AAV- CYP46A1 shRNA delivery is associated with cholesterol accumulation in cell membranes and strong neuronal toxicity leading to severe endoplasmic reticulum stress and neuronal death with hippocampal atrophy. Interestingly a reduction of only 30 to 50% in CYP46 levels induces amyloid beta accumulation and hyperphosphorylation of Tau protein in the hippocampus, a phenotype resembling Alzheimer's disease. CYP46A1 inhibition in AD mice with amyloid pathology leads to accelerated toxicity with major amyloid accumulation, rapid neuronal death and seizures, evidencing the toxic loop between cholesterol metabolism impairment and amyloid production 249, 250.

On the contrary, CYP46 overexpression in AD models improves cognition and decreases pathology in the brain. Injection of an AAV vector coding for the enzyme CYP46A1 restores cholesterol metabolism, decreases amyloid beta accumulation and plaque formation in the different AD models 249, 251 (Fig. 6). Importantly, this beneficial effect of AAV-CYP46A1 delivery is demonstrated not only in three different amyloid models but also in Tau22 mice 252. Dendritic spine density are restored, together with electrophysiological parameters (LTC), contributing to the correction of memory deficits in these mice. In parallel, results from studies in Huntington mouse model demonstrated that overexpressing CYP46A1 restores deficient cholesterol metabolism, behaviour deficits and neuropathological hallmarks confirming the link between brain cholesterol impairment and neuronal function253.

