Alzheimer's Disease

Cover image: White matter atrophy in AD. See page 125, chapter 8 for details.

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Edited by

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FOREWORD

Alzheimer's disease (AD) is the sixth leading cause of death in the USA. Globally about 50 million individuals have AD or related dementias. With the increasing average age of humans worldwide, the total number of people with dementia is projected to reach 82 million by 2030 and 152 million by 2050. Despite its prevalence, AD is the only cause of death among the top 10 causes of death globally for which no effective pharmaceutical agents exist to halt or slow down the disease progression. By some estimates, AD and related dementias are the single most expensive medical condition. In 2019, direct costs of AD in the USA will be ~$290 billion, which is expected to rise to ~$1.1 trillion by 2050 if no treatments are developed. Hence, there is a tremendous imperative to gain a better understanding of the pathogenesis of AD and to develop effective treatments. AD is a complex, multifactorial disease, which is unique to humans. AD is defined neuropathologically by the accumulation of amyloid β (Aβ) into extracellular plaques in the brain parenchyma and in the vasculature (known as congophilic amyloid angiopathy [CAA]), and abnormally phosphorylated tau that accumulates intraneuronally forming neurofibrillary tangles (NFTs). Pathological aggregation of phosphorylated tau and Aβ occurs in a sequential process. Monomers first aggregate into oligomers intraneuronally that then further aggregate into the fibrils observed in amyloid plaques and NFTs. This pathology then spreads in a characteristic brain topography that is distinct for NFTs and plaques. This process develops over many years, with a preclinical period of two to three decades, the onset of which is modulated by apolipoprotein E (apoE) genotype, as well as other genetic and environmental risk factors.

This book integrates considerable expertise from a wide range of authors from different disciplines. It includes clinicians through to translational and basic scientists. In aggregate, this book provides a comprehensive and up-to-date overview of AD. It covers the heterogeneous underlying AD pathology, with a review of genetic and proteomic approaches to better understand the disease. In addition, there is an extensive review of various potential contributing factors to the emergence of AD, as well as a discussion of novel biomarkers and potential effective therapeutic approaches. I trust that these reviews will be of value to clinicians and health professionals caring for patients with AD, and will provide a comprehensive and thought-provoking introduction to young investigators interested in translational aspects of the AD and related dementias field.

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PREFACE

Alzheimer's disease (AD) is the most common cause of dementia. The term "dementia" is derived from the Latin word *demens*, meaning "being out of one's mind," and has been used since the 13th century. AD has been recognized as a distinct entity since the publication of Alzheimer's description of a patient with presenile dementia in 1906. The first biochemical identification of amyloid beta (Aβ) as the major component of amyloid plaques, a key neuropathological lesion in AD, was published in 1984 with the seminal work of Dr. George Glenner. The latter discovery led to the amyloid cascade hypothesis of AD, with a focus on developing amyloid directed therapeutic approaches. The latter have all failed in clinical trials thus far. More recently, there is growing body of genetic, transcriptomic, and proteomic data pointing to the complexity of AD pathogenesis. This has resulted in a greater diversity of therapeutic approaches being attempted—in effect, resulting in "more shots on goal," with the prospect that at least some of these approaches will be efficacious. Hence, despite the many failures of AD therapeutic clinical trials, this is a hopeful time in AD research. There is a growing anticipation that our greater understanding of the underlying multifactorial pathogenesis of AD will result in effective therapeutic interventions in the near future.

In this book, we present reviews with the most current information on several critical aspects of AD, providing the readers with a broad picture of the underlying neuropathology, genetics, proteomics, risk factors, novel biomarkers, and potential interventions. Chapters 1–5 discuss the underlying AD pathogenesis using genomic and proteomic approaches, linking diverse pathways that can lead to complex metabolic dysfunction. Chapter 6 reviews the potential role of trace metals in AD, while Chapter 7 examines the diversity of Aβ species involved in AD pathology. Chapter 8 discusses the contributions of white matter degeneration in AD. Chapter 9 examines the potential intriguing role of the brain-gut-microbiota axis in mediating AD. Chapters 10 and 11 discuss potential biomarkers for AD, such as deficits in ocular exploration and early language impairments, respectively. Chapters 12–15 examine possible novel preventative and/or therapeutic methodologies such as exercise, optimizing depression therapy, and diverse psychosocial interventions.

We would like to thank all the authors for their diligent work in contributing toward this book. The 15 chapters review diverse facets of AD, which together paint a comprehensive picture of the pathogenesis, associated risk factors, novel biomarkers, and potential therapeutic targets. We believe that this book will encourage readers to delve deeper into this field and take up the critical challenge of working toward effective treatments for AD and related dementias.

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A New Perspective on Alzheimer's Disease as a Brain Expression of a Complex Metabolic Disorder

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Abstract: Alzheimer's disease (AD) is an irredeemable chronic neurodegenerative disorder and the predominant cause of dementia. The disease progression is associated with the deposition of amyloid plaques and formation of neurofibrillary tangles in the brain, yet clinical dementia is the end and culminating stage of the enduring pathology. Recent evidence suggests that AD is characterized by distinctive abnormalities apparent on systemic, histological, macromolecular, and biochemical levels. Besides the well-described characteristic profuse neurofibrillary tangles, dystrophic neurites, and Aβ deposits, the AD pathology includes substantial neuronal loss, inflammation, extensive DNA damage, considerable mitochondrial malfunction, impaired energy metabolism, and chronic oxidative stress. Moreover, severe metabolic dysfunction leading to oxidative stress is a possible cause and hallmark of AD that is apparent decades before the disease manifestation. State-of-the-art metabolomics studies have proved that arginine and branched-chain amino acids metabolism disturbances accompany AD and contribute to its pathogenesis. Repetitive failures to find an efficient anti-amyloid or anti-Tau treatment, which would face the challenges of the complex AD pathology, led to the hypothesis that hyperphosphorylated Tau and deposited

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Aβ proteins are hallmarks, not the ultimate causes of AD. Accordingly, the modern scientific vision of AD etiology and pathogenesis must reach beyond the hallmarks and look for alternative strategies and areas of research.

Keywords: Alzheimer's disease; arginase; arginine; branched-chain amino acids; oxidative stress; urea cycle

INTRODUCTION

Alzheimer's disease (AD) is a severe chronic neurodegenerative disorder and the leading cause of dementia (1). The gradual progression of cognitive decline is associated with characteristic brain atrophy, amyloid plaques deposition, and neurofibrillary tangles (NFT) formation (2). More than 100 years ago, AD has been described as an extremely rare pathology and, in fact, it was uncommon before the baby-boomers began to reach retirement age. Nowadays, it looks like one of the most significant medical, social, and economic challenges that faces the 21st century. Growing life-expectancy, high sugar and fat diet, and sedentary lifestyle have led to an epidemic-like and exponential dissemination of the disease within various social and national strata. Today, more than 50 million individuals suffer from the stage of AD that we refer to as dementia worldwide, and this number is expected to triple by 2050 (3).

Despite a century-long rigorous investigation, there is no complete scientific consensus regarding the causes of AD. The prevailing current view among scientists centers upon the amyloid cascade hypothesis (4, 5). However, growing clinical and empirical evidence points to extremely complex systemic pathophysiology accompanying AD-associated cognitive impairment and even contributing to its development decades prior to the clinical manifestation (6, 7).

The recent introduction of novel biomarkers for early detection and clinical management of AD has improved the diagnostic precision and qualification of neuropathology. The new techniques provide practical tools for more objective assessment of the treatment outcomes and early therapeutic strategy correction, with emphasis on the molecular mechanisms of the disease. Moreover, this systems-level approach identifies sex and age-specific differences and further advances the development of personalized medicine.

AD is an incredibly complex illness, which constitutes a combination of numerous interrelated pathological events that include neurovascular, inflammatory, bioenergetic, and systemic metabolic processes. In addition to the classic, distinctive hallmarks, the disease is characterized by systemic abnormalities and brain metabolic aberrations, which are evident at molecular and biochemical levels. Accordingly, the typical contemporary description of AD-related pathology includes neuroinflammation, activation of apoptosis, mitochondrial dysfunction, metabolic impairment, and chronic oxidative stress.

Notably, oxidative damage is considered to be the earliest event in AD pathology. Reliable data demonstrate an inverse correlation between levels of oxidative damage and both beta-amyloid (Aβ) deposition and duration of dementia (8). Moreover, the formation of intraneuronal NFT is associated

with reduced oxidative damage as well, which further supports the view that the onset of oxidative damage is an early event in AD pathogenesis.

Causes of brain oxidative stress include brain hypoperfusion due to advanced atherosclerosis or endothelial dysfunction, traumatic brain injury (TBI), infections, autoimmune disorders, insulin resistance (IR), and other diseases leading to neuroinflammation. Of note, the very first AD patient autopsy disclosed substantial brain atrophy and apparent arteriosclerosis (9). Recent converging evidence suggests that chronic cerebral hypoperfusion follows progressive aging due to cerebral atherosclerosis and endothelial dysfunction (10). These two interrelated pathological processes lead to brain energy crisis and trigger the characteristic neurodegeneration (11) (Figure 1).

Initial studies with focal ischemic insults and chronic cerebral hypoperfusion in rats have shown increased amyloid precursor protein (APP) translation levels followed by local Aβ deposition in the brain parenchyma (12). Furthermore, chronic vascular insufficiency induces cleavage of the APP into Aβ-sized fragments in a rodent model (13). The used model of chronic blood-vessel occlusion demonstrated progressive accumulation of Aβ peptide in the aged rats. Of note,

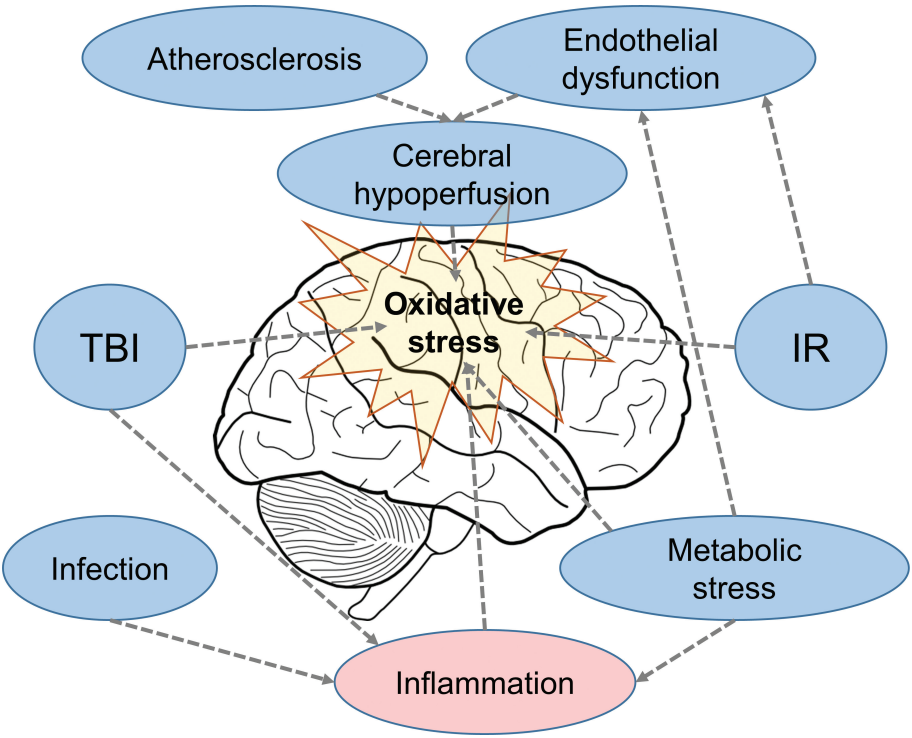

Figure 1 Main pathogenic factors of AD development. Cerebral hypoperfusion, metabolic stress, traumatic brain injury (TBI), and insulin resistance (IR) are the main causes of AD development.

Aβ deposition pattern displayed a gradual shift from neurons to the extracellular matrix, mimicking the characteristics of sporadic AD. The described hypoxia-induced response is attributed to a significant increase in the activities of APP amyloidogenic proteases (β- and γ-secretases), although nonamyloidogenic α-secretase activity declines (14). Additionally, proinflammatory cytokines, and in particular tumor necrosis factor (TNF), prompt transcription of the *APP* gene via direct regulation of its promoter, which leads to overproduction and deposition of Aβ (15).

Brain tissue dreprivation of arginine has been suggested as a possible pathogenic mechanism leading to oxidative damage (16). Arginine deprivation leads to endothelial nitric oxide synthase (NOS3) substrate deficiency and enzymatic "uncoupling" due to induced arginase activation (16, 17). Uncoupling changes NOS3 enzymatic profile radically. As an alternative to oxidizing arginine to citrulline and NO, uncoupled enzyme reduces molecular oxygen to superoxide anion, which leads to neuronal oxidative stress (18).

Additionally, recent metabolomics studies have indicated characteristic branched-chain amino acids (BCAAs) deficiency as a metabolic signature of AD (19). BCAAs play an important role in glutamine/glutamate brain metabolism and provide nitrogen for at least one-third of the cerebral glutamate (20). Therefore, perturbations of BCAAs levels have a substantial impact on brain function and tip the scale between excitation and inhibition. Of note, BCAAs supplementation has been intensively investigated preclinically, demonstrating a therapeutic potential in different animal models of atherosclerosis (21), obesity (22), metabolic syndrome (23), and AD (24).

According to our model (Figure 2), AD is a spectrum of disorders, which has a mutual downstream pathway and pattern of manifestation with deviant biological reactions that eventually culminate in clinical dementia. We comprehend brain amyloidogenesis as a natural evolutionary conserved reaction to oxidative and metabolic stresses, which can be induced by numerous factors including nutrient imbalances. This view corresponds with the notion of Aβ antioxidant functions in the aging and AD brain (27) and an influential concept, which deals with intraneuronal accumulation of Aβ, is a protective cellular mechanism to cope with oxidative insults (28). Moreover, amyloid aggregation and formation of extracellular amyloid plaques, where amyloid is in an insoluble form, are also an adaptive mechanism of the brain (29). This bioprocess reduces the concentration of the soluble toxic oligomeric and fibrillar species, which impair synaptic function and induce an inflammatory response. For that reason, there is a gradual reduction of Aβ concentration in the cerebrospinal fluid (CSF) of AD patients (30), the index that is inversely correlated with the cognitive decline (31).

EARLY- AND LATE-ONSET AD ARE TWO DIFFERENT ENTITIES

Advanced age is the main risk factor of AD (32). Nevertheless, its early onset is relatively common, representing about 5% of all cases (33). The very first case described by Alois Alzheimer was a woman who died at just 55 years from the disease. In fact, this case should be classified as an early-onset AD (EOAD) incident. This form is defined in the literature by clinical symptoms appearing

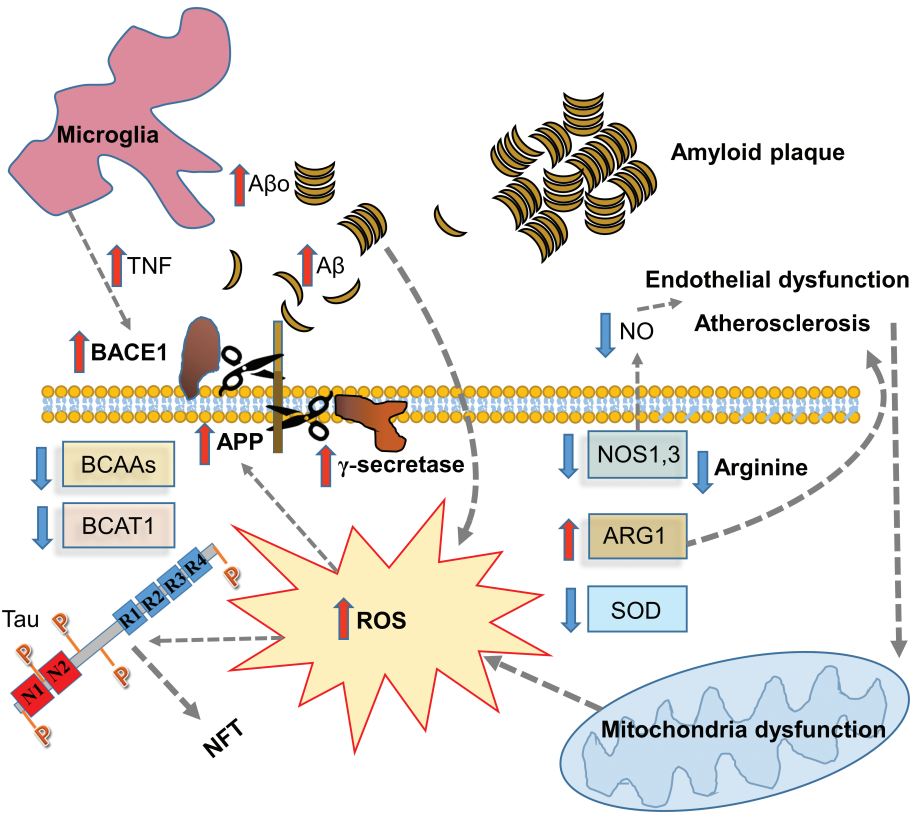

Figure 2 Proposed model of AD pathogenesis. The diagram presents oxidative stress induced by mitochondrial dysfunction, inflammation, or metabolic stress as the principal pathogenic AD event. Oxidative stress leads to an elevation in ROS levels and accumulation of oxidation products in neurons, which results in overexpression and increased processing of *APP* gene and eventually plaque formation (25), hyperphosphorylation of Tau and NFT pathology, which in turn produces more ROS and results in neurodegeneration and cellular death (26). Aβ directly induces the production of ROS and further exacerbates oxidative stress and impairs endogenous antioxidant system including the activity of SODs.

before the age of 61 years (34). Generally, EOAD is inherited following dominant Mendelian fashion, although it represents a genetically heterogeneous group (35). Epidemiological data suggest that autosomal dominant familial AD (FAD) with PSEN1, PSEN2, and APP mutations accounts for about 0.5% of all AD cases (36); yet, the share of familial form in the group of EOAD rises to 13% (34). Late-onset AD (LOAD) demonstrates a high heritability, with much more genes implicated in its development; however, the progression of LOAD is believed to be driven by a combination of genetic and environmental factors (37), and thus it remains to be principally idiopathic.

EOAD frequently manifests with distinguishable cognitive profile from LOAD. No evident amnesia characterizes the disease course, sometimes presenting just with language discrepancies, apraxia, and other uncommon functional

deficits (38). Moreover, a specific memory impairment itself presents distinct patterns in EOAD and LOAD cases, with significantly more impaired semantic memory in LOAD than in EOAD patients (39). Recent objective data have demonstrated that EOAD CSF and fludeoxyglucose F 18 (^{18}F-FDG) positron emission tomography (PET) features substantially contrast with LOAD. CSF t-Tau shows significantly higher levels in EOAD patients (40). Moreover, ^{18}F-FDG PET scans of EOAD patients present the asymmetric patterns of hypometabolism with a localization that prominently differs from LOAD. Likewise, dementia severity in relatively young patients strongly correlates with amyloid plaques burden, but this direct relation progressively weakens with age and even disappears in the ninth decade of life (41). Of note, the term "dementia" itself is an umbrella term for cognitive impairment that interferes with one's ability to conduct routine daily affairs, and therefore extremely varies between different social groups.

Remarkably, at a molecular level, the Aβ oligomeric subtypes show a distinct pattern in each of AD forms. Amyloid pentameric species in the insoluble fraction are more abundant in EOAD than in LOAD (42). Additionally, elevated inflammatory markers, together with impaired renal function, distinguish LOAD, which points to substantial differences in pathogenesis and development between the two clinical forms of the disease (43).

These observations indicate that EOAD afflicting presenile populations represents a categorically separate pathological entity, which is characterized by distinctive pathophysiological mechanisms accountable for its unambiguous genetic background and uncommon clinical manifestation. In our opinion, only this form presents classic AD or presenile dementia of Alzheimer type.

In opposition to presenile form, the etiology of LOAD disease is much more heterogeneous, with a combined contribution of numerous genetic, age-related, and environmental factors. Unlike EOAD, LOAD often presents in comorbidity with diabetes and hypertension (44). Of note, no two patients have the same combination of the disease-related factors; therefore, the clinical appearance and the treatment strategies for this form have to be accurately personalized. Moreover, despite the apparent differences between presenile and senile forms of AD, taxonomically they are still the same illness. In our opinion, LOAD is a syndrome, but not a stand-alone disease. It is a concurrence of correlated with each other convergent symptoms, and concurrence literally means syndrome (45). Thus, we suggest that the presence of the same confluent hallmarks, which characterize the EOAD and LOAD, does not reflect their mutual etiology and pathogenesis; therefore, attempts to link these two forms of AD to a single common causative agent are futile. For that reason, this chapter deals only with more common LOAD, which has a distinct metabolic signature.

LOAD AS A SYSTEMIC METABOLIC DISORDER

Converging evidence points to severe metabolic dysfunction as a leading cause and hallmark of AD (46). State-of-the-art metabolomics and imaging studies dealing with the immense complexity of the AD phenotype have disclosed this aspect of the disease. Gradual decline in cerebral metabolic rate is one of the earliest indicators that distinguish patients with mild cognitive impairment (MCI) and

poses the clinical suspicion of prodromal AD (47), which suggests a key role of metabolic dysfunction in initial mechanisms of AD development. Likewise, advanced analyses of the brain tissue could detect explicit metabolic perturbations associated with AD, both in humans (48) and mice (49).

A recent human postmortem unbiased lipidomics and metabolomics study has disclosed 34 metabolites, which distinguish frontal cortices' composition of AD patients from healthy controls (50). The authors identified six biochemical pathways, which are significantly altered in AD brains. The list of the pathways by their significance rate includes alanine, aspartate, and glutamate metabolism; arginine and proline metabolism; cysteine and methionine metabolism; glycine, serine, and threonine metabolism; purine metabolism; and pantothenate and CoA biosynthesis (50).

Metabolomics profiling of human plasma, which combines high-resolution mass spectrometry and advanced chemometrics and pathway enrichment analysis, indicates differentially affected polyamine and arginine metabolism in MCI subjects converting to AD (51).

Animal studies support and advance these findings. Multivariate statistical analysis of metabolite profiles of the brain, liver, and kidney tissues from APP/PS1 and wild-type (WT) mice indicates systemic nature of AD-associated pathophysiology (52). Liver and kidney samples from 6-month-old mice were fingerprinted using a high-throughput multi-platform metabolomics approach based on gas chromatography/mass spectrometry and reversed-phase liquid chromatography. Several observations pointed to the systemic character of the disorder with severely impaired glucose metabolism, mitochondrial dysfunction, and abnormal metabolism of BCAAs (52). Another longitudinal research performed in APP/PS1 transgenic and wild-type mice (6, 8, 10, 12, and 18 months of age) with deep profiling of the brain and plasma metabolome proved severely disturbed polyamines and BCAAs metabolism (53).

Growing clinical evidence points to a widespread AD-related systemic disorder characterized by severely affected peripheral parenchymal organs and blood in similar magnitude as the brain (54). Remarkably, the scope and features of AD-associated metabolic abnormalities resemble advanced pathology observed in obese and diabetic patients (55). These common aberrations led to the hypothesis that AD represents a unique form of diabetes. A novel term "type 3 diabetes" has been coined and accepted in the scientific literature (56). This term reflects a substantial overlap at molecular and biochemical levels between AD and diabetes mellitus type 2 (57). Diabetic elderly patients were shown to develop extensive vascular abnormalities, which are associated with classic AD pathology (58). Likewise, recent data evidently and causatively relate obesity and AD (59).

Remarkably, several common treatment strategies for the abovementioned metabolic diseases are extremely effective, which proves mutual pathophysiology. Various preclinical and clinical studies have verified that a long list of drugs that are conventional in the treatment of diabetes, atherosclerosis, and other metabolic disorders improves the overall status, behavioral and cellular functions of AD patients. For instance, insulin-based therapy has emerged as a promising approach to halt AD-associated cognitive decline (60). Wang et al. evidenced a substantial effect of metformin upon neurogenesis and spatial memory acquisition in mice (61). A significant neuroprotective effect of metformin was demonstrated in rodents on a high-fat diet (HFD) (62). A recent meta-analysis study has proved

that metformin use is associated with reduced risk of dementia in patients with diabetes (63). In order to evaluate the potentials of the drug as a disease-modifying medicine in AD, a randomized 2-month-long placebo-controlled crossover study was performed, verifying the metformin-associated improvement in executive functioning (64).

Additionally, animal and human studies with thiazolidinediones have shown the potential to treat AD and diabetes. The treatment improves memory via facilitation of synaptic transmission and reduction of neurodegeneration (65, 66). Likewise, a broad variety of antioxidants are shown to be promising in atherosclerosis, AD (67), and diabetes mellitus (68). It is worth mentioning that chronic curcumin treatment improves the function of insulin-producing β-cells, reduces Aβ-associated cytotoxicity, mitigates Tau protein hyperphosphorylation, and alleviates neurodegeneration (69, 70).

Curiously, despite the lack of consensus about the AD etiology and pathogenesis, and absence of disease-modifying therapy, the disease prevalence in the western world has declined gradually over the last two decades (71). Several population-based studies have suggested that despite the growing absolute number of elderly people with dementia, age-specific risk of dementia is declining (72). In the USA alone, the proportion of elderly people with dementia has decreased by about 24% between 2000 and 2012 (73). A similar trend was observed in England between 1991 and 2011 (74).

One possible explanation for the phenomenon might be recent considerable achievements in treatments of cardiovascular diseases and diabetes. The innovative widespread prevention and treatment strategies for these disorders include intensive medication with novel effective medicines. Therefore, the progress in the control of main dementia risk factors substantially assisted in reducing the prevalence of dementia among the target age groups.

Recent evidence suggests that AD-associated cognitive impairment is the outcome of extremely complex pathophysiology. In the light of new findings, more thorough consideration of the complexity of AD as a syndrome is required. Moreover, strategies targeting β-amyloid or Tau protein are not adequate to cure the disease; therefore, attempts to treat single hallmarks of AD, such as plaques and tangles, are futile.

A CONTRIBUTION OF THE UREA CYCLE AND POLYAMINE METABOLIC PATHWAY IN THE DEVELOPMENT OF AD

The human brain weighs just 2% of the entire body weight but consumes about a fifth of the total glucose-derived energy, and consequently is highly vulnerable to oxidative stress (75). Neurons particularly are strongly dependent upon oxidative phosphorylation as an energy source, compared to other cells. As a general rule, oxidative stress increases with aging (76), which is followed by escalation of protein oxidation and extensive lipid peroxidation in susceptible organs and, particularly, in the brain. In the course of the progression of age-related or AD-related neurodegeneration, neurons gradually lose their capacity of maintaining an appropriate redox balance. This imbalance leads to progressive accumulation of reactive oxygen species (ROS), mitochondrial dysfunction, and,

eventually, to neuronal injury (77). Moreover, Aβ deposits are directly associated with the free-radical generation, forming a vicious circle of AD pathogenesis (78, 79) (Figure 2).

Antioxidants are capable of transferring electrons to and from oxidizing agents, inhibiting free radicals production and reducing potential cell damages (80). Generally, antioxidants are classified into enzymatic agents (superoxide dismutase [SOD], catalase, glutathione peroxidase, glutathione reductase, etc.) and non-enzymatic agents (coenzyme Q10, carotenoids, vitamins E and C, and arginine) (81).

Arginine is a potent free radical scavenger (82) and protects neurons against oxidative stress through its antioxidant potentials (83). Its cationic nature contributes to the unique protonative properties and ability to react directly with the superoxide anion radical (84). Thus, arginine and its derivatives regulate membrane peroxidation processes (85). Although the human cells are capable of synthesizing arginine, its external supplementation is necessary for infants and the elderly, making arginine conditionally essential (86). Moreover, some clinical conditions lead to depletion of endogenous arginine resources, which escalates the demand for it. Among these conditions are severe infections, burns, wounds, intensive physical activity, and sterility (87).

Several recent studies have explored the association between age-related cognitive function decline and aberrations in brain arginine metabolism. An animal study has disclosed altered arginine metabolic profile even prior to any memory deficit (88), which proves the parallel development of brain arginine metabolism aberrations and behavioral deficits in AD mice. Moreover, behavioral deficits and brain profile alternations follow the changes in plasma arginine metabolic profile, which advocates the use of arginine-centric antemortem biomarkers for the early diagnosis of AD (88). More recent data from the same laboratory have demonstrated a significantly altered brain arginine metabolism in a mouse model of tauopathy (89). Noticeable changes were observed in ornithine, polyamines, and glutamate concentrations, which further suggest a shift of arginine metabolism to the direction of arginase–polyamine pathway in AD rodent models brain.

Additional evidence indicates severe arginine metabolism disturbances in various brain areas and points to significantly escalated arginase activity in the hippocampi of AD patients (90). Another study has reported decreased levels of arginine in the cortices of AD patients (91). Moreover, innovative capillary electrophoresis–mass spectrometry metabolomics investigations of AD patients' CSF detect a decline in arginine levels (92, 93). Remarkably, urine levels of arginine in amnestic MCI patients are also significantly lower than in normal controls (94). Additionally, these patients demonstrate a reduced global arginine bioavailability ratio, the index, which is positively correlated with the Mini-Mental Status Examination score, making urinary arginine levels a potential diagnostic biomarker for MCI. Of note, numerous animal studies have further implicated altered arginine metabolism in the pathogenesis of AD (95, 96).

Arginine was shown to mitigate hydrogen peroxide-induced apoptosis and protect against $A\beta_{(25\text{-}35)}$-induced toxicity in cultured PC-12 cells (97). The amino acid supplementation improves cognitive function in demented elderly (98). Additionally, its administration within 30 min of a stroke significantly decreases the frequency and severity of symptoms (99).

Arginine overcomes biological barriers via ubiquitously expressed high-affinity permeases or cationic amino acid transporters (CATs), which are involved in the transport of the cationic amino acids (arginine, lysine, histidine, etc.) (100). Arginine is generally transported from the circulating blood into the brain via CAT1, which is excessively expressed at the blood–brain barrier (BBB) (101, 102). The amino acid influx transport in the rat model has been proven to be saturable with a Michaelis–Menten constant (Km) value of 56 μM. Of note, the physiological serum concentration of arginine is about 170 μM in rodents and about 100 μM in men (103). Consequently, the capacity of its transport system is substantially limited (104) that makes traditional arginine supplement insufficient to demonstrate all of its possible effects. Therefore, the pharmacological targeting of enzymes that metabolize arginine in order to improve its availability is a likely beneficial method to treat neurological conditions (17).

Arginase cleaves arginine to produce urea and ornithine at the last step of the urea cycle, which generally protects the cells against ammonia toxicity, while ornithine and its downstream derivatives participate in collagen formation, induce cell proliferation, and influence other vital physiological processes (Figure 3). A substantial increase in arginase activity contributes to vascular dysfunction in the atherosclerotic (apolipoprotein E deficient) mice by interfering in the function of the neurovascular unit, which leads to BBB leakage and neuroinflammation (105).

Arginine is the immediate precursor of NO and other bioactive molecules (Figure 3). Nitric oxide synthases (NOSs) utilize arginine as a substrate to produce NO and citrulline (106). Consequently, the bioavailability of arginine is a regulating factor for NO synthesis (107). Arginine-derived NO serves as a potent antioxidant agent protecting cells from damage caused by ROS (108). It causes vasodilation and improves blood supply to neurons, which reduce their susceptibility to oxidative stress (109). Likewise, NO moderates Ca^{2+} influx into the neurons, protecting them from excitotoxicity (110). Remarkably, under physiological conditions, the molecule represents a key endothelium protective factor (111) but becomes detrimental under oxidative stress. Substrate deficiency leads to NOS3 uncoupling and deviation from NO synthesis, converting it to a superoxide-producing enzyme (112). A significant reduction of NOS activity in AD brains, with a decrease in the levels of NOS1 and NOS3 proteins, has been reported (90). Moreover, AD-associated arginase overactivation substantially limits mutual substrate availability, and is followed by a decrease in NO production (113). This mechanism is particularly important in the statuses with limited extracellular resources of arginine, like advanced age, for example.

The arginase expression is induced by various stimuli, including cytokines, catecholamines, lipopolysaccharide, TNF, oxidized low-density lipoprotein, and hypoxia (114, 115). In the brains of the AD model mice, arginase 1 (Arg1) was shown to be not only localized in the cells but also distributed in the extracellular space. In the hippocampus, it displays a spatial correlation with Aβ deposition, and Iba1 expression (16). In addition, activation of arginase 2 (Arg2) is associated with translocation from the mitochondria to the cytosol (116, 117) (Figure 3).

Recent evidence points to escalated *Arg2* gene expression in AD brains (118). Moreover, Arg2 deficiency reduces the rate of hyperoxia-mediated retinal neurodegeneration (119), suggesting the contribution of arginase in the neuronal

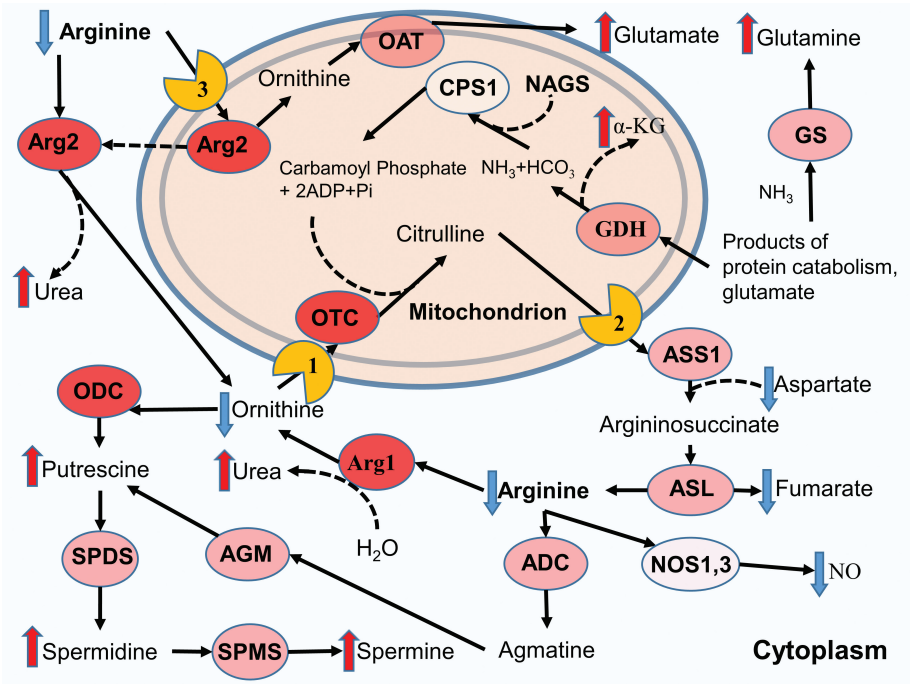

Figure 3 Arginine metabolic pathways and their deviations in AD brain. Arginine is primary substrate metabolized by nitric oxide synthases (NOS1 and NOS3 under physiological conditions), arginase 1 (Arg1) and arginine decarboxylase (ADC). Arginase 2 (Arg2) plays a role in extra-urea cycle arginine metabolism. Arginine is cleaved by arginase to form urea and ornithine at the final step of the urea cycle. The urea cycle consists of N-acetylglutamate synthase (NAGS), which is an allosteric cofactor for catalytic enzyme carbamoyl phosphate synthase (CPS1), and other four catalytic enzymes: ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS1), argininosuccinate lyase (ASL), and arginase 1 (Arg1). NAGS, CPS1 and OTC are localized in the mitochondria, while ASS1, ASL, and ARG1 are in the cytosol. Two enzymes: OTC and CPS1 are present in very low concentrations in the human brain. As a result, the brain urea cycle is not efficient and to remove ammonia and relies on alternative glutamine synthesis by glutamine synthetase (GS). Glutamine concentration rises in AD brain. Glutamate dehydrogenase (GDH) catalyzes the deamination of glutamate to α-ketoglutarate (α-KG), which concentrations also increase in AD brain, and ammonia (NH3). The mitochondrial ornithine transporter (1), citrin (2), and the mitochondrial cationic amino acid transporter type 1 (3). Red arrows indicate elevated levels; blue arrows designate reduced ones. The intensity of the circles' color reflects the level of activation (arbitrary scale). Argininosuccinate lyase (ASL), argininosuccinate synthetase (ASS), nitric oxide (NO), ornithine decarboxylase (ODC), spermidine synthase (SPDS), spermine synthase (SPMS), agmatinase (AGM), ornithine aminotransferase (OAT).

degeneration via overactivation of the N-methyl-D-aspartate receptors (120). Accordingly, targeting Arg2 has been proposed as a means of decelerating age-related diseases treatment (121).

Inhibition of ornithine decarboxylase (ODC) with α-difluoromethylornithine has been proved to be neuroprotective in a rodent model of AD (16). The authors speculated that arginine deprivation is a critical AD pathogenic factor, which eventually leads to neuronal death and cognitive deficits. We have hypothesized

that upregulation of arginase activity and consequent arginine and NO deficiency, in the brain areas characterized by excessive amyloid deposition, contribute to the clinical manifestation of AD (17). Accordingly, we targeted arginase, but not ODC, with its uncompetitive inhibitor, norvaline, to ameliorate the symptoms of the disease (117).

Norvaline has been proven to be a potent inhibitor of urea synthesis in isolated rat liver cells (122). It also inhibits arginase in vivo via negative feedback inhibition mechanism due to its structural similarity with ornithine (123). Moreover, the inhibition process is enantiomer dependent because its stereoisomer, D-norvaline, does not affect NO production (113). The potency of norvaline to amplify the rate of NO production has been evidenced in vitro (113). In addition, the substance was effectively used in a rat model of artificial metabolic syndrome (23).

Remarkably, norvaline also effectively inhibits ornithine transcarbamylase (OTC) activity, the mitochondrial enzyme converting ornithine to citrulline (124) (Figure 3). OTC is extensively expressed in AD brains, but not in controls, which is followed by about ninefold increase in OTC activity in the CSF (125). Epidemiological studies have revealed that single nucleotide polymorphism of the *OTC* gene promoter is associated with AD morbidity, suggesting that the *OTC* gene is a minor genetic AD determinant (126). *OTC* activation leads to apparent ornithine deficiency (90) and, in turn, arginase activation via product inhibition insufficiency. Thus, the vicious circle of metabolic changes acts in the AD brain (Figure 3). Accordingly, norvaline is capable of correcting the AD-related arginine metabolism aberrations by inhibiting two central enzymes of the urea cycle.