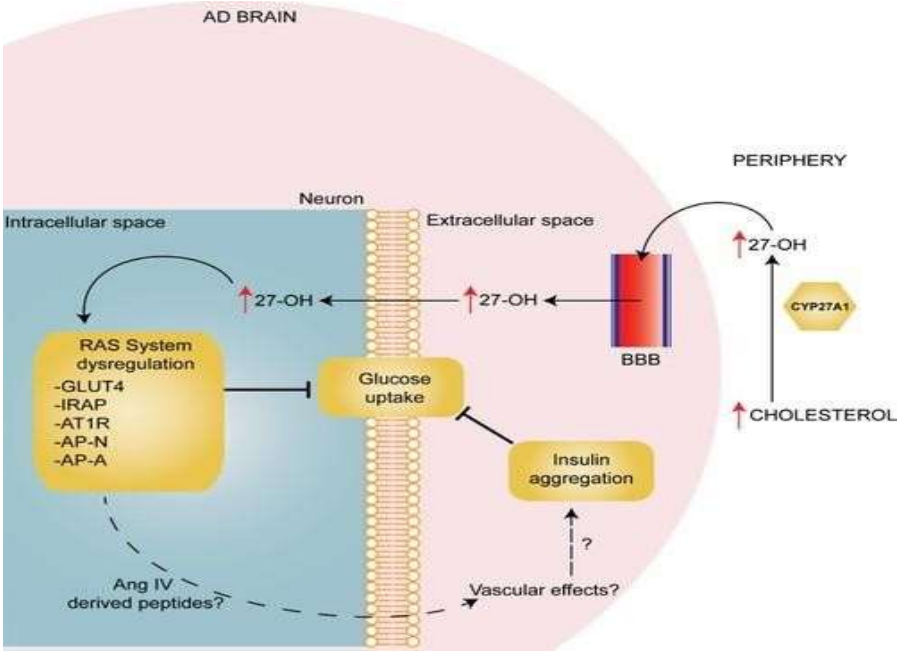


***Alterations of the renin-angiotensin system in the brain in AD***

In an effort to identify the mechanisms by which high levels of 27-OH produce neuronal damage, we reported high levels of 27-OH increase the renin-angiotensin system activity in HFD fed mice (Fig. 8) 276. These results were proven translatable when found that patients with MCI and AD also present increased angiotensin (AGT) and angiotensin-converting enzyme (ACE) in the brain 277. Going back to the animal models to clarify the mechanisms of action of 27-OH in the brain, the Cyp27TG mice were used, a transgenic mouse model overexpressing CYP27A1 to produce 5 times more 27-OH systemically. These animals also show cognitive impairment at 12 months old together with reduced glucose uptake in the brain 273, 278. The mechanisms leading to reduced glucose uptake in these mice is mediated by an over-activation of the RAS system, leading to an imbalance between the angiotensin isoforms AngIII and IV 273. The balance between these forms is also modified by the catabolism of AngIV by aminopeptidases, which are modulated importantly by 27-OH, particularly aminopeptidase-A (AP-A) and aminopeptidase-N (AP-N). In CYP27TG brains, elevated 27-OH increases AP-N, which cleaves AngIV thus decreasing its levels. AngIV under physiological conditions downregulates the abundance of insulin-regulated aminopeptidase (IRAP) in the brain 279, but under high 27-OH levels, AP-N degrades AngIV allowing increased IRAP activity 273.

Elevated 27-OH levels not only increase IRAP activity but also decrease the levels of the glucose transporter GLUT4, which is regulated negatively by AngIII. In CYP27TG brains, Ang III is elevated due to increased AP-A activity (converting AngII to AngIII), which in turn downregulates GLUT4 273. Together with increased IRAP activity, GLUT4 downregulation leads to reduced glucose uptake by neurons (Fig. 7). These mechanisms have been confirmed in vitro by knock-down experiments and they explain the alterations observed in patients with altered RAS markers in the CSF.

Taken together, these results point CYP27A1 as a druggable target to prevent the effects of peripheral hypercholesterolaemia in the blood, in opposition to statins or ACE inhibitors. A recent clinical trial with atorvastatin in SPG5 patients did not decrease 27-OH levels in patient′s CSF, whilst effectively decreasing them in the blood together with cholesterol, meaning that statins cannot effectively normalize 27-OH in the brain in the short term 272. CYP27A1-specific inhibitors, such as anastrozole, have been proposed as therapeutics for AD 280; however, to our knowledge, no clinical trial is testing the effect of these compounds in AD risk or cognition.




**Symptoms**

Memory loss is the key symptom of Alzheimer's disease. Early signs include difficulty remembering recent events or conversations. But memory gets worse and other symptoms develop as the disease progresses.

At first, someone with the disease may be aware of having trouble remembering things and thinking clearly. As symptoms get worse, a family member or friend may be more likely to notice the issues.

Brain changes associated with Alzheimer's disease lead to growing trouble with.

**Memory**

Everyone has memory lapses at times, but the memory loss associated with Alzheimer's disease persists and gets worse. Over time, memory loss affects the ability to

function at work or at home.

**People with Alzheimer's disease may**

- Repeat statements and questions over and over.
- Forget conversations, appointments or events.
- Misplace items, often putting them in places that don't make sense.
- Get lost in places they used to know well.
- Eventually forget the names of family members and everyday objects.
- Have trouble finding the right words for objects, expressing thoughts or taking part in conversations.

**Thinking and reasoning**

Alzheimer's disease causes difficulty concentrating and thinking, especially about abstract concepts such as numbers.

Doing more than one task at once is especially difficult. It may be challenging to manage finances, balance checkbooks and pay bills on time. Eventually, a person with Alzheimer's disease may be unable to recognize and deal with numbers.

**Making judgments and decisions**

Alzheimer's disease causes a decline in the ability to make sensible decisions and judgments in everyday situations. For example, a person may make poor choices in social settings or wear clothes for the wrong type of weather. It may become harder for someone to respond to everyday problems. For example, the person may not know how to handle food burning on the stove or decisions when driving.

**Planning and performing familiar tasks**

Routine activities that require completing steps in order become a struggle. This may include planning and cooking a meal or playing a favorite game. Eventually, people with advanced Alzheimer's disease forget how to do basic tasks such as dressing and bathing.

**Changes in personality and behavior**

Brain changes that occur in Alzheimer's disease can affect moods and behaviors. Problems may include the following

- Depression.
- Loss of interest in activities.
- Social withdrawal.
- Mood swings.
- Distrust in others.
- Anger or aggression.
- Changes in sleeping habits.
- Wandering.
- Loss of inhibitions.
- Delusions, such as believing something has been stolen.