A PUTATIVE ROLE OF BCCAS IN THE DEVELOPMENT OF AD

BCAAs are the amino acids possessing branched aliphatic side-chains. There are three proteinogenic BCAAs—valine, leucine, and isoleucine, which are essential amino acids—and several non-proteinogenic BCAAs, including 2-aminoisobutyric acid and 2-aminopentanoic acid (norvaline) (127). It is worth mentioning that the vast majority of essential amino acids are metabolized in the liver; however, BCAAs escape the first-pass hepatic catabolism and are mainly oxidized in skeletal muscles, adipose tissue, and the brain (128).

BCAAs catabolism initiates with a transamination reaction catalyzed by the branched-chain aminotransferases (BCATs). The family of BCATs consists of two isoforms: mitochondrial BCAT2 and cytosolic BCAT1. BCATs are mutual to all three BCAAs, and transamination by BCATs is the exclusive reaction for BCAAs only. The products of the reaction are glutamate and three different branched-chain α-ketoacids (BCKAs). Of note, other non-proteinogenic BCAAs (for instance, norvaline) are competent to be substrates for BCAT in rodents and humans (129).

There are indications that BCAAs play a different role in the brain compared to other tissues. BCAT1 and BCAT2 are expressed prominently in the brain cells, where the enzymes maintain the continuous supply of the principal excitatory neurotransmitter glutamate. Remarkably, BCAT1 is present predominantly in neurons, while the appearance of BCAT2 is limited to astrocytes (130, 131).

In the mammalian brain, BCAAs are involved in several vital processes. Among them are key neurotransmitters' metabolism, protein synthesis, and energy production (132). Glutamate is the principal excitatory neurotransmitter of the mammalian brain (133), and its concentrations are substantially higher in brain than in plasma (134). Glutamate does not cross the BBB in considerable quantities, except in regions with fenestrated capillaries (135); therefore, neuronal glutamate has to be continually synthesized from constantly accessible and reliable precursors. Its synthesis requires an efficient amino group donor, which is transported rapidly into the brain and is readily transaminated. BCAAs meet these needs optimally. Their unique properties and availability allow them to play a central role in glutamate metabolism. It was estimated that at least one-third of the cerebral glutamate contains nitrogen derived from the BCAAs (20). Consequently, perturbations in the levels of BCAAs meaningfully influence the whole function of the central nervous system, and the balance of excitation and inhibition, in particular.

González-Domínguez et al. utilized gas chromatography coupled with mass spectrometry to profile low-molecular-weight metabolites in serum of newly diagnosed sporadic AD patients who had not received any medication yet (136). Alterations of 23 metabolites were detected, including significantly decreased valine levels. In a more recent study including hundreds of participants conducted by Toledo et al., lower plasma valine levels were shown to correlate with the rate of cognitive decline. Likewise, the coefficient for valine was negatively associated with actual ventricular volume changes. Accordingly, an increase in valine concentration was associated with a significantly decreased risk of AD (137). Another study by Tynkkynen et al. utilized innovative profiling of blood metabolites via nuclear magnetic resonance and mass spectrometry (19). Remarkably, lower levels of all three BCAAs were strongly associated with an increased risk of dementia and AD in a combined meta-analysis with a replication sample.

In our original studies in a rodent model of AD, we provided the mice with arginase inhibitor non-proteinogenic BCAA norvaline, which is an isoform of valine (117). The animals treated with norvaline demonstrated significantly improved spatial memory acquisition, associated with an increase in hippocampal spine density, and reduced neuroinflammation. Moreover, the rate of the brain amyloidosis was significantly diminished due to a reduction in the expression levels of the APP, which was followed by a significant increase in [Cu-Zn] superoxide dismutase levels, suggesting improvement of the internal antioxidant mechanisms (24). Further investigations will shed light on the potential of BCAAs to halt AD progression.

CONCLUSION

Scientific society has already sought a potent AD-modifying medication for more than a century. Unfortunately, its best efforts have been to no avail. A cornucopia of agents has been trialed, hoping to preclude the impending calamity, but with no conclusive results. The primary cause of the continual failures is the misleading and highly controversial hypothesis, which besets the development of adequate AD therapy.

The chronic absence of an AD-modifying drug, despite multibillion dollar research and development investment, puzzles the best scientific minds and enigmatizes the entire field of knowledge. Continual failure to rise to the challenges of the multifaceted AD pathology and offer an efficient disease-modifying therapy predicated upon the dominant during the last 30 years amyloid cascade hypothesis, with aggressive anti-amyloid or anti-Tau treatments, led to the suggestion that hyperphosphorylated Tau and deposited Aβ proteins are just hallmarks and not the ultimate causes of AD (139, 140). Accordingly, treatment strategies targeting beta-amyloid or Tau protein are not competent to cure the disease (140). As a result, a novel trend in academic research and preclinical drug development is directed toward the discovery of therapeutic agents targeting altered brain metabolism and energetics (141). Recently proposed novel strategies based on a universal approach to the problem of AD and a progressive vision of the disease etiology and pathogenesis reaching beyond the conventional hallmarks provide a hope to halt the looming epidemic.

Current metabolomics techniques are based on a comprehensive understanding of AD pathophysiology, which is predicated upon the detailed knowledge of its peculiarities, the disease onset coincidences, and the precise order of the pathology development. In this context, an emerging metabolic hypothesis of AD, which is strongly supported by empirical evidence, and treats the classic hallmarks of the disease as the epiphenomena of the major complex pathology, has promising potential to offer a competent therapeutic solution.

Moreover, this new concept proposes a novel approach to the clinical classification and the treatment strategy for two distinct forms of AD. There is a consensus about the considerable differences between EOAD and LOAD. These two forms of AD have dissimilar courses, different genetic backgrounds and clinical manifestations, and are followed by unrelated metabolic impairments. Therefore, they have to be treated as separate entities.

In categorizing LOAD as a brain expression of a systemic complex metabolic disorder, which shares similarities and pathogenic pathways with diabetes mellitus, obesity, and atherosclerosis, we suggest common treatment and preventive strategies for all these pathologies. Therefore, regular physical and mental activity, diet, blood glucose, cholesterol levels monitoring and regulation, and antioxidants supplementation have particular importance in AD prevention and treatment. Moreover, novel emerging potent medicines, which have been successfully trialed in patients with various systemic metabolic diseases, might be extremely effective in AD patients as well.

We suggest that inclusive AD treatment strategies, targeting both brain and systemic abnormalities, are more effective than strategies that target CNS abnormalities alone. Such approaches should include an auxiliary intervention into the metabolic pathways and personalized correction of misbalances. Likewise, the systemic management of AD comorbidities and mutual risk factors is a central part of preventive AD therapy.

In addition, we argue that, in the case of LOAD, early prevention is the best healthcare salutary strategy. Consequently, the most critical current objectives are the empowerment of people with the ability to change their lifestyle, and the arming of doctors with the appropriate tools and medicines to halt the AD development.

Conflict of interest: The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this chapter.

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REFERENCES

1. Alzheimer's Association. 2016 Alzheimer's disease facts and figures. Alzheimers Dement. 2016;12(4):1–80.
2. Goedert M, Spillantini MG. A century of Alzheimer's disease. Science. 2006;314(5800):777–81. http://dx.doi.org/10.1126/science.1132814
3. Christina P. World Alzheimer report 2018. The state of the art of dementia research: New frontiers. London: Alzheimer's Disease International (ADI); 2018.
4. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 1991;12(10):383–8. http://dx.doi.org/10.1016/0165-6147(91)90609-V
5. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. Science. 1992;256(5054):184–5. http://dx.doi.org/10.1126/science.1566067
6. Sonntag KC, Ryu WI, Amirault KM, Healy RA, Siegel AJ, McPhie DL, et al. Late-onset Alzheimer's disease is associated with inherent changes in bioenergetics profiles. Sci Rep. 2017;7(1):14038. http://dx.doi.org/10.1038/s41598-017-14420-x
7. Wilkins JM, Trushina E. Application of metabolomics in Alzheimer's disease. Front Neurol. 2018;8:719. http://dx.doi.org/10.3389/fneur.2017.00719
8. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, et al. Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol. 2001; 60(8):759–67. http://dx.doi.org/10.1093/jnen/60.8.759
9. Pearce JMS. Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2000;68:348. http://dx.doi.org/10.1136/jnnp.68.3.348
10. De La Torre JC. Cerebral hemodynamics and vascular risk factors: Setting the stage for Alzheimer's disease. J Alzheimers Dis. 2012;32(3):553–67. http://dx.doi.org/10.3233/JAD-2012-120793
11. De La Torre JC. Pathophysiology of neuronal energy crisis in Alzheimer's disease. Neurodegener Dis. 2008;5(3–4):126–32. http://dx.doi.org/10.1159/000113681
12. Kalaria RN, Bhatti SU, Lust WD, Perry G. The amyloid precursor protein in ischemic brain injury and chronic hypoperfusion. Ann N Y Acad Sci. 1993;695:190–3. http://dx.doi.org/10.1111/j.1749-6632.1993.tb23050.x
13. Bennett SAL, Pappas BA, Stevens WD, Davidson CM, Fortin T, Chen J. Cleavage of amyloid precursor protein elicited by chronic cerebral hypoperfusion. Neurobiol Aging. 2000;21(2):207–14. http://dx.doi.org/10.1016/S0197-4580(00)00131-7
14. Salminen A, Kauppinen A, Kaarniranta K. Hypoxia/ischemia activate processing of amyloid precursor protein: Impact of vascular dysfunction in the pathogenesis of Alzheimer's disease. J Neurochem. 2017;140(4):536–49. http://dx.doi.org/10.1111/jnc.13932
15. Lahiri DK, Chen D, Vivien D, Ge YW, Greig NH, Rogers JT. Role of cytokines in the gene expression of amyloid β-protein precursor: Identification of a 5'-UTR-binding nuclear factor and its implications in Alzheimer's disease. J Alzheimers Dis. 2003;5(2):81–90. http://dx.doi.org/10.3233/JAD-2003-5203
16. Kan MJ, Lee JE, Wilson JG, Everhart AL, Brown CM, Hoofnagle AN, et al. Arginine deprivation and immune suppression in a mouse model of Alzheimer's disease. J Neurosci. 2015;35(15):5969–82. http://dx.doi.org/10.1523/JNEUROSCI.4668-14.2015
17. Polis B, Samson AO. Arginase as a potential target in the treatment of Alzheimer's disease. Adv Alzheimers Dis. 2018;7:119–40. http://dx.doi.org/10.4236/aad.2018.74009

18. Loscalzo J. An experiment of nature: Genetic L-arginine deficiency and NO insufficiency. J Clin Investig. 2001;108(5):663–4. http://dx.doi.org/10.1172/JCI13848
19. Tynkkynen J, Chouraki V, van der Lee SJ, Hernesniemi J, Yang Q, Li S, et al. Association of branched-chain amino acids and other circulating metabolites with risk of incident dementia and Alzheimer's disease: A prospective study in eight cohorts. Alzheimers Dement. 2018;14(6):723–3. http://dx.doi.org/10.1016/j.jalz.2018.01.003
20. Yudkoff M. Brain metabolism of branched-chain amino acids. Glia. 1997;21(1):92–8. http://dx.doi.org/10.1002/(SICI)1098-1136(199709)21:1%3C92::AID-GLIA10%3E3.0.CO;2-W
21. Zhao Y, Dai XY, Zhou Z, Zhao GX, Wang X, Xu MJ. Leucine supplementation via drinking water reduces atherosclerotic lesions in apoE null mice. Acta Pharmacol Sin. 2016;37(2):196–203. http://dx.doi.org/10.1038/aps.2015.88
22. Macotela Y, Emanuelli B, Bång AM, Espinoza DO, Boucher J, Beebe K, et al. Dietary leucine – An environmental modifier of insulin resistance acting on multiple levels of metabolism. PLoS One. 2011;6(6):e21187. http://dx.doi.org/10.1371/journal.pone.0021187
23. El-Bassossy HM, El-Fawal R, Fahmy A, Watson ML. Arginase inhibition alleviates hypertension in the metabolic syndrome. Br J Pharmacol. 2013;169(3):693–703. http://dx.doi.org/10.1111/bph.12144
24. Polis B, Srikanth K, Gurevich V, Gil-Henn H, Samson A. L-Norvaline, a new therapeutic agent against Alzheimer's disease. Neural Regen Res. 2019;14(9):1562–72. http://dx.doi.org/10.4103/1673-5374.255980
25. Muche A, Arendt T, Schliebs R. Oxidative stress affects processing of amyloid precursor protein in vascular endothelial cells. PLoS One. 2017;12(6):e0178127. http://dx.doi.org/10.1371/journal.pone.0178127
26. Melov S, Adlard PA, Morten K, Johnson F, Golden TR, Hinerfeld D, et al. Mitochondrial oxidative stress causes hyperphosphorylation of tau. PLoS One. 2007;2(6):e536. http://dx.doi.org/10.1371/journal.pone.0000536
27. Smith MA, Casadesus G, Joseph JA, Perry G. Amyloid-β and τ serve antioxidant functions in the aging and Alzheimer brain. Free Radic Biol Med. 2002;33(9):1194–9. http://dx.doi.org/10.1016/S0891-5849(02)01021-3
28. Nunomura A, Tamaoki T, Tanaka K, Motohashi N, Nakamura M, Hayashi T, et al. Intraneuronal amyloid β accumulation and oxidative damage to nucleic acids in Alzheimer disease. Neurobiol Dis. 2010;37(3):731–7. http://dx.doi.org/10.1016/j.nbd.2009.12.012
29. Castellani RJ, Lee HG, Siedlak SL, Nunomura A, Hayashi T, Nakamura M, et al. Reexamining Alzheimer's disease: Evidence for a protective role for amyloid-β protein precursor and amyloid-β. J Alzheimers Dis. 2009;18(2):447–52. http://dx.doi.org/10.3233/JAD-2009-1151
30. Motter R, Vigo-Pelfrey C, Kholodenko D, Barbour R, Johnson-Wood K, Galasko D, et al. Reduction of β-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. Ann Neurol. 1995;38(4):643–8. http://dx.doi.org/10.1002/ana.410380413
31. Haapalinna F, Paajanen T, Penttinen J, Kokki H, Kokki M, Koivisto AM, et al. Low cerebrospinal fluid amyloid-beta concentration is associated with poorer delayed memory recall in women. Dement Geriatr Cogn Dis Extra. 2016;6(2):303–12. http://dx.doi.org/10.1159/000446425
32. Guerreiro R, Bras J. The age factor in Alzheimer's disease. Genome Med. 2015;7:106. http://dx.doi.org/10.1186/s13073-015-0232-5
33. Zhu X, Tan L, Wang H, Jiang T, Cao L, Wang C, et al. Rate of early onset Alzheimer's disease: A systematic review and meta-analysis. Ann Transl Med. 2015;3(3):38.
34. Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, et al. Early-onset autosomal dominant Alzheimer disease: Prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet. 1999;65(3):664–70. http://dx.doi.org/10.1086/302553
35. Smith M. Genetics of Alzheimer's disease. In: Dementia. 5th ed. David Ames, John T. O'Brien, Alistair Burns. Editors. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2017;519–528.
36. Finckh U, Kuschel C, Anagnostouli M, Patsouris E, Pantes GV, Gatzonis S, et al. Novel mutations and repeated findings of mutations in familial Alzheimer disease. Neurogenetics. 2005;6(2):85–9. http://dx.doi.org/10.1007/s10048-005-0211-x

37. Barber RC. The genetics of Alzheimer's disease. Scientifica (Cairo). 2012;2012:246210. http://dx.doi.org/10.6064/2012/246210
38. Smits LL, Pijnenburg YAL, Koedam ELGE, Van Der Vlies AE, Reuling IEW, Koene T, et al. Early onset alzheimer's disease is associated with a distinct neuropsychological profile. J Alzheimer's Dis. 2012;30(1):101–8. http://dx.doi.org/10.3233/JAD-2012-111934
39. Joubert S, Gour N, Guedj E, Didic M, Guériot C, Koric L, et al. Early-onset and late-onset Alzheimer's disease are associated with distinct patterns of memory impairment. Cortex. 2016;74:217–32. http://dx.doi.org/10.1016/j.cortex.2015.10.014
40. Chiaravalloti A, Koch G, Toniolo S, Belli L, Di Lorenzo F, Gaudenzi S, et al. Comparison between early-onset and late-onset Alzheimer's disease patients with amnestic presentation: CSF and 18F-FDG PET study. Dement Geriatr Cogn Dis Extra. 2016;6(1):108–19. http://dx.doi.org/10.1159/000441776
41. Prohovnik I, Perl DP, Davis KL, Libow L, Lesser G, Haroutunian V. Dissociation of neuropathology from severity of dementia in late-onset Alzheimer disease. Neurology. 2006;66(1):49–55. http://dx.doi.org/10.1212/01.wnl.0000191298.68045.50
42. Dorothée G, Bottlaender M, Moukari E, De Souza LC, Maroy R, Corlier F, et al. Distinct patterns of antiamyloid-β antibodies in typical and atypical Alzheimer disease. Arch Neurol. 2012;69(9):1181–5. http://dx.doi.org/10.1001/archneurol.2012.604
43. Panegyres PK, Chen H-Y. Differences between early and late onset Alzheimer's disease. Am J Neurodegener Dis. 2013;2(4):300–6.
44. Poblador-Plou B, Calderón-Larrañaga A, Marta-Moreno J, Hancco-Saavedra J, Sicras-Mainar A, Soljak M, et al. Comorbidity of dementia: A cross-sectional study of primary care older patients. BMC Psychiatry. 2014;14:84. http://dx.doi.org/10.1186/1471-244X-14-84
45. Albert D. Dorland's illustrated medical dictionary. 32nd ed. Saunders; 2011.
46. De La Monte SM, Tong M. Brain metabolic dysfunction at the core of Alzheimer's disease. Biochem Pharmacol. 2014;88(4):548–59. http://dx.doi.org/10.1016/j.bcp.2013.12.012
47. Pagani M, Nobili F, Morbelli S, Arnaldi D, Giuliani A, Öberg J, et al. Early identification of MCI converting to AD: A FDG PET study. Eur J Nucl Med Mol Imaging. 2017;44(12):2042–52. http://dx.doi.org/10.1007/s00259-017-3761-x
48. Inoue K, Tsutsui H, Akatsu H, Hashizume Y, Matsukawa N, Yamamoto T, et al. Metabolic profiling of Alzheimer's disease brains. Sci Rep. 2013;3:2364. http://dx.doi.org/10.1038/srep02364
49. Salek RM, Xia J, Innes A, Sweatman BC, Adalbert R, Randle S, et al. A metabolomic study of the CRND8 transgenic mouse model of Alzheimer's disease. Neurochem Int. 2010;56(8):937–47. http://dx.doi.org/10.1016/j.neuint.2010.04.001
50. Paglia G, Stocchero M, Cacciatore S, Lai S, Angel P, Alam MT, et al. Unbiased metabolomic investigation of Alzheimer's disease brain points to dysregulation of mitochondrial aspartate metabolism. J Proteome Res. 2016;15(2):608–18. http://dx.doi.org/10.1021/acs.jproteome.5b01020
51. Graham SF, Chevallier OP, Elliott CT, Hölscher C, Johnston J, Mcguinness B, et al. Untargeted metabolomic analysis of human plasma indicates differentially affected polyamine and L-arginine metabolism in mild cognitive impairment subjects converting to Alzheimer's disease. PLoS One. 2015;10(3):1–16. http://dx.doi.org/10.1371/journal.pone.0119452
52. González-Domínguez R, García-Barrera T, Vitorica J, Gómez-Ariza JL. Metabolomic investigation of systemic manifestations associated with Alzheimer's disease in the APP/PS1 transgenic mouse model. Mol Biosyst. 2015;11(9):2429–40. http://dx.doi.org/10.1039/C4MB00747F
53. Pan X, Nasaruddin MB, Elliott CT, McGuinness B, Passmore AP, Kehoe PG, et al. Alzheimer's disease-like pathology has transient effects on the brain and blood metabolome. Neurobiol Aging. 2016;38:151–63. http://dx.doi.org/10.1016/j.neurobiolaging.2015.11.014
54. Maarouf CL, Walker JE, Sue LI, Dugger BN, Beach TG, Serrano GE. Impaired hepatic amyloid-beta degradation in Alzheimer's disease. PLoS One. 2018;13(9):e0203659. http://dx.doi.org/10.1371/journal.pone.0203659
55. Mittal K, Mani RJ, Katare DP. Type 3 diabetes: Cross talk between differentially regulated proteins of type 2 diabetes mellitus and Alzheimer's disease. Sci Rep. 2016;6:25589. http://dx.doi.org/10.1038/srep25589

56. Kandimalla R, Thirumala V, Reddy PH. Is Alzheimer's disease a type 3 diabetes? A critical appraisal. Biochim Biophys Acta Mol Basis Dis. 2017 May;1863(5):1078–89. http://dx.doi.org/10.1016/j.bbadis.2016.08.018
57. De La Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. J Diabetes Sci Technol. 2008;2(6):1101–13. http://dx.doi.org/10.1177/193229680800200619
58. Ahtiluoto S, Polvikoski T, Peltonen M, Solomon A, Tuomilehto J, Winblad B, et al. Diabetes, Alzheimer disease, and vascular dementia: A population-based neuropathologic study. Neurology. 2010;75(13):1195–202. http://dx.doi.org/10.1212/WNL.0b013e3181f4d7f8
59. Alford S, Patel D, Perakakis N, Mantzoros CS. Obesity as a risk factor for Alzheimer's disease: Weighing the evidence. Obesity Rev. 2018;19(2):269–80. http://dx.doi.org/10.1111/obr.12629
60. Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: A pilot clinical trial. Arch Neurol. 2012;69(1):29–38. http://dx.doi.org/10.1001/archneurol.2011.233
61. Wang J, Gallagher D, Devito LM, Cancino GI, Tsui D, He L, et al. Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. Cell Stem Cell. 2012;11(1):23–35. http://dx.doi.org/10.1016/j.stem.2012.03.016
62. Asadbegi M, Yaghmaei P, Salehi I, Ebrahim-Habibi A, Komaki A. Neuroprotective effects of metformin against Aβ-mediated inhibition of long-term potentiation in rats fed a high-fat diet. Brain Res Bull. 2016;121:178–85. http://dx.doi.org/10.1016/j.brainresbull.2016.02.005
63. Campbell JM, Stephenson MD, De Courten B, Chapman I, Bellman SM, Aromataris E. Metformin use associated with reduced risk of dementia in patients with diabetes: A systematic review and meta-analysis. J Alzheimer's Dis. 2018;65(4):1225–36. http://dx.doi.org/10.3233/JAD-180263
64. Koenig AM, Mechanic-Hamilton D, Xie SX, Combs MF, Cappola AR, Xie L, et al. Effects of the insulin sensitizer metformin in Alzheimer disease: Pilot data from a randomized placebo-controlled crossover study. Alzheimer Dis Assoc Disord. 2017;31(2):107–13. http://dx.doi.org/10.1097/WAD.0000000000000202
65. Gad ES, Zaitone SA, Moustafa YM. Pioglitazone and exenatide enhance cognition and downregulate hippocampal beta amyloid oligomer and microglia expression in insulin-resistant rats. Can J Physiol Pharmacol. 2016;94(8):819–28. http://dx.doi.org/10.1139/cjpp-2015-0242
66. Galimberti D, Scarpini E. Pioglitazone for the treatment of Alzheimer's disease. Expert Opin Investig Drugs. 2017;26(1):97–101. http://dx.doi.org/10.1080/13543784.2017.1265504
67. Zhao Y, Zhao B. Natural antioxidants in prevention and management of Alzheimer's disease. Front Biosci Elit. 2012;4:794–808. http://dx.doi.org/10.2741/e419
68. Ruhe RC, McDonald RB. Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. J Am Coll Nutr. 2001 Oct;20(5 Suppl):363S–9S; discussion 381S–3S. http://dx.doi.org/10.1080/07315724.2001.10719169
69. Huang HC, Zheng BW, Guo Y, Zhao J, Zhao JY, Ma XW, et al. Antioxidative and neuroprotective effects of curcumin in an Alzheimer's disease rat model co-treated with intracerebroventricular streptozotocin and subcutaneous D-galactose. J Alzheimers Dis. 2016;52(3):899–911. http://dx.doi.org/10.3233/JAD-150872
70. Tang M, Taghibiglou C, Liu J. The mechanisms of action of curcumin in Alzheimers disease. J Alzheimers Dis. 2017;58(4):1003–16. http://dx.doi.org/10.3233/JAD-170188
71. Roehr S, Pabst A, Luck T, Riedel-heller SG. Is dementia incidence declining in high-income countries? A systematic review and meta-analysis. Clin Epidemiol. 2018;10:1233–47. http://dx.doi.org/10.2147/CLEP.S163649
72. Langa KM. Is the risk of Alzheimer's disease and dementia declining? Alzheimers Res Ther. 2015;7(1):34. http://dx.doi.org/10.1186/s13195-015-0118-1
73. Langa KM, Larson EB, Crimmins EM, Faul JD, Levine DA, Kabeto MU, et al. A comparison of the prevalence of dementia in the United States in 2000 and 2012. JAMA Intern Med. 2017;2800(1):51–8. http://dx.doi.org/10.1001/jamainternmed.2016.6807
74. Matthews FE, Arthur A, Barnes LE, Bond J, Jagger C, Robinson L, et al. A two-decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of

England: Results of the cognitive function and ageing study I and II. Lancet. 2013;382(9902):1405–12. http://dx.doi.org/10.1016/S0140-6736(13)61570-6
75. Shetty PK, Galeffi F, Turner DA. Cellular links between neuronal activity and energy homeostasis. Front Pharmacol. 2012;3:43. http://dx.doi.org/10.3389/fphar.2012.00043
76. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239–47. http://dx.doi.org/10.1038/35041687
77. Chen X, Guo C, Kong J. Oxidative stress in neurodegenerative diseases. Neural Regen Res. 2012;7(5):376–85.
78. Mclellan ME, Kajdasz ST, Hyman BT, Bacskai BJ. In vivo imaging of reactive oxygen species specifically associated with thioflavine s-positive amyloid plaques by multiphoton microscopy. J Neurosci. 2003;23(6):2212–17. http://dx.doi.org/10.1523/JNEUROSCI.23-06-02212.2003
79. Perry G, Taddeo MA, Nunomura A, Zhu X, Zenteno-Savin T, Drew KL, et al. Comparative biology and pathology of oxidative stress in Alzheimer and other neurodegenerative diseases: Beyond damage and response. Comp Biochem Physiol C Toxicol Pharmacol. 2002;133(4):507–13. http://dx.doi.org/10.1016/S1532-0456(02)00119-9
80. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol. 2003;17(1):24–38. http://dx.doi.org/10.1002/jbt.10058
81. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother. 2005;59(7):365–73. http://dx.doi.org/10.1016/j.biopha.2005.07.002
82. Ahmad A, Sattar MZA, Rathore HA, Hussain AI, Khan SA, Fatima T, et al. Antioxidant activity and free radical scavenging capacity of L-arginine and NaHS: A comparative in vitro study. Acta Pol Pharm Drug Res. 2015;72(2):245–52.
83. Hosseini M, Anaeigoudari A, Beheshti F, Soukhtanloo M, Nosratabadi R. Protective effect against brain tissues oxidative damage as a possible mechanism for beneficial effects of L-arginine on lipopolysaccharide induced memory impairment in rats. Drug Chem Toxicol. 2018;41(2):175–81. http://dx.doi.org/10.1080/01480545.2017.1336173
84. Wallner S, Hermetter A, Mayer B, Wascher TC. The alpha-amino group of L-arginine mediates its antioxidant effect. Eur J Clin Invest. 2001;31(2):98–102. http://dx.doi.org/10.1046/j.1365-2362.2001.00771.x
85. Milyutina N. Antiradical and antioxidant effect of arginine and its action on lipid peroxidation in hypoxia. Bull Exp Biol Med. 1990;110(9):263–5. http://dx.doi.org/10.1007/BF00840280
86. Wu G, Jaeger LA, Bazer FW, Rhoads JM. Arginine deficiency in preterm infants: Biochemical mechanisms and nutritional implications. J Nutr Biochem. 2004;15(8):442–51. http://dx.doi.org/10.1016/j.jnutbio.2003.11.010
87. Hristina K, Langerholc T, Trapecar M. Novel metabolic roles of L-arginine in body energy metabolism and possible clinical applications. J Nutr Health Aging. 2014;18(2):213–18. http://dx.doi.org/10.1007/s12603-014-0015-5
88. Bergin D, Jing Y, Mockett B, Zhang H, Abraham W, Liu P. Altered plasma arginine metabolome precedes behavioural and brain arginine metabolomic profile changes in the APPswe/PS1ΔE9 mouse model of Alzheimer's disease. Transl Psychiatry. 2018;18(2):213–18. http://dx.doi.org/10.1038/s41398-018-0149-z
89. Vemula P, Jing Y, Zhang H, Hunt JB, Sandusky-Beltran LA, Lee DC, et al. Altered brain arginine metabolism in a mouse model of tauopathy. Amino Acids. 2019;51(3):513–28. http://dx.doi.org/10.1007/s00726-018-02687-x
90. Liu P, Fleete MS, Jing Y, Collie ND, Curtis MA, Waldvogel HJ, et al. Altered arginine metabolism in Alzheimer's disease brains. Neurobiol Aging. 2014;35(9):1992–2003. http://dx.doi.org/10.1016/j.neurobiolaging.2014.03.013
91. Gueli MC, Taibi G. Alzheimer's disease: Amino acid levels and brain metabolic status. Neurol Sci. 2013;34(9):1575–9. http://dx.doi.org/10.1007/s10072-013-1289-9

92. Ibanez C, Simo C, Martin-Alvarez PJ, Kivipelto M, Winblad B, Cedazo-Minguez A, et al. Toward a predictive model of Alzheimer's disease progression using capillary electrophoresis-mass spectrometry metabolomics. Anal Chem. 2012;84(20):8532–40. http://dx.doi.org/10.1021/ac301243k
93. Fonteh AN, Harrington RJ, Tsai A, Liao P, Harrington MG. Free amino acid and dipeptide changes in the body fluids from Alzheimer's disease subjects. Amino Acids. 2007;32(2):213–24. http://dx.doi.org/10.1007/s00726-006-0409-8
94. Zhang Y, Tang Y, Dammer E, Liu J, Zhao Y, Zhu L, et al. Dysregulated urinary arginine metabolism in older adults with amnestic mild cognitive impairment. Front Aging Neurosci. 2019;11:90. http://dx.doi.org/10.3389/fnagi.2019.00090
95. Liu P, Jing Y, Collie ND, Campbell SA, Zhang H. Pre-aggregated Aβ25-35 alters arginine metabolism in the rat hippocampus and prefrontal cortex. Neuroscience. 2011;193:269–82. http://dx.doi.org/10.1016/j.neuroscience.2011.07.054
96. Bergin DH, Jing Y, Zhang H, Liu P. A single intracerebroventricular Aβ25-35 infusion leads to prolonged alterations in arginine metabolism in the rat hippocampus and prefrontal cortex. Neuroscience. 2015;298:367–79. http://dx.doi.org/10.1016/j.neuroscience.2015.04.034
97. Fonar G, Polis B, Maltsev A, Samson AO. Intracerebroventricular administration of L-arginine improves spatial memory acquisition in triple transgenic mice via reduction of oxidative stress and apoptosis. Transl Neurosci. 2018;9:43–53. http://dx.doi.org/10.1515/tnsci-2018-0009
98. Ohtsuka Y, Nakaya J. Effect of oral administration of L-arginine on senile dementia. Am J Med. 2000;108(5):439. http://dx.doi.org/10.1016/S0002-9343(99)00396-4
99. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Tanabe Y, et al. L-arginine improves the symptoms of strokelike episodes in MELAS. Neurology. 2005;64(4):710–12. http://dx.doi.org/10.1212/01.WNL.0000151976.60624.01
100. Hatzoglou M, Fernandez J, Yaman I, Closs E. Regulation of cationic amino acid transport: The story of the CAT-1 transporter. Annu Rev Nutr. 2004;24:377–99. http://dx.doi.org/10.1146/annurev.nutr.23.011702.073120
101. O'Kane RL, Viña JR, Simpson I, Zaragozá R, Mokashi A, Hawkins RA. Cationic amino acid transport across the blood-brain barrier is mediated exclusively by system y+. Am J Physiol Endocrinol Metab. 2006;291(2):E412–19. http://dx.doi.org/10.1152/ajpendo.00007.2006
102. Tachikawa M, Hosoya K. Transport characteristics of guanidino compounds at the blood-brain barrier and blood-cerebrospinal fluid barrier: Relevance to neural disorders. Fluids Barriers CNS. 2011;8(1):13. http://dx.doi.org/10.1186/2045-8118-8-13
103. Stoll J, Wadhwani KC, Smith QR. Identification of the cationic amino acid transporter (system y+) of the rat blood-brain barrier. J Neurochem. 1993;60(5):1956–9. http://dx.doi.org/10.1111/j.1471-4159.1993.tb13428.x
104. Shin WW, Fong WF, Pang SF, Wong PC. Limited blood brain barrier transport of polyamines. J Neurochem. 1985;44(4):1056–9. http://dx.doi.org/10.1111/j.1471-4159.1985.tb08724.x
105. Badaut J, Copin JC, Fukuda AM, Gasche Y, Schaller K, da Silva RF. Increase of arginase activity in old apolipoprotein-E deficient mice under Western diet associated with changes in neurovascular unit. J Neuroinflammation. 2012;9:132. http://dx.doi.org/10.1186/1742-2094-9-132
106. Nelson DL, Cox MM. Lehninger principles of biochemistry. 3rd ed. New York: Wort Publishers; 2004.
107. Balez R, Ooi L. Getting to NO Alzheimer's disease: Neuroprotection versus neurotoxicity mediated by nitric oxide. Oxid Med Cell Longev. 2016;2016:3806157. http://dx.doi.org/10.1155/2016/3806157
108. Wink DA, Hanbauer I, Krishna MC, DeGraff W, Gamson J, Mitchell JB. Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. Proc Natl Acad Sci USA. 1993;90(21):9813–17. http://dx.doi.org/10.1073/pnas.90.21.9813
109. Durante W, Johnson FK, Johnson RA. Arginase: A critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol. 2007;34(9):906–11. http://dx.doi.org/10.1111/j.1440-1681.2007.04638.x
110. Ditlevsen DK, Køhler LB, Berezin V, Bock E. Cyclic guanosine monophosphate signalling pathway plays a role in neural cell adhesion molecule-mediated neurite outgrowth and survival. J Neurosci Res. 2007;85(4):703–11. http://dx.doi.org/10.1002/jnr.21175
111. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res. 2017;120(4):713–35. http://dx.doi.org/10.1161/CIRCRESAHA.116.309326

112. Li H, Förstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. Curr Opin Pharmacol. 2013;13(2):161–7. http://dx.doi.org/10.1016/j.coph.2013.01.006
113. Chang CI, Liao JC, Kuo L. Arginase modulates nitric oxide production in activated macrophages. Am J Physiol. 1998;274(1):H342–8. http://dx.doi.org/10.1152/ajpheart.1998.274.1.H342
114. Ryoo S, Bhunia A, Chang F, Shoukas A, Berkowitz DE, Romer LH. OxLDL-dependent activation of arginase II is dependent on the LOX-1 receptor and downstream RhoA signaling. Atherosclerosis. 2011;214(2):279–87. http://dx.doi.org/10.1016/j.atherosclerosis.2010.10.044
115. Lange PS, Langley B, Lu P, Ratan RR. Novel roles for arginase in cell survival, regeneration, and translation in the central nervous system. J Nutr. 2004;134(10 Suppl):2812S–17S; discussion 2818S–19S. http://dx.doi.org/10.1093/jn/134.10.2812S
116. Pandey D, Bhunia A, Oh YJ, Chang F, Bergman Y, Kim JH, et al. OxLDL triggers retrograde translocation of arginase2 in aortic endothelial cells via ROCK and mitochondrial processing peptidase. Circ Res. 2014;115(4):450–9. http://dx.doi.org/10.1161/CIRCRESAHA.115.304262
117. Polis B, Kolluru DS, Elliott E, Gil-Henn H, Samson AO. L-norvaline reverses cognitive decline and synaptic loss in a murine model of Alzheimer's disease. Neurotherapeutics. 2018;15(4):1036–54. http://dx.doi.org/10.1007/s13311-018-0669-5
118. Hansmannel F, Sillaire A, Kamboh MI, Lendon C, Pasquier F, Hannequin D, et al. Is the urea cycle involved in Alzheimer's disease? J Alzheimer's Dis. 2010;21(3):1013–21. http://dx.doi.org/10.3233/JAD-2010-100630
119. Narayanan SP, Xu Z, Putluri N, Sreekumar A, Lemtalsi T, Caldwell RW, et al. Arginase 2 deficiency reduces hyperoxia-mediated retinal neurodegeneration through the regulation of polyamine metabolism. Cell Death Dis. 2014;5(2):e1075. http://dx.doi.org/10.1038/cddis.2014.23
120. Pernet V, Bourgeois P, Di Polo A. A role for polyamines in retinal ganglion cell excitotoxic death. J Neurochem. 2007;103(4):1481–90. http://dx.doi.org/10.1111/j.1471-4159.2007.04843.x
121. Xiong Y, Yepuri G, Montani JP, Ming XF, Yang Z. Arginase-II deficiency extends lifespan in mice. Front Physiol. 2017;8:682. http://dx.doi.org/10.3389/fphys.2017.00682
122. Rognstad R. Sources of ammonia for urea synthesis in isolated rat liver cells. BBA Gen Subj. 1977;496(2):249–54. http://dx.doi.org/10.1016/0304-4165(77)90306-3
123. Saheki T, Sato Y, Takada S, Katsunuma T. Regulation of urea synthesis in rat liver. J Biochem. 1979;86(3):745–50. http://dx.doi.org/10.1093/oxfordjournals.jbchem.a132579
124. Bode AM, Foster JD, Nordlieon RC. Glycogenesis from glucose and ureagenesis in isolated perfused rat livers. Biochemistry. 1994 Mar;269(11):7879–86.
125. Bensemain F, Hot D, Ferreira S, Dumont J, Bombois S, Maurage C, et al. Evidence for induction of the ornithine transcarbamylase expression in Alzheimer's disease. Mol Psychiatry. 2009;14(1):106–16. http://dx.doi.org/10.1038/sj.mp.4002089
126. Hansmannel F, Lendon C, Pasquier F, Dumont J, Hannequin D, Chapuis J, et al. Is the ornithine transcarbamylase gene a genetic determinant of Alzheimer's disease? Neurosci Lett. 2009;449(1):76–80. http://dx.doi.org/10.1016/j.neulet.2008.10.081
127. Li C, Salditt T. Structure of magainin and alamethicin in model membranes studied by x-ray reflectivity. Biophys J. 2006;91(9):3285–300. http://dx.doi.org/10.1529/biophysj.106.090118
128. Brosnan JT, Brosnan ME. Branched-chain amino acids: Enzyme and substrate regulation. J Nutr. 2006;91(9):3285–300.
129. Hall TR, Wallin R, Reinhart GD, Hutson SM. Branched chain aminotransferase isoenzymes. Purification and characterization of the rat brain isoenzyme. J Biol Chem. 1993;268(5):3092–8.
130. Bixel MG, Hutson SM, Hamprecht B. Cellular distribution of branched-chain amino acid aminotransferase isoenzymes among rat brain glial cells in culture. J Histochem Cytochem. 1997;45(5):685–94. http://dx.doi.org/10.1177/002215549704500506
131. Bixel MG, Shimomura Y, Hutson SM, Hamprecht B. Distribution of key enzymes of branched-chain amino acid metabolism in glial and neuronal cells in culture. J Histochem Cytochem. 2001;49(3):407–18. http://dx.doi.org/10.1177/002215540104900314
132. Fernstrom J. Branched-chain amino acids and brain function. J Nutr. 2005;135(6 Suppl):1539S–46S. http://dx.doi.org/10.1093/jn/135.6.1539S
133. Meldrum BS. Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. J Nutr. 2000;130(4S Suppl):1007S–15S. http://dx.doi.org/10.1093/jn/130.4.1007S

134. Hawkins RA. The blood-brain barrier and glutamate. Am J Clin Nutr. 2009;90(3):867S–74S. http://dx.doi.org/10.3945/ajcn.2009.27462BB
135. Price MT, Olney JW, Lowry OH, Buchsbaum S. Uptake of exogenous glutamate and aspartate by circumventricular organs but not other regions of brain. J Neurochem. 1981;36(5):1774–80. http://dx.doi.org/10.1111/j.1471-4159.1981.tb00430.x
136. González-Domínguez R, García-Barrera T, Gómez-Ariza JL. Metabolite profiling for the identification of altered metabolic pathways in Alzheimer's disease. J Pharm Biomed Anal. 2015;107:75–81. http://dx.doi.org/10.1016/j.jpba.2014.10.010
137. Toledo JB, Arnold M, Kastenmüller G, Chang R, Baillie RA, Han X, et al. Metabolic network failures in Alzheimer's disease: A biochemical road map. Alzheimers Dement. 2017;13(9):965–984. http://dx.doi.org/10.1016/j.jalz.2017.01.020
138. Morris GP, Clark IA, Vissel B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol Commun. 2014;2(1):135. http://dx.doi.org/10.1186/s40478-014-0135-5
139. Makin S. The amyloid hypothesis on trial. Nature. 2018;559(7715):S4–S7. http://dx.doi.org/10.1038/d41586-018-05719-4
140. Canter RG, Penney J, Tsai LH. The road to restoring neural circuits for the treatment of Alzheimer's disease. Nature. 2016;539(7628):187–96. http://dx.doi.org/10.1038/nature20412
141. Godyń J, Jończyk J, Panek D, Malawska B. Therapeutic strategies for Alzheimer's disease in clinical trials. Pharmacol Rep. 2016;68(1):127–38. http://dx.doi.org/10.1016/j.pharep.2015.07.006

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Gene Ontology: A Resource for Analysis and Interpretation of Alzheimer's Disease Data

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Abstract: Gene Ontology (GO) is a universal resource for analyses and interpretation of high-throughput biological datasets. GO is developed and curated by several different groups, based at scientific institutions around the world, working together under the auspices of the GO Consortium. GO annotations capture biological functional knowledge by associating gene products with GO terms. GO term and gene product records all have computer-readable accession numbers; therefore, these annotations can be easily used for analyses of large datasets while retaining human-readable labels. The UCL Functional Gene Annotation group focuses on GO annotation of human gene products. Our group has led initiatives to systematically annotate proteins and microRNAs across specific biomedical fields, and our current biocuration effort, funded by the Alzheimer's Research UK foundation, is focused on dementia and Alzheimer's disease. Our group has also contributed to the development and revision of the ontology describing neurological domains of biology. Here we present an overview of GO and explain how our work, as well as the work of other members of the GO Consortium, is improving the neurological domains of the GO resource. These biocuration efforts will benefit the dementia and Alzheimer's research community by rendering GO more suitable for analyses of neurological datasets.

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INTRODUCTION

Several genes associated with monogenic Alzheimer's disease (AD) have been identified (1); however, the disease can also be caused by polygenic and environmental risk factors (1, 2). To understand the cellular processes and risk factors associated with AD, numerous transcriptomic, proteomic, and genome-wide association (GWA) studies have been conducted (3–5). Researchers are now turning to pathway-based GWA analysis and Next Generation Sequencing (NGS) to identify the genes contributing to the "missing heritability" (6, 7).

The process of finding gene variants that are causative, or modifiers, of disease is often time-consuming. Bioinformatics-based analyses can aid the identification of AD risk variants, based on the variant's association with a gene product implicated in neurobiological processes and pathways impaired in dementia. Such approaches are reliant on bioinformatics resources, including Gene Ontology (GO) (8, 9), KEGG (10), Reactome (11), and molecular interaction databases (12, 13). These resources provide connections between gene products and biological pathways or networks, which are relevant to AD. The end result of these analyses is the identification of both the risk variant and the candidate gene associated with the risk (14, 15). In addition, considerable research is now focused on the selection of biomarkers for AD (16), and the creation of biomarker panels is likely to be more successful if it is known what biological pathways the candidate biomarkers have in common.

GENE ONTOLOGY

The majority of analyses of high-throughput approaches rely on high-quality annotation data (4, 5) because these bridge the gap between data collation and data analysis (4, 17). Gene annotation datasets provide functional knowledge about gene products, such as proteins or microRNAs, in a computationally accessible format, thus these data can be exploited by systems biology investigators. The main resources used to identify significantly enriched pathways in "omics" studies are those provided by GO (8, 9), KEGG (10), Reactome (11), and protein interaction databases (12, 13). GO annotation data are frequently used because it can describe a gene product's role in a process or its location in a cell, even when the basic molecular activity of this gene product is still under investigation (Figure 1) (18). In contrast, Reactome and KEGG provide very specific information about the molecular function of a gene product within a pathway, with the "reaction" catalyzed or facilitated by each gene product clearly identified within a pathway diagram. Consequently, gene products whose role has not been fully elucidated cannot be included in these resources. Furthermore, although the human and mammalian phenotype ontologies (HPO, MP) (19) are being used to interpret NGS data, understanding how multiple genes contribute to a single

Gene Product	Symbol	GO Term	Evidence	Annotation Extension
UniProtKB:O95782	AP2A1	GO:0050750 low-density lipoprotein particle receptor binding	ECO:0000353 IPI	activated_by (CHEBI:64646) more...
UniProtKB:P30533	LRPAP1	GO:0150093 amyloid-beta clearance by transcytosis	ECO:0000315 IMP	has_input (UniProtKB:P05067:PRO_0000000093) more...
UniProtKB:Q00610	CLTC	GO:0072583 clathrin-dependent endocytosis	ECO:0000315 IMP	has_input (UniProtKB:Q07954) more...
UniProtKB:Q00610	CLTC	GO:0050750 low-density lipoprotein particle receptor binding	ECO:0000353 IPI	activated_by (CHEBI:64646) more...
UniProtKB:Q00610	CLTC	GO:0150093 amyloid-beta clearance by transcytosis	ECO:0000315 IMP	has_input (UniProtKB:P05067:PRO_0000000093) more...
UniProtKB:Q07954	LRP1	GO:0006898 receptor-mediated endocytosis	ECO:0000315 IMP	has_input (UniProtKB:P05067:PRO_0000000093) more...
UniProtKB:Q13492	PICALM	GO:0072583 clathrin-dependent endocytosis	ECO:0000315 IMP	has_input (UniProtKB:Q07954) more...
UniProtKB:Q13492	PICALM	GO:0050750 low-density lipoprotein particle receptor binding	ECO:0000353 IPI	activated_by (CHEBI:64646) more...
UniProtKB:Q15109	AGER	GO:0016324 apical plasma membrane	ECO:0000314 IDA	part_of (CL:0002144) more...

Figure 1 A selection of Gene Ontology annotations. This list of Gene Ontology annotations was downloaded from the QuickGO browser (37). All of these annotations, based on the experimental data presented by Zhao et al. (20), were created by the UCL Functional Gene Annotation group. The annotations were filtered by 'PMID:26005850'. The columns, in order from left to right, are as follows: Symbol, HGNC-approved gene symbol; GO term, GO term identifier and name; Evidence, one of the many Evidence and Conclusion Ontology (ECO) codes (38) associated with each GO annotation to indicate the type of experiments that support the annotation (IDA, Inferred from Direct Assay; IMP, Inferred from Mutant Phenotype; IPI, Inferred from Physical Interaction); Annotation Extension, additional information about the annotations, for example, the location of the function (occurs_in CL:0002144, capillary endothelial cell), or the entity that activates the function (activated_by CHEBI:64646, amyloid-beta polypeptide 40).

disease or phenotype will require resources, such as GO, that describe the cellular roles of these genes.

The GO resource (8, 9) is maintained, curated, and made available through the concerted efforts of the GO Consortium, whose aim is to provide both an ontology of terms and gene product annotations. Consequently, the GO Consortium includes skilled biocuration scientists, ontology editors, and software engineers. The ontology enables the description of attributes of gene products, including proteins, macromolecular complexes, and noncoding RNAs, in three key domains: *molecular function*, *biological process*, and *cellular component*. Fully defined computer-readable GO terms are used by the GO Consortium annotation groups, including our Functional Gene Annotation group at UCL, to create links (annotations) between GO terms and gene products across many species, based on published scientific findings, providing a computable and traceable summary of individual experiments. GO terms are used to describe gene products by their molecular functions (e.g., *scavenger receptor activity*), the biological processes they contribute toward (e.g., *microtubule cytoskeleton organization*), and their subcellular locations (e.g., *extracellular region*). For instance, GO curators have contributed 46 GO annotations based on experimental evidence presented by Zhao et al. (20), of which a selection is presented in Figure 1.

The gene product annotations contributed by GO biocurators are regularly submitted to the GO knowledgebase, where the most current and complete collection of GO terms and annotations is publicly available to all users (9).

Providers of bioinformatics tools, such as g:Profiler (21), Cytoscape (22), or DAVID (23), import GO data into their tools for use in enrichment analyses of large datasets. Therefore, the association of GO terms with gene product records (to create annotations) and the use of GO annotation data in analysis tools together enable groups of similarly annotated gene products, within an "omics" dataset, to be identified as significantly enriched (18, 24, 25). Thus, dysregulated pathways, functions, and macromolecular complexes can be identified within high-throughput datasets. However, GO annotation is a continuously ongoing initiative with certain biological aspects annotated more thoroughly than others. Insufficient annotation of key biological processes and pathways relevant to dementia can hinder the interpretation of outcomes from GWA studies, microarray, and proteomic approaches to dissect AD and other AD-relevant diseases (26). Consequently, these analyses may identify partial protein networks or only general GO terms as enriched in the dataset, for example *mitochondrion* (27) and *calcium-mediated signaling* (4). Having recognized this deficit, the Functional Gene Annotation group at UCL have, for the last 5 years, focused on the annotation of gene products relevant to Parkinson's and Alzheimer's diseases (26, 28, 29). This has led to substantial improvements in the representation of processes such as mitophagy, amyloid precursor protein processing, oxidative stress, and tau-associated processes.

Improving the GO to represent dementia-relevant processes

The GO is structured as directed graphs, with each GO term having a unique term name, for example, *phosphatidylcholine-sterol O-acyltransferase activity*, *proteasomal protein catabolic process*, or *high-density lipoprotein particle*, and a definition (Figure 2) as well as a computer-readable numerical identifier. In addition, the ontology is a dynamic resource, with the ontology itself continually being expanded and refined to capture current knowledge. Although GO terms exist which describe most gene products' processes, functions, and locations, many of these terms are very general and are not specific enough to fully describe the role of AD-associated gene products. The UCL Functional Gene Annotation group has begun to address this issue through the development of the ontology to provide more specific and descriptive GO terms, by improving the existing term definitions and by revising the existing ontology structure (26, 28, 29). The association of these more specific GO terms prevents the loss of valuable descriptions of gene products, based on experimental information, that would have been unavailable if the more general GO term had been applied. For example, we have improved the ontology domains describing the unfolded protein response (UPR) (28), autophagy (29), and neuron projection development (26). These improvements have led to an expansion of the number of GO terms describing these processes, as well as revision of relationships between terms within the ontology. All of these biological processes have relevance to AD as well as Parkinson's disease and other neurological conditions.

Although GO terms are categorized into three key domains, as introduced above, revisions in one domain are often done in conjunction with another domain describing the same biological niche. For instance, our work on *neuron projection development* (26), a biological process GO term, resulted, first, in

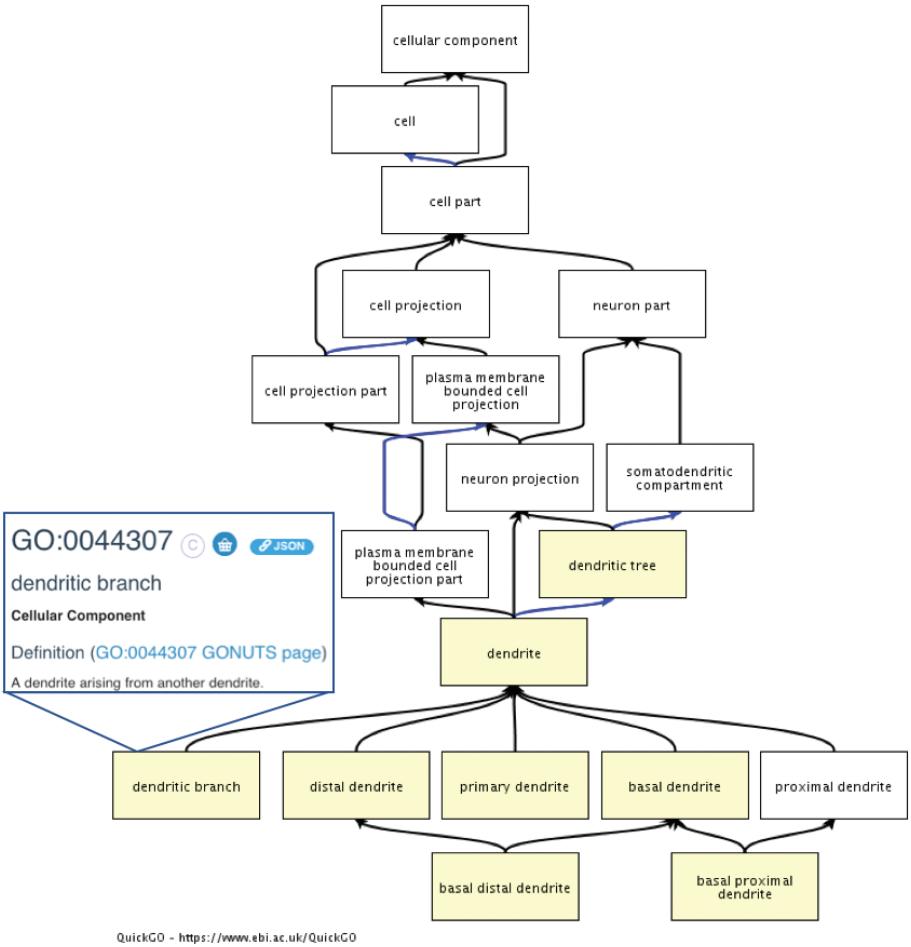

Figure 2 A selection of the dendrite Gene Ontology graph. This figure was generated by the QuickGO browser (37) and shows the *is_a* (black arrows) and *part_of* (blue arrows) hierarchy of just a small number of terms within the *dendrite* branch of the ontology; currently, the dendritic GO domain has 36 terms. The general term *dendrite* is used to group different types of dendrites, for example, *primary dendrite* and *distal dendrite* are both more descriptive child terms of *dendrite*. The definition (as displayed in QuickGO) of one of the GO terms, *dendritic branch*, is also included. The yellow highlighted terms were contributed by the UCL Functional Gene Annotation group.

contribution of new, more descriptive, GO terms, such as *neuron projection arborization*, *dendrite morphogenesis*, or *dendrite arborization*. Yet, simultaneously, we also improved the *dendrite* branch of the cellular component GO aspect, as shown in Figure 2. Similarly, curation of the autophagy (29) processes led to not only generation of highly specific *biological process* GO terms, but also resulted in revisions of related *cellular component* terms, such as *autophagosome*, *amphisome*, or *late endosome*. Thus, enhancing one ontology branch within a specific domain

of GO is often done in conjunction with improvements in other branches and domains, consequently enriching the ontology resource more broadly.

The neuroscience research community will also have benefited from curation work of the SYSCILIA research Consortium, which involved revisions and improvements to cilia-related biology in GO, resulting in contribution of 50 new GO terms (30). Among others, ciliary dysfunction has been shown to affect Sonic hedgehog signaling in the brain, a pathway with demonstrated implications in Alzheimer's (31), Parkinson's (32), and Huntington's (33) diseases. Consequently, revisions and new contributions to the ciliary niche will have improved the representation of cilia biology in GO and, therefore, resulted in more informative analyses of neurological datasets with changes in ciliary proteins.

Another ongoing biocuration initiative with direct relevance to elucidation of Alzheimer's data is the SynGO project and the associated synaptic GO portal (34). SynGO is a collaboration between the Stanley Center for Psychiatric Research at the Broad Institute (Cambridge, MA, USA), the Center for Neurogenomics and Cognitive Research at the Vrije Universiteit (Amsterdam, The Netherlands), and the GO Consortium, thus combining the efforts of experts in synapse biology and GO biocurators to generate the best possible representation of synapse biology in GO.

The UniProt Knowledgebase (EMBL-EBI, Cambridge, UK) has also been improving the representation of Alzheimer's data in their resource, an initiative which includes GO annotation as well as IntAct (13) curation of protein–protein interaction and/or curation of disease variants, as a part of a project funded by the National Institutes of Health (USA). The ultimate goal of this project is to create an online AD portal with thoroughly annotated and easily searchable information on the disease and biological pathways impaired in dementia (35). Importantly, all biocuration scientists, aiming to improve the representation of dementia-relevant biology in GO, work together under the auspices of the GO Consortium, thus ensuring GO annotation consistency and quality.

The creation of GO annotations

There are two major approaches that rely on concerted efforts of skilled biologists and software engineers (36), which result in high-quality GO annotations: manual techniques that depend on the knowledge and expertise of biocuration scientists and computational methods that generate annotations, for instance, based on sequence similarity algorithms. Every annotation is attributed to an identified reference, often a publication identifier, such as PMID, and each annotation must indicate what kind of evidence supports the association between the gene product and the GO term (Figure 1).

The computational annotation approach is a high-throughput and efficient method of associating high-level terms to a large number of gene products across all genomes. These annotations are often assigned, based on specific protein domains with known functions or cellular locations, or based on orthology to a manually curated gene product. However, to provide more specific annotations, GO biocurators read the published scientific literature and use the published data to manually associate highly descriptive GO terms to gene products. Consequently, complete, highly detailed annotation of the processes and networks

that a single gene product is involved in may take a considerable time. Depending on the number of published papers describing the gene product, a curator will annotate an average of 1–3 experimental papers per day.

Furthermore, as there is no limit to the number of GO annotations that can be assigned to a gene product record, it is possible to describe the many different roles that the gene product may have, depending on the cell type it is expressed in, the developmental stage of the organism, and the environmental stimuli the cell is responding to. The UCL Functional Gene Annotation group takes an unusual approach to annotation, in that we usually focus on annotation of a specific process involving a number of gene products, such as amyloid precursor protein processing, rather than working through an unrelated set of gene products. This enables us to develop a better understanding of the biology and apply a consistent annotation approach to all gene products involved in the process, thus providing depth to the annotations. In addition, at UCL we annotate full papers, whereas some groups will curate only the information in a paper that is relevant to a specific prioritized gene. This approach enables us to provide annotations to a large number of relevant gene products, involved in a specific process, which may not be included in the list of annotation priorities. For example, after completing the annotation of 84 proteins and protein complexes, prioritized for annotation as part of the amyloid-beta or tau projects, we had, in total, annotated 526 proteins and complexes (26).

Furthermore, in response to the research community's needs (39), at UCL our annotation procedure involves inclusion of annotation extensions (40) to capture information about the cell and tissue types in which a particular gene product is active, as well as the specific target of a protein or a microRNA. These detailed annotations provide critical knowledge for biomarkers, diagnostics, and drug discovery and will be of considerable value to the research community and allow users of GO to query a variety of data. For example, a GO user could investigate all targets of a particular protein ubiquitin ligase, or, more specifically, search for all proteins involved in catabolic pathways in microglial cells. Unfortunately, although biocurators have been contributing the annotation extension data for over 6 years, there are no tools that are using this data, and only a few browsers display it (9, 41). In the near future, the annotation extension information will be ported to Gene Ontology Causal Activity Modeling (GO-CAM) (42).

Gene products annotated using GO

Historically, GO was used specifically for annotation of proteins. Recently, the GO Consortium has extended the range of gene products that are annotated; rather than only annotating proteins some members of the GO Consortium are now annotating protein complexes (43) and microRNAs (26, 44, 45). To curate these entities, it has been necessary to create new identifiers (43, 46) and develop strict guidelines to ensure that a consistent annotation approach is applied. For example, there are many papers describing the coregulation of a microRNA or a set of microRNAs with the transcription of a panel of mRNAs and implying that these microRNAs therefore regulate the coregulated mRNAs. Such data do not comply with quality standards implemented by the GO Consortium and are not being captured as GO annotations (44). Instead microRNA GO annotations are

contributed based on more precise low-throughput functional experiments, involving microRNA mimics or knockdown, followed by an assessment of the expression of a panel of specific mRNAs. In addition, reporter assay data, confirming a direct interaction between a microRNA and an mRNA, are being captured using specific GO terms (e.g., *mRNA binding involved in posttranscriptional gene silencing*). Furthermore, in these cases the annotation extension will be used to capture the identifier of the targeted mRNA. The resulting interaction data are not only available in the GO annotation files, but also within the EBI-GOA-miRNA dataset from the PSICQUIC web server (45).

The impact of improving the GO resource on data interpretation

By creating an open access dataset of high-quality annotations, which describe the cellular role of those proteins and microRNAs that contribute to pathways dysregulated in AD, the GO provides an invaluable resource for researchers. GO annotations are incorporated into over 50 functional analysis tools, the majority of which are freely available, such as g:Profiler (21), PANTHER (47), Cytoscape (22), and DAVID (23), but others are subscription based, such as Ingenuity Pathway Analysis (QIAGEN Bioinformatics) (48) and MetaCore (Clarivate Analytics) (49). These tools, and many other functional analysis tools, are used by researchers to analyze a variety of high-throughput data, including transcriptomic (4, 50–53), proteomic (5, 54, 55), and GWA (6, 14, 56) data. In addition, existing pipelines ensure that the GO annotations are included in widely used public resources such as UniProt (57), NCBI Gene (58), Ensembl (59), RNAcentral (46), and even Wikipedia. GO annotations associated with individual protein, RNA, or macromolecular complex records are used by researchers to extract a synopsis of the cellular role of a gene product. These gene summaries have many uses in research, for example, they can help guide researchers to the most likely candidate gene associated with a risk locus (14, 18). However, it is the use of GO for the interpretation of data from high-throughput analyses where this resource can be exploited to the full.

The quality of the GO annotations used in the analysis of large biological datasets will determine how informative the outcomes of this analysis will be. Without highly descriptive annotations the analysis can only identify GO functions, processes, or location that are not very specific, such as *site of polarized growth*, *wound healing*, and *cell migration* (54). The identification of more informative enriched terms is dependent not only on the presence of highly descriptive GO terms describing biological knowledge, but also on the association of these terms with a sufficient number of gene products to enable the term to be detected as significantly enriched. A recent meta-analysis of late-onset AD, that included over 94,000 individuals, identified over 100 new risk loci, associated with amyloid-beta and tau processes, as well as immune response pathways and lipid processing (14). This meta-analysis took a wide range of approaches to identify new risk loci, one of which was the use of the pathway analysis software, MAGMA (60), and GO annotation files (36). The GO terms *plasma lipoprotein particle assembly*, *reverse cholesterol transport*, *regulation of amyloid precursor catabolic process*, and *activation of immune response* were identified as processes with relevance to AD. The first three of these GO terms provide a good description of the processes involved, whereas the last term *activation of immune*

response is too general to really give an indication of the mechanism involved. This is likely to reflect the considerable investment in annotation of cardiovascular (18, 61, 62) and nervous system genes (26, 28), and the lack of focused annotation of the immune system. In addition, papers describing the immune system are often highly detailed and more challenging for biocurators without a background in immunology to fully annotate (63). Thus, the annotation of immune-associated pathways does not reflect the volume of literature and knowledge in this domain.

Another study aimed to elucidate protein expression in different brain regions in Alzheimer's cases relative to controls to provide a broader understanding of molecular pathways impaired in dementia (64). In this study, GO analysis was used to identify the *biological processes* that had the largest numbers of differentially expressed proteins associated with them. A wide variety of processes were identified, in this way, including *regulation of apoptosis* associated with the hippocampus and *protein transport* associated with the cerebellum and cingulate gyrus, therefore allowing researchers to identify new routes for potential therapeutic interventions.

GO term enrichment analysis has also been implemented in pilot studies aiming to identify biomarkers associated with dementia, which can be detected using noninvasive methods in easily accessible bodily fluids, such as blood (65) and urine (66). For instance, Chouliaras et al. (65) used GO enrichment together with KEGG pathway analysis to demonstrate the relationships between the identified blood biomarkers with neurological processes and neuronal components. Significantly enriched GO terms included *regulation of amyloid-beta formation* and *amyloid-beta binding*, *main axon*, and *ion channel complex*, whereas KEGG pathways included *glutamatergic synapse* and *Alzheimer's disease*, thus confirming their relevance to cognitive impairments.

Similarly, Watanabe et al. (66) used GO term enrichment and KEGG pathway analyses to delineate the roles of proteins differentially expressed in urine of Alzheimer's patients relative to healthy controls to identify a urine biomarker signature, which could be used for noninvasive diagnostic purposes. *Lipoprotein metabolism*, *heat shock protein 90 signaling pathway*, and *matrix metalloproteinase signaling pathway* as well as *redox regulation by thioredoxin* were among the molecular pathways with the highest enrichment scores, providing evidence for impairment of vascular processes key to the development of dementia (66). In addition, Watanabe et al. (66) also supplemented their functional GO and KEGG analyses with an *interrelation* network analysis to determine, which of the proteins differentially expressed in the Alzheimer's urine samples interact with each other (either directly or via an intermediate). This network analysis of molecular relationships enabled these researchers to further elucidate which GO biological processes and KEGG pathways should be prioritized in future studies and whether they correlate with and confirm other findings. An alternative approach to using GO annotations is to visualize them on an interaction network. This provides the researcher with an overview of the contribution that a network, or part of the network, makes to a particular process or the cellular location of the interacting entities, as shown in the example in Figure 3. Thus, the use of multiple, interoperable, annotation resources provides the opportunity to fully exploit and interrogate individual datasets.

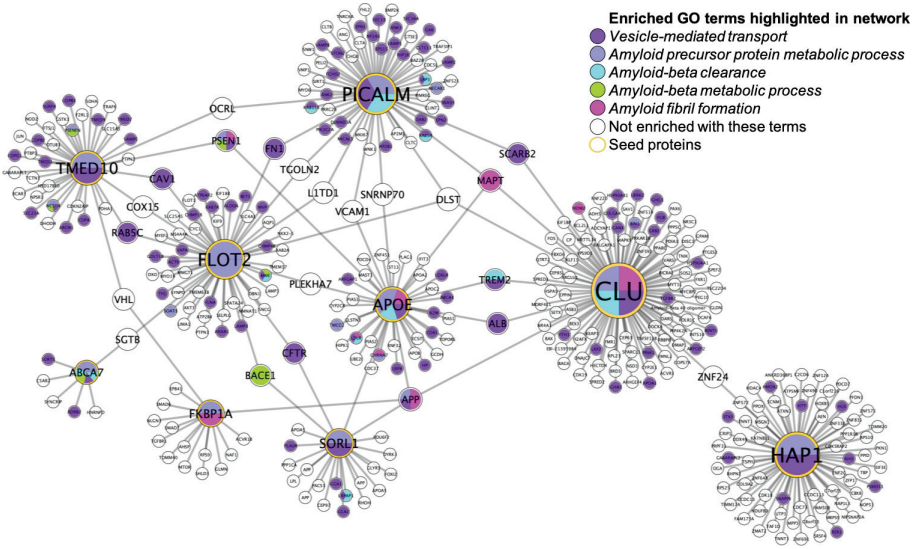

Figure 3 Network of proteins identified in Alzheimer's disease meta-analysis. Nine proteins identified in an Alzheimer's disease meta-analysis (14) due to their association with the GO term "regulation of amyloid precursor protein catabolic process" were used to seed an interaction network using Cytoscape (22) and five files available on the PSICQUIC web server (67) (IntAct, BHF-UCL, UniProt, MINT, and EBI-GOA-non-IntAct). The seed proteins are outlined in yellow. The network was analyzed using Golorize (68), BiNGO (69), and GO ontology and annotation files (36), as described in Denny et al. (29) (downloaded March 29, 2019). The proteins associated with a selection of the enriched GO terms (or one of their child terms, including regulation child terms) are shown in the network.

The above examples demonstrate how continuous, systematic, and consistent improvements to the GO resource, including contribution of new descriptive GO terms, and their association with gene products in the form of GO annotations, impact more informative outcomes of analyses of high-throughput datasets. The functional analyses relying on GO allow researchers to, first, plan and design further studies leading to a better understanding of the molecular mechanisms underlying dementia, and, second, to develop noninvasive diagnostic methods, which collectively will help to improve the management and treatment of AD.

CONCLUSION

The GO resource (8, 9, 70) adds value to published experimental data by creating computer-readable annotations that describe specific functions of a gene product, such as protein, complex, or noncoding RNA, and the biological processes and pathways it contributes to. This benefits all biological areas, including the AD field, on which the UCL Functional Gene Annotation team has recently been focusing their biocuration efforts. Gene annotations using GO terms enable groups of gene products, with similar cellular roles or locations in the cell, to be

easily identified within a dataset, such as a list of differentially expressed genes from AD cases. Thus, dysregulated pathways, functions, and macromolecular complexes can be identified within high-throughput datasets using GO annotation data and functional enrichment tools. GO annotation data are therefore needed for pathway construction, enrichment analyses and interpretation of large-scale datasets (3–5) and to inform biomarker selection decisions (27), and can also be used to identify novel drug targets or novel repurposing of drugs. Furthermore, the AD-focused and comprehensive efforts of the UCL Functional Gene Annotation team have improved and continue to improve the GO resource, enhancing its applicability to this neurobiological research domain and facilitating analyses and interpretation of AD *big data*.

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REFERENCES

1. Carmona S, Hardy J, Guerreiro R. The genetic landscape of Alzheimer disease. Handb Clin Neurol. 2018;395–408.
2. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol. 2011 Sep;10(9):819–28.
3. Patel H, Dobson RJB, Newhouse SJ. A meta-analysis of Alzheimer's disease brain transcriptomic data. J Alzheimer's Dis. 2019 Apr 23;68(4):1635–56.
4. Cooper-Knock J, Kirby J, Ferraiuolo L, Heath PR, Rattray M, Shaw PJ. Gene expression profiling in human neurodegenerative disease. Nat Rev Neurol. 2012;8(9):518–30.
5. Kang M-G, Byun K, Kim JH, Park NH, Heinsen H, Ravid R, et al. Proteogenomics of the human hippocampus: The road ahead. Biochim Biophys Acta. 2015 Jul;1854(7):788–97.
6. Guio-Vega GP, Forero DA. Functional genomics of candidate genes derived from genome-wide association studies for five common neurological diseases. Int J Neurosci. 2017 Feb 17;127(2):118–23.
7. Ebbert MTW, Ridge PG, Kauwe JSK. Bridging the gap between statistical and biological epistasis in Alzheimer's disease. Biomed Res Int. 2015;2015:1–7.
8. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000;25(1):25–9.

9. The Gene Ontology Consortium. The Gene Ontology resource: 20 years and still GOing strong. Nucleic Acids Res. 2019 Jan 8;47(D1):D330–8.
10. Kotera M, Hirakawa M, Tokimatsu T, Goto S, Kanehisa M. The KEGG databases and tools facilitating omics analysis: latest developments involving human diseases and pharmaceuticals. Methods Mol Biol. 2012;802:19–39.
11. Haw R, Hermjakob H, D'Eustachio P, Stein L. Reactome pathway analysis to enrich biological discovery in proteomics data sets. Proteomics. 2011;11(18):3598–613.
12. Kerrien S, Aranda B, Breuza L, Bridge A, Broackes-Carter F, Chen C, et al. The IntAct molecular interaction database in 2012. Nucleic Acids Res. 2012;40(Database issue):D841–6.
13. Orchard S, Ammari M, Aranda B, Breuza L, Briganti L, Broackes-Carter F, et al. The MIntAct project – IntAct as a common curation platform for 11 molecular interaction databases. Nucleic Acids Res. 2014;42(Database issue):D358–63.
14. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. Nat Genet. 2019 Mar 28;51(3):414–30.
15. Holmans P, Moskvina V, Jones L, Sharma M, Vedernikov A, Buchel F, et al. A pathway-based analysis provides additional support for an immune-related genetic susceptibility to Parkinson's disease. Hum Mol Genet. 2013;22(5):1039–49.
16. Hrubešová K, Fousková M, Habartová L, Fišar Z, Jirák R, Raboch J, et al. Search for biomarkers of Alzheimer's disease: Recent insights, current challenges and future prospects. Clin Biochem. 2019;72:39–51.
17. Lovering RC, Dimmer EC, Talmud PJ. Improvements to cardiovascular Gene Ontology. Atherosclerosis. 2009;205(1):9–14.
18. Lovering RC, Roncaglia P, Howe DG, Lauleder kind SJF, Khodiyar VK, Berardini TZ, et al. Improving interpretation of cardiac phenotypes and enhancing discovery with expanded knowledge in the Gene Ontology. Circ Genom Precis Med. 2018;11(2):e001813.
19. Bone WP, Washington NL, Buske OJ, Adams DR, Davis J, Draper D, et al. Computational evaluation of exome sequence data using human and model organism phenotypes improves diagnostic efficiency. Genet Med. 2016 Jun 12;18(6):608–17.
20. Zhao Z, Sagare AP, Ma Q, Halliday MR, Kong P, Kisler K, et al. Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance. Nat Neurosci. 2015 Jul 25;18(7):978–87.
21. Reimand J, Arak T, Adler P, Kolberg L, Reisberg S, Peterson H, et al. g:Profiler-a web server for functional interpretation of gene lists (2016 update). Nucleic Acids Res. 2016;44(W1):W83–9.
22. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics. 2011;27(3):431–2.
23. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44–57.
24. Manzoni C, Denny P, Lovering RC, Lewis PA. Computational analysis of the LRRK2 interactome. PeerJ. 2015;3:e778.
25. Evans IM, Kennedy SA, Paliashvili K, Santra T, Yamaji M, Lovering RC, et al. Vascular endothelial growth factor (VEGF) promotes assembly of the p130Cas interactome to drive endothelial chemotactic signaling and angiogenesis. Mol Cell Proteomics. 2017;16(2):168–80.
26. Kramarz B, Roncaglia P, Meldal BHM, Huntley RP, Martin MJ, Orchard S, et al. Improving the Gene Ontology resource to facilitate more informative analysis and interpretation of Alzheimer's disease data. Genes (Basel). 2018;9(12):593.
27. Levine AJ, Miller JA, Shapshak P, Gelman B, Singer EJ, Hinkin CH, et al. Systems analysis of human brain gene expression: mechanisms for HIV-associated neurocognitive impairment and common pathways with Alzheimer's disease. BMC Med Genomics. 2013 Feb 13;6(1):4.
28. Foulger RE, Denny P, Hardy J, Martin MJ, Sawford T, Lovering RC. Using the Gene Ontology to annotate key players in Parkinson's disease. Neuroinformatics. 2016;14(3):297–304.
29. Denny P, Feuermann M, Hill DP, Lovering RC, Plun-Favreau H, Roncaglia P. Exploring autophagy with Gene Ontology. Autophagy. 2018 Mar 04;14(3):419–36.
30. Roncaglia P, van Dam TJP, Christie KR, Nacheva L, Toedt G, Huynen MA, et al. The Gene Ontology of eukaryotic cilia and flagella. Cilia. 2017 Dec 16;6(1):10.

31. Vorobyeva AG, Saunders AJ. Amyloid-β interrupts canonical sonic hedgehog signaling by distorting primary cilia structure. Cilia. 2018 Dec 17;7(1):5.
32. Dhekne HS, Yanatori I, Gomez RC, Tonelli F, Diez F, Schüle B, et al. A pathway for Parkinson's disease LRRK2 kinase to block primary cilia and sonic hedgehog signaling in the brain. Elife. 2018 Nov 6;7.
33. Kaliszewski M, Knott AB, Bossy-Wetzel E. Primary cilia and autophagic dysfunction in Huntington's disease. Cell Death Differ. 2015 Sep 10;22(9):1413–24.
34. Koopmans F, van Nierop P, Andres-Alonso M, Byrnes A, Cijsouw T, Coba MP, et al. SynGO: An evidence-based, expert-curated knowledge base for the synapse. Neuron. 2019;103(2):217–234.e4.
35. Lussi YC, Breuza L, Casas CC, Georghiou G, Magrane M, Masson P, et al. UniProtKB and Alzheimer's disease: Linking molecular defects to disease phenotype. 2019 Mar 21 [accessed 2019 Jun 5];8. Available from: https://f1000research.com/posters/8-316.
36. Huntley RP, Sawford T, Mutowo-Meullenet P, Shypitsyna A, Bonilla C, Martin MJ, et al. The GOA database: Gene Ontology annotation updates for 2015. Nucleic Acids Res. 2015;43(Database issue):D1057–63.
37. Binns D, Dimmer E, Huntley R, Barrell D, O'Donovan C, Apweiler R. QuickGO: A web-based tool for Gene Ontology searching. Bioinformatics. 2009;25(22):3045–6.
38. Chibucos MC, Siegele DA, Hu JC, Giglio M. The Evidence and Conclusion Ontology (ECO): Supporting GO annotations. Methods Mol Biol. 2017;1446:245–59.
39. Khatri P, Sirota M, Butte AJ. Ten years of pathway analysis: Current approaches and outstanding challenges. PLoS Comput Biol. 2012;8(2):e1002375.
40. Huntley RP, Harris MA, Alam-Faruque Y, Blake JA, Carbon S, Dietze H, et al. A method for increasing expressivity of Gene Ontology annotations using a compositional approach. BMC Bioinformatics. 2014;15:155.
41. Barrell D, Dimmer E, Huntley RP, Binns D, O'Donovan C, Apweiler R. The GOA database in 2009 – An integrated Gene Ontology annotation resource. Nucleic Acids Res. 2009;37(Database issue):D396–403.
42. Thomas PD, Hill DP, Mi H, Osumi-Sutherland D, Van Auken K, Carbon S, et al. Gene Ontology causal activity modeling (GO-CAM) moves beyond GO annotations to structured descriptions of biological functions and systems. Nat Genet. 2019;51(10):1429–1433.
43. Meldal BH, Forner-Martinez O, Costanzo MC, Dana J, Demeter J, Dumousseau M, et al. The Complex Portal – An encyclopaedia of macromolecular complexes. Nucleic Acids Res. 2015;43(Database issue):D479–84.
44. Huntley RP, Sitnikov D, Orlic-Milacic M, Balakrishnan R, D'Eustachio P, Gillespie ME, et al. Guidelines for the functional annotation of microRNAs using the Gene Ontology. RNA. 2016;22(5):667–76.
45. Huntley RP, Kramarz B, Sawford T, Umrao Z, Kalea A, Acquaah V, et al. Expanding the horizons of microRNA bioinformatics. RNA. 2018;24(8):1005–17.
46. RNAcentral Consortium. RNAcentral: An international database of ncRNA sequences. Nucleic Acids Res. 2015;43(Database issue):D123–9.
47. Mi H, Poudel S, Muruganujan A, Casagrande JT, Thomas PD. PANTHER version 10: Expanded protein families and functions, and analysis tools. Nucleic Acids Res. 2016;44(D1):D336–42.
48. Kramer A, Green J, Pollard J Jr., Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014;30(4):523–30.
49. Dubovenko A, Nikolsky Y, Rakhmatulin E, Nikolskaya T. Functional analysis of OMICs data and small molecule compounds in an integrated "knowledge-based" platform. Methods Mol Biol. 2017;1613:101–24.
50. Avramopoulos D, Szymanski M, Wang R, Bassett S. Gene expression reveals overlap between normal aging and Alzheimer's disease genes. Neurobiol Aging. 2011;32(12):2319 e27–34.
51. Brooks LRK, Mias GI. Data-driven analysis of age, sex, and tissue effects on gene expression variability in Alzheimer's disease. Front Neurosci. 2019 Apr 24;13:392.
52. Newman M, Hin N, Pederson S, Lardelli M. Brain transcriptome analysis of a familial Alzheimer's disease-like mutation in the zebrafish presenilin 1 gene implies effects on energy production. Mol Brain. 2019 Dec 3;12(1):43.

53. Carbajosa G, Malki K, Lawless N, Wang H, Ryder JW, Wozniak E, et al. Loss of Trem2 in microglia leads to widespread disruption of cell coexpression networks in mouse brain. Neurobiol Aging. 2018 Sep;69:151–66.
54. Perreau VM, Orchard S, Adlard PA, Bellingham SA, Cappai R, Ciccotosto GD, et al. A domain level interaction network of amyloid precursor protein and Abeta of Alzheimer's disease. Proteomics. 2010;10(12):2377–95.
55. Karri V, Ramos D, Martinez JB, Odena A, Oliveira E, Coort SL, et al. Differential protein expression of hippocampal cells associated with heavy metals (Pb, As, and MeHg) neurotoxicity: Deepening into the molecular mechanism of neurodegenerative diseases. J Proteomics. 2018 Sep 15;187:106–25.
56. Shahbaba B, Shachaf CM, Yu Z. A pathway analysis method for genome-wide association studies. Stat Med. 2012;31(10):988–1000.
57. The UniProt Consortium UniProt: The universal protein knowledgebase. Nucleic Acids Res. 2017;45(D1):D158–69.
58. Brown GR, Hem V, Katz KS, Ovetsky M, Wallin C, Ermolaeva O, et al. Gene: A gene-centered information resource at NCBI. Nucleic Acids Res. 2015 Jan 28;43(D1):D36–42.
59. Flicek P, Amode MR, Barrell D, Beal K, Brent S, Carvalho-Silva D, et al. Ensembl 2012. Nucleic Acids Res. 2012;40(Database issue):D84–90.
60. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: Generalized gene-set analysis of GWAS data. PLoS Comput Biol. 2015;11(4):e1004219.
61. Alam-Faruque Y, Huntley RP, Khodiyar VK, Camon EB, Dimmer EC, Sawford T, et al. The impact of focused Gene Ontology curation of specific mammalian systems. PLoS One. 2011;6(12):e27541.
62. Khodiyar VK, Hill DP, Howe D, Berardini TZ, Tweedie S, Talmud PJ, et al. The representation of heart development in the Gene Ontology. Dev Biol. 2011;354(1):9–17.
63. Lovering RC, Camon EB, Blake JA, Diehl AD. Access to immunology through the Gene Ontology. Immunology. 2008;125(2):154–60.
64. Xu J, Patassini S, Rustogi N, Riba-Garcia I, Hale BD, Phillips AM, et al. Regional protein expression in human Alzheimer's brain correlates with disease severity. Commun Biol. 2019 Dec 4;2(1):43.
65. Chouliaras L, Pishva E, Haapakoski R, Zsoldos E, Mahmood A, Filippini N, et al. Peripheral DNA methylation, cognitive decline and brain aging: pilot findings from the Whitehall II imaging study. Epigenomics. 2018 May;10(5):585–95.
66. Watanabe Y, Hirao Y, Kasuga K, Tokutake T, Semizu Y, Kitamura K, et al. Molecular network analysis of the urinary proteome of Alzheimer's disease patients. Dement Geriatr Cogn Dis Extra. 2019 Feb 8;9(1):53–65.
67. Orchard S, Albar JP, Deutsch EW, Eisenacher M, Binz PA, Martinez-Bartolome S, et al. From proteomics data representation to public data flow: A report on the HUPO-PSI workshop September 2011, Geneva, Switzerland. Proteomics. 2012;12(3):351–5.
68. Garcia O, Saveanu C, Cline M, Fromont-Racine M, Jacquier A, Schwikowski B, et al. GOlorize: A Cytoscape plug-in for network visualization with Gene Ontology-based layout and coloring. Bioinformatics. 2007;23(3):394–6.
69. Maere S, Heymans K, Kuiper M. BiNGO: A Cytoscape plugin to assess overrepresentation of Gene Ontology categories in biological networks. Bioinformatics. 2005;21(16):3448–9.
70. Gene Ontology Consortium. Gene Ontology Consortium: Going forward. Nucleic Acids Res. 2015;43(Database issue):D1049–56.

3

Using Proteomics to Understand Alzheimer's Disease Pathogenesis

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Abstract: Our current understanding of the molecular changes that drive Alzheimer's disease (AD) pathogenesis is incomplete. Unbiased, mass-spectrometry-based proteomic studies provide an efficient and comprehensive way to quantitatively examine thousands of proteins at once using microscopic amounts of human brain tissue. Recently, the number of proteomic studies that examine protein changes in AD brain tissue has been increasing. This chapter reviews the different proteomic approaches currently being used to identify pathological protein changes in AD brain tissue including bulk tissue studies that examine protein changes throughout the progression of AD, studies of the insoluble proteome in AD, studies using proteomics to examine selective vulnerability in AD, studies of the amyloid plaque and neurofibrillary tangle proteome, studies of the synaptic proteome, and studies of the interactome of beta amyloid and tau. Combined, these complementary proteomic approaches provide increased understanding about the protein changes that occur in the AD brain. Results from these proteomic studies provide an excellent resource for future hypothesis-driven targeted studies and will help identify new biomarkers of disease and new drug targets for AD.

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INTRODUCTION

Alzheimer's disease (AD) is a complex, multifactorial disease. Various genetic, environmental, and lifestyle risk factors have been associated with the development of AD; however, none of these have been shown to definitively cause late onset AD (1). AD is diagnosed at autopsy by the presence of characteristic neuropathology: amyloid plaques and neurofibrillary tangles (NFTs), which primarily consist of aggregated beta amyloid (Aβ) and hyperphosphorylated tau, respectively (2). The development of these neuropathological lesions is associated with increased neuroinflammation, synaptic loss, neurodegeneration, and ultimately the development of cognitive impairment that clinically characterizes AD. Imaging and biomarker studies suggest that AD begins ~20 years before the development of dementia, resulting in a long preclinical stage of disease before clinical symptoms are apparent (3).

There are still significant gaps in our understanding about the molecular mechanisms that underlie the pathogenesis of AD. For example, we do not know what causes AD, what factors drive the development of neuropathology, what factors cause the development of cognitive impairment, or what factors are responsible for the considerable heterogeneity in the rate of progression in people with AD. A greater understanding of all of these factors is essential for the development of effective therapeutics and discovery of new biomarkers for AD. New therapeutics are particularly needed for AD as the previous record for AD clinical trials has been very poor: 99.6% of AD clinical trials have failed, and currently, no disease-modifying treatment is available. This high failure rate has been attributed to various factors including starting treatment too late in the disease process, having the wrong drug targets, or relying too heavily on results from preclinical studies that use animal models of AD that poorly reflect human disease (4–6).

THE BENEFITS OF USING PROTEOMICS TO STUDY PROTEIN CHANGES IN AD BRAIN TISSUE

Traditionally, studies examining the molecular mechanisms that drive AD pathogenesis have used a targeted, hypothesis-driven approach that focuses on select proteins of interest. This approach has uncovered many of the major players involved in AD pathogenesis, most notably identifying beta amyloid (Aβ) as the major protein present in amyloid plaques (7, 8), identifying tau as the major protein present in NFTs (9), and identifying apolipoprotein E (apoE) as the most significant genetic risk factor for late onset AD (10, 11). However, using a targeted approach precludes the discovery of novel disease-associated proteins and limits the ability to understand these protein changes in the broad context of AD.

Unbiased, hypothesis-free "omics" studies such as genomic, transcriptomic, epigenetic and proteomic studies offer a comprehensive, highly efficient way to identify genes or proteins that are involved in the pathogenesis of AD. The high-throughput nature of "omics" studies means that they can be performed using microscopic amounts of human tissue samples, which are essential to study when examining diseases that are unique to humans, such as AD (12). Genomic and epigenetic studies have successfully identified new genetic risk factors for late-onset AD and have provided the basis for new hypothesis-driven studies examining how these genetic variants and epigenetic changes are involved in AD (13–16). Unbiased, mass-spectrometry-based proteomic studies of human AD brain tissue are essential to complement these genomic studies, particularly given that proteins are the druggable targets in AD. Furthermore, there is a poor correlation between RNA expression and protein levels in AD brain tissue; therefore, transcriptomic or genomic studies do not provide a complete picture of the pathogenic changes in the AD brain (17). Using mass-spectrometry-based proteomics to study the pathogenesis of AD has many advantages including the following: thousands of protein differences can be quantified simultaneously using microscopic amounts of brain tissue, the unbiased nature of these studies permits the discovery of novel proteins involved in AD pathogenesis, and proteomics can detect post-translational modifications on proteins (e.g. phosphorylation, oxidation, and ubiquitination) that are known to have an important pathological role in AD. The large amount of data generated in proteomic studies provides a comprehensive, bird's eye view of all protein differences that occur in AD, which can provide insight into the molecular mechanisms that cause AD at a network/systems level, which is particularly useful when studying complex diseases like AD (18, 19). Mass-spectrometry-based proteomic studies have been limited in the past by technical and financial constraints; however, these factors have recently become less restrictive, and consequently, the number of proteomics studies using AD brain tissue has increased.

AD PROTEOMIC STUDIES USING BULK TISSUE HOMOGENATES

The majority of proteomic studies examining AD brain tissue have defined the proteomic changes between AD and age-matched, cognitively normal controls using bulk tissue samples. In these studies, proteomics is used to compare protein expression between AD and controls in brain homogenate, usually limited to one vulnerable brain region. Early liquid chromatography-mass spectrometry (LC-MS) studies generated preliminary findings about protein differences between AD and control brains, but were typically restricted by small sample sizes and therefore struggled to detect protein differences after correcting for multiple comparisons (20–26). More recent studies have included a larger number of samples, which are consequently sufficiently powered to detect hundreds of protein differences in AD brains (17, 27–34). Encouragingly, meta-analysis of these recent studies shows that many of the significantly altered proteins in AD brains are consistent, leading to increased confidence that these altered proteins are relevant to the pathogenesis of AD.

The most comprehensive studies have been conducted by researchers at Emory University, USA (17, 30, 31). Their studies primarily examined protein differences in the frontal cortex throughout the progression of AD, specifically comparing protein levels in advanced AD, asymptomatic AD (also referred to as preclinical AD), and age-matched cognitively normal subjects. Combined, these studies identified hundreds of protein differences present at different stages of AD. They found that the number of protein differences steadily increased with disease progression, suggesting that the number of protein differences is reflective of increased dysfunction involving more pathways as AD progresses. Their analysis allowed the identification of subsets of proteins that were exclusively altered in the symptomatic phase of AD and those that were altered prior to the onset of clinical symptoms. For example, they showed that proteins involved in synaptic function and synaptogenesis progressively decreased throughout AD, starting before clinical symptoms were present. They also showed that altered RNA metabolism and increased inflammation were present in AD brains in the earliest stages of disease prior to cognitive impairment. In contrast, astrocyte and microglia proteins increased in late stage AD and showed a strong correlation with the number of NFTs present. A consequent study by the same group specifically focused on the protein differences present in AD cases stratified by ApoE genotype (33). ApoE is the major genetic risk factor for late onset AD (35, 36). The three alleles of ApoE (apoE2, apoE3, and apoE4) confer different risk for AD: apoE4 increases risk for AD and apoE2 decreases risk for AD. Their proteomic results suggested that apoE may confer risk in AD through a combination of effects on inflammation, metabolism, and cerebral vasculature, and using their proteomic approach, they were able to pinpoint the specific proteins involved (33).

One important factor to be mindful of when interpreting and comparing proteomic studies is the type of tissue lysis method used prior to mass spectrometry. Different lysis methods enrich for different populations of proteins or even different pools of the same protein. For example, soluble and insoluble forms of the same protein may require different lysis methods for detection. Therefore, the use of various lysis methods can complicate meta-analysis of multiple proteomic studies as the same proteins are not always detected by each lysis method. However, one advantage of using varied lysis methods is that combined analysis of proteomic studies that use various lysis methods provides a richer view of molecular changes in the AD brain. For example, some studies have specifically examined differences in the insoluble proteome in AD (21, 25, 30, 37), which enriches for proteins that are associated with the insoluble plaques or NFTs in AD, as well as other proteins that are independently prone to insolubility in the AD brain. Of these studies, Hales et al. provide the most comprehensive analysis of insoluble protein changes in AD (30). Interestingly, they showed that the number of differentially expressed proteins in the insoluble fraction increased with disease severity and that many of these insoluble proteins were involved in mitochondrial function, which is known to be decreased in AD (30). Other recent studies have used proteomics to answer specific questions about which proteins are primarily affected by post-translational modifications in AD. Two recent studies have used enrichment strategies to identify all proteins that are phosphorylated and ubiquitinated in AD (38, 39). These studies showed that the number of ubiquitinated proteins was much higher in AD brains than in control brains, which is consistent

with the accumulation of insoluble and misfolded proteins during AD and reflects the proteolytic stress present in AD (38). Examination of phosphorylated proteins confirmed that tau was the most highly phosphorylated protein in AD in comparison to controls and also identified an additional 142 proteins that were phosphorylated to a greater extent in AD brains (39).

THE USE OF PROTEOMICS TO UNDERSTAND SELECTIVE VULNERABILITY IN AD

One of the most striking features of AD is that specific brain regions are particularly vulnerable to the development of amyloid plaques, NFTs, and neurodegeneration, while other regions are comparatively resistant to pathology. Why this occurs is still unknown. However, various factors have been proposed to contribute to vulnerability including: gene expression, long axonal projections or large neuronal size, being an excitatory neuron, containing low levels of calcium buffering proteins, or containing high levels of metastable subproteome proteins that are prone to aggregate in times of stress (40–42). Defining a particular brain region as vulnerable or resistant in AD can be complex, as it depends on the neuropathological factor you use to define vulnerability. This is important because some brain regions are preferentially vulnerable to developing amyloid plaques, while others are preferentially vulnerable to developing NFTs or neurodegeneration, and the presence of these different types of neuropathology does not always correlate. In general, regions that are particularly vulnerable to the development of AD include the hippocampus, entorhinal cortex, basal forebrain, and locus coeruleus. Comparatively resistant regions include the cerebellum and occipital cortex.

Transcriptomics studies suggest that there is likely a distinct protein signature of vulnerable neurons in AD (43); however, this has not yet been comprehensively examined at the protein level. Proteomic studies of selective vulnerability in AD are complex as additional variables need to be considered in their experimental design. For example, basal protein differences between different brain regions have to be accounted for when interpreting protein differences that appear to be associated with increased vulnerability to AD. This is particularly important when comparing brain regions that are morphologically different such as the cerebellum and the hippocampus. Disparate basal protein expression between brain regions complicates interpretation of results as protein differences could be due to either basal brain region differences or AD associated differences. Accounting for these variables is possible, but ultimately results in large, complex studies that require a large number of samples to perform all analyses with sufficient power.

The majority of bulk tissue proteomic studies have only analyzed one or two brain regions, usually focusing only on vulnerable brain regions, meaning that they cannot be used to examine the protein changes associated with selective vulnerability in AD. Two recent studies have aimed to fill this knowledge gap. Xu et al. (44) performed the most extensive analysis of proteomic changes in the AD brain that are associated with selective vulnerability. They compared protein expression in three highly affected regions (hippocampus, entorhinal cortex, and

cingulate gyrus), two lightly affected regions (sensory cortex and motor cortex), and one comparatively unaffected region (cerebellum). As expected, the majority of protein differences between AD and control brains were observed in the highly affected regions, and these protein changes were reflective of increased innate and adaptive immune responses in the brain and increased apoptosis. The fewer protein differences in the lightly affected regions appeared to be reflective of early stage pathology, suggesting that the same molecular changes that drive pathogenesis of AD eventually spread to these lightly affected regions. Intriguingly, the cerebellum actually showed a large number of protein differences between AD and controls, even more so than the lightly affected regions. However, these protein differences appeared to be reflective of potentially protective molecular changes such as increased expression of proteins associated with growth factors, increased oxidative defense proteins, and decreased transfer RNA synthetases. Mendonca et al. (45) also performed a comprehensive study looking at the proteomic differences in brain regions preferentially vulnerable to tau pathology in AD. They compared the proteome in AD and controls in two brain regions that are highly vulnerable to the development of NFTs (parahippocampal cortex and entorhinal cortex) and two brain regions that are moderately vulnerable to the development of NFTs (temporal cortex and frontal cortex). In doing so, they generated a complex dataset that will be useful for future data mining studies examining the protein changes associated with tau pathology in AD. In the future, expanding these studies to include comparisons between multiple brain regions at multiple stages of AD will be useful for defining the protein differences that underlie selective vulnerability in AD and to definitively determine whether mildly affected regions show molecular changes that are similar to those in early stage AD.

PROTEOMICS OF NEUROPATHOLOGICAL FEATURES PRESENT IN AD

Other groups, including my own, have recognized the importance of performing localized proteomic studies that specifically focus on disease-associated neuropathological features or specific cell populations. Using a localized approach that focuses specifically on areas highly affected by disease has the potential to reveal protein differences between AD and controls that are particularly relevant to pathogenesis. We have focused our efforts on using a localized proteomics approach to determine the proteome of amyloid plaques, NFTs, and vulnerable neurons. In this approach, neuropathological features or vulnerable neuron populations are microdissected from sections of human brain tissue, and their protein composition is analyzed using mass spectrometry (46–48). A key advantage of our approach is that it can be performed using formalin-fixed paraffin-embedded (FFPE) tissue. This is important because the majority of human tissue specimens available for research are FFPE blocks of tissue that are collected and used during autopsy. Therefore, developing a method that is compatible with FFPE tissue greatly increases the feasibility of human tissue studies, particularly those using rare or unique cases. A second key advantage of our method is that it can be performed using microscopic amounts of tissue. We have successfully performed

proteomics using as little as 1.5 mm^2 of tissue, which is the equivalent of approximately 550 amyloid plaques or 4000 NFTs. This number of plaques and tangles can typically be collected using <4 tissue sections, showing just how little tissue is required for these studies. But the most important aspect of our approach is that we can quantify over a 1000 proteins at once using these microscopic tissue samples, which therefore provide a comprehensive analysis of the proteins that are associated with neuropathological features in the AD brain and proteins that are associated with selective vulnerability of specific neuronal populations. For example, we showed that amyloid plaques consistently contained hundreds of proteins in addition to Aβ and that many of these were novel proteins that had not previously been associated with AD (49, 50). Importantly, we also showed that the protein composition of amyloid plaques was significantly different in people with rapidly progressive AD in comparison to typical sporadic AD, suggesting that different molecular mechanisms may underlie plaque development in different subtypes of disease. We also recently examined the proteome of NFTs and identified over 500 proteins in NFTs in addition to tau, many of which were novel (51). These examples show the power of an unbiased localized proteomics approach to efficiently identify hundreds of proteins that are associated with amyloid plaques or NFTs. These findings can be used as the basis for future targeted studies that aim to determine the mechanistic involvement of these proteins in AD.

One example protein that we discovered using proteomics and have since followed up on in a targeted study is secernin-1. Very little is known about the function of secernin-1, and no study has previously associated secernin-1 with AD. We identified secernin-1 as a novel amyloid plaque protein in our previous proteomic study of amyloid plaques (49). We have since performed a comprehensive neuropathological study of secernin-1 accumulation in early and late stage AD (52). Surprisingly, we found that secernin-1 abundantly and specifically accumulated in NFTs in AD and that its presence in amyloid plaques was limited to accumulation in the dystrophic neurites present in neuritic plaques. Co-immunoprecipitation showed that secernin-1 directly interacted with phosphorylated tau in AD brains, suggesting that it could have an important role in mediating the toxic actions of tau in AD. Intriguingly, secernin-1 colocalized with phosphorylated tau aggregates only in AD and not in other neurodegenerative diseases that also show the presence of aggregated phosphorylated tau including Pick's disease, progressive supranuclear palsy, and corticobasal degeneration. This suggests that secernin-1 could be a new potential biomarker that discriminates between AD and other tauopathies. These results show that localized proteomics studies are capable of identifying new biomarkers of disease and new potential drug targets. Secernin-1 is just one example protein from a list of many new potential candidates that we have identified in our proteomics studies that can be examined in future mechanistic studies.

A small number of other groups have also used a similar localized proteomics approach to study the proteome of neuropathological features or vulnerable cell populations/brain regions in AD. Two small studies have examined the proteome of human amyloid plaques (53, 54), and two small studies have examined the proteome of human NFTs (55, 56). Three other studies have examined the proteome of cerebral amyloid angiopathy (CAA), which is present when Aβ pathologically accumulates in blood vessels (57–59). Combined, all of these studies provide preliminary data that have hinted at new proteins that are associated

with these neuropathological features; however, the small number of cases included in these initial studies means that further studies are needed to provide a comprehensive understanding of the proteome of amyloid plaques, NFTs, and CAA. Future studies examining larger numbers of cases that are stratified by AD subtype will be very informative in helping to identify proteins that have a particular interaction with neuropathological features and to determine whether proteins associated with these neuropathological features are different between subtypes of AD.

PROTEOMICS OF SYNAPTIC FRACTIONS IN AD

Synapse loss is an early feature of AD that closely correlates with cognitive impairment (60–63). Understanding the synaptic protein changes in AD could help us understand what is driving this process. Multiple studies have been completed that have analyzed the proteome of synaptosomes and post-synaptic density in control human brains, which have been nicely combined in a recent meta-analysis (64). However, a comprehensive analysis of the synaptic proteome in AD has not yet been performed. Preliminary results have been generated that analyzed the proteome of synaptosomes (65, 66) or post-synaptic density fractions (67). However, the small sample sizes used in these studies (between n = 2 and n = 6) mean that their findings are not yet definitive. Other studies have used their bulk tissue homogenate results to look specifically at synaptic protein changes (34, 68); however, these results could potentially miss differences in low abundance synaptic proteins. To date, all studies examining synaptic protein differences in AD have compared advanced AD and controls. Given that synaptic loss is an early feature of AD, it would be particularly useful to determine the protein changes that contribute to synapse loss in either mild cognitive impairment or preclinical AD. Larger studies examining differences in the synaptic proteome in early AD are currently ongoing in the field, and these will likely provide a greater overview of the specific protein changes that contribute to synaptic loss in AD. Future results detailing the synaptic protein differences in AD will be very interesting given that it has been recently suggested that synaptic proteins in the cerebrospinal fluid may also be excellent new biomarkers for early AD (64, 69).

ANALYSIS OF THE Aβ OR TAU INTERACTOME IN AD

Another useful proteomics approach to study AD pathogenesis is using affinity purification-mass spectrometry to identify the proteins that interact with toxic Aβ or tau species in AD. In this approach, particular species of Aβ or tau are isolated from human brain samples using antibodies. Proteins that interact with Aβ or tau are isolated at the same time, and mass spectrometry is used to identify these interacting proteins. This is a powerful approach because it allows the efficient and comprehensive examination of all proteins that interact with Aβ or tau in an unbiased manner. It can also determine which proteins interact with particular species Aβ or tau, which is important as some species are more toxic than others.

Results from these studies have the potential to increase our understanding about how Aβ and tau are involved in the pathogenesis of AD and could lead to the discovery of new drug targets.

Despite Aβ being the predominant focus of AD research for decades, there is a surprisingly limited number of studies that have used affinity purification mass spectrometry to examine the Aβ interactome. One possible reason for this is that it is difficult to find an appropriate antibody that specifically recognizes Aβ and not its longer precursor protein (amyloid precursor protein; APP). Accordingly, a number of studies have instead examined the interactome of APP in mouse brain tissue (70, 71) and in cells expressing human APP (72, 73). However, despite this limitation, there have been two recent studies that have developed alternative ways to examine the Aβ interactome. The first study isolated aggregated Aβ complexes from human brain samples using a non-specific Aβ antibody (that also recognizes APP), but limited their downstream proteomic analysis to only those proteins present in the insoluble fraction, with the assumption that the resulting interacting proteins were limited to those present in insoluble Aβ-containing aggregates rather than APP (37). The second study used a more traditional approach of binding recombinant monomeric Aβ42 or oligomeric Aβ42 to beads that were then used to pull down interacting proteins from human brain samples (74). Combined, these studies identified over 100 proteins that interact with Aβ, including some proteins that preferentially interacted with oligomeric Aβ in comparison to monomeric Aβ. However, more studies are needed in the future that compare the interactome of different Aβ species (such as Aβ40, Aβ42, and pyroglutamate modified Aβ) and that determine the endogenous pathological interactions present in AD brain tissue, as these may be different than those present in artificial in vitro experiments.

To date, all studies examining the tau interactome have used total tau antibodies that identify proteins that interact with all tau species. Two studies have examined using human brain tissue (37, 75), while others have examined tau interactors in mouse brains (76–79) and in cells expressing human tau (80). These studies found that different isoforms or domains of tau regulate different protein interactions, identified the major protein families that tau preferentially binds to, and identified new potential drug targets for preventing tau toxicity. However, one limitation of these studies is that using a total tau antibody results in the identification of all proteins that interact with both physiological and pathological tau in the brain, therefore making it difficult to determine which interactions are specific to the pathological phosphorylated tau species present in AD brains. Therefore, we have recently completed the first study of the phosphorylated tau interactome in human AD brain samples (51). Our results showed that phosphorylated tau in AD brains preferentially interacted with neuronal proteins, which is consistent with the intraneuronal location of phosphorylated tau in AD. We found that phosphorylated tau particularly interacted with proteins associated with two of the main protein degradation systems in the cell: the ubiquitin–proteasome system and the phagosome–lysosome system. The specific proteins involved suggested that phosphorylated tau may be potentially interfering with degradation of proteins by the proteasome and may contribute to lysosomal dysfunction in AD via interference with vacuolar ATPase proton pumps that are responsible

for acidification of lysosomes. Impairment of both of these processes has been previously associated with AD (81–83); however, this is the first study to show that tau may be involved in this process. This is an example of the informative nature of interactome studies, showing that they can provide a complete and unbiased overview of the pathogenic brain changes that occur in AD that are directly linked to a specific toxic protein species.

Going forward, performing these studies in a systematic manner that directly compares the interactome of multiple Aβ or tau species will help determine which protein interactions are particularly important for disease progression. Determining these key interactions that drive toxicity and that drive the formation of plaques or NFTs will help identify new potential drug targets for AD.

CONCLUSION

In conclusion, proteomics studies using human tissue are very useful for increasing our understanding about the pathogenesis of AD. The combined proteomic results from studies described above provide a powerful resource for generating new hypotheses about the cause of AD. Unbiased, proteomic studies using AD brain tissue have been previously limited by concerns about cost, technical limitations, and the assumption that very large samples sizes are required to counteract the large inter-patient variability in AD. However, recent studies have shown that hundreds of significant protein differences can be detected using sample sizes as low as 5 when comparing AD and controls. Larger sample sizes appear to be required when comparing different stages of AD (e.g., preclinical AD vs. advanced AD) or different subtypes of disease (e.g., rapidly progressive AD vs. sporadic AD); however, even in these studies, 20 samples/group are sufficient to identify hundreds of protein differences between groups. These results show that discovery proteomic studies using AD brain tissue are feasible. Importantly, meta-analysis of proteomics studies using AD brain tissue shows that many altered proteins in AD brain tissues are consistent between studies, therefore also validating these findings.

Going forward, it will be useful to expand the scope of these previous studies. Focusing on localized proteomics changes, either in neuropathological features, vulnerable neuron populations, or synaptic fractions, has the potential to greatly increase our understanding about what protein changes drive the development of neuropathology or neurodegeneration in these particularly affected regions. Systematic examination of the proteins that interact with specific species of Aβ or tau will help identify how these two proteins cause toxicity in AD. Results from localized or interactome studies have the potential to identify new drug targets or biomarkers of disease that are directly associated with AD neuropathology. Determining the protein changes that occur throughout the progression of AD is also particularly important to examine in future studies: the ideal drug targets for AD are pathological changes that occur in the earliest stages of disease; therefore proteomic studies that characterize protein changes in preclinical AD or mild cognitive impairment should be a priority.

Combined, proteomic studies are capable of providing a roadmap of protein changes that are associated with AD. These studies pinpoint the protein networks

that are most involved in disease as well as the specific proteins that are involved. Overall, these studies provide an excellent resource for future hypothesis-driven targeted studies that will hopefully help identify new biomarkers of disease and will help in the development of new drugs for AD.

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REFERENCES

1. Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. Lancet. 2017;390(10113):2673–734. http://dx.doi.org/10.1016/S0140-6736(17)31363-6
2. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener. 2019;14(1):32. http://dx.doi.org/10.1186/s13024-019-0333-5
3. Sperling R, Mormino E, Johnson K. The evolution of preclinical Alzheimer's disease: Implications for prevention trials. Neuron. 2014;84(3):608–22. http://dx.doi.org/10.1016/j.neuron.2014.10.038
4. Wisniewski T, Drummond E. Developing therapeutic vaccines against Alzheimer's disease. Expert Rev Vaccines. 2016;15(3):401–15. http://dx.doi.org/10.1586/14760584.2016.1121815
5. Herline K, Drummond E, Wisniewski T. Recent advancements toward therapeutic vaccines against Alzheimer's disease. Expert Rev Vaccines. 2018;7(8):707–21. http://dx.doi.org/10.1080/14760584.2018.1500905
6. Cummings J. Lessons learned from Alzheimer disease: Clinical trials with negative outcomes. Clin Transl Sci. 2018;11(2):147–52. http://dx.doi.org/10.1111/cts.12491
7. Glenner GG, Wong CW. Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 1984;120(3):885–90. http://dx.doi.org/10.1016/S0006-291X(84)80190-4
8. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and down syndrome. Proc Natl Acad Sci U S A. 1985;82(12):4245–9. http://dx.doi.org/10.1073/pnas.82.12.4245
9. Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem. 1986;261(13):6084–9.
10. Wisniewski T, Frangione B. Apolipoprotein E. A pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett. 1992;135(2):235–8. http://dx.doi.org/10.1016/0304-3940(92)90444-C
11. Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K. Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res. 1991;541(1):163–6. http://dx.doi.org/10.1016/0006-8993(91)91092-F
12. Drummond E, Wisniewski T. Alzheimer's disease: Experimental models and reality. Acta Neuropathol. 2017;133(2):155–75. http://dx.doi.org/10.1007/s00401-016-1662-x

13. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nat Genet. 2019;51(3):414–30. http://dx.doi.org/10.1038/s41588-019-0358-2
14. Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. Nature Genet. 2019;51(3):404–13. http://dx.doi.org/10.1038/s41588-018-0311-9
15. De Jager PL, Srivastava G, Lunnon K, Burgess J, Schalkwyk LC, Yu L, et al. Alzheimer's disease: Early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat Neurosci. 2014;17(9):1156–63. http://dx.doi.org/10.1038/nn.3786
16. Lunnon K, Smith R, Hannon E, De Jager PL, Srivastava G, Volta M, et al. Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat Neurosci. 2014;17(9):1164–70. http://dx.doi.org/10.1038/nn.3782
17. Seyfried NT, Dammer EB, Swarup V, Nandakumar D, Duong DM, Yin L, et al. A multi-network approach identifies protein-specific co-expression in asymptomatic and symptomatic Alzheimer's disease. Cell Syst. 2017;4(1):60–72.e4. http://dx.doi.org/10.1016/j.cels.2016.11.006
18. Drummond E, Goni F, Liu S, Prelli F, Scholtzova H, Wisniewski T. Potential novel approaches to understand the pathogenesis and treat Alzheimer's disease. J Alzheimer's Dis JAD. 2018;64(Suppl 1): S299–312. http://dx.doi.org/10.3233/JAD-179909
19. Wisniewski T, Drummond E. Future Horizons in Alzheimer's Disease Research. In: David B. Teplow, editor. Molecular biology of neurodegenerative diseases: Visions for the future. Prog Mol Biol Transl Sci. Elsevier; 2019;168:223–241. http://dx.doi.org/10.1016/bs.pmbts.2019.08.001.
20. Castano EM, Maarouf CL, Wu T, Leal MC, Whiteside CM, Lue LF, et al. Alzheimer disease periventricular white matter lesions exhibit specific proteomic profile alterations. Neurochem Int. 2013;62(2):145–56. http://dx.doi.org/10.1016/j.neuint.2012.12.001
21. Gozal YM, Duong DM, Gearing M, Cheng D, Hanfelt JJ, Funderburk C, et al. Proteomics analysis reveals novel components in the detergent-insoluble subproteome in Alzheimer's disease. J Proteome Res. 2009;8(11):5069–79. http://dx.doi.org/10.1021/pr900474t
22. Musunuri S, Wetterhall M, Ingelsson M, Lannfelt L, Artemenko K, Bergquist J, et al. Quantification of the brain proteome in Alzheimer's disease using multiplexed mass spectrometry. J Proteome Res. 2014;13(4):2056–68. http://dx.doi.org/10.1021/pr401202d
23. Musunuri S, Kultima K, Richard BC, Ingelsson M, Lannfelt L, Bergquist J, et al. Micellar extraction possesses a new advantage for the analysis of Alzheimer's disease brain proteome. Anal Bioanal Chem. 2015;407(4):1041–57. http://dx.doi.org/10.1007/s00216-014-8320-8
24. Donovan LE, Higginbotham L, Dammer EB, Gearing M, Rees HD, Xia Q, et al. Analysis of a membrane-enriched proteome from postmortem human brain tissue in Alzheimer's disease. Proteomics Clin Appl. 2012;6(3–4):201–11. http://dx.doi.org/10.1002/prca.201100068
25. Bai B, Hales CM, Chen PC, Gozal Y, Dammer EB, Fritz JJ, et al. U1 small nuclear ribonucleoprotein complex and RNA splicing alterations in Alzheimer's disease. Proc Natl Acad Sci U S A. 2013;110(41):16562–7. http://dx.doi.org/10.1073/pnas.1310249110
26. Begcevic I, Kosanam H, Martinez-Morillo E, Dimitromanolakis A, Diamandis P, Kuzmanov U, et al. Semiquantitative proteomic analysis of human hippocampal tissues from Alzheimer's disease and age-matched control brains. Clin Proteomics. 2013;10(1):5. http://dx.doi.org/10.1186/1559-0275-10-5
27. Andreev VP, Petyuk VA, Brewer HM, Karpievitch YV, Xie F, Clarke J, et al. Label-free quantitative LC-MS proteomics of Alzheimer's disease and normally aged human brains. J Proteome Res. 2012;11(6):3053–67. http://dx.doi.org/10.1021/pr3001546
28. Hondius DC, van Nierop P, Li KW, Hoozemans JJ, van der Schors RC, van Haastert ES, et al. Profiling the human hippocampal proteome at all pathologic stages of Alzheimer's disease. Alzheimers Dementia. 2016;12(6):654–68. http://dx.doi.org/10.1016/j.jalz.2015.11.002
29. Ho Kim J, Franck J, Kang T, Heinsen H, Ravid R, Ferrer I, et al. Proteome-wide characterization of signalling interactions in the hippocampal CA4/DG subfield of patients with Alzheimer's disease. Sci Rep. 2015;5:11138. http://dx.doi.org/10.1038/srep11138
30. Hales CM, Dammer EB, Deng Q, Duong DM, Gearing M, Troncoso JC, et al. Changes in the detergent-insoluble brain proteome linked to amyloid and tau in Alzheimer's disease progression. Proteomics. 2016;16(23):3042–53. http://dx.doi.org/10.1002/pmic.201600057

31. Johnson ECB, Dammer EB, Duong DM, Yin L, Thambisetty M, Troncoso JC, et al. Deep proteomic network analysis of Alzheimer's disease brain reveals alterations in RNA binding proteins and RNA splicing associated with disease. Mol Neurodegener. 2018;13(1):52. http://dx.doi.org/10.1186/s13024-018-0282-4
32. Zhang Q, Ma C, Gearing M, Wang PG, Chin LS, Li L. Integrated proteomics and network analysis identifies protein hubs and network alterations in Alzheimer's disease. Acta Neuropathol Commun. 2018;6(1):19. http://dx.doi.org/10.1186/s40478-018-0524-2
33. Dai J, Johnson ECB, Dammer EB, Duong DM, Gearing M, Lah JJ, et al. Effects of APOE genotype on brain proteomic network and cell type changes in Alzheimer's disease. Front Mol Neurosci. 2018;11:454. http://dx.doi.org/10.3389/fnmol.2018.00454
34. Bereczki E, Branca RM, Francis PT, Pereira JB, Baek JH, Hortobagyi T, et al. Synaptic markers of cognitive decline in neurodegenerative diseases: A proteomic approach. Brain. 2018;141(2):582–95. http://dx.doi.org/10.1093/brain/awx352
35. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, et al. Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A. 1993;90(5):1977–81. http://dx.doi.org/10.1073/pnas.90.5.1977
36. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr., et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet. 1994;7(2):180–4. http://dx.doi.org/10.1038/ng0694-180
37. Ayyadevara S, Balasubramaniam M, Parcon PA, Barger SW, Griffin WS, Alla R, et al. Proteins that mediate protein aggregation and cytotoxicity distinguish Alzheimer's hippocampus from normal controls. Aging Cell. 2016;15(5):924–39. http://dx.doi.org/10.1111/acel.12501
38. Abreha MH, Dammer EB, Ping L, Zhang T, Duong DM, Gearing M, et al. Quantitative analysis of the brain ubiquitylome in Alzheimer's disease. Proteomics. 2018;18(20):e1800108. http://dx.doi.org/10.1002/pmic.201800108
39. Dammer EB, Lee AK, Duong DM, Gearing M, Lah JJ, Levey AI, et al. Quantitative phosphoproteomics of Alzheimer's disease reveals cross-talk between kinases and small heat shock proteins. Proteomics. 2015;15(2–3):508–19. http://dx.doi.org/10.1002/pmic.201400189
40. Mrdjen D, Fox EJ, Bukhari SA, Montine KS, Bendall SC, Montine TJ. The basis of cellular and regional vulnerability in Alzheimer's disease. Acta Neuropathol. 2019; 138(5):729–749. http://dx.doi.org/10.1007/s00401-019-02054-4
41. Fu H, Hardy J, Duff KE. Selective vulnerability in neurodegenerative diseases. Nature Neurosci. 2018;21(10):1350–8. http://dx.doi.org/10.1038/s41593-018-0221-2
42. Mattsson N, Schott JM, Hardy J, Turner MR, Zetterberg H. Selective vulnerability in neurodegeneration: Insights from clinical variants of Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2016;87(9):1000–4. http://dx.doi.org/10.1136/jnnp-2015-311321
43. Grothe MJ, Sepulcre J, Gonzalez-Escamilla G, Jelistratova I, Scholl M, Hansson O, et al. Molecular properties underlying regional vulnerability to Alzheimer's disease pathology. Brain. 2018;141(9):2755–71. http://dx.doi.org/10.1093/brain/awy189
44. Xu J, Patassini S, Rustogi N, Riba-Garcia I, Hale BD, Phillips AM, et al. Regional protein expression in human Alzheimer's brain correlates with disease severity. Commun Biol. 2019;2:43. http://dx.doi.org/10.1038/s42003-018-0254-9
45. Mendonca CF, Kuras M, Nogueira FCS, Pla I, Hortobagyi T, Csiba L, et al. Proteomic signatures of brain regions affected by tau pathology in early and late stages of Alzheimer's disease. Neurobiol Dis. 2019;130:104509. http://dx.doi.org/10.1016/j.nbd.2019.104509
46. Drummond E, Nayak S, Pires G, Ueberheide B, Wisniewski T. Isolation of amyloid plaques and neurofibrillary tangles from archived Alzheimer's disease tissue using laser-capture microdissection for downstream proteomics. Methods Mol Biol. 2018;1723:319–34. http://dx.doi.org/10.1007/978-1-4939-7558-7_18
47. Drummond E, Nayak S, Ueberheide B, Wisniewski T. Localized proteomics of individual neurons isolated from formalin-fixed, paraffin embedded tissue sections using laser capture microdissection. In: Martinez ES, Fernández J, editors. Current proteomic approaches applied to brain function. New York: Humana Press; 2017. p. 289–301.

48. Drummond ES, Nayak S, Ueberheide B, Wisniewski T. Proteomic analysis of neurons microdissected from formalin-fixed, paraffin-embedded Alzheimer's disease brain tissue. Sci Rep. 2015;5:15456. http://dx.doi.org/10.1038/srep15456
49. Drummond E, Nayak S, Faustin A, Pires G, Hickman RA, Askenazi M, et al. Proteomic differences in amyloid plaques in rapidly progressive and sporadic Alzheimer's disease. Acta Neuropathol. 2017;133(6):933–54. http://dx.doi.org/10.1007/s00401-017-1691-0
50. Drummond E, Wisniewski T. The use of localized proteomics to identify the drivers of Alzheimer's disease pathogenesis. Neural Regen Res. 2017;12(6):912–13. http://dx.doi.org/10.4103/1673-5374.208570
51. Drummond E, Pires G, MacMurray, C, Askenazi M, Nayak S, Bourdon M, Ueberheide B, Wisniewski T. Phosphorylated tau interactome in the human Alzheimer's disease brain. Alzheimer's and Dementia. 2019;15(7):1517. https://doi.org/10.1016/j.jalz.2019.08.077
52. Pires G, McElligott S, Drusinsky S, Halliday G, Potier MC, Wisniewski T, Drummond E. Secernin-1 is a novel phosphorylated tau binding protein that accumulates in Alzheimer's disease and not in other tauopathies. Acta Neuropathol Commun. 2019. In Press. http://dx.doi.org/10.1186/s40478-019-0848-6
53. Xiong F, Ge W, Ma C. Quantitative proteomics reveals distinct composition of amyloid plaques in Alzheimer's disease. Alzheimers Dementia. 2019;15(3):429–40. http://dx.doi.org/10.1016/j.jalz.2018.10.006
54. Liao L, Cheng D, Wang J, Duong DM, Losik TG, Gearing M, et al. Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. J Biol Chem. 2004;279(35):37061–8. http://dx.doi.org/10.1074/jbc.M403672200
55. Wang Q, Woltjer RL, Cimino PJ, Pan C, Montine KS, Zhang J, et al. Proteomic analysis of neurofibrillary tangles in Alzheimer disease identifies GAPDH as a detergent-insoluble paired helical filament tau binding protein. FASEB J. 2005;19(7):869–71. http://dx.doi.org/10.1096/fj.04-3210fje
56. Minjarez B, Valero Rustarazo ML, Sanchez del Pino MM, Gonzalez-Robles A, Sosa-Melgarejo JA, Luna-Munoz J, et al. Identification of polypeptides in neurofibrillary tangles and total homogenates of brains with Alzheimer's disease by tandem mass spectrometry. J Alzheimers Dis JAD. 2013;34(1):239–62. http://dx.doi.org/10.3233/JAD-121480
57. Manousopoulou A, Gatherer M, Smith C, Nicoll JAR, Woelk CH, Johnson M, et al. Systems proteomic analysis reveals that clusterin and tissue inhibitor of metalloproteinases 3 increase in leptomeningeal arteries affected by cerebral amyloid angiopathy. Neuropathol Appl Neurobiol. 2017;43(6):492–504. http://dx.doi.org/10.1111/nan.12342
58. Inoue Y, Ueda M, Tasaki M, Takeshima A, Nagatoshi A, Masuda T, et al. Sushi repeat-containing protein 1: A novel disease-associated molecule in cerebral amyloid angiopathy. Acta Neuropathol. 2017;134(4):605–17. http://dx.doi.org/10.1007/s00401-017-1720-z
59. Hondius DC, Eigenhuis KN, Morrema THJ, van der Schors RC, van Nierop P, Bugiani M, et al. Proteomics analysis identifies new markers associated with capillary cerebral amyloid angiopathy in Alzheimer's disease. Acta Neuropathol Commun. 2018;6(1):46. http://dx.doi.org/10.1186/s40478-018-0540-2
60. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002;298(5594):789–91. http://dx.doi.org/10.1126/science.1074069
61. Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology. 2007;68(18):1501–8. http://dx.doi.org/10.1212/01.wnl.0000260698.46517.8f
62. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, et al. Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol. 1991;30(4):572–80. http://dx.doi.org/10.1002/ana.410300410
63. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. Ann Neurol. 1990;27(5):457–64. http://dx.doi.org/10.1002/ana.410270502
64. Lleo A, Nunez-Llaves R, Alcolea D, Chiva C, Balateu-Panos D, Colom-Cadena M, et al. Changes in synaptic proteins precede neurodegeneration markers in preclinical Alzheimer's disease cerebrospinal fluid. Mol Cell Proteomics. 2019;18(3):546–60. http://dx.doi.org/10.1074/mcp.RA118.001290
65. Chang RY, Etheridge N, Nouwens AS, Dodd PR. SWATH analysis of the synaptic proteome in Alzheimer's disease. Neurochem Int. 2015;87:1–12. http://dx.doi.org/10.1016/j.neuint.2015.04.004
66. Chang RY, Nouwens AS, Dodd PR, Etheridge N. The synaptic proteome in Alzheimer's disease. Alzheimers Dementia. 2013;9(5):499–511. http://dx.doi.org/10.1016/j.jalz.2012.04.009

67. Zhou J, Jones DR, Duong DM, Levey AI, Lah JJ, Peng J. Proteomic analysis of postsynaptic density in Alzheimer's disease. Clin Chim Acta. 2013;420:62–8. http://dx.doi.org/10.1016/j.cca.2013.03.016
68. Sweet RA, MacDonald ML, Kirkwood CM, Ding Y, Schempf T, Jones-Laughner J, et al. Apolipoprotein E*4 (APOE*4) genotype is associated with altered levels of glutamate signaling proteins and synaptic coexpression networks in the prefrontal cortex in mild to moderate Alzheimer disease. Mol Cell Proteomics. 2016;15(7):2252–62. http://dx.doi.org/10.1074/mcp.M115.056580
69. Duits FH, Brinkmalm G, Teunissen CE, Brinkmalm A, Scheltens P, Van der Flier WM, et al. Synaptic proteins in CSF as potential novel biomarkers for prognosis in prodromal Alzheimer's disease. Alzheimers Res Ther. 2018;10(1):5. http://dx.doi.org/10.1186/s13195-017-0335-x
70. Bai Y, Markham K, Chen F, Weerasekera R, Watts J, Horne P, et al. The in vivo brain interactome of the amyloid precursor protein. Mol Cell Proteomics. 2008;7(1):15–34. http://dx.doi.org/10.1074/mcp.M700077-MCP200
71. Kohli BM, Pflieger D, Mueller LN, Carbonetti G, Aebersold R, Nitsch RM, et al. Interactome of the amyloid precursor protein APP in brain reveals a protein network involved in synaptic vesicle turnover and a close association with synaptotagmin-1. J Proteome Res. 2012;11(8):4075–90. http://dx.doi.org/10.1021/pr300123g
72. Hosp F, Vossfeldt H, Heinig M, Vasiljevic D, Arumughan A, Wyler E, et al. Quantitative interaction proteomics of neurodegenerative disease proteins. Cell Rep. 2015;11(7):1134–46. http://dx.doi.org/10.1016/j.celrep.2015.04.030
73. Andrew RJ, Fisher K, Heesom KJ, Kellett KAB, Hooper NM. Quantitative interaction proteomics reveals differences in the interactomes of amyloid precursor protein isoforms. J Neurochem. 2019;149(3):399–412. http://dx.doi.org/10.1111/jnc.14666
74. Wang H, Muiznieks LD, Ghosh P, Williams D, Solarski M, Fang A, et al. Somatostatin binds to the human amyloid beta peptide and favors the formation of distinct oligomers. eLife. 2017;6:pii: e28401. http://dx.doi.org/10.7554/eLife.28401
75. Meier S, Bell M, Lyons DN, Ingram A, Chen J, Gensel JC, et al. Identification of novel tau interactions with endoplasmic reticulum proteins in Alzheimer's disease brain. J Alzheimers Dis JAD. 2015;48(3):687–702. http://dx.doi.org/10.3233/JAD-150298
76. Liu C, Song X, Nisbet R, Gotz J. Co-immunoprecipitation with tau isoform-specific antibodies reveals distinct protein interactions and highlights a putative role for 2N tau in disease. J Biol Chem. 2016;291(15):8173–88. http://dx.doi.org/10.1074/jbc.M115.641902
77. Maziuk BF, Apicco DJ, Cruz AL, Jiang L, Ash PEA, da Rocha EL, et al. RNA binding proteins co-localize with small tau inclusions in tauopathy. Acta Neuropathol Commun. 2018;6(1):71. http://dx.doi.org/10.1186/s40478-018-0574-5
78. Wang P, Joberty G, Buist A, Vanoosthuyse A, Stancu IC, Vasconcelos B, et al. Tau interactome mapping based identification of Otub1 as Tau deubiquitinase involved in accumulation of pathological Tau forms in vitro and in vivo. Acta Neuropathol. 2017;133(5):731–49. http://dx.doi.org/10.1007/s00401-016-1663-9
79. Stefanoska K, Volkerling A, Bertz J, Poljak A, Ke YD, Ittner LM, et al. An N-terminal motif unique to primate tau enables differential protein-protein interactions. J Biol Chem. 2018;293(10):3710–19. http://dx.doi.org/10.1074/jbc.RA118.001784
80. Gunawardana CG, Mehrabian M, Wang X, Mueller I, Lubambo IB, Jonkman JE, et al. The human tau interactome: Binding to the ribonucleoproteome, and impaired binding of the proline-to-leucine mutant at position 301 (P301L) to chaperones and the proteasome. Mol Cell Proteomics. 2015;14(11):3000–14. http://dx.doi.org/10.1074/mcp.M115.050724
81. Keck S, Nitsch R, Grune T, Ullrich O. Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. J Neurochem. 2003;85(1):115–22. http://dx.doi.org/10.1046/j.1471-4159.2003.01642.x
82. Keller JN, Hanni KB, Markesbery WR. Impaired proteasome function in Alzheimer's disease. J Neurochem. 2000;75(1):436–9. http://dx.doi.org/10.1046/j.1471-4159.2000.0750436.x
83. Colacurcio DJ, Nixon RA. Disorders of lysosomal acidification-the emerging role of v-ATPase in aging and neurodegenerative disease. Ageing Res Rev. 2016;32:75–88. http://dx.doi.org/10.1016/j.arr.2016.05.004

4

Common Proteomic and Genomic Contribution to Ischemic Brain Damage and Alzheimer's Disease

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Abstract: Ischemic brain damage is associated with the deposition of folding proteins, such as all fragments of the amyloid protein precursor and tau protein, in the intra- and extracellular spaces of neurons. In this chapter, we summarize the protein changes associated with Alzheimer's disease and their gene expression (amyloid protein precursor and tau protein) after cerebral ischemia and their role in the ischemic etiology of Alzheimer's disease. Recent advances in understanding the ischemic etiology of Alzheimer's disease have revealed dysregulation of *amyloid protein precursors, β-secretase, presenilin 1 and 2, autophagy, mitophagy*, *apoptosis*, and *tau protein* genes after ischemic brain injury. However, reduced expression of mRNA of the α-secretase in cerebral ischemia causes neurons to be less resistant to injury. In this chapter, we present the latest evidence that Alzheimer's disease-related proteins and their genes play a key role in brain damage with ischemia-reperfusion and that ischemic episode is an essential and leading provider of Alzheimer's disease development. Understanding the underlying processes of linking Alzheimer's disease-related proteins and their genes in brain ischemia

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injury with the risk of developing Alzheimer's disease will provide the most significant goals for therapeutic development to date.

Keywords: Alzheimer's disease; amyloid; brain ischemia; secretases; tau protein

INTRODUCTION

Newer studies show that brain ischemia with reperfusion can be associated with a fully developed Alzheimer's disease (1, 2). It is generally suggested that cerebral ischemia triggers Alzheimer's disease, and an ischemic change in the permeability of the blood–brain barrier additionally causes amyloid transportation from the blood to the brain, and this is the last element that causes the full-bloom sporadic Alzheimer's disease (3, 4). Ischemic human stroke and experimental ischemia-reperfusion brain injury are serious, life-threatening neuropathological episodes with severe complications such as post-ischemic cognitive impairment and physical disability (5–12). The evidence to date suggests that there is a potential compatibility among neuropathogenesis of brain ischemia and Alzheimer's disease. First, clinical observations have shown that Alzheimer's disease is a contributing factor to the development of ischemic brain damage and vice versa (13). Second, ischemic brain injury and Alzheimer's disease have the same risk factors like hypertension, hyperlipidemia, diabetes, and age. Third, experimental ischemia-reperfusion brain injury produces a stereotypical pattern of selective death/loss of neurons in the hippocampus with severe brain atrophy, which is similar to the neuropathology observed in Alzheimer's disease, indicating active, slowly progressive neuropathological processes (14–18). Fourth, inflammatory changes appear to play a key role in the progression of brain ischemia and Alzheimer's disease (19). Fifth, the data indicate that ischemic brain damage can cause the pathology of proteins typical for Alzheimer's disease by inducing the generation and deposition of the β-amyloid peptide and other fragments of amyloid protein precursor (16, 20–22). Finally, studies show that tau protein dysfunction also plays a key role in regulating brain ischemia-reperfusion episodes (23–33). Together, these results point to common proteomic and genomic factors in ischemic brain injury and Alzheimer's disease in the neuropathological processes.

In this chapter, we present the current knowledge about the dysregulation of genes involved in the amyloidogenic processing of the amyloid protein precursor, which is associated with the generation of the β-amyloid peptide in the brain after ischemia. In addition, we pay attention to whether the signal pathway of the amyloid protein precursor is involved in the induced ischemic death of neurons in the CA1 area of the hippocampus and medial temporal cortex. Also, we take into account the importance of ischemic gene expression associated with Alzheimer's disease, such as autophagy, mitophagy, and apoptosis during clinical onset, progression and maturation of brain injury after ischemia in the etiology of Alzheimer's disease. With regard to the latest exciting discoveries after brain ischemia injury, we combine data from the proteomic and genomic point of view. In recent years, several researchers have documented that brain ischemia-reperfusion episode is an important element in the development of Alzheimer's disease and plays a key role in proteomic and genomic (e.g., amyloid protein precursor, amyloid

processing secretases, autophagy, mitophagy, caspase 3, and tau protein) changes of this disorder (1, 2, 31). Below we summarize the latest evidence that Alzheimer's disease-related proteins and their genes play an essential role in brain ischemia-reperfusion injury, and ischemic episode is a necessary and most important supplier for the start and progress of the full development of sporadic Alzheimer's disease.

AMYLOID STAINING AND BLOOD LEVEL AFTER BRAIN ISCHEMIA

Although a significant progress has been made in research on the pathogenicity of amyloid in Alzheimer's disease, the underlying molecular amyloid machinery affecting neurodegeneration after ischemic brain injury is unclear. Herein we present the existing facts regarding amyloidogenic processing of the amyloid protein precursor into amyloid during brain injury due to ischemia and reperfusion, which is associated with the production and accumulation of the N- and C-terminal of amyloid protein precursor and amyloid in the brain. The appearance of an elevated level of β-amyloid peptide in the blood and its staining in the brain after ischemic injury sheds new light on a better understanding of the role of amyloid in the development of neurological deficits following an ischemic episode.

In animals

Different fragments of the amyloid protein precursor staining were observed in the extra- and intracellular spaces after experimental ischemic brain injury (15, 20, 34–37). In animals that survived up to 6 months after brain ischemia with recirculation in the extracellular space of the hippocampus, brain cortex, white matter, and around the lateral ventricles, the N- and C-terminal deposits of the amyloid protein precursor and the β-amyloid peptide were observed (16, 20). The accumulation of different parts of the amyloid protein precursor in various cells, such as the neuronal, glial, microglia, oligodendrocyte, endothelial, pericyte, and ependymal cells, has also been found (15, 20, 38–42). Especially astrocytes around microvessels showed intense staining of many very long, thin processes that adhered to or embraced capillaries. More than 6 months of survival after cerebral ischemia, only the C-terminal staining of the amyloid protein precursor and the β-amyloid peptide was observed (16). Accumulation of β-amyloid peptide in response to transient focal ischemic brain injury does not appear to be a temporary phenomenon, as diffuse β-amyloid peptide deposits turn into plaque about 9 months after ischemic episode (43). After ischemia–reperfusion brain injury, the β-amyloid peptide arises as a result of neuronal ischemic damage (34), and it is likely that this peptide with its own neurotoxic activity further affects ischemic neurons.

In humans

Examination of human ischemic brains has shown that ischemia is associated with the accumulation of β-amyloid peptide in brain tissue (44–46). Studies have

shown both diffuse and senile β-amyloid peptide plaques in areas of the brain prone to ischemia, at arterial border zones and cortex after focal and global cerebral ischemia (44–46). The middle layers of the cerebral cortex, which are very susceptible to ischemic injury, were most commonly affected by amyloid (44–46). The number of amyloid plaques in brain tissue correlated positively with age (44–46). In brains after global cerebral ischemia with a survival of up to 1 month, strong staining of the β-amyloid peptide in neurons and perivascular areas was found (45). The staining of neurons depended on the area of the brain. Neurons from the cerebral cortex and hippocampus were the most intensely stained. The ependymal and epithelial cells were also stained on the β-amyloid peptide. Not all brains had senile amyloid plaques in the cerebral cortex. The cerebral white and gray matter vessels were surrounded by β-amyloid peptide deposits. Deposits in the perivascular space looked like cuffs. In some brains, the walls of the meningeal and cortical vessels were stained for the β-amyloid peptide. Accumulation of amyloid in the perivascular blood vessel space of the blood–brain barrier suggested that the β-amyloid peptide was derived from blood. Some evidence to support this hypothesis comes from clinical studies showing that the β-amyloid peptide in the blood has been elevated in patients after ischemic brain injury (22, 47). According to another study, β-amyloid peptides 1–40 and 1–42 staining were found in the human hippocampus after ischemia (21). This intense staining of various β-amyloid peptides may contribute to the progression of ischemic hippocampus neurodegeneration.

In the brains of patients after global cerebral ischemia caused by cardiac arrest, the immunostaining of the receptor for advanced glycation end products was located both in the cells of the choroid plexus epithelium and in the ependymal cells bordering the brain ventricles (48). These cells form both the cerebrospinal fluid–brain barrier and the blood–cerebrospinal fluid barrier. The β-amyloid peptide was noted by staining in the blood vessels of the choroid plexus and in the basal membrane of the choroid plexus epithelium (48). The data showed that the choroid plexus epithelium and the lining cells, equipped with a receptor for advanced glycation end products, play not only a significant role in the accumulation of the β-amyloid peptide in the brain parenchyma but also are a place where amyloid can be removable.

After ischemic brain injury in humans due to cardiac arrest, approximately 70-fold increase in beta-amyloid peptide 1–42 in the serum was found (22). The level of amyloid growth correlated negatively with the complete clinical outcome after ischemic brain injury, which in turn probably reflects the severity of ischemic damage (22). The data confirm that brain ischemia may play a key role in the amyloidogenic processing of the amyloid protein precursor.

TAU PROTEIN STAINING, PHOSPHORYLATION, AND BLOOD LEVEL AFTER BRAIN ISCHEMIA

Although there has been significant progress recently in research on the pathogenicity of the tau protein in Alzheimer's disease, the basic molecular processes associated with the tau protein that affect neurodegeneration after ischemic brain trauma have not been finally clarified. In this analysis, we show that both

ischemia–reperfusion brain damage and the permeability of the blood–brain barrier after ischemia induce tau protein dysfunction. As a result, we suggest that modifications of the tau protein by phosphorylation are dangerous for microtubule activity, especially in neurons, and are involved in the development of irreversible neuropathology in the ischemic brain with Alzheimer's disease dementia.

In animals

Early experimental studies documented tau protein staining in neuronal and glial cells in the hippocampus, thalamus, and cortex after permanent and focal brain ischemia (36, 49–53). The modified tau protein was also observed in microglial cells both in ischemic penumbra and in brain tissue, respectively, after focal and global cerebral ischemia (29, 54). The above data showed that some neuronal and glial cells had changes in the tau protein after ischemic brain damage (52), which may be the main pathological stage of ischemic processes in these cells (53). Another study revealed that tau protein alone can block the transport of amyloid protein precursor in neurons, which leads to the accumulation of the amyloid protein precursor in the body of neuronal cells (55).

Studies have also shown that the phosphorylation patterns of tau protein differed in different models of cerebral ischemia (32). The tau protein was dephosphorylated after total and focal cerebral ischemia (51, 52, 56). During total brain ischemia, the tau protein was dephosphorylated, and during recirculation, it was re-phosphorylated and accumulated in the brain tissue (56). Transient local ischemic brain injury in rats with 24-h recirculation induces site-specific hyperphosphorylation of tau protein (57). An experimental combination of reversible total brain ischemia with hyperhomocysteinemia resulted in an approximate 700-fold increase in the number of hyperphosphorylated positive tau protein neurons in the cerebral cortex compared to control conditions (31). Recent studies indicate that following brain ischemia, hyperphosphorylated tau protein in cortical neurons is integrated with apoptosis (24, 27, 29, 30, 54, 57, 58). Khan et al. (30) showed an increase in the production of paired helical filaments of tau protein after forebrain ischemia in mice. Wen et al. (24, 57, 58) provided evidence that brain ischemia with recirculation is involved in neurofibrillary tangle-like development after local ischemic cerebral injury. Finally, tau protein dysfunction, a typical hallmark of Alzheimer's disease, worsens experimental ischemic brain damage *via* tau protein-mediated iron export (59) and excitotoxicity depending on the tau protein (28, 60).

In humans

Early studies have shown that tau protein staining in neurons and glia is present in the hippocampus, thalamus, and cerebral cortex in the human brain after ischemia (61–63). The modified tau protein was also observed in microglial cells (63). It was noted that microglial cells' tau protein passes independent of phosphorylation modification following cerebral ischemia with recirculation in humans (63). Finally, in one of the studies, many neurofibrillary tangle-bearing neurons were observed in the nucleus basalis of Meynert ipsilateral to a massive focal cerebral infarction (23).

Tau protein was detected in human plasma after complete ischemic brain injury with two peaks after 2 and 4 days, which probably indicates the degree of neuronal damage after ischemia–reperfusion episode (25). The observed bimodal changes in the tau protein in the blood are consistent with the 2 types of neuronal death: first, by necrosis, and second, by delayed neuronal death (26). The presented research suggests that the increase in blood tau protein can be used as a biomarker to assess neurological damage to the brain after ischemia (25, 26).

mRNAs ASSOCIATED WITH THE AMYLOID PROTEIN PRECURSOR AFTER BRAIN ISCHEMIA

Due to the fact that there are some new data in the literature in human and animal studies regarding changes in amyloid protein precursor following ischemia–reperfusion brain damage in this part of the review, we present the first steps in mRNA studies related to the metabolism of the amyloid protein precursor after various types of brain ischemia. This indicates that there is urgent need for data on the new causative pathological role of amyloid in cerebral ischemia, which molecule presumably has an irreversible effect on the post-ischemic outcome.

mRNA of the amyloid protein precursor

After experimental focal ischemic brain damage with reperfusion, the mRNA level of the amyloid protein precursor increased both in the core and in the penumbra, by 150 and 200%, respectively, in 1 week of recirculation (64, 65). In addition, after permanent local ischemic brain injury without recirculation, the mRNA domain of the Kunitz-type protease inhibitor domain-containing amyloid protein precursor in the cortex of the brain was induced for 3 weeks (66). Also after transient local cerebral ischemia, the amyloid protein precursors, 770 and 751 mRNAs, were induced within 1 week of recirculation (67). In addition, 1 h after local ischemic brain damage in ovariectomized rats, the increased mRNA level of the amyloid protein precursor was observed in ischemic brain structures (64). In contrast, estrogen treatment reduces the mRNA level of the amyloid protein precursor in the ischemic brain (64). These data suggest that estrogen therapy can be used to lower the mRNA of the amyloid protein precursor after the ischemic episode.

mRNA of enzymes metabolizing the amyloid protein precursor

The amyloid protein precursor is metabolized by α-secretase, and this process is a non-amyloidogenic process. After experimental ischemic brain injury, the level of α-secretase mRNA decreases (68, 69). The second process is called amyloidogenic process, and the amyloid protein precursor is metabolized by β-secretase and γ-secretase to produce the β-amyloid peptide (70). Some studies have demonstrated that ischemic episode of the brain activates the production and activity of β-secretase after ischemia (71–74). Another study showed changes in mRNA levels of three enzymes that metabolize the amyloid protein precursor: β-secretase,

cathepsin B, and glutaminyl cyclase, which increased in the cortex and hippocampus after ischemia (75).

Three days after ischemic brain injury, the highest level of presenilin 1 mRNA was observed in the neuronal cells of CA3 area of the hippocampus (76). This observation suggests that an elevated level of presenilin 1 mRNA probably is associated with the response of neuronal cells to ischemia. In another study, the increased level of presenilin 1 mRNA showed the maximum growth in the striatum, cortex, and hippocampus after focal ischemic brain damage (77). In the above study, the increased level of presenilin 1 mRNA was greater on the side opposite to local ischemic brain injury. This observation may reflect the disappearance of brain neurons on the ipsilateral side. The mRNA of presenilin 1, which increased after brain ischemia (76, 77), is involved in the production of the β-amyloid peptide by the γ-secretase complex (70, 78). The above data will help understand the progressive neuronal disappearance following the ischemia–reperfusion episode of the brain and the slow, prolonged accumulation of the β-amyloid peptide in ischemic brain tissue (16).

EXPRESSION OF GENES INVOLVED IN THE PRODUCTION OF AMYLOID AFTER BRAIN ISCHEMIA

The ischemic–reperfusion episode of the brain is undoubtedly one of the most common multifactorial forms of neurodegeneration, including many pathological processes occurring during ischemia and recirculation and gradually spreading to various areas of the brain. It seems that the ischemic event in humans and animals is associated with the development of Alzheimer's disease type of neurodegenerative pathology, such as the accumulation of all parts of the amyloid protein precursor after its processing in the amyloidogenic process and dysregulation of Alzheimer's disease-related genes involved in this process. Progress in understanding new proteomic and genomic processes caused by ischemic brain damage in various brain structures that have not yet been fully elucidated will result in new strategies for the treatment of neurodegeneration of the Alzheimer's disease type with full-blown dementia due to ischemia.

CA1 area of the hippocampus and medial temporal cortex

In the CA1 region of the hippocampus and temporal cortex, the expression of the *amyloid protein precursor* gene was below the control value within 2 days after ischemia (79, 80). In the above areas, 7 and 30 days after cerebral ischemia–reperfusion, the expression of the *amyloid protein precursor* gene was above the control value (79, 80). The expression of the β-*secretase* gene increased above the control value following brain ischemia injury in the CA1 area of the hippocampus 2 to 7 days after recirculation (79). But, 30 days after brain ischemia, the expression of the β-*secretase* gene was below the control value (79). The expression of the β-*secretase* gene was above the control value in the temporal cortex after 2 days from ischemic episode (80). The expression of the β-*secretase* gene was reduced in the temporal cortex 7 and 30 days after

ischemia (80). In the CA1 region, the expression of the *presenilin 1* and 2 gene was increased 2 and 7 days after ischemia (79). But, 30 days post-ischemic injury, the gene expression of *presenilin 1* and 2 was below the control value (79). In the temporal cortex, *presenilin 1* gene expression was lowered below the control value, but *presenilin 2* was above the control value 2 days after ischemia (81). Seven days after ischemia, the gene expression of presenilin 1 was reduced, and presenilin 2 was elevated (81). Thirty days post-ischemia, the expression of *presenilin 1* gene was above the control value, and *presenilin 2* was below the control value (81).

EXPRESSION OF THE *TAU PROTEIN* GENE IN THE CA1 AREA AFTER BRAIN ISCHEMIA

In the neurons of CA1 area of the hippocampus, the tau protein encoding gene expression increased above the control value on the 2nd day after brain ischemia (33). On the 7th day of reperfusion after ischemic episode, the gene expression oscillated in the range of control values (33). On the 30th day of recirculation after brain ischemia, the expression of the *tau protein* gene was below the control value (33). The statistical significance of changes in the expression of the *tau protein* gene after brain ischemia–reperfusion injury in rats was between 2 and 7, and 2 and 30 days of recirculation (33).

EXPRESSION OF GENES INVOLVED IN THE DIRECT DEATH OF NEURONS AFTER BRAIN ISCHEMIA

One of the risk factors of Alzheimer's disease is aging, and for that reason, a large number of scientists believe that the main cause of Alzheimer's disease is brain ischemia closely related to age. It seems that brain injury caused by ischemia and reperfusion facilitates the development of irreversible neurodegeneration similar to Alzheimer's disease as a result of neuronal death, synaptic dysfunction, inflammatory changes, white matter damage, and general brain atrophy, which changes are closely related to genes involved in neuronal death in Alzheimer's disease. Despite the years of expansion, the amyloid Alzheimer's disease theory has not solved the etiology of the disorder (82), and the current research suggests that brain ischemia leads to neurodegeneration of Alzheimer's disease through numerous terminal events, such as dysregulation of genes that cause cell death in various brain structures of varying intensity. Understanding the basic pathological pathways causing proteomic and genomic changes associated with Alzheimer's disease and induced by cerebral ischemia will help in the development of neurodegenerative dementia treatment after ischemia.

CA1 area of the hippocampus

Expression of the *autophagy* gene in the CA1 region of the hippocampus after brain ischemia with 2, 7, and 30 days of recirculation was within the control

limits (83). Two days after ischemic brain injury, the expression of the *mitophagy* gene in the CA1 region increased above the control value. Seven and 30 days after ischemia–reperfusion injury of the brain, the gene expression was within the control range. Overexpression of the *caspase 3* gene in the CA1 region was observed after 2 and 7 days of recirculation. However, 30 days after ischemic brain injury, the gene expression was below the control value.

Medial temporal cortex

Two days after ischemic brain injury, *autophagy* gene expression increased above the control value in the medial temporal cortex (84). However, 7 and 30 days after ischemia–reperfusion brain injury, *autophagy* gene expression decreased. Two days after cerebral ischemia, *mitophagy* gene expression decreased below the control value (84). Nevertheless, 7 and 30 days after ischemic brain injury, the expression of the *mitophagy* gene increased above the control value. Two days after cerebral ischemia, the expression of *caspase 3* gene decreased below the control value (84). However, 7 and 30 days after brain injury due to ischemia and reperfusion, *caspase 3* gene expression increased above the control value.

THE RELATIONSHIP BETWEEN IRON DYSHOMEOSTASIS AND AMYLOIDOGENESIS

Both the amyloid protein precursor and iron play a key role in brain neurodegeneration due to Alzheimer's disease and cerebral ischemia (59, 85–88). Alzheimer's disease is primarily characterized by the deposition of amyloid plaques and the formation of neurofibrillary tangles which co-localize with iron (88). Under physiological conditions, the amyloid protein precursor is processed primarily on the non-amyloidogenic pathway by α-secretase, thereby producing the neuroprotective ectodomain of the soluble amyloid protein precursor α and the carboxy-terminal fragment α. Alternatively, a small pool of amyloid protein precursor is processed by the amyloidogenic pathway using β-secretase, thereby producing a soluble amyloid protein precursor β and carboxy-terminal fragment β. The carboxy-terminal fragment β is further cleaved by γ-secretase, resulting in β-amyloid peptides.

Iron is gradually deposited in selected areas of the brain during Alzheimer's disease, as well as in the course of ischemic neurodegeneration (59, 85–88). In the brain, iron is present in neurons, oligodendrocytes, astroglia, and microglia cells. Excess iron is associated with oxidative stress and neuronal damage because iron accumulation in neurons can cause free radical production and mitochondrial dysfunction and ultimately lead to neuronal death. In addition, iron can induce hyperphosphorylation and aggregation of tau protein. Deficiency of tau protein leads to iron accumulation, which is associated with impaired transport of the amyloid protein precursor to the cell membrane (59, 87). Therefore, iron accumulation in brain cells must be strictly regulated to maintain basic cellular function and avoid cytotoxicity. The evidence obtained confirms the role of the amyloid protein precursor in maintaining iron homeostasis

in brain tissue (86). It has been demonstrated that the amyloid protein precursor and soluble amyloid protein precursor α facilitate iron outflow by stabilizing the iron exporter ferroportin 1 on the cell membrane (86, 87). In contrast, ablation of the amyloid protein precursor in neurons causes iron retention (87), while the knockout of the amyloid protein precursor in mice causes iron accumulation in the brain (86). While the amyloid protein precursor affects iron export, the inverse is also true because iron modulates the metabolism of the amyloid protein precursor (86). Iron and interleukin 1 levels in cells regulate translation of the amyloid protein precursor by acting on an iron-responsive element found in the 5' untranslated region of the amyloid protein precursor mRNA (87, 89). Iron has also been shown to affect the processing of the amyloid protein precursor and the production of β-amyloid peptides. In addition, the activation of α-secretase and β-secretase is proteolytically modulated by furin; furin protein levels are reduced under conditions of excess iron, which promotes β-secretase activity, thereby promoting amyloidogenesis (87). Iron and inflammation promote amyloid toxicity (87). Recent experimental studies showed that: (i) iron overload increased retention in the neurons of the soluble amyloid protein precursor α, (ii) iron overload reduced the extracellular levels of the soluble amyloid protein precursor α and β-amyloid peptide, and (iii) the direct molecular target of iron is β-secretase (86).

Given the key physiological and pathological role of the amyloid protein precursor and its cleavage products in the brain, it is likely that iron overload may affect neuronal activity, interfering with the normal processing of the amyloid protein precursor. Although it is unclear what mechanism causes abnormal intracellular retention of the soluble amyloid protein precursor α, there is evidence that cell accumulation of the soluble amyloid protein precursor α may be due to intracellular cleavage of the amyloid protein precursor by α secretase or the internalization of the extracellular soluble amyloid protein precursor α by cell surface receptors (90, 91). Together, evidence of the beneficial role of the secreted soluble amyloid protein precursor α indicates that iron overload mediates the decrease in secreted soluble amyloid protein precursor α, which can lead to harmful consequences. This possibility is particularly important in neurological diseases, given that the secretion of the soluble amyloid protein precursor α affects many brain disorders, including Alzheimer's disease and cerebral ischemia (85–88). In addition, it has recently been suggested that the loss of β-amyloid peptide function, rather than its accumulation, plays a pathogenic role in Alzheimer's disease (92). In summary, iron overload affected nonamyloidogenic as well as amyloidogenic metabolism of the neuronal amyloid protein precursor. In addition, it was confirmed that the soluble amyloid protein precursor α is an endogenous inhibitor of β-secretase activity, potentially affecting the production of β-amyloid peptide (86). As iron directly inhibits β-secretase activity, it is likely that increased iron primarily inhibits β-secretase and the amyloidogenic pathway and promotes the non-amyloidogenic pathway and retention of the soluble amyloid protein precursor α. β-secretase activity is then inhibited by the growth of the soluble amyloid protein precursor α (86). These abnormal iron-induced changes form a *vicious circle* that leads to dysregulation of the processing of amyloid protein precursors in neurons.

CONCLUSION

Although there are reasonable doubts about the effects of cerebral ischemia on the development of Alzheimer's disease, the mounting evidence on the ischemic theory of Alzheimer's disease should not be ignored. Ignoring the numerous scientifically substantiated clinical and experimental data on the connection between brain ischemia and Alzheimer's disease will hamper not only the proper understanding of the disease mechanism but also the development of complementary and alternative strategies for the treatment and management of Alzheimer's disease. The conclusions drawn from the study of ischemia-induced Alzheimer's disease-associated proteins and their genes in the hippocampus and the medial temporal cortex, which contribute to the death of neurons, the production of the β-amyloid peptide, and neurofibrillary tangle-like formation, are important for the development of treatment goals in the therapy of Alzheimer's disease. As deposits of amyloid and tau protein may not be the cause in the pathogenesis of Alzheimer's disease, further research is needed in this field. Animal models of cerebral ischemia seem to be a useful experimental approach in determining the role of folding proteins and their genes in the neurodegenerative process of sporadic Alzheimer's disease.

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REFERENCES

1. Salminen A, Kauppinen A, Kaarniranta K. Hypoxia/ischemia activate processing of amyloid precursor protein: Impact of vascular dysfunction in the pathogenesis of Alzheimer's disease. J Neurochem. 2017;140:536–49. http://dx.doi.org/10.1111/jnc.13932
2. Pluta R. Brain ischemia: Alzheimer's disease mechanisms. New York: Nova Science Publishers, Inc.; 2019. 311 p.
3. Pluta R, Jabłoński M, Ułamek-Kozioł M, Kocki J, Brzozowska J, Januszewski S, et al. Sporadic Alzheimer's disease begins as episodes of brain ischemia and ischemically dysregulated Alzheimer's disease genes. Mol Neurobiol. 2013;48:500–15. http://dx.doi.org/10.1007/s12035-013-8439-1
4. Pluta R, Ułamek-Kozioł M, Czuczwar SJ. Brain ischemia: Alzheimer's disease mechanisms. New York: Nova Science Publishers, Inc.; 2019. Chapter XII, Shared genomic and proteomic contribution to brain ischemia and Alzheimer's disease: Ischemic etiology of Alzheimer's disease. p. 209–49.

5. Kiryk A, Pluta R, Figiel I, Mikosz M, Ułamek M, Niewiadomska G, et al. Transient brain ischemia due to cardiac arrest causes irreversible long-lasting cognitive injury. Behav Brain Res. 2011;219:1–7. http://dx.doi.org/10.1016/j.bbr.2010.12.004
6. De la Tremblaye PB, Plamondon H. Impaired conditioned emotional response and object recognition are concomitant to neuronal damage in the amygdale and perirhinal cortex in middle-aged ischemic rats. Behav Brain Res. 2011;219:227–233. http://dx.doi.org/10.1016/j.bbr.2011.01.009
7. Li J, Wang YJ, Zhang M, Fang CQ, Zhou HD. Cerebral ischemia aggravates cognitive impairment in a rat model of Alzheimer's disease. Life Sci. 2011;89:86–92. http://dx.doi.org/10.1016/j.lfs.2011.04.024
8. Brainin M, Tuomilehto J, Heiss WD, Bornstein NM, Bath PM, Teuschl Y, et al. Post Stroke Cognition Study Group. Post-stroke cognitive decline: An update and perspectives for clinical research. Eur J Neurol. 2015;22:229–38. http://dx.doi.org/10.1111/ene.12626
9. Cohan CH, Neumann JT, Dave KR, Alekseyenko A, Binkert M, Stransky K, et al. Effect of cardiac arrest on cognitive impairment and hippocampal plasticity in middle-aged rats. PLoS One. 2015;10:e0124918. http://dx.doi.org/10.1371/journal.pone.0124918
10. Surawan J, Areemit S, Tiamkao S, Sirithanawuthichai T, Saensak S. Risk factors associated with post-stroke dementia: A systematic review and meta-analysis. Neurol Int. 2017;9:7216. http://dx.doi.org/10.4081/ni.2017.7216
11. Bivard A, Lillicrap T, Maréchal B, Garcia-Esperon C, Holliday E, Krishnamurthy V, et al. Transient ischemic attack results in delayed brain atrophy and cognitive decline. Stroke. 2018;49:384–90. http://dx.doi.org/10.1161/STROKEAHA.117.019276
12. Kim JH, Lee Y. Dementia and death after stroke in older adults during a 10-year follow-up: Results from a competing risk model. J Nutr Health Aging. 2018;22:297–301. http://dx.doi.org/10.1007/s12603-017-0914-3
13. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease: The Nun Study. JAMA. 1997;277:813–17. http://dx.doi.org/10.1001/jama.1997.03540340047031
14. Hossmann KA, Schmidt-Kastner R, Grosse Ophoff B. Recovery of integrative central nervous function after one hour global cerebro-circulatory arrest in normothermic cat. J Neurol Sci. 1987;77:305–20. http://dx.doi.org/10.1016/0022-510X(87)90130-4
15. Pluta R. The role of apolipoprotein E in the deposition of β-amyloid peptide during ischemia–reperfusion brain injury. A model of early Alzheimer's disease. Ann N Y Acad Sci. 2000;903:324–34. http://dx.doi.org/10.1111/j.1749-6632.2000.tb06383.x
16. Pluta R, Ułamek M, Jabłoński M. Alzheimer's mechanisms in ischemic brain degeneration. Anat Rec. 2009;292:1863–81. http://dx.doi.org/10.1002/ar.21018
17. Pluta R, Januszewski S, Jabłoński M, Ułamek M. Factors in creepy delayed neuronal death in hippocampus following brain ischemia–reperfusion injury with long-term survival. Acta Neurochir. 2010;106(Suppl.):37–41. http://dx.doi.org/10.1007/978-3-211-98811-4_5
18. Pluta R, Ułamek M, Jabłoński M. Consideration of the ischaemic basis and treatment of Alzheimer's disease. Folia Neuropathol. 2010;48:11–26.
19. Sekeljic V, Bataveljic D, Stamenkovic S, Ułamek M, Jabłoński M, Radenovic L, et al. Cellular markers of neuroinflammation and neurogenesis after ischemic brain injury in the long-term survival rat model. Brain Struct Funct. 2012;217:411–20. http://dx.doi.org/10.1007/s00429-011-0336-7
20. Pluta R, Kida E, Lossinsky AS, Golabek AA, Mossakowski MJ, Wisniewski HM. Complete cerebral ischemia with short-term survival in rats induced by cardiac arrest: I. Extracellular accumulation of Alzheimer's β-amyloid protein precursor in the brain. Brain Res. 1994;649:323–8. http://dx.doi.org/10.1016/0006-8993(94)91081-2
21. Qi J, Wu H, Yang Y, Wand D, Chen Y, Gu Y, et al. Cerebral ischemia and Alzheimer's disease: The expression of amyloid-β and apolipoprotein E in human hippocampus. J Alzheimers Dis. 2007;12:335–41. http://dx.doi.org/10.3233/JAD-2007-12406
22. Zetterberg H, Mörtberg E, Song L, Chang L, Provuncher GK, Patel PP, et al. Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid β levels in humans. PLoS One. 2011;6(12):e28263. http://dx.doi.org/10.1371/journal.pone.0028263
23. Kato T, Hirano A, Katagiri T, Sasaki H, Yamada S. Neurofibrillary tangle formation in the nucleus basalis of Meynert ipsilateral to a massive cerebral infarct. Ann Neurol. 1988;23:620–3. http://dx.doi.org/10.1002/ana.410230617

24. Wen Y, Yang SH, Liu R, Perez EJ, Brun-Ziukemagel AM, Koulen P, et al. Cdk5 is involved in NFT-like tauopathy induced by transient cerebral ischemia in female rats. Biochim Biophys Acta. 2007;1772:473–83. http://dx.doi.org/10.1016/j.bbadis.2006.10.011
25. Mörtberg E, Zetterberg H, Nordmark J, Blennow K, Catry C, Decraemer H, et al. Plasma tau protein in comatose patients after cardiac arrest treated with therapeutic hypothermia. Acta Anaesthesiol Scand. 2011;55:1132–8. http://dx.doi.org/10.1111/j.1399-6576.2011.02505.x
26. Randall J, Mörtberg E, Provuncher GK, Fournier DR, Duffy DC, Rubertsson S, et al. Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: Results of a pilot study. Resuscitation. 2013;84:351–6. http://dx.doi.org/10.1016/j.resuscitation.2012.07.027
27. Majd S, Power JH, Koblar SA, Grantham HJ. Early glycogen synthase kinase-3 and protein phosphatase 2A independent tau dephosphorylation during global brain ischaemia and reperfusion following cardiac arrest and the role of the adenosine monophosphate kinase pathway. Eur J Neurosci. 2016;44:1987–97. http://dx.doi.org/10.1111/ejn.13277
28. Bi M, Gladbach A, van Eersel J, Ittner A, Przybyla M, van Hummel A, et al. Tau exacerbates excitotoxic brain damage in an animal model of stroke. Nat Commun. 2017;8:473. http://dx.doi.org/10.1038/s41467-017-00618-0
29. Fujii H, Takahashi T, Mukai T, Tanaka S, Hosomi N, Maruyama H, et al. Modifications of tau protein after cerebral ischemia and reperfusion in rats are similar to those occurring in Alzheimer's disease – Hyperphosphorylation and cleavage of 4- and 3-repeat tau. J Cereb Blood Flow Metab. 2017;37:2441–57. http://dx.doi.org/10.1177/0271678X16668889
30. Khan S, Yuldasheva NY, Batten TFC, Pickles AR, Kellett KAB, Saha S. Tau pathology and neurochemical changes associated with memory dysfunction in an optimized murine model of global cerebral ischaemia – A potential model for vascular dementia? Neurochem Int. 2018;118:134–44. http://dx.doi.org/10.1016/j.neuint.2018.04.004
31. Kovalska M, Tothova B, Kovalska L, Tatarkova Z, Kalenska D, Tomascova A, et al. Association of induced hyperhomocysteinemia with Alzheimer's disease-like neurodegeneration in rat cortical neurons after global ischemia-reperfusion injury. Neurochem Res. 2018;43:1766–78. http://dx.doi.org/10.1007/s11064-018-2592-x
32. Pluta R, Ułamek-Kozioł M, Januszewski S, Czuczwar SJ. Tau protein dysfunction after brain ischemia. J Alzheimers Dis. 2018;66:429–37. http://dx.doi.org/10.3233/JAD-180772
33. Pluta R, Bogucka-Kocka A, Ułamek-Kozioł M, Bogucki J, Kocki J, Czuczwar SJ. Ischemic tau protein gene induction as an additional key factor driving development of Alzheimer's phenotype changes in CA1 area of hippocampus in an ischemic model of Alzheimer's disease. Pharmacol Rep. 2018;70:881–4. http://dx.doi.org/10.1016/j.pharep.2018.03.004
34. Ishimaru H, Ishikawa K, Haga S, Shoji M, Ohe Y, Haga C, et al. Accumulation of apolipoprotein E and β-amyloid-like protein in a trace of the hippocampal CA1 pyramidal cell layer after ischaemic delayed neuronal death. Neuro Rep. 1996;7:3063–7. http://dx.doi.org/10.1097/00001756-199611250-00054
35. Lin B, Schmidt-Kastner R, Busto R, Ginsberg MD. Progressive parenchymal deposition of β-amyloid precursor protein in rat brain following global cerebral ischemia. Acta Neuropathol. 1999;97:359–68. http://dx.doi.org/10.1007/s004010050999
36. Sinigaglia-Coimbra R, Cavalheiro EA, Coimbra CG. Postischemic hyperthermia induces Alzheimer-like pathology in the rat brain. Acta Neuropathol. 2002;103:444–52. http://dx.doi.org/10.1007/s00401-001-0487-3
37. Jabłoński M, Maciejewski R, Januszewski S, Ułamek M, Pluta R. One year follow up in ischemic brain injury and the role of Alzheimer factors. Physiol Res. 2011;60(Suppl. 1):S113–19.
38. Banati RB, Gehrmann J, Wießner C, Hossmann KA, Kreutzberg GW. Glial expression of the β-amyloid precursor protein (APP) in global ischemia. J Cereb Blood Flow Metab. 1995;15:647–54. http://dx.doi.org/10.1038/jcbfm.1995.80
39. Palacios G, Mengod G, Tortosa A, Ferrer I, Palacios JM. Increased β-amyloid precursor protein expression in astrocytes in the gerbil hippocampus following ischaemia: Association with proliferation of astrocytes. Eur J Neurosci. 1995;7:501–10. http://dx.doi.org/10.1111/j.1460-9568.1995.tb00346.x
40. Nihashi T, Inao S, Kajita Y, Kawai T, Sugimoto T, Niwa M, et al. Expression and distribution of beta amyloid precursor protein and beta amyloid peptide in reactive astrocytes after transient middle cerebral artery occlusion. Acta Neurochir. 2001;143:287–95. http://dx.doi.org/10.1007/s007010170109

41. Badan I, Platt D, Kessler C, Popa-Wagner A. Temporal dynamics of degenerative and regenerative events associated with cerebral ischemia in aged rats. Gerontology. 2003;49:356–65. http://dx.doi.org/10.1159/000073763
42. Badan I, Dinca I, Buchhold B, Suofu Y, Walker L, Gratz M, et al. Accelerated accumulation of N- and C-terminal beta APP fragments and delayed recovery of microtubule-associated protein 1B expression following stroke in aged rats. Eur J Neurosci. 2004;19:2270–80. http://dx.doi.org/10.1111/j.0953-816X.2004.03323.x
43. Van Groen T, Puurunen K, Maki HM, Sivenius J, Jolkkonen J. Transformation of diffuse beta-amyloid precursor protein and beta-amyloid deposits to plaques in the thalamus after transient occlusion of the middle cerebral artery in rats. Stroke. 2005;36:1551–6. http://dx.doi.org/10.1161/01.STR.0000169933.88903.cf
44. Jendroska K, Poewe W, Daniel SE, Pluess J, Iwerssen-Schmidt H, Paulsen J, et al. Ischemic stress induces deposition of amyloid beta immunoreactivity in human brain. Acta Neuropathol. 1995;90:461–6. http://dx.doi.org/10.1007/BF00294806
45. Wiśniewski HM, Maślińska D. Beta-protein immunoreactivity in the human brain after cardiac arrest. Folia Neuropathol. 1996;34:65–71.
46. Jendroska K, Hoffmann OM, Patt S. Amyloid β peptide and precursor protein (APP) in mild and severe brain ischemia. Ann N Y Acad Sci. 1997;826:401–5. http://dx.doi.org/10.1111/j.1749-6632.1997.tb48492.x
47. Lee PH, Bang OY, Hwang EM, Lee JS, Joo US, Mook-Jung I, et al. Circulating beta amyloid peptide is elevated in patients with acute ischemic stroke. J Neurol Transm. 2005;112:1371–9. http://dx.doi.org/10.1007/s00702-004-0274-0
48. Maślińska D, Laure-Kamionowska M, Taraszewska A, Deręgowski K, Maśliński S. Immunodistribution of amyloid beta protein (Aβ) and advanced glycation end-product receptors (RAGE) in choroid plexus and ependyma of resuscitated patients. Folia Neuropathol. 2011;49:295–300.
49. Dewar D, Graham DI, Teasdale GM, McCulloch J. Alz-50 and ubiquitin immunoreactivity is induced by permanent focal cerebral ischaemia in the cat. Acta Neuropathol. 1993;86:623–9. http://dx.doi.org/10.1007/BF00294302
50. Dewar D, Graham DI, Teasdale GM, McCulloch J. Cerebral ischemia induces alterations in tau and ubiquitin proteins. Dementia. 1994;5:168–73. http://dx.doi.org/10.1159/000106716
51. Geddes JW, Schwab C, Craddock S, Wilson JL, Pettigrew LC. Alterations in tau immunostaining in the rat hippocampus following transient cerebral ischemia. J Cereb Blood Flow Metab. 1994;14:554–64. http://dx.doi.org/10.1038/jcbfm.1994.69
52. Dewar D, Dawson D. Tau protein is altered by focal cerebral ischaemia in the rat: An immunohistochemical and immunoblotting study. Brain Res. 1995;684:70–8. http://dx.doi.org/10.1016/0006-8993(95)00417-O
53. Irving EA, Yatsushiro K, McCulloch J, Dewar D. Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: Involvement of free radicals. J Cereb Blood Flow Metab. 1997;17:612–22. http://dx.doi.org/10.1097/00004647-199706000-00003
54. Majd S, Power JH, Koblar SA, Grantham HJM. Introducing a developed model of reversible cardiac arrest to produce global brain ischemia and its impact on microtubule-associated protein tau phosphorylation at Ser396. Int J Neurol Neurother. 2016;3:040. http://dx.doi.org/10.23937/2378-3001/3/1/1040
55. Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol. 2002;156:1051–63. http://dx.doi.org/10.1083/jcb.200108057
56. Mailliot C, Podevin-Dimster V, Rosenthal RE, Sergeant N, Delacourte A, Fiskum G, et al. Rapid tau protein dephosphorylation and differential rephosphorylation during cardiac arrest-induced cerebral ischemia and reperfusion. J Cereb Blood Flow Metab. 2000;20:543–9. http://dx.doi.org/10.1097/00004647-200003000-00013
57. Wen Y, Yang S, Liu R, Simpkins JW. Transient cerebral ischemia induces site-specific hyperphosphorylation of tau protein. Brain Res. 2004;1022:30–8. http://dx.doi.org/10.1016/j.brainres.2004.05.106
58. Wen Y, Yang S, Liu R, Brun-Zinkernagel AM, Koulen P, Simpkins JW. Transient cerebral ischemia induces aberrant neuronal cell cycle re-entry and Alzheimer's disease-like tauopathy in female rats. J Biol Chem. 2007;279:22684–92. http://dx.doi.org/10.1074/jbc.M311768200

59. Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, et al. Tau mediated iron export prevents ferroptotic damage after ischemic stroke. Mol Psychiatry. 2017;22:1520–30. http://dx.doi.org/10.1038/mp.2017.171
60. Pluta R, Salińska E, Puka M, Stafiej A, Lazarewicz JW. Early changes in extracellular amino acids and calcium concentrations in rabbit hippocampus following complete 15-min cerebral ischemia. Resuscitation. 1988;16:193–210. http://dx.doi.org/10.1016/0300-9572(88)90046-9
61. Uchihara T, Tsuchiya K, Kondo H, Hayama T, Ikeda K. Widespread appearance of Alz-50 immunoreactive neurons in the human brain with cerebral infarction. Stroke. 1995;26:2145–8. http://dx.doi.org/10.1161/01.STR.26.11.2145
62. Irving EA, Nicoll J, Graham DI, Dewar D. Increased tau immunoreactivity in oligodendrocytes following human stroke and head injury. Neurosci Lett. 1996;213:189–92. http://dx.doi.org/10.1016/0304-3940(96)12856-1
63. Uchihara T, Nakamura A, Arai T, Ikeda K, Tsuchiya K. Microglial tau undergoes phosphorylation independent modification after ischemia. Glia. 2004;45:180–7. http://dx.doi.org/10.1002/glia.10318
64. Shi J, Panickar KS, Yang SH, Rabbani O, Day AL, Simpkins JW. Estrogen attenuates over-expression of beta-amyloid precursor protein messenger RNA in an animal model of focal ischemia. Brain Res. 1998;810:87–92. http://dx.doi.org/10.1016/S0006-8993(98)00888-9
65. Shi J, Yang SH, Stubley L, Day AL, Simpkins JW. Hypoperfusion induces overexpression of β-amyloid precursor protein mRNA in a focal ischemic rodent model. Brain Res. 2000;853:1–4. http://dx.doi.org/10.1016/S0006-8993(99)02113-7
66. Abe K, Tanzi RE, Kogure K. Selective induction of Kunitz-type protease inhibitor domain-containing amyloid precursor protein mRNA after persistent focal ischemia in rat cerebral cortex. Neurosci Lett. 1991;125:172–4. http://dx.doi.org/10.1016/0304-3940(91)90020-T
67. Koistinaho J, Pyykonen I, Keinanen R, Hokfelt T. Expression of β-amyloid precursor protein mRNAs following transient focal ischaemia. Neuro Rep. 1996;7:2727–31. http://dx.doi.org/10.1097/00001756-199611040-00064
68. Nalivaeva NN, Fisk L, Kochkina EG, Plesneva SA, Zhuravin IA, Babusikova E, et al. Effect of hypoxia/ischemia and hypoxic preconditioning/reperfusion on expression of some amyloid-degrading enzymes. Ann N Y Acad Sci. 2004;1035:21–33. http://dx.doi.org/10.1196/annals.1332.002
69. Yan FL, Zhang J, Guan XN, Hong Z. mRNA expression and activity of ADAM17 in hippocampus after chronic cerebral hypoperfusion: Experiment with aged rats. Zhonghua Yi Xue Za Zhi. 2007;87:2515–17.
70. Pluta R, Furmaga-Jabłońska W, Maciejewski R, Ułamek-Kozioł M, Jabłoński M. Brain ischemia activates β- and γ-secretase cleavage of amyloid precursor protein: Significance in sporadic Alzheimer's disease. Mol Neurobiol. 2013;47:425–34. http://dx.doi.org/10.1007/s12035-012-8360-z
71. Blasko I, Beer R, Bigl M, Apelt J, Franz G, Rudzki D, et al. Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease β-secretase (BACE-1). J Neural Transm. 2004;111:523–36. http://dx.doi.org/10.1007/s00702-003-0095-6
72. Chen XH, Siman R, Iwata A, Meaney DF, Trojanowski JQ, Smith DH. Long-term accumulation of amyloid-β, β-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. Am J Pathol. 2004;165:357–71. http://dx.doi.org/10.1016/S0002-9440(10)63303-2
73. Wen Y, Onyewuchi O, Yang S, Liu R, Simpkins JW. Increased beta-secretase activity and expression in rats following transient cerebral ischemia. Brain Res. 2004;1009:1–8. http://dx.doi.org/10.1016/j.brainres.2003.09.086
74. Chuang CM, Hsieh CL, Lin HY, Lin JG. Panax Notoginseng Burk attenuates impairment of learning and memory functions and increases ED1, BDNF and beta-secretase immunoreactive cells in chronic stage ischemia-reperfusion injured rats. Am J Chin Med. 2008;36:685–93. http://dx.doi.org/10.1142/S0192415X08006156
75. Ye J, Pi R, Mao X, Chen X, Qin J, Xu S, et al. Alterations in mRNA expression of BACE1, cathepsin B, and glutaminyl cyclase in mice ischemic brain. Neuro Rep. 2009;20:1456–60. http://dx.doi.org/10.1097/WNR.0b013e328332024a
76. Tanimukai H, Imaizumi K, Kudo T, Katayama T, Tsuda M, Takagi T, et al. Alzheimer-associated presenilin-1 gene is induced in gerbil hippocampus after transient ischemia. Mol Brain Res. 1998;54:212–18. http://dx.doi.org/10.1016/S0169-328X(97)00337-9

77. Pennypacker KR, Hernandez H, Benkovic S, Morgan DG, Willing AE, Sanberg PR. Induction of presenilins in the rat brain after middle cerebral arterial occlusion. Brain Res Bull. 1999;48:539–43. http://dx.doi.org/10.1016/S0361-9230(99)00031-3
78. Polavarapu R, An J, Zhang C, Yepes M. Regulated intramembrane proteolysis of the low-density lipoprotein receptor-related protein mediates ischemic cell death. Am J Pathol. 2008;172:1355–62. http://dx.doi.org/10.2353/ajpath.2008.070975
79. Kocki J, Ułamek-Kozioł M, Bogucka-Kocka A, Januszewski S, Jabłoński M, Gil-Kulik P, et al. Dysregulation of amyloid precursor protein, β-secretase, presenilin 1 and 2 genes in the rat selectively vulnerable CA1 subfield of hippocampus following transient global brain ischemia. J Alzheimers Dis. 2015;47:1047–56. http://dx.doi.org/10.3233/JAD-150299
80. Pluta R, Kocki J, Ułamek-Kozioł M, Petniak A, Gil-Kulik P, Januszewski S, et al. Discrepancy in expression of β-secretase and amyloid-β protein precursor in Alzheimer-related genes in the rat medial temporal lobe cortex following transient global brain ischemia. J Alzheimers Dis. 2016;51:1023–31. http://dx.doi.org/10.3233/JAD-151102
81. Pluta R, Kocki J, Ułamek-Kozioł M, Bogucka-Kocka A, Gil-Kulik P, Januszewski S, et al. Alzheimer-associated presenilin 2 gene is dysregulated in rat medial temporal lobe cortex after complete brain ischemia due to cardiac arrest. Pharmacol Rep. 2016;68:155–61. http://dx.doi.org/10.1016/j.pharep.2015.08.002
82. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14:535–62. http://dx.doi.org/10.1016/j.jalz.2018.02.018
83. Ułamek-Kozioł M, Kocki J, Bogucka-Kocka A, Januszewski S, Bogucki J, Czuczwar SJ, et al. Autophagy, mitophagy and apoptotic gene changes in the hippocampal CA1 area in a rat ischemic model of Alzheimer's disease. Pharmacol Rep. 2017;69:1289–94. http://dx.doi.org/10.1016/j.pharep.2017.07.015
84. Ułamek-Kozioł M, Kocki J, Bogucka-Kocka A, Petniak A, Gil-Kulik P, Januszewski S, et al. Dysregulation of autophagy, mitophagy, and apoptotic genes in the medial temporal lobe cortex in an ischemic model of Alzheimer's disease. J Alzheimers Dis. 2016;54:113–21. http://dx.doi.org/10.3233/JAD-160387
85. Eid R, Arab NTT, Greenwood MT. Iron mediated toxicity and programmed cell death: A review and a re-examination of existing paradigms. Biochim Biophys Acta Mol Cell Res 2017;1864:399–430. http://dx.doi.org/10.1016/j.bbamcr.2016.12.002
86. Chen Y, Chen W, Huang X, Xu Y, Zhang H. Iron dysregulates APP processing accompanying with sAPPα cellular retention and β-secretase inhibition in rat cortical neurons. Acta Pharmacol Sin. 2018;39:177–83. http://dx.doi.org/10.1038/aps.2017.113
87. Masaldan S, Bush AI, Devos D, Rolland AS, Moreau C. Striking while the iron is hot: Iron metabolism and ferroptosis in neurodegeneration. Free Radic Biol Med. 2019;133:221–33. http://dx.doi.org/10.1016/j.freeradbiomed.2018.09.033
88. Ndayisaba A, Kaindlstorfer C, Wenning GK. Iron in neurodegeneration – Cause or consequence? Front Neurosci. 2019;13:180. http://dx.doi.org/10.3389/fnins.2019.00180
89. Bandyopadhyay S, Rogers JT. Alzheimer's disease therapeutics targeted to the control of amyloid precursor protein translation: Maintenance of brain iron homeostasis. Biochem Pharmacol. 2014;88:486–94. http://dx.doi.org/10.1016/j.bcp.2014.01.032
90. Kounnas MZ, Moir RD, Rebeck GW, Bush AI, Argraves WS, Tanzi RE, et al. LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-amyloid precursor protein and mediates its degradation. Cell. 1995;82:331–40. http://dx.doi.org/10.1016/0092-8674(95)90320-8
91. Shin RW, Saido TC, Maeda M, Kitamoto T. Novel alpha-secretase cleavage of Alzheimer's amyloid beta precursor protein in the endoplasmic reticulum of COS7 cells. Neurosci Lett. 2005;376:14–19. http://dx.doi.org/10.1016/j.neulet.2004.11.032
92. Kepp KP. Alzheimer's disease due to loss of function: A new synthesis of the available data. Prog Neurobiol. 2016;143:36–60. http://dx.doi.org/10.1016/j.pneurobio.2016.06.004

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Contributing Factors of Neurodegeneration in Alzheimer's Disease

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Abstract: Alzheimer's Disease (AD) affects at least 5.7 million Americans, and it is the sixth leading cause of death in the United States. At the onset, patients experience minor memory problems. Next, impairments in speech and motor function manifest as a limitation to well-being and independence. Slowing this pandemic rise is critical, since AD also bears a huge socioeconomical burden. Unfortunately, there is limited prevention and no effective cure has been found, as all clinical trials for promising AD drugs have failed thus far. The pathological hallmarks of AD include amyloid-β plaques (Aβ), neurofibrillary tangles (NFT), and neuroinflammation. Other factors include *APOE4* and environmental stressors, such as metal dyshomeostasis, which contribute to AD pathogenesis. Herein, we review major contributing factors involved in AD pathophysiology. Deeper understanding of associated molecular mechanisms underlying AD pathogenesis is critical for developing novel AD theranostics.

Keywords: Amyloid-β; amyloid precursor protein; metals; β-site of APP cleaving enzyme 1 (BACE1 or β-secretase); neurotoxicity; NF-κB; presenilins

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is the most represented form of dementia. It is the 3rd most common disease affecting the population, inflicting at least 5.7 million Americans as the trend continues to rise at a pandemic rate. It is the leading cause of age-dependent disability on a global scale (1). AD reduces the quality of a patient's life, as irreversible cognitive decline becomes apparent due to pathological and morphological changes such as cortical atrophy, neuroinflammation, loss of synaptic connections, and cellular death (2) leaving the individual dependent on significant care, as their memories and motor function deteriorate.

Fortunately, technological advances have afforded researchers the ability to characterize neuronal loss in the hippocampus and cortices (3). Additional work has acknowledged perspectives on multifaceted complexities that have linked risk-associated genes and environmental factors to these differences (4). For example, increased exposure to air pollution, chemicals, and ionizing radiation is harmful (5, 6) and potentially contributes to dementia-related diseases. Unfortunately, AD has no efficacious treatments, and thus, the disease is a critical health concern and has incurred a colossal socioeconomic burden. Recently, the Alzheimer's Association reported the cost as $236 billion and is projected to rise to $1.1 trillion in 2050 (1). Therefore, identifying an accurate diagnosis and effective treatment is urgent.

The hallmarks of AD are evident, with neuroinflammation and aggregated Aβ plaques followed by neurofibrillary tangles (NFT). In fact, recent studies observed plaque deposits within cognitively normal individuals up to 20 years before the onset of cognitive decline (7). Why Aβ fibrils aggregate into plaques has yet to be elucidated; however, there is evidence that its exacerbated presence is toxic to neuronal cells. For example, Aβ inhibits respiratory function, reduces ATP levels (8), and leads to mitochondrial dysfunction (9). In vitro studies of PC12 cells observed depolarization of the mitochondrial membrane potential and decreased activity of mitochondrial electron transport chain complexes. As Aβ aggregates, it leads to signaling impairments causing the cells to undergo apoptosis. Anti-Aβ drugs tested in human clinical trial have failed to produce promising results. As such, the credibility of the amyloid hypothesis has been questioned, and the true role of Aβ is currently being investigated.

AMYLOID PRECURSOR PROTEIN

Amyloid plaques, or the insoluble Aβ peptides, in the brain form through the cleavage of amyloid precursor protein (APP) by the b-site of APP cleaving enzyme 1 (BACE1 or β-secretase) and γ-secretase (10, 11). APP is located on chromosome 21, and it is a type I transmembrane protein involved in secretory and endocytic processes (12). It contains a metal-binding domain, heparin, collagen, laminin, and a protease inhibitor domain (13). Although the function of APP is unclear, there is evidence to suggest that the ectodomain of APP may be involved in cell adhesion, trophic support, cell growth, and differentiation of neuronal stem cells (14). Conversely, the intracellular domain may modulate mitochondrial function (15).

APP can be processed through two pathways: the amyloidogenic pathway and the non-amyloidogenic pathway. In the amyloidogenic pathway, β-secretase cleaves APP at amino acid 671 releasing APP β (sAPPβ). Next, the CTF99 embedded in the plasma membrane is cleaved by γ-secretase, made up of 4 subunits (16, 17), including the catalytic domains *Presenilin1* gene (*PS1*) and *Presenilin2* (*PS2*) (18). BACE1 is a rate-limiting step for Aβ production, and knockout studies result in complete inhibition of Aβ generation (19). In the non-amyloidogenic pathway, APP is cleaved by α-secretase at amino acid 687, releasing soluble APPα (sAPPα). The remaining protein, CTF83 is cleaved by γ-secretase releasing a soluble p3 fragment. α-secretase belongs to a family of single-pass transmembrane and secretes zinc-containing endopeptidases that are dominant in neurons (20). Aggregated Aβ function in normal physiology remains to be elucidated (19); however, Aβ disrupts postsynaptic trafficking in glutamate receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (21) and N-Methyl-D-Aspartate (NMDA) receptors (22). Their actions may be important for learning and memory, and synaptic plasticity (23–25). Aβ has also been shown to modulate the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) through interaction with KCC2 (26). A study by Senechal et al. investigated APP knockout mice and discovered dysregulated long-term potentiation (LTP) and learning deficits (27, 28). Moreover, theta–gamma oscillation phase–amplitude coupling was also diminished in regions of the parietal cortex and hippocampus compared to the wild type (27). As such, the function of APP is complex, and data so far have linked the role of the protein in neurite growth (29–31), axon guidance (32), and neuronal cell adhesion (33).

β-SITE OF APP CLEAVING ENZYME 1

β-site of APP cleaving enzyme 1 (BACE1) is a major drug target for therapy (34) because its expression correlates not only with the onset of AD but also with glucose intolerance. Importantly, this downstream effect is a risk factor for diabetes. Studies on mouse models which inhibit BACE1 expression resulted in improvements in glucose homeostasis, lowered leptin levels, and decreased hypothalamic inflammation (35, 36). However, depletion of BACE1 leads to other harmful effects as evidence suggests it important in regulating adult hippocampal neurons responsible for memory (37, 38) and other important neuronal processes. For example, mice models that possess faulty BACE1 expression result in deficits in synaptic transmission and plasticity in the hippocampal region (39). Furthermore, the cell adhesion molecule Neuregulin-1 (Nrg1), which must be cleaved by BACE1, mediates radial migration of glutamatergic and GABAergic neurons. It is also responsible for myelination and synaptic plasticity (40) and is required for the formation of new synapses while strengthening existing ones. Interestingly, BACE1 null mice result in a reduction of Nrg1 cleavage, resulting in characteristics of schizophrenia (41). Similarly, Sez6 is a protein that is concentrated in areas associated with morphological plasticity. This includes areas within the hippocampus and cerebellum in postnatal brains. Sez6 is also cleaved by BACE1 and mediates dendritic arborization of cortical neurons (42) which is critical for neuronal transfer of information. Thus, defective BACE1 leads

to poor motor coordination, weak balance, and cognitive deficits. Lastly, BACE1 deficiency also affects Jagged-1 (Jag1) that regulates astrogenesis/neurogenesis through Notch signaling pathway (43, 44) and contributes to memory formation. Therefore, suppression of this enzyme is a double-edged sword and more research is needed to help in AD patients.

GENETIC RISK FACTORS OF ALZHEIMER DISEASE PATHOLOGY

There are two forms of AD: sporadic and familial. The majority of the cases (approximately 95%) are classified as sporadic late-onset AD (LOAD), while about 5% are classified as familial early-onset l AD (EOAD) with an autosomal dominant inheritance pattern. Sporadic AD is influenced by complex genetic variants combined with environmental factors (45). However, there is little evidence to define how this occurs. Early onset is caused by rare mutations in three genes located on chromosome 21 (46, 47) and chromosome 14 (48). The summary of genetic mutations implicated in LOAD is shown in Table 1 and EOAD is given in Table 2 (45).

TABLE 1 **A summary of genetic mutations implicated in LOAD**

Gene	Protein	Chromosome	Risk change %	Proposed molecular phenotype
APOE	Apolipoprotein E	19q13	~400–1500%	Clearance of Aβ Lipid metabolism
ABCA7	ATP-binding cassette subfamily A member 7	19p13.3	~20%	Lipid metabolism Cellular signaling
BINI	Bridgin integrator 1	2q14	~15%	Production of Aβ Clearance of Aβ Cellular signaling
CR1	Complement component (3b/4b) receptor 1	1q32	~15%	Clearance of Aβ Innate immunity
PICALM	Phosphadylinositol-binding clathrin assembly molecule	11q14	~15%	Production of Aβ Clearance of Aβ Cellular signaling
CD2AP	CD2-associated protein	6p12.3	~10%	Cellular Signaling
CD33	CD33 (Siglec 3)	19q13.3	~10%	Innate immunity Degradation of
CLU	Clusterin	8p21.1	~10%	Clearance of Aβ Innate immunity
EPHA1	EPH receptor A1	7q34	~10%	Cellular signaling Innate immunity
ATXN1	Ataxin 1	6p22.3	NA	Production of Aβ

TABLE 2 A summary of genetic mutations implicated in EAOD

Gene	Protein	Chromosome	Molecular phenotype
APP	Amyloid β protein precursor	21q21	Increased $A\beta_{42}/A\beta_{40}$ ratio Increased Aβ production Increased Aβ aggregation
PSEN1	Presenilin 1	14q24	Increased $A\beta_{42}/A\beta_{40}$ ratio
PSEN2	Presenilin 2	1q31	Increased $A\beta_{42}/A\beta_{40}$ ratio

Mutations in *APP, Presenilin1* (*PS1*), and *Presenilin2* (*PS2*) genes have been integral in the development of AD as they cause a disruption in the ratio of Aβ42 production (49). In normal physiology, presenilins, needed for the production of Aβ peptides via both β- and γ-secretases-mediated cleavage (50, 51), are responsible for autosomal transmission and the promotion of amyloid plaque. *PS1* regulates calcium homeostasis and mediates neurotransmission (52, 53). The largest amount of mutations occurs for *PS1* at an estimate of 200 mutants, whereas *APP* and *PS2* have 10–25 mutants on the AD and frontotemporal dementia mutation database.