**Preserved skills**

Despite major changes to memory and skills, people with Alzheimer's disease are able to hold on to some skills even as symptoms get worse. Preserved skills may include reading or listening to books, telling stories, sharing memories, singing, listening to music, dancing, drawing, or doing crafts.

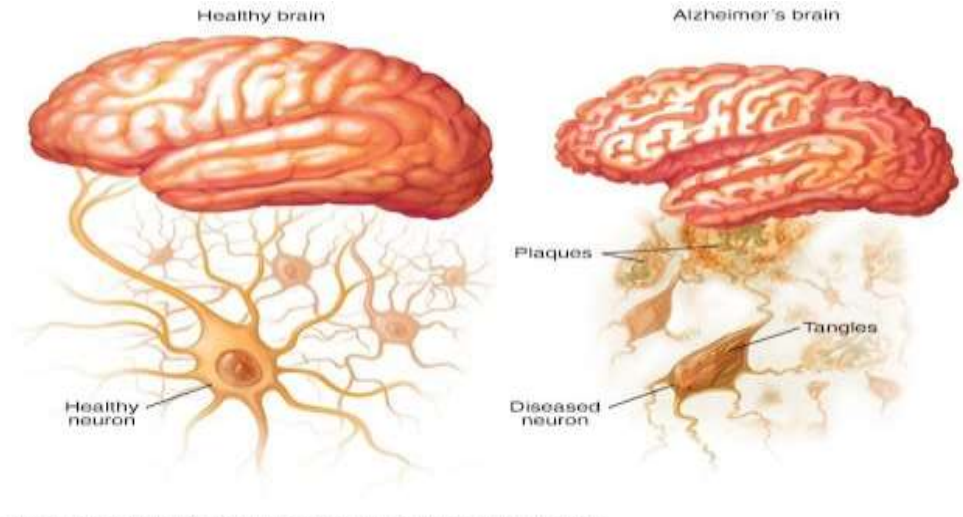
These skills may be preserved longer because they're controlled by parts of the brain affected later in the course of the disease.

**When to see a doctor**

A number of conditions can result in memory loss or other dementia symptoms. Some of those conditions can be treated. If you are concerned about your memory or other thinking skills, talk to your health care provider.

If you are concerned about thinking skills you observe in a family member or friend, talk about your concerns and ask about going together to talk to a provider.

**Causes**






**Healthy brain and brain with Alzheimer's disease Enlarge image**

The exact causes of Alzheimer's disease aren't fully understood. But at a basic level, brain proteins fail to function as usual. This disrupts the work of brain cells, also called neurons, and triggers a series of events. The neurons become damaged and lose connections to each other. They eventually die.

Scientists believe that for most people, Alzheimer's disease is caused by a combination of genetic, lifestyle and environmental factors that affect the brain over time. In less than 1% of cases, Alzheimer's is caused by specific genetic changes that almost guarantee a person will develop the disease. In these cases, the disease usually begins in middle age.

Researchers trying to understand the cause of Alzheimer's disease are focused on the role of two proteins:

- Plaques. Beta-amyloid is a fragment of a larger protein. When these fragments clump together, they appear to have a toxic effect on neurons and to disrupt communication between brain cells. These clumps form larger deposits called amyloid plaques, which also include other cellular debris.
- Tangles. Tau proteins play a part in a brain cell's internal support and transport system to carry nutrients and other essential materials. In Alzheimer's disease, tau proteins change shape and organize into structures called neurofibrillary tangles. The tangles disrupt the transport system and cause damage to cells.

**Risk factors**

**Age**

Increasing age is the greatest known risk factor for Alzheimer's disease. Alzheimer's isn't a part of typical aging. But as you grow older, the chances of developing it increases.

One study found that every year there were four new diagnoses per 1,000 people ages 65 to 74. Among people ages 75 to 84, there were 32 new diagnoses per 1,000 people. For those 85 and older, there were 76 new diagnoses per 1,000 people.

**Family history and genetics**

The risk of developing Alzheimer's is somewhat higher if a first-degree relative — your parent or sibling — has the disease. Just how genes among families affect the risk is largely unexplained, and the genetic factors are likely complex.