Meta-analysis revealed at least 15 potential loci where variations may predispose one to developing AD (46). However, a particular gene appears to be the most burdensome, the *ApoE* gene. It has four different isoforms: ApoEe1, ApoEe2, ApoEe3, and ApoEe4. Apolipoprotein E (ApoE) regulates synaptic function, promotes plasticity, increases the number of dendritic spines, and regulates protein trafficking across neurons (54). It is responsible for the regulation of triglyceride and cholesterol metabolism. Binding of lipidated ApoE facilitates Aβ uptake in an isoform-dependent manner, and inhibited clearance contributes to Aβ accumulation. One variant of *ApoE* gene has been identified as the largest risk factor for late-onset AD through computational analysis (55–57). It is important to note that possessing ApoEe4 over the e3 (common) or e2 (other variant) alleles is not enough to cause AD but it acts as a determinant which increases overtime as the patients ages (45). Analyses reveal that a heterozygous pair increases AD by threefold, whereas a homozygous pair increases the risk by 15-fold (58). It is thought to be the least effective in binding to, and facilitating the uptake of, Aβ. Additionally, its strong ties to neurovascular dysfunction further confirm its contribution in AD manifestation (56). The allele can be investigated for potential biomarkers and to unearth new targets for AD drug discovery due to significant clinical and neurobiological correlations. Among them, *ApoE e4* allele and low CSF level of Aβ42 have been reported (59). Patients with the *e4 allele* tend to present with early-onset memory impairment, decrease in global cognitive function, and weak episodic memory (60). Interestingly, the *ApoE* e2 variant seems to reduce the risk of dementia compared to the common e3 allele, despite its association with an increased amyloid burden (56). Overall, monitoring *ApoE* gene can play an important role in understanding the AD pathophysiology and be used as an assessment tool for at-risk patients.

Other genes that have a strong association with late-onset AD include SORL1, which mediates protein trafficking (61), and ACE, which regulates blood pressure (62). Furthermore, testing a single nucleotide for any association with disease

pathology can be accomplished through GWAS technology. One avenue leads to the discovery of GRB2, which mediates tau phosphorylation and has a high affinity for APP and the presinilins (63, 64). Other findings identified ATXN1, which affects Aβ levels by modulating β-secretase levels and cleavage of APP (65), and BIN1 (66), which is highly expressed in the central nervous system and plays a role in receptor-mediated endocytosis (67). Furthermore, ADAM10 mutations have impaired enzyme activity and lead to the onset of AD in the elderly (67). Lastly, CD33 has been an interesting discovery because it helps strengthen that Aβ acts as an AMP (11, 68–70).

THE NEUROIMMUNE SYSTEM

There are many challenges in understanding the complexity of inflammation in relation to AD in order to develop appropriate therapeutics. Clinical analysis of AD patients exhibited chronic neuroinflammation, insufficient energy metabolism, and redox stress in postmortem brains (71). These observations have been replicated in both animal and cell culture models. Increased inflammatory cascade by microglia has been observed in areas of Aβ deposits and activation of NF-κB (72, 73).

Due to their high affinity for Aβ deposits, understanding the role of microglia may help identify therapeutic targets. In brief, microglia are recognized as the brain macrophage and play an integral role in housekeeping. Upon signal detection, they act to remove debris, toxins, pathogens, and apoptotic neurons (74, 75) by releasing a cascade of inflammatory factors. As such, they release reactive oxygen species and Th1 cytokines including interleukin 1-beta (IL-1β), IL-6, tumor necrosis factor alpha (TNF-α), and interferon-gamma (76) to ramp up the immune system. Furthermore, they are integral in upregulating MCHII complexes, leading to an inflammatory cascade in innate immune response in many disorders such as Parkinson's disease, HIV, and multiple sclerosis (77–80). In AD brain, microglia are constantly aggregated around Aβ plaques (81) to form a barrier between healthy tissue and areas of injured or infected tissue. Since there is no evidence to suggest microglia can degrade Aβ, they undergo a state of compromised phagocytosis, in which the semi-degraded Aβ are ultimately expelled from the microglial cell (82) causing a dysregulation of homeostasis. Extended exposure to Aβ leads to disrupted calcium homeostasis within astrocytic cells, which also leads to degeneration of neurons (83).

NF-κB regulates the expression of more than 400 genes (72) and can be induced by ROS, interleukinIL-1β, TNF-α, bacterial lipopolysaccharides (LPS), isoproterenol, and ionizing radiation (73, 84). Its activation is dependent on growth factors and the neurotransmitter, glutamate (85). Thus, NF-κB plays an important role in DNA transcription and cellular survival. In general, high levels of NF-κB expression are associated with normal aging and upregulate microglial activity (85–87). This overexpression increases the susceptibility for AD through upregulating *BACE1* and *APP* genes (88).

Furthermore, rodent models have demonstrated the outcomes of unregulated NF-κB, resulting in a destructive feedback loop (89, 90). For example, mice that overexpressed NF-κB had clinical signs of increased apoptosis in the hippocampal

region through triggering TNF-α and iNOS when exposed to neurotoxins (91, 92). Moreover, drosophila studies that overexpressed NF-κB in the hypothalamus-like pars intercerebralis resulted in deficits in learning, inadequate memory consolidation, and increase in mortality rates compared to the controls (93). Upon clinical analysis, imaging studies resulted in severe neurodegeneration (94).

Studies regarding the relationship between AD and lifestyle choices concluded that an increased risk of AD was associated with diabetes, high blood pressure, and smoking (95–97). Type 2 diabetes mellitus (T2DM) increases a patient's risk of developing AD by over 50% (45), and it affects the increase of Aβ pathology by its ability to upregulate NF-κB and the expression of BACE1 (98, 99). As researchers continue their efforts in drug therapeutic development, alternative approaches have been sought, including cognitive exercises that have improved the production of dopamine and vitamin C (100, 101). Rats that were subjected to proinflammatory diets, and adhered to aerobic exercise, resulted in attenuated NF-κB expression in the liver and muscles. Similarly, regular exercise resulted in an increase of endurance, cognition, and performance (102–104). Unfortunately, standard models are not adequate in analyzing the effect of nutrition on the onset of AD, and no study to date can definitively state the relationship (101).

BLOOD–BRAIN BARRIER

The blood–brain barrier (BBB) plays a vital role in the longevity of an individual's health, and it is responsible for the clearance of Aβ; thus, any insult that compromises the integrity of BBB can cause neuronal cells damage (105–107). New studies have observed the progression of AD along with compromised BBB (108). This negative effect is alarming as any damage to the neurovascular unit (NVU) results in toxic substance leaking into the CNS circulating in the blood. In fact, the mechanism of transporting Aβ out of brain is impaired in AD patients, which contributes substantially to its accumulation (107). One example is the dysfunction of P-glycoprotein (Pgp) (109) resulting in increased deposits and age-associated cognitive impairments. Furthermore, mediating glucose transport for neuronal functionality is integral for astrocytes and neurons, and expression of GLUTs is downregulated in patients. This decreases brain energy supply as confirmed by brain imaging studies (110). Other risks associated with an impaired BBB lead to insufficient nutrient supply and toxin removal, and altered protein expression, all impacting and upregulating the role of neurodegeneration (111, 112). Although it is not elucidated how the mechanism works, therapeutic interventions in alleviating the disease progression are necessary. Recent findings conclude that AD risk factors can be modulated with lifestyle changes in regard to increase in educational levels, exercise, and healthy dietary choices (113–115).

METALS

Strong evidence suggests that biometals in the brains of AD patients are insufficiently maintained, thereby promoting cognitive loss. Due to their structure and function, the proteins that play a role in AD pathophysiology have capabilities

of interacting with metals, especially zinc and copper. Other transitional metals include lead, aluminum, and iron, which may negatively impact human health if the homeostasis is not maintained (116, 117). Neuronal damage can occur due to dysregulation of integral metals needed to maintain brain function. The accumulation of Cu ions has been identified around plaques in postmortem AD brains (118), suggesting the impact of Cu on AD progression. As such, excessive dietary Cu on high-cholesterol diet in rabbits and AD mouse model induces hallmark pathologies. Research has found that chronic exposure to Cu contributes to an increased risk of AD by facilitating Aβ accumulation (118).

Additionally, zinc regulates many proteins such as SNAP25, PSD95, AMPA receptors, and NMDA receptors. ZnT3, a zinc transporter, allows for the release of zinc from neurons into synapses and is involved in cognition and memory. The disruption in mechanism results in cognitive decline (120). Likewise, AD mouse brains have irregular protein levels of CamKII, spinophilin, NMDA receptors, and BDNF (119, 121). Interestingly, AD transgenic mice studies indicated Aβ amyloid aggregated in areas of high Fe, Cu, and Zn levels, indicating accumulation of metals within the brain promotes the aggregation of the Aβ peptides (122, 123). Recent studies have found that APP can regulate iron levels in the brain by removing it from cells, similar to ceruloplasmin. In AD, this activity is decreased by 70% in cortical tissue (122, 123). Tau knockout mice lacked the ability to clear out iron and developed age-dependent neurodegeneration. Rescue studies provided clues that quinoline activity may be a possible therapeutic for AD (124).

PBT2, currently in clinical trials, is a disease modifying drug that does not act like a chelator but as an ionophore (119, 121). Administration of PBT2 for 12 weeks improved mild forms of AD cases through executive function and composite cognitive z-scores and reduced the levels of Aβ in cerebrospinal fluid (125, 126). Other studies also showed increased neurite outgrowth in vitro and decreased tau phosphorylation (121, 127).

Iron is critical for maintaining neuronal tissue and is involved in the synthesis of myelin and neurotransmitters. Conversely, excessive accumulation can enhance Aβ production and tau dysfunction leading to neuronal cell death. Parallel to how iron increases expression of ferritin and ferroportin, iron also increases the processing of APP (128, 129). This causes formation of senile plaques and leads to oxidative stress, resulting in oligomerization and more Aβ generation (130). Iron dysregulation increases NFT (131) creating an iron-rich population within oxidatively stressed environments (132). Quantitative mapping that displays an increase in iron loading shows a strong predictor for cognitive decline. The disruption of iron levels affects neuronal populations within the hippocampus through Fenton and Haber–Weiss reactions (133), producing oxidative lipids that further increase the neurotoxicity and AD pathogenesis (134). As stated, NFT is the integral for trafficking APP to neuronal membrane to facilitate iron efflux from neurons (122, 135), and thus, the loss of tau expression increases the risk for cognitive loss and cortical atrophy in mice (124).

The effects of aluminum on neurodegeneration have attracted attention since it can cause mitochondrial dysfunction and ATP depletion at the cellular level, and decline in memory and cognitive performance on a psychiatric level (136, 137). It can also cause apoptosis in neurons (138). Biopsy studies have confirmed elevated levels of aluminum in LOAD brains, possible source being drinking water (139).

CONCLUSION

AD significantly reduces patients' quality of life. Therefore, there is an urgent need to develop early detection diagnostics and preventive measures to slow the progress of the onset until the discovery of a cure. Serial failures of clinical trials for AD experimental drugs have led us to reevaluate the pathology of this devastating disease and to embark on further understanding of the underlying AD pathophysiology and associated contributing factors. Agents against targets such as BACE1 and APP amyloidosis have proved to be ineffective against AD progression so far. Therefore, further studies in AD pathogenic mechanisms and future utility of artificial intelligence (AI)-based drug discovery tools may aid in developing novel theranostic agents for AD (140, 141).

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REFERENCES

1. Alzheimer's Association. 2016 Alzheimer's disease facts and figures. Alzheimers Dement. 2016;12(4):459–509. http://dx.doi.org/10.1016/j.jalz.2016.03.001
2. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002;298(5594):789–91. http://dx.doi.org/10.1016/j.jalz.2016.03.001
3. Hossini AM, Megges M, Prigione A, Lichtner B, Toliat MR, Wruck W, et al. Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. BMC Genomics. 2015;16:84. http://dx.doi.org/10.1186/s12864-015-1262-5
4. Yuede CM, Timson BF, Hettinger JC, Yuede KM, Edwards HM, Lawson JE, et al. Interactions between stress and physical activity on Alzheimer's disease pathology. Neurobiol Stress. 2018;8:158–71. http://dx.doi.org/10.1016/j.ynstr.2018.02.004
5. Calderon-Garciduenas L, Vojdani A, Blaurock-Busch E, Busch Y, Friedle A, Franco-Lira M, et al. Air pollution and children: Neural and tight junction antibodies and combustion metals, the role of barrier breakdown and brain immunity in neurodegeneration. J Alzheimers Dis. 2015;43(3):1039–58. http://dx.doi.org/10.3233/JAD-141365
6. Oudin A, Forsberg B, Adolfsson AN, Lind N, Modig L, Nordin M, et al. Traffic-related air pollution and dementia incidence in Northern Sweden: A longitudinal study. Environ Health Perspect. 2016;124(3):306–12. http://dx.doi.org/10.1289/ehp.1408322
7. Burns JM, Swerdlow RH. Backwaters and rapids on the amyloid river. Neurology. 2013;80(10):878–9. http://dx.doi.org/10.1212/WNL.0b013e3182840d14
8. Rhein V, Baysang G, Rao S, Meier F, Bonert A, Muller-Spahn F, et al. Amyloid-beta leads to impaired cellular respiration, energy production and mitochondrial electron chain complex activities in

human neuroblastoma cells. Cell Mol Neurobiol. 2009;29(6–7):1063–71. http://dx.doi.org/10.1007/s10571-009-9398-y
9. Swerdlow RH. Mitochondria and cell bioenergetics: Increasingly recognized components and a possible etiologic cause of Alzheimer's disease. Antioxid Redox Signal. 2012;16(12):1434–55. http://dx.doi.org/10.1089/ars.2011.4149
10. Zhang H, Ma Q, Zhang YW, Xu H. Proteolytic processing of Alzheimer's beta-amyloid precursor protein. J Neurochem. 2012;120(Suppl 1):9–21. http://dx.doi.org/10.1111/j.1471-4159.2011.07519.x
11. Bertram L, Tanzi RE. The genetic epidemiology of neurodegenerative disease. J Clin Invest. 2005;115(6):1449–57. http://dx.doi.org/10.1172/JCI24761
12. Matsui T, Ingelsson M, Fukumoto H, Ramasamy K, Kowa H, Frosch MP, et al. Expression of APP pathway mRNAs and proteins in Alzheimer's disease. Brain Res. 2007;1161:116–23. http://dx.doi.org/10.1016/j.brainres.2007.05.050
13. Dawkins E, Small DH. Insights into the physiological function of the beta-amyloid precursor protein: Beyond Alzheimer's disease. J Neurochem. 2014;129(5):756–69. http://dx.doi.org/10.1111/jnc.12675
14. Young-Pearse TL, Chen AC, Chang R, Marquez C, Selkoe DJ. Secreted APP regulates the function of full-length APP in neurite outgrowth through interaction with integrin beta1. Neural Dev. 2008;3:15. http://dx.doi.org/10.1186/1749-8104-3-15
15. Muller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. Prog Neurobiol. 2008;85(4):393–406. http://dx.doi.org/10.1016/j.pneurobio.2008.05.002
16. Bolduc DM, Montagna DR, Gu Y, Selkoe DJ, Wolfe MS. Nicastrin functions to sterically hinder gamma-secretase-substrate interactions driven by substrate transmembrane domain. Proc Natl Acad Sci U S A. 2016;113(5):E509–18. http://dx.doi.org/10.1073/pnas.1512952113
17. Holmes O, Paturi S, Selkoe DJ, Wolfe MS. Pen-2 is essential for gamma-secretase complex stability and trafficking but partially dispensable for endoproteolysis. Biochemistry. 2014;53(27):4393–406. http://dx.doi.org/10.1021/bi500489j
18. Zhang YW, Thompson R, Zhang H, Xu H. APP processing in Alzheimer's disease. Mol Brain. 2011;4:3. http://dx.doi.org/10.1186/1756-6606-4-3
19. Kumar DK, Choi SH, Washicosky KJ, Eimer WA, Tucker S, Ghofrani J, et al. Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. Sci Transl Med. 2016;8(340):340ra72. http://dx.doi.org/10.1126/scitranslmed.aaf1059
20. Kuhn PH, Wang H, Dislich B, Colombo A, Zeitschel U, Ellwart JW, et al. ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. EMBO J. 2010;29(17):3020–32. http://dx.doi.org/10.1038/emboj.2010.167
21. Gu Z, Liu W, Yan Z. {beta}-Amyloid impairs AMPA receptor trafficking and function by reducing Ca2+/calmodulin-dependent protein kinase II synaptic distribution. J Biol Chem. 2009;284(16):10639–49. http://dx.doi.org/10.1074/jbc.M806508200
22. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al. Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci. 2005;8(8):1051–8. http://dx.doi.org/10.1038/nn1503
23. Hick M, Herrmann U, Weyer SW, Mallm JP, Tschape JA, Borgers M, et al. Acute function of secreted amyloid precursor protein fragment APPs alpha in synaptic plasticity. Acta Neuropathol. 2015;129(1):21–37. http://dx.doi.org/10.1007/s00401-014-1368-x
24. Taylor CJ, Ireland DR, Ballagh I, Bourne K, Marechal NM, Turner PR, et al. Endogenous secreted amyloid precursor protein-alpha regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. Neurobiol Dis. 2008;31(2):250–60. http://dx.doi.org/10.1016/j.nbd.2008.04.011
25. Preat T, Goguel V. Role of drosophila amyloid precursor protein in memory formation. Front Mol Neurosci. 2016;9:142. http://dx.doi.org/10.3389/fnmol.2016.00142
26. Doshina A, Gourgue F, Onizuka M, Opsomer R, Wang P, Ando K, et al. Cortical cells reveal APP as a new player in the regulation of GABAergic neurotransmission. Sci Rep. 2017;7(1):370. http://dx.doi.org/10.1038/s41598-017-00325-2
27. Zhang X, Zhong W, Brankack J, Weyer SW, Muller UC, Tort AB, et al. Impaired theta-gamma coupling in APP-deficient mice. Sci Rep. 2016;6:21948. http://dx.doi.org/10.1038/srep21948

28. Senechal Y, Kelly PH, Dev KK. Amyloid precursor protein knockout mice show age-dependent deficits in passive avoidance learning. Behav Brain Res. 2008;186(1):126–32. http://dx.doi.org/10.1016/j.bbr.2007.08.003
29. Hoareau C, Borrell V, Soriano E, Krebs MO, Prochiantz A, Allinquant B. Amyloid precursor protein cytoplasmic domain antagonizes reelin neurite outgrowth inhibition of hippocampal neurons. Neurobiol Aging. 2008;29(4):542–53. http://dx.doi.org/10.1016/j.neurobiolaging.2006.11.012
30. Rama N, Goldschneider D, Corset V, Lambert J, Pays L, Mehlen P. Amyloid precursor protein regulates netrin-1-mediated commissural axon outgrowth. J Biol Chem. 2012;287(35):30014–23. http://dx.doi.org/10.1074/jbc.M111.324780
31. Chen K, Lu H, Gao T, Xue X, Wang C, Miao F. Synergic interaction between amyloid precursor protein and neural cell adhesion molecule promotes neurite outgrowth. Oncotarget. 2016;7(12):14199–206. http://dx.doi.org/10.18632/oncotarget.7348
32. Wang B, Li H, Mutlu SA, Bowser DA, Moore MJ, Wang MC, et al. The amyloid precursor protein is a conserved receptor for slit to mediate axon guidance. eNeuro. 2017;4(3):ENEURO.0185-17.2017. http://dx.doi.org/10.1523/ENEURO.0185-17.2017
33. Sosa LJ, Caceres A, Dupraz S, Oksdath M, Quiroga S, Lorenzo A. The physiological role of the amyloid precursor protein as an adhesion molecule in the developing nervous system. J Neurochem. 2017;143(1):11–29. http://dx.doi.org/10.1111/jnc.14122
34. Evin G, Hince C. BACE1 as a therapeutic target in Alzheimer's disease: Rationale and current status. Drugs Aging. 2013;30(10):755–64. http://dx.doi.org/10.1007/s40266-013-0099-3
35. Meakin PJ, Harper AJ, Hamilton DL, Gallagher J, McNeilly AD, Burgess LA, et al. Reduction in BACE1 decreases body weight, protects against diet-induced obesity and enhances insulin sensitivity in mice. Biochem J. 2012;441(1):285–96. http://dx.doi.org/10.1042/BJ20110512
36. Zhao WQ, Townsend M. Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease. Biochim Biophys Acta. 2009;1792(5):482–96. http://dx.doi.org/10.1016/j.bbadis.2008.10.014
37. Chatila ZK, Kim E, Berle C, Bylykbashi E, Rompala A, Oram MK, et al. BACE1 regulates proliferation and neuronal differentiation of newborn cells in the adult hippocampus in mice. eNeuro. 2018;5(4):pii: ENEURO.0067-18.2018. http://dx.doi.org/10.1523/ENEURO.0067-18.2018
38. Vassar R. BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. Alzheimers Res Ther. 2014;6(9):89. http://dx.doi.org/10.1186/s13195-014-0089-7
39. Dominguez D, Tournoy J, Hartmann D, Huth T, Cryns K, Deforce S, et al. Phenotypic and biochemical analyses of BACE1- and BACE2-deficient mice. J Biol Chem. 2005;280(35):30797–806. http://dx.doi.org/10.1074/jbc.M505249200
40. Mei L, Nave KA. Neuregulin-ERBB signaling in the nervous system and neuropsychiatric diseases. Neuron. 2014;83(1):27–49. http://dx.doi.org/10.1016/j.neuron.2014.06.007
41. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, et al. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet. 2002;71(4):877–92. http://dx.doi.org/10.1086/342734
42. Gunnersen JM, Kim MH, Fuller SJ, De Silva M, Britto JM, Hammond VE, et al. Sez-6 proteins affect dendritic arborization patterns and excitability of cortical pyramidal neurons. Neuron. 2007;56(4):621–39. http://dx.doi.org/10.1016/j.neuron.2007.09.018
43. Hu X, He W, Luo X, Tsubota KE, Yan R. BACE1 regulates hippocampal astrogenesis via the Jagged 1-Notch pathway. Cell Rep. 2013;4(1):40–9. http://dx.doi.org/10.1016/j.celrep.2013.06.005
44. Gaiano N, Fishell G. The role of notch in promoting glial and neural stem cell fates. Annu Rev Neurosci. 2002;25:471–90. http://dx.doi.org/10.1146/annurev.neuro.25.030702.130823
45. Tanzi RE. The genetics of Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(10):pii: a006296. http://dx.doi.org/10.1101/cshperspect.a006296
46. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. Science. 2002;297(5580):353–6. http://dx.doi.org/10.1126/science.1072994
47. Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML, et al. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science. 1987;235(4791):880–4. http://dx.doi.org/10.1126/science.2949367

48. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature. 1995;375(6534):754–60. http://dx.doi.org/10.1038/375754a0
49. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: A genetic perspective. Cell. 2005;120(4):545–55. http://dx.doi.org/10.1016/j.cell.2005.02.008
50. De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature. 1998;391(6665):387–90. http://dx.doi.org/10.1038/34910
51. Kounnas MZ, Danks AM, Cheng S, Tyree C, Ackerman E, Zhang X, et al. Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. Neuron. 2010;67(5):769–80. http://dx.doi.org/10.1016/j.neuron.2010.08.018
52. Bezprozvanny I, Hiesinger PR. The synaptic maintenance problem: Membrane recycling, Ca2+ homeostasis and late onset degeneration. Mol Neurodegener. 2013;8:23. http://dx.doi.org/10.1186/1750-1326-8-23
53. Mattson MP. ER calcium and Alzheimer's disease: In a state of flux. Sci Signal. 2010;3(114):pe10. http://dx.doi.org/10.1126/scisignal.3114pe10
54. Dumanis SB, Cha HJ, Song JM, Trotter JH, Spitzer M, Lee JY, et al. ApoE receptor 2 regulates synapse and dendritic spine formation. PLoS One. 2011;6(2):e17203. http://dx.doi.org/10.1371/journal.pone.0017203
55. Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. Nat Rev Neurol. 2013;9(2):106–18. http://dx.doi.org/10.1038/nrneurol.2012.263
56. Holtzman DM, Herz J, Bu G. Apolipoprotein E and apolipoprotein E receptors: Normal biology and roles in Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(3):a006312. http://dx.doi.org/10.1101/cshperspect.a006312
57. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013;45(12):1452–8. http://dx.doi.org/10.1038/ng.2802
58. McGuinness B, Carson R, Barrett SL, Craig D, Passmore AP. Apolipoprotein epsilon4 and neuropsychological performance in Alzheimer's disease and vascular dementia. Neurosci Lett. 2010;483(1):62–6. http://dx.doi.org/10.1016/j.neulet.2010.07.063
59. Sun X, Nicholas J, Walker A, Wagner MT, Bachman D. APOE genotype in the diagnosis of Alzheimer's disease in patients with cognitive impairment. Am J Alzheimers Dis Other Demen. 2012;27(5):315–20. http://dx.doi.org/10.1177/1533317512452037
60. Liu F, Pardo LM, Schuur M, Sanchez-Juan P, Isaacs A, Sleegers K, et al. The apolipoprotein E gene and its age-specific effects on cognitive function. Neurobiol Aging. 2010;31(10):1831–3. http://dx.doi.org/10.1016/j.neurobiolaging.2008.09.015
61. Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet. 2007;39(2):168–77. http://dx.doi.org/10.1038/ng1943
62. Kehoe PG, Russ C, McIlory S, Williams H, Holmans P, Holmes C, et al. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. Nat Genet. 1999;21(1):71–2. http://dx.doi.org/10.1038/5009
63. Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, et al. GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. Neuron. 2007;54(5):713–20. http://dx.doi.org/10.1016/j.neuron.2007.05.022
64. Nizzari M, Venezia V, Repetto E, Caorsi V, Magrassi R, Gagliani MC, et al. Amyloid precursor protein and Presenilin1 interact with the adaptor GRB2 and modulate ERK 1,2 signaling. J Biol Chem. 2007;282(18):13833–44. http://dx.doi.org/10.1074/jbc.M610146200
65. Zhang C, Browne A, Child D, Divito JR, Stevenson JA, Tanzi RE. Loss of function of ATXN1 increases amyloid beta-protein levels by potentiating beta-secretase processing of beta-amyloid precursor protein. J Biol Chem. 2010;285(12):8515–26. http://dx.doi.org/10.1074/jbc.M109.079079
66. Deming Y, Li Z, Kapoor M, Harari O, Del-Aguila JL, Black K, et al. Genome-wide association study identifies four novel loci associated with Alzheimer's endophenotypes and disease modifiers. Acta Neuropathol. 2017;133(5):839–56. http://dx.doi.org/10.1007/s00401-017-1685-y

67. Pant S, Sharma M, Patel K, Caplan S, Carr CM, Grant BD. AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd1 in endocytic recycling. Nat Cell Biol. 2009;11(12):1399–410. http://dx.doi.org/10.1038/ncb1986
68. Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, et al. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One. 2010;5(3):e9505. http://dx.doi.org/10.1371/journal.pone.0009505
69. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011;43(5):429–35. http://dx.doi.org/10.1038/ng.803
70. Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011;43(5):436–41. http://dx.doi.org/10.1038/ng.801
71. Vaz FNC, Fermino BL, Haskel MVL, Wouk J, de Freitas GBL, Fabbri R, et al. The relationship between copper, iron, and selenium levels and Alzheimer disease. Biol Trace Elem Res. 2018;181(2):185–91. http://dx.doi.org/10.1007/s12011-017-1042-y
72. Chow SK, Yu D, Macdonald CL, Buibas M, Silva GA. Amyloid beta-peptide directly induces spontaneous calcium transients, delayed intercellular calcium waves and gliosis in rat cortical astrocytes. ASN Neuro. 2010;2(1):e00026. http://dx.doi.org/10.1042/AN20090035
73. Karunaweera N, Raju R, Gyengesi E, Munch G. Plant polyphenols as inhibitors of NF-kappaB induced cytokine production-a potential anti-inflammatory treatment for Alzheimer's disease? Front Mol Neurosci. 2015;8:24. http://dx.doi.org/10.3389/fnmol.2015.00024
74. Bessis A, Bechade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. Glia. 2007;55(3):233–8. http://dx.doi.org/10.1002/glia.20459
75. Caldero J, Brunet N, Ciutat D, Hereu M, Esquerda JE. Development of microglia in the chick embryo spinal cord: Implications in the regulation of motoneuronal survival and death. J Neurosci Res. 2009;87(11):2447–66. http://dx.doi.org/10.1002/jnr.22084
76. Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM, Jr., et al. Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia. 2001;35(1):72–9. http://dx.doi.org/10.1002/glia.1072
77. Garden GA. Microglia in human immunodeficiency virus-associated neurodegeneration. Glia. 2002;40(2):240–51. http://dx.doi.org/10.1002/glia.10155
78. Muzio L, Martino G, Furlan R. Multifaceted aspects of inflammation in multiple sclerosis: The role of microglia. J Neuroimmunol. 2007;191(1–2):39–44. http://dx.doi.org/10.1016/j.jneuroim.2007.09.016
79. Cameron B, Landreth GE. Inflammation, microglia, and Alzheimer's disease. Neurobiol Dis. 2010;37(3):503–9. http://dx.doi.org/10.1016/j.nbd.2009.10.006
80. McGeer PL, Itagaki S, McGeer EG. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. Acta Neuropathol. 1988;76(6):550–7. http://dx.doi.org/10.1007/BF00689592
81. Walker DG, Lue LF. Investigations with cultured human microglia on pathogenic mechanisms of Alzheimer's disease and other neurodegenerative diseases. J Neurosci Res. 2005;81(3):412–25. http://dx.doi.org/10.1002/jnr.20484
82. Majumdar A, Chung H, Dolios G, Wang R, Asamoah N, Lobel P, et al. Degradation of fibrillar forms of Alzheimer's amyloid beta-peptide by macrophages. Neurobiol Aging. 2008;29(5):707–15. http://dx.doi.org/10.1016/j.neurobiolaging.2006.12.001
83. Kounatidis I, Ligoxygakis P. Drosophila as a model system to unravel the layers of innate immunity to infection. Open Biol. 2012;2(5):120075. http://dx.doi.org/10.1098/rsob.120075
84. Wang L, Kounatidis I, Ligoxygakis P. Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer. Front Cell Infect Microbiol. 2014;3:113. http://dx.doi.org/10.3389/fcimb.2013.00113
85. Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, Baltimore D. NF-kappa B functions in synaptic signaling and behavior. Nat Neurosci. 2003;6(10):1072–8. http://dx.doi.org/10.1038/nn1110
86. Kounatidis I, Chtarbanova S, Cao Y, Hayne M, Jayanth D, Ganetzky B, et al. NF-kappaB immunity in the brain determines fly lifespan in healthy aging and age-related neurodegeneration. Cell Rep. 2017;19(4):836–48. http://dx.doi.org/10.1016/j.celrep.2017.04.007

87. Sadagurski M, Cady G, Miller RA. Anti-aging drugs reduce hypothalamic inflammation in a sex-specific manner. Aging Cell. 2017;16(4):652–60. http://dx.doi.org/10.1111/acel.12590
88. Chen CH, Zhou W, Liu S, Deng Y, Cai F, Tone M, et al. Increased NF-kappaB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. Int J Neuropsychopharmacol. 2012;15(1):77–90. http://dx.doi.org/10.1017/S1461145711000149
89. Baker RG, Hayden MS, Ghosh S. NF-kappaB, inflammation, and metabolic disease. Cell Metab. 2011;13(1):11–22. http://dx.doi.org/10.1016/j.cmet.2010.12.008
90. Purkayastha S, Zhang G, Cai D. Uncoupling the mechanisms of obesity and hypertension by targeting hypothalamic IKK-beta and NF-kappaB. Nat Med. 2011;17(7):883–7. http://dx.doi.org/10.1038/nm.2372
91. Niu YL, Zhang WJ, Wu P, Liu B, Sun GT, Yu DM, et al. Expression of the apoptosis-related proteins caspase-3 and NF-kappaB in the hippocampus of Tg2576 mice. Neurosci Bull. 2010;26(1):37–46. http://dx.doi.org/10.1007/s12264-010-6122-3
92. Rehman SU, Shah SA, Ali T, Chung JI, Kim MO. Anthocyanins reversed D-galactose-induced oxidative stress and neuroinflammation mediated cognitive impairment in adult rats. Mol Neurobiol. 2017;54(1):255–71. http://dx.doi.org/10.1007/s12035-015-9604-5
93. Zhang Y, Liu G, Yan J, Zhang Y, Li B, Cai D. Metabolic learning and memory formation by the brain influence systemic metabolic homeostasis. Nat Commun. 2015;6:6704. http://dx.doi.org/10.1038/ncomms7704
94. Li X, Song D, Leng SX. Link between type 2 diabetes and Alzheimer's disease: From epidemiology to mechanism and treatment. Clin Interv Aging. 2015;10:549–60. http://dx.doi.org/10.2147/CIA.S74042
95. Durazzo TC, Mattsson N, Weiner MW, Alzheimer's disease neuroimaging I. Smoking and increased Alzheimer's disease risk: A review of potential mechanisms. Alzheimers Dement. 2014;10(3 Suppl):S122–45. http://dx.doi.org/10.1016/j.jalz.2014.04.009
96. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging (Albany NY). 2017;9(2):419–46. http://dx.doi.org/10.18632/aging.101168
97. Bangen KJ, Gu Y, Gross AL, Schneider BC, Skinner JC, Benitez A, et al. Relationship between type 2 diabetes mellitus and cognitive change in a multiethnic elderly cohort. J Am Geriatr Soc. 2015;63(6):1075–83. http://dx.doi.org/10.1111/jgs.13441
98. Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature. 2012;488(7409):96–9. http://dx.doi.org/10.1038/nature11283
99. Vassar R, Kuhn PH, Haass C, Kennedy ME, Rajendran L, Wong PC, et al. Function, therapeutic potential and cell biology of BACE proteases: Current status and future prospects. J Neurochem. 2014;130(1):4–28. http://dx.doi.org/10.1111/jnc.12715
100. Yang J, Zhang X, Zhu Y, Lenczowski E, Tian Y, Yang J, et al. The double-edged role of copper in the fate of amyloid beta in the presence of anti-oxidants. Chem Sci. 2017;8(9):6155–64. http://dx.doi.org/10.1039/C7SC01787A
101. Gustafson DR, Clare Morris M, Scarmeas N, Shah RC, Sijben J, Yaffe K, et al. New perspectives on Alzheimer's disease and nutrition. J Alzheimers Dis. 2015;46(4):1111–27. http://dx.doi.org/10.3233/JAD-150084
102. Botezelli JD, Coope A, Ghezzi AC, Cambri LT, Moura LP, Scariot PP, et al. Strength training prevents hyperinsulinemia, insulin resistance, and inflammation independent of weight loss in fructose-fed animals. Sci Rep. 2016;6:31106. http://dx.doi.org/10.3233/JAD-150084
103. Minegishi Y, Haramizu S, Misawa K, Shimotoyodome A, Hase T, Murase T. Deletion of nuclear factor-kappaB p50 upregulates fatty acid utilization and contributes to an anti-obesity and high-endurance phenotype in mice. Am J Physiol Endocrinol Metab. 2015;309(6):E523–33. http://dx.doi.org/10.1152/ajpendo.00071.2015
104. Ropelle ER, Flores MB, Cintra DE, Rocha GZ, Pauli JR, Morari J, et al. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol. 2010;8(8):pii: e1000465. http://dx.doi.org/10.1371/journal.pbio.1000465

105. van de Haar HJ, Burgmans S, Hofman PA, Verhey FR, Jansen JF, Backes WH. Blood-brain barrier impairment in dementia: Current and future in vivo assessments. Neurosci Biobehav Rev. 2015;49:71–81. http://dx.doi.org/10.1016/j.neubiorev.2014.11.022
106. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol. 2009;118(1):103–13. http://dx.doi.org/10.1007/s00401-009-0522-3
107. Sagare AP, Bell RD, Zlokovic BV. Neurovascular dysfunction and faulty amyloid beta-peptide clearance in Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(10):pii: a011452. http://dx.doi.org/10.1101/cshperspect.a011452
108. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. Neurobiol Dis. 2017;107:41–56. http://dx.doi.org/10.1016/j.nbd.2016.07.007
109. van Assema DM, Lubberink M, Bauer M, van der Flier WM, Schuit RC, Windhorst AD, et al. Blood-brain barrier P-glycoprotein function in Alzheimer's disease. Brain. 2012;135(Pt 1):181–9. http://dx.doi.org/10.1093/brain/awr298
110. Chen Z, Zhong C. Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: Implications for diagnostic and therapeutic strategies. Prog Neurobiol. 2013;108:21–43. http://dx.doi.org/10.1016/j.pneurobio.2013.06.004
111. Goncalves A, Ambrosio AF, Fernandes R. Regulation of claudins in blood-tissue barriers under physiological and pathological states. Tissue Barriers. 2013;1(3):e24782. http://dx.doi.org/10.4161/tisb.24782
112. Carmeliet P, De Strooper B. Alzheimer's disease: A breach in the blood-brain barrier. Nature. 2012;485(7399):451–2. http://dx.doi.org/10.1038/485451a
113. McCartney DL, Stevenson AJ, Walker RM, Gibson J, Morris SW, Campbell A, et al. Investigating the relationship between DNA methylation age acceleration and risk factors for Alzheimer's disease. Alzheimers Dement (Amst). 2018;10:429–37. http://dx.doi.org/10.1016/j.dadm.2018.05.006
114. Livingston G, Frankish H. A global perspective on dementia care: A Lancet Commission. Lancet. 2015;386(9997):933–4. http://dx.doi.org/10.1016/S0140-6736(15)00078-1
115. Horvath S, Ritz BR. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging (Albany NY). 2015;7(12):1130–42. http://dx.doi.org/10.18632/aging.100859
116. Ayton S, Lei P, Bush AI. Metallostasis in Alzheimer's disease. Free Radic Biol Med. 2013;62:76–89. http://dx.doi.org/10.1016/j.freeradbiomed.2012.10.558
117. Bush AI. The metal theory of Alzheimer's disease. J Alzheimers Dis. 2013;33(Suppl 1):S277–81.
118. James SA, Churches QI, de Jonge MD, Birchall IE, Streltsov V, McColl G, et al. Iron, copper, and zinc concentration in abeta plaques in the APP/PS1 mouse model of Alzheimer's disease correlates with metal levels in the surrounding neuropil. ACS Chem Neurosci. 2017;8(3):629–37. http://dx.doi.org/10.1021/acschemneuro.6b00362
119. Adlard PA, Bica L, White AR, Nurjono M, Filiz G, Crouch PJ, et al. Metal ionophore treatment restores dendritic spine density and synaptic protein levels in a mouse model of Alzheimer's disease. PLoS One. 2011;6(3):e17669. http://dx.doi.org/10.1371/journal.pone.0017669
120. Miller Y, Ma B, Nussinov R. Zinc ions promote Alzheimer Abeta aggregation via population shift of polymorphic states. Proc Natl Acad Sci U S A. 2010;107(21):9490–5. http://dx.doi.org/10.1073/pnas.0913114107
121. Adlard PA, Cherny RA, Finkelstein DI, Gautier E, Robb E, Cortes M, et al. Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Abeta. Neuron. 2008;59(1):43–55. http://dx.doi.org/10.1016/j.neuron.2008.06.018
122. Duce JA, Tsatsanis A, Cater MA, James SA, Robb E, Wikhe K, et al. Iron-export ferroxidase activity of beta-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. Cell. 2010;142(6):857–67. http://dx.doi.org/10.1016/j.cell.2010.08.014
123. Smith MA, Harris PL, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A. 1997;94(18):9866–8. http://dx.doi.org/10.1073/pnas.94.18.9866
124. Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. Nat Med. 2012;18(2):291–5. http://dx.doi.org/10.1038/nm.2613
125. Lannfelt L, Blennow K, Zetterberg H, Batsman S, Ames D, Harrison J, et al. Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: A phase IIa,

double-blind, randomised, placebo-controlled trial. Lancet Neurol. 2008;7(9):779–86. http://dx.doi.org/10.1016/S1474-4422(08)70167-4
126. Faux NG, Ritchie CW, Gunn A, Rembach A, Tsatsanis A, Bedo J, et al. PBT2 rapidly improves cognition in Alzheimer's disease: Additional phase II analyses. J Alzheimers Dis. 2010;20(2):509–16. http://dx.doi.org/10.3233/JAD-2010-1390
127. Crouch PJ, Savva MS, Hung LW, Donnelly PS, Mot AI, Parker SJ, et al. The Alzheimer's therapeutic PBT2 promotes amyloid-beta degradation and GSK3 phosphorylation via a metal chaperone activity. J Neurochem. 2011;119(1):220–30. http://dx.doi.org/10.1111/j.1471-4159.2011.07402.x
128. Rogers JT, Randall JD, Cahill CM, Eder PS, Huang X, Gunshin H, et al. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem. 2002;277(47):45518–28. http://dx.doi.org/10.1074/jbc.M207435200
129. Cho HH, Cahill CM, Vanderburg CR, Scherzer CR, Wang B, Huang X, et al. Selective translational control of the Alzheimer amyloid precursor protein transcript by iron regulatory protein-1. J Biol Chem. 2010;285(41):31217–32. http://dx.doi.org/10.1074/jbc.M110.149161
130. Zhang L, Zhao B, Yew DT, Kusiak JW, Roth GS. Processing of Alzheimer's amyloid precursor protein during H2O2-induced apoptosis in human neuronal cells. Biochem Biophys Res Commun. 1997;235(3):845–8. http://dx.doi.org/10.1006/bbrc.1997.6698
131. Ledesma MD, Avila J, Correas I. Isolation of a phosphorylated soluble tau fraction from Alzheimer's disease brain. Neurobiol Aging. 1995;16(4):515–22.
132. Jin Jung K, Hyun Kim D, Kyeong Lee E, Woo Song C, Pal Yu B, Young Chung H. Oxidative stress induces inactivation of protein phosphatase 2A, promoting proinflammatory NF-kappaB in aged rat kidney. Free Radic Biol Med. 2013;61:206–17. http://dx.doi.org/10.1016/0197-4580(95)00075-P
133. Ayton S, Fazlollahi A, Bourgeat P, Raniga P, Ng A, Lim YY, et al. Cerebral quantitative susceptibility mapping predicts amyloid-beta-related cognitive decline. Brain. 2017;140(8):2112–19. http://dx.doi.org/10.1093/brain/awx137
134. Gamblin TC, King ME, Kuret J, Berry RW, Binder LI. Oxidative regulation of fatty acid-induced tau polymerization. Biochemistry. 2000;39(46):14203–10. http://dx.doi.org/10.1021/bi001876l
135. McCarthy RC, Park YH, Kosman DJ. sAPP modulates iron efflux from brain microvascular endothelial cells by stabilizing the ferrous iron exporter ferroportin. EMBO Rep. 2014;15(7):809–15. http://dx.doi.org/10.15252/embr.201338064
136. Giorgianni CM, D'Arrigo G, Brecciaroli R, Abbate A, Spatari G, Tringali MA, et al. Neurocognitive effects in welders exposed to aluminium. Toxicol Ind Health. 2014;30(4):347–56. http://dx.doi.org/10.1177/0748233712456062
137. Inan-Eroglu E, Ayaz A. Is aluminum exposure a risk factor for neurological disorders? J Res Med Sci. 2018;23:51. http://dx.doi.org/10.4103/jrms.JRMS_921_17
138. Bharathi, Vasudevaraju P, Govindaraju M, Palanisamy AP, Sambamurti K, Rao KS. Molecular toxicity of aluminium in relation to neurodegeneration. Indian J Med Res. 2008;128(4):545–56.
139. McLachlan DR, Bergeron C, Smith JE, Boomer D, Rifat SL. Risk for neuropathologically confirmed Alzheimer's disease and residual aluminum in municipal drinking water employing weighted residential histories. Neurology. 1996;46(2):401–5. http://dx.doi.org/10.1212/WNL.46.2.401
140. Carpenter KA, Cohen DS, Jarrell JT, Huang X. Deep learning and virtual drug screening. Future Med Chem. 2018. http://dx.doi.org/10.4155/fmc-2018-0314
141. Carpenter KA, Huang X. Machine learning-based virtual screening and its applications to Alzheimer's drug discovery: A review. Curr Pharm Des. 2018;24(28):3347–58. http://dx.doi.org/10.2174/1381612824666180607124038

6

The Role of Trace Metals in Alzheimer's Disease

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Abstract: The extracellular aggregation of insoluble protein deposits of amyloid-β (Aβ) into plaques and the hyperphosphorylation of the intracellular protein tau leading to neurofibrillary tangles are the main pathological hallmarks of Alzheimer's disease (AD). Both Aβ and tau are metal-binding proteins. Essential trace metals such as zinc, copper, and iron play important roles in healthy brain function but altered homeostasis and distribution have been linked to neurodegenerative diseases and aging. In addition, the presence of non-essential trace metals such as aluminum has been associated with AD. Trace metals and abnormal metal metabolism can influence protein aggregation, synaptic signaling pathways, mitochondrial function, oxidative stress levels, and inflammation, ultimately resulting in synapse dysfunction and neuronal loss in the AD brain. Herein we provide an overview of metals and metal-binding proteins and their pathophysiological role in AD.

Keywords: Amyloid beta; copper; iron; metal-binding; zinc

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INTRODUCTION

Essential trace metals and those with biological functions (biometals) play a vital role in many physiological processes in the human body. As free ion, some of them can participate in cellular signaling pathways, while bound to proteins they may have a structural or regulatory role in protein folding and function. The fact that about 10% of the genes in the human genome encode for proteins with zinc (Zn)-binding motif points to the evident dependency of biological processes on this trace metal. This number of Zn-binding proteins is not even accounting for Zn coordinated between two proteins in protein–protein interactions (1). In addition to Zn, several proteins and processes depend on other essential trace metals, the most important of which are iron (Fe), manganese (Mn), copper (Cu), and selenium (Se) (a metalloid). The average human body contains about 4.2 g Fe, 2.3 g Zn, 0.072 g Cu, 0.015 g Se, and 0.012 g Mn (2). However, the distribution of trace metals can vary depending on the organ considered. In the human brain, Fe is the most prevalent trace metal, which can be found both as heme (bound to hemoglobin in blood) and non-heme Fe. Heme-bound Fe may be a major contributor to the overall concentration. Therefore, Zn, the second most prevalent metal, may play an even more prominent role in the brain, which is underlined by its function as neurotransmitter/neuromodulator (3). Additionally, within the brain, some trace metals are enriched in particular brain regions (Figure 1). For example, the hippocampus is a brain region that is high in Zn, while the nucleus caudatus has higher levels of Fe than several other brain regions (4, 5). This unequal concentration of trace elements in different tissues demands a tightly regulated distribution. Given that charged molecules such as metal ions cannot freely pass the cellular membrane, a plethora of transport proteins evolved, with very specific regional and also developmentally and environmentally dependent expression. Especially, the regulation of metal concentrations in the brain faces a tight control at the level of the blood–brain barrier (BBB), a barrier composed of endothelial cells of the brain capillaries, pericytes, astrocytes, and the basement membrane (6). Together, they form a functional unit, mediating the exchange of trace metals between neurons, capillaries, and glia, while protecting against neurotoxicity of non-essential trace metals or excessive levels of essential trace elements. A specific set of transporters allows the crossing of trace metals into the brain. For example, only for Zn, 24 different transport proteins are known in humans (7), which allow the establishment of zinc homeostasis in tissues.

The maintenance of a balance between biometals is complicated by the influence different metals have on each other. Their concentration is regulated by complex interactions between trace metal ions and their ligands. For example, due to their physicochemical nature, Zn and Cu are known to compete for the binding sites of some transporters and metal-binding proteins, resulting in an antagonistic relationship, where low levels of Zn increase Cu levels and vice versa (8). Due to these interactions, the loss of, or increase in, one trace metal can lead to the establishment of a completely new biometal profile affecting many other trace metals of a system (9).

Metal homeostasis can be challenged in many ways. In fact, our body is not only exposed to essential trace metals. Through the environment (e.g., air, food,

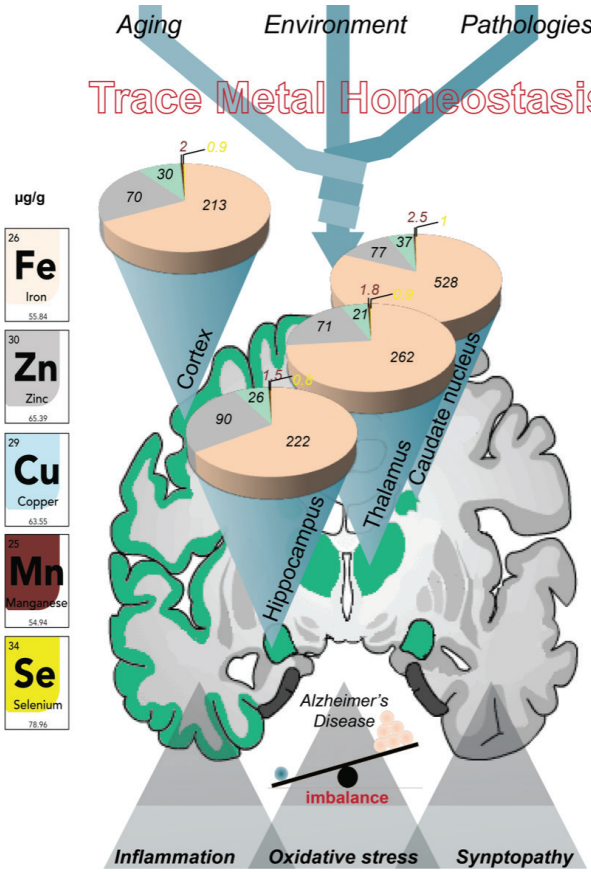

Figure 1 Trace metal concentration in different brain regions. Within the brain, trace metals are unequally concentrated in different brain regions. The figure shows the concentrations in µg/g brain tissue in the human cortex, hippocampus, thalamus, and caudate nucleus for Fe, Zn, Cu, Mn, and Se. This metal homeostasis can be challenged by environmental factors, the presence of pathologies such as aggregates of metal-binding proteins, and aging.

medical devices, and cosmetic products), other nonessential trace elements such as lead (Pb), mercury (Hg), and aluminum (Al) may enter our system. Indeed, these metals are present in all humans at low levels (e.g., 0.060 g Al, 0.012 g Pb, and 0.006 g Hg). Some of these metals are currently reported to have no or little effect on the body [e.g., titanium (Ti)], while others can produce adverse effects even at concentrations slightly above the normal background levels [e.g., Pb, Hg, and cadmium (Cd)]. These toxic effects are usually due to a chemical nature similar to that of an essential metal that allows binding to metal-binding sites of metal transporters and other proteins, leading to competition with essential trace metals. However, toxic metals are often not able to produce the biological effect of essential metals, and therefore, they act antagonistically. Recent evidences put synaptic signaling, synapse formation and

plasticity, oxidative stress, inflammation, and protein aggregation at the forefront of disease-relevant processes caused by abnormal trace metal homeostasis (Figure 1).

The enrichment of biometals in a tissue may occur through several mechanisms, such as mutations in metal import and export proteins, proteins buffering metals through transient binding (e.g. metallothioneins) (10), and also the abnormal accumulation of metal-binding proteins that occurs in several neurodegenerative diseases such as Parkinson's disease (alpha-synuclein protein) (11) and Alzheimer's disease (AD).

In AD, a contribution of abnormal trace metal homeostasis and signaling has been extensively reported (9). However, changes in trace metals' levels in AD are complex and can rarely be directly associated with systemic alterations that can be measured in easily accessible biosamples such as serum. The most likely reason for this is the ability of senile plaques to sequester specific metal ions that in turn become mislocalized instead of decreasing or increasing systemically. Cu, Zn, and, to a lesser extent, Fe are known to associate with senile plaques made of beta-amyloid (Aβ) protein (see below). It is hypothesized that this association causes several biological effects. For example, sequestration of Cu and Zn into plaques leads to an abnormal distribution of these metals, initially resulting in a deficiency of Cu and Zn in the vicinity of plaques (12, 13) and not throughout the whole brain.

Therefore, findings concerning alterations in metal ions in AD are highly dependent on the tissue and resolution used for analysis. Regarding essential metals, although results vary in some studies, Mn, Cu, Fe, and Zn seem to show an inverse correlation with senile plaque load and thus a decrease in the cerebrospinal fluid (CSF) (14) of AD patients.

The accumulation of trace elements, including Al, Pb, Hg, Cu, and Fe, has been implicated in AD through an increase in oxidative stress (15). In particular, a disruption in the homeostasis of Cu and Fe, two redox-active metals, may increase lipid peroxidation, and the oxidative damage to neurofibrillary tangles (NFTs), senile plaques, and nucleic acids (16). Oxidative stress is induced by an imbalance in the redox state, involving the generation of excessive reactive oxygen species (ROS) or the dysfunction of the antioxidant system (17). Cu is a potent mediator of the highly reactive hydroxyl radical (OH•) and is highly concentrated in senile plaques. Consequently, Cu contributes to the increase of oxidative stress in AD. In addition, increased levels of Fe, transferrin, and ferritin may contribute to NFT formation, possibly due to the binding of Fe to the tau protein. In the brain, oxidative stress may cause serious damage via several mechanisms, including the release of excitatory amino acids and neurotoxicity (18). Although Zn is redox-inert, Zn signaling plays a role in the regulation of proteins (e.g., enzymes kinases and phosphatases) controlling redox-signaling pathways. Therefore, while not acting as an electron donor, Zn plays a role in redox biology, where zinc, in general, is considered as an antioxidant. However, these indirect antioxidant-like effects are present only in certain conditions and both a lack and excess of Zn can result in pro-oxidant effects (19).

In addition to changes in trace metal homeostasis resulting from AD, it is likely that alterations may also facilitate and trigger the development of AD pathology. For example, it has been shown that the levels of Zn decrease during

aging as a result of more restricted food choice (20, 21), which may facilitate inflammatory processes (22), increase oxidative stress (19), and decrease memory as seen in several animal models for Zn deficiency (23). More importantly, key proteins involved in the etiology of AD, and especially early-onset AD (familial AD), such as amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2), have been shown to bind to or regulate metals. For example, PS are important for cellular Cu and Zn turnover (24). Further, metals have been shown to interact with the two major disease-related proteins of AD, namely Aβ and tau.

METAL INTERACTIONS WITH APP

The APP is expressed in various tissues of the human body, in particular in the brain. Its general function within the brain has been linked to neurite outgrowth and neuronal cell migration (25). However, it becomes increasingly evident that APP can be considered to act as a metalloprotein, which is involved in the regulation of Cu, Fe, and ferroxidase homeostasis (26). Recent studies indicate furthermore that metals are involved in the proteolytic processing of APP.

APP displays two putative metal-binding sides, which are located within the E1 (124–189, APP770 numbering) and E2 (376–554) domains (27, 28). Cu binds to APP between residues 142 and 166 (29). It has been demonstrated that the two Cu-binding residues 149 and 151 are involved in the metabolism, folding and stability, and homodimerization of APP (30). Besides, Cu ions have been shown to promote cell surface localization of APP (31). Furthermore, it has been shown that cellular Cu levels can influence the expression of APP in vitro at both gene and protein levels (32).

APP also displays an evolutionary conserved Zn-binding site between amino acid positions 170 and 188 (33, 34). The binding of Zn to APP has been reported to play a similar role as Cu-binding in the homodimerization of APP (35) (Figure 2A).

Fe is involved in the direct regulation of APP translation. The APP mRNA displays an Fe response element (IRE) in its 5'-untranslated region (5'-UTR) sequence (36), and APP levels increase after a rise in cytosolic free Fe levels (37). Additionally, APP has been suggested to be involved in Fe export in the brain through the stabilization of ferroportin (Fpn). Deletion of APP in vitro in primary neurons impairs Fe export, which can be fully restored by the addition of APP (38).

Aβ is derived from APP by the sequential proteolysis by β- and γ-secretases. Metals have also been shown to indirectly influence Aβ generation by modifying the proteolytic processing of APP (28). Interestingly, all three secretases (α, β, and γ) involved in the enzymatic cleavage of APP interact with metal ions. The enzymatic activity of the α-secretase TACE is regulated by a "cysteine switch" motif, which is based on an intramolecular bond between cysteine (Cys) and a Zn atom in its catalytic site (39). Furthermore, the major β-secretase involved in APP processing displays a Cu-binding site in its C-terminal domain (40). Further, Zn has been shown to enhance the synthesis of PS1, the active subunit of the γ-secretase (41).

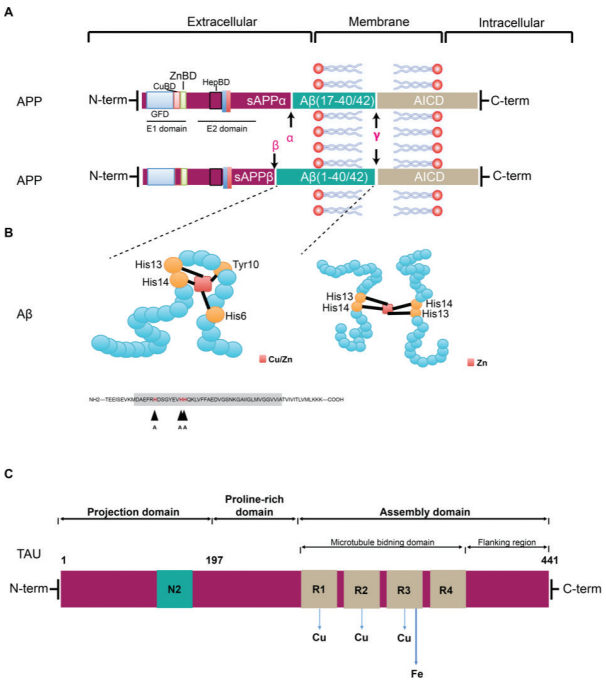

Figure 2 **APP cleavage and APP and tau metal-binding sites.** A) Cleavage of APP by α- and γ-secretases creates the sAPPα and $A\beta_{17\text{-}40/42}$ fragments (upper panel). Cleavage by β- and γ-secretases creates the sAPPβ and $A\beta_{1\text{-}40/42}$ fragments (lower panel). The N-terminal part of APP contains a CuBD, copper-binding domain; ZnBD, zinc-binding domain; HepBD, heparin-binding domain; GFD, growth factor-like domain. **B)** Two of several possibilities of metal interaction with Aβ. Below: Sequence of Aβ, 3 His in the Aβ peptide domain of human wild type (wt) Aβ are important for metal coordination**. C)** Representation of the three main domains studied in tau protein. The picture shows the binding sites of Cu and Fe, respectively, through R1, R2, and R3 regions in the MTB ensuring the binding to the microtubules.

METAL INTERACTIONS WITH AMYLOID-BETA (Aβ)

The Aβ domain of APP is another region that can directly bind Zn and Cu ions; however until now, there is no evidence that this region interacts with metal ions prior to the enzymatic cleavage mediated by α-, β-, and γ- secretases (42) (Figure 2B).

Aggregation of Aβ into insoluble fibrils is a key pathological event in AD and is mediated by the interactions of Aβ with metals, in particular, Zn, Cu, and Fe. Early studies have shown that in particular, the histidine (His) residues in Aβ are responsible for the metal-mediated aggregation of Aβ (43). Interestingly, in mice and rats, the same His residues are not present, which might explain why these animals are more resistant to the amyloid pathology compared to other mammals (44).

The strong chelation properties of Aβ of Zn, Cu, and Fe explain the enrichment of these ions in amyloid plaques and suggest that one potential pathological influence of Aβ might be to sequester metal ions (45) and, through increasing concentrations of redox-active Cu and Fe ions in amyloid plaques, to promote oxidative stress.

Zinc and Amyloid-Beta

Zn is a factor contributing to the neurotoxicity of Aβ through the stabilization of amyloid fibrils (46). Various coordination sites have been proposed for the binding of zinc to Aβ with particular importance of His13 and His6. In vitro studies demonstrated that Zn induces the rapid and extensive aggregation of synthetic Aβ (34, 47), which might act as a seeding factor in the formation of amyloid plaques. In support of these studies, high levels of Zn have been found in the senile plaques of postmortem tissue of AD patients (45) and plaques of genetic AD mouse models. Interestingly, Aβ deposits fail to develop into mature plaques in the cerebellum where vesicular Zn is absent. Scavenging of Zn ions through Aβ may itself be a pathomechanism of AD. A locally decreasing Zn level in the vicinity of plaques contributes to synapse loss (13).

Copper and Amyloid-Beta

Homeostasis is fundamental for all metal ions, but for Cu it is critical because this metal is redox-active and can catalyze and activate O_2, generating reactive oxygen species (ROS) involved in oxidative damage. The soluble monomeric Aβ displays three high-affinity His Cu-binding sites (His6, His13, and His14), which along with the N-terminal amino group and aspartate form a tetragonal complex with Cu ions (48, 49). Cu(II) has been demonstrated to play a crucial role in the formation of β-sheet structures, which are thought to be a preliminary step of the toxic aggregates of the fibrillar form of Aβ. Thus, Cu binding to Aβ has been proposed to play a major role in the neurotoxicity of Aβ. In line with this, a series of studies have reported that Cu chelators rapidly induce the inhibition of Aβ accumulation in transgenic AD mouse models (50, 51).

Other essential trace metals and Amyloid-Beta

Because of Fe^{3+} hydroxide species precipitation, the binding of Fe^{3+} to Aβ seems implausible. Instead, Asp1, Glu3, and the three His residues (His6, His13, and His14) are involved in binding of Fe^{2+}. However, iron mineral deposits in the cortical tissue may occur in vivo and contain magnetite (Fe_3O_4). They have been found in tissue extracted from human AD brain and brain from APP/PS1 transgenic mice (52). The aggregation state of Aβ appears to affect iron redox cycle and consequently may lead to the release of free radicals via Fenton chemistry. Interestingly, the degree of altered iron accumulations in AD is correlated with the amount of Aβ plaque pathology. However, these changes appear to occur after the development of the AD pathological hallmarks (53). Increased aggregation of

Aβ has been observed through the down-regulation of the enzymes that regulate the degradation of extracellular Aβ deposits induced by high Mn levels (54).

Toxic trace metals and Amyloid-Beta

Several studies have suggested that Al interacts with Aβ. Al has been detected in both the Aβ plaques and NFTs. Treatment of neuronal cultures with Al resulted in a marked accumulation of Aβ aggregates in vitro. However, the relevance of this for AD pathology in vivo is currently not well understood (48). Similarly, Hg exposure has been shown to promote the accumulation of Aβ deposits in vitro. Cd, like Mn, has been reported to reduce the expression of Aβ-degrading enzymes, resulting in an increased Aβ accumulation(48).

METAL INTERACTIONS WITH TAU

In AD, Tau aggregates due to hyperphosphorylation, abnormal splicing, or mutation in the tau encoding gene (55, 56). AD is the most common tauopathy among degenerative brain diseases. The tauprotein, with a molecular weight between 50 and 68 kDa, is encoded by a single gene (*MAPT*) located on chromosome 17q21 in humans. Tau is mainly localized in neuronal axons, but also dendrites (57), in the central nervous system (CNS). Tau is a microtubule-associated protein (MAP) implicated in the stabilization and integrity of microtubules (MT) in neurons, and its activity is regulated through a phosphorylation-dependent mechanism (58). In physiological conditions, tau is phosphorylated (facilitating the disassociation of the protein from the MT) and dephosphorylated (promoting the binding with MT) through the activity of tau kinases and phosphatases (59). In the human brain, under developmental control, six different isoforms of tau exist with a variation in size from 352 to 441 amino acids. The isoforms differ in the inclusion or exclusion of N repeats (0N or 1N or 2N) at the amino-terminal region and for the presence of three (3R) or four (4R) MT-binding domain (MTB) repeats (R) in the carboxyl-terminal part of the molecule (60).

The major domains identified in the tau protein are the *projection domain*, situated in the acidic N-terminal part, and the *assembly domain,* localized in the basic C-terminal domain. The two domains with the opposite charge are separated from one another by the *proline (Pro)-rich region,* situated in the middle part of the protein. Here, tau interacts with proteins containing an SH3 domain. Further, the Pro-rich region is the target of different Pro-directed kinases and also FYN-tyrosine kinases (61). The assembly domain, through R1–R4 repeat regions and flanking domain, binds microtubules and supports their assembly. This domain is the key in the regulation of the phosphorylation state of the tau protein (62). On the contrary, the projection part does not interact with microtubules but projects away from their surface interacting with other cytoskeletal elements, mitochondria or the neuronal plasma membrane (63, 64).

Phosphorylation of tau plays a crucial role in the pathogenesis of AD (65, 66) introducing negative charge(s) that promote an electrostatic interaction with metal ions (67) (Figure 2C). Tau abnormal phosphorylation leads to an abnormal structure, that is, polymerized into paired helical filaments (PHFs) which may further

aggregate to form NFTs, assuming the shape of a toxic protein deprived of the biological functions typical of the MAP family. It was reported that in AD brains, NFTs include metals, confirming an association between endogenous redox-active transition metals and metal-binding sites in tau (68). Binding of a series of metal ions including the essential biometals, Zn, Cu, Fe, Mg, and Mn, and non-essential trace metals, Pb, Cd, Hg, and Al, may promote tau hyperphosphorylation and induce tau aggregation. In contrast, Fe and lithium (Li) reduce the abnormal phosphorylation of tau (48). For this reason, biometal homeostasis is essential, and the disruption of this balance may play a key role in the pathogenesis of AD.

Zinc and Tau

A disruption in the Zn homeostasis leads to a series of pathogenic conditions in the AD brain, including the formation of NFTs composed of hyperphosphorylated tau. Recent studies show that Zn is involved in the mechanism of tau hyperphosphorylation via two different interactions: in vitro, Zn can directly affect tau at serine (Ser) and Pro sites, at threonine (Thr) and Pro sites or via two Cys residues: C291 and C322 (69). At the same time, Zn can indirectly hyperphosphorylate tau protein, by activating kinase and phosphatase pathways, for example activating Raf/mitogen protein kinase and inhibiting phosphatases such as PP2A (70, 71).

These two independent ways of action have different effects on tau toxicity. It has been demonstrated that the direct interaction between tau and Zn plays an important role in tau toxicity: after removing the Zn-binding site, tau toxicity is completely abolished, assuming that the toxic effect of tau necessitates both the presence of hyperphosphorylation and Zn bond. Tau hyperphosphorylation pathways appear to be less toxic, compared to tau toxicity that occurs from the direct binding between tau and Zn (72). Recently, it has been discovered that Zn could be considered a catalyst, accelerating the aggregation of tau-R3 complexes and, at the same time, promoting the formation of tau oligomers (73, 74). Thus, correct Zn homeostasis in AD is fundamental because abnormally high concentrations of this mineral induce the development of granular tau aggregates, while abnormally low concentrations of Zn lead to amyloid fibril formation (75).

Copper and Tau

A high concentration of Cu (0.4 mM) was reported in amyloid plaques and NFTs. Thereby, NFT may be linked to high levels of redox-active Cu (68). Besides, Cu is involved in tau hyperphosphorylation by activating the cyclin-dependent kinase (CDK)5/p25 complex. Tau hyperphosphorylation resulting from the activation of GSK-3β kinase by Cu is controversial: some studies suggest that GSK-3β kinase is activated by Cu (76, 77), while other studies propose that GSK-3β kinase may not necessarily be involved in the abnormal phosphorylation of the protein (78). The binding between tau and Cu is highly selective. Studies revealed that the full-length Human Tau40 isoform (K32) can bind one Cu for each monomer (1:1 binding stoichiometry) with a dissociation constant (K_d) close to 1 μM via two Cys residues. The sequences mediating the binding of Cu are ^{287}VQSKCGS^{293} and ^{310}YKPVDLSKVTSKCGS^{324}. An analysis conducted by circular dichroism and nuclear magnetic resonance (NMR) spectroscopy showed only limited formation

of aggregates after binding Cu because the addition of Cu to K32 does not affect the secondary structure, and thus, tau remains mostly disordered (79). In vitro, it has been demonstrated that Cu can bind different tau fragments containing diverse MTBR such as R1 and R2, showing alterations in the secondary structure (80, 81). Furthermore, the interactions between tau R2 and Cu lead to the production of H_2O_2 (82). The repeat R3 can be associated with more than one Cu ion via two His residues (83).

The role of Cu-binding to tau remains controversial, although some studies suggest that the binding between Cu and tau inhibits the formation of abnormal aggregates in vitro (78, 81). For example, increasing intracellular Cu levels by the addition of Cu–bis (thiosemicarbazone) complexes, inhibits tau hyperphosphorylation (76).

Iron and Tau

Fe dysregulation is linked to oxidative stress in tauopathies. Fe, as Zn and Cu, interacts with some of the isoforms of the tau protein, causing irreversible structural changes. The result of this interaction is protein aggregation and/or oxidative stress, through the Fenton reaction, perpetuating a condition of cellular damage. Analysis of postmortem AD brains shows increased Fe levels in several brain regions (84).

In the human body, Fe is available in two oxidation states: Fe^{3+} (redox-inert state) that is stored in ferritin and Fe^{2+} (redox-active). The iron status associated with NFTs in AD is Fe^{3+}, which can induce the aggregation of hyperphosphorylated tau. Fe-binding sites using His residues have been identified in tau (85). The hyperphosphorylated status of tau may not involve Fe^{3+} interacting with the protein, but Thr phosphorylation can regulate the interaction between tau and Fe^{2+} (86). Thus, the phosphorylation level of tau causes conformational changes of tau to mediate tau–Fe interactions (87). In addition to a direct interaction, Fe induces tau hyperphosphorylation, both in vitro and in vivo, by activating the CDK5/p25 complex and GSK-3β and MAP kinases (88). This evidence suggests a possible role of iron involved as a co-factor for tau aggregation.

Other essential trace metals and Tau

In AD patients, Mg levels appear lower (540–625 μg/g) compared to the physiologic range (620–680 μg/g) (89). In vivo, data obtained from an AD transgenic mouse model show that Mg increases the phosphorylation of the GSK-3β kinase at Ser9, which in turn reduces the hyperphosphorylation of tau protein (90). Additionally, in postmortem brains of patients affected by AD, the level of Mn appears to be higher (91). An increase of Mn levels is related to abnormal tau aggregation and its hyperphosphorylation, mediated by GSK-3β kinase (92).

Toxic trace metals and Tau

Al is the most widely exogenous metal ion distributed in the environment. As Fe^{3+}, Al^{3+} is a trivalent cation that influences protein phosphorylation of tau (93, 94). Recent data show that Al can promote the formation of sodium dodecyl

sulfate (SDS)–resistant tau oligomers after tau phosphorylation (95). The role of Al in AD has been intensively investigated since NFT-like deposits were discovered in mammalian brains after intracerebral Al injection (96). Al has been shown promoting tau aggregation through the down-regulation of PP2A activity and an increase of CDK5 and GSK-3β kinase levels (97). Thus, Al, although not directly binding to tau, may have a role as co-factor in AD (98).

Further, heavy metals such as Cd, Pb, and Hg have been implicated in AD pathology (99). Data show that Cd is involved in the formation of NFTs (100, 101). Both in cell models and in in vivo studies, Cd increases the activation of GSK-3β kinase, causing the hyperphosphorylation of tau (102). Similarly, Pb has been reported to modulate tau aggregation by increasing the activity of CDK5/p25 complex and GSK-3β kinase (103). Hg was demonstrated to inhibit tubulin that has a very high-affinity binding-site for Hg (104). Once Hg binds tubulin, the structural integrity of the protein is impaired. The final result of this interaction between Hg and tubulin is the formation of NFTs (105). Also, Hg is involved in tau hyperphosphorylation; the mechanism starts with the oxidative stress induced by Hg, ultimately affecting tau phosphorylation status (106).

OTHER METAL-BINDING PROTEINS AND THEIR ROLE IN AD

Several of the effects of an altered trace metal status in AD such as increased oxidative stress, neuroinflammation, and effects on synapses are mediated by excess or lack of trace metals for binding to proteins other than Aβ and tau. Together with several other factors in AD, oxidative stress leads to an activation of the immune system. The immune system is highly dependent on trace metal biology. Especially, Zn signaling seems to be a key mediator of inflammatory responses. For example, the activity of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), a major regulator of pro-inflammatory cytokines such as interleukins (IL) (107), is regulated, among others, by Zn through Zn binding of the IKK (IκB kinase) complex member IKKβ (108). Further, the formation of senile plaques made of Aβ aggregates stimulates inflammasomes, such as the NLRP3 (nucleotide-binding domain and leucine-rich repeat-containing family, pyrin domain-containing-3) inflammasome that detects the inflammatory Aβ aggregates and responds by forming active IL-1β through secreting caspase-1 (Casp-1) (109). IL-1β acts as an inflammatory cytokine (110), which leads to the creation of an inflammatory environment around the plaque. This ultimately decreases plaque degradation and destruction by microglia cells. Zn deficiency and/or high Cu levels facilitate NLRP3 inflammasome activation (111) and thereby the production of IL-1β in macrophages (112).

Initially, Pro-IL-1β is expressed in response to damage-associated molecular patterns (DAMPs) that bind to pattern recognition receptors (PRRs) on the macrophage to upregulate pro-inflammatory gene expression. Inflammation, protein misfolding, and aggregation, as well as neurodegeneration, lead to increased levels of so-called alarmins or DAMPs that include several cytokines including those from the S100 family. The S100 proteins are engaged in classical calcium-activated signaling but recent work has shown their involvement in new biochemical mechanisms in the brain related to the prevention of protein aggregation (113) and

sensing of neuronal Ca and Zn levels (114). Therefore, S100 alarmins are implicated in the maintenance of protein homeostasis (proteostasis) and metal ion homeostasis (metallostasis) in the brain. Upon activation and at high (μM) concentrations, S100 proteins act as extracellular cytokines via RAGE (receptor for advanced glycation end-products) mediated signaling. RAGE persistent engagement increases S100 extracellular levels via NF-κB activation resulting in a positive feedback cycle (115). Glial S100B and S100A9 proteins show increased expression in response to several risk factors for AD, including aging (116). Interestingly, S100B undergoes metal-binding-induced conformational changes and thereby delays the onset of Aβ aggregation by interacting with $A\beta_{1-42}$ monomers inhibiting primary nucleation (113). However, high levels of S100B can elicit alterations in intracellular Zn concentrations (114).

The increase in S100B proteins and accumulation of Aβ as a factor for trace metal imbalances also has direct effects on Zn signaling at excitatory glutamatergic synapses. It has been shown that the dynamics of major postsynaptic scaffold proteins of these synapses (SHANK2 and SHANK3) are dependent on Zn availability (117). Studies have shown that SHANK platform disassembly is linked to the molecular pathology of AD (118, 119), and recent research confirmed that the progressive accumulation of Aβ results in decreased Zn concentrations at the synapse, which in turn leads to disruption of SHANK3 scaffold formation, and ultimately, loss of synapses (13). Thus, Zn sequestration by protein aggregates in AD may be a contributor to the cognitive impairments caused by the loss of synapses through trapping synaptic Zn rather than through neurodegeneration in general (120). In addition, NMDA receptors at synapses are Zn-binding proteins (121). Increased trapping of Zn lowers the inhibitory activity of Zn on the NMDAR. Excessive stimulation of receptors at the excitatory synapse has been linked to neuronal death through excitotoxicity leading to chronic neurodegeneration in AD (122). Together, these metal-imbalance-driven signaling pathways create a vicious cycle leading to increased inflammation, oxidative stress, and neuronal damage (Figure 3).

METAL DETECTION FOR AD DIAGNOSIS

Several metal bioimaging strategies have been developed not only to examine the distribution of metals in human clinical AD brain tissue and AD mouse models but also to diagnose and monitor the progression of AD (123). In the clinical setting, the most common imaging tool is magnetic resonance imaging (MRI). This technique focuses on Fe due to its magnetic properties and its abundance in the brain. The latest MRI technology provides sufficient resolution to detect regional differences and has the major advantage that it can be applied to living patients rather than being a tool for postmortem analysis only (124). The presence of localized Fe can be detected by MRI (T2). However, although detecting general metal dyshomeostasis, MRI has limitations in visualizing metal-loaded plaques directly with high resolution. Further development of metal-based compounds or compounds visualizing metal homeostasis, such as a Cu-64-labelled–*bis* (thiosemicarbazonato) complex for clinical application in positron emission tomography (PET), and improvement