A better understood genetic factor is a form of the apolipoprotein E (APOE) gene. A form of the gene, APOE e4, increases the risk of Alzheimer's disease. About 25% to 30% of the population carries an APOE e4 allele. But not everyone with this form of the gene develops the disease.

**Down syndrome**

Many people with Down syndrome develop Alzheimer's disease. This is likely related to having three copies of chromosome 21. Chromosome 21 is the gene involved in the production of the protein that leads to the creation of beta-amyloid. Beta-amyloid fragments can become plaques in the brain. Symptoms tend to appear 10 to 20 years earlier in people with Down syndrome than they do for the general population.

**Sex**

Overall there are more women with the disease because they tend to live longer than men.

**Mild cognitive impairment**

Someone with mild cognitive impairment (MCI) has a decline in memory or other thinking skills that is greater than usual for the person's age. But the decline doesn't prevent the person from functioning in social or work environments.

However, people with MCI have a significant risk of developing dementia.

When MCI affects mainly memory, the condition is more likely to progress to dementia due to Alzheimer's disease. A diagnosis of MCI offers people the chance to put a greater focus on healthy lifestyle changes and to come up with strategies to make up for memory loss. They also can schedule regular health care appointments to monitor symptoms.

**Head trauma**

Several large studies found that people age 50 years or older who had a traumatic brain injury (TBI) had an increased risk of dementia and Alzheimer's disease. The risk is even higher in people with more-severe and multiple TBIs. Some studies found that the risk may be greatest within the first six months to two years after the injury.

**Air pollution**

Studies in animals have found that air pollution particulates can speed the breakdown of the nervous system. And human studies have found that air pollution exposure — especially from traffic exhaust and burning wood — is linked to a greater dementia risk.

**Excessive alcohol consumption**

Drinking large amounts of alcohol has long been known to cause brain changes. Several large studies and reviews found that alcohol use disorders were linked to an increased risk of dementia — early-onset dementia in particular.

**Poor sleep patterns**

Research has shown that poor sleep patterns, such as trouble falling asleep or staying asleep, are linked to an increased risk of Alzheimer's disease.

**Lifestyle and heart health**
Research has shown that the same risk factors associated with heart disease also may increase the risk of dementia. It's unclear if these factors increase risk of dementia by worsening Alzheimer's changes in the brain or by leading to brain vascular changes.

**They include**
**Lack of exercise**
- Obesity.
- Smoking or exposure to secondhand smoke.
- High blood pressure.
- High cholesterol.
- Poorly controlled type 2 diabetes.

Complications
Alzheimer's symptoms such as memory loss, language loss, impaired judgment and other brain changes can make it harder to manage other health conditions. A person with Alzheimer's disease may not be able to:
- Tell someone about being in pain.
- Explain symptoms of another illness.
- Follow a treatment plan.
- Explain medicine side effects.

As Alzheimer's disease moves into its last stages, brain changes begin to affect physical functions. The changes can affect the ability to swallow, balance, and control bowel and bladder movements. These effects can lead to other health problems such as:
- Inhaling food or liquid into the lungs.
- Flu, pneumonia and other infections.
- Falls.
- Fractures.
- Bedsores.
- Poor nutrition or dehydration.
- Constipation or diarrhea.
- Dental problems such as mouth sores or tooth decay.

**Prevention**
Alzheimer's disease is not a preventable condition. However, a number of lifestyle risk factors can be modified.

Evidence suggests that taking steps to reduce the risk of cardiovascular disease may also lower your risk of developing dementia.

**To follow heart-healthy lifestyle choices that may reduce the risk of dementia**
- Exercise regularly.
- Eat a diet of fresh produce, healthy oils and foods low in saturated fat, such as a Mediterranean diet.
- Follow treatment guidelines to manage high blood pressure, diabetes and high cholesterol.
- If you smoke, ask your health care provider for help to quit.

One large, long-term study done in Finland found that making lifestyle changes helped reduce cognitive decline among people who were at risk of dementia. Those in the study were given individual and group sessions that focused on diet, exercise and social activities.

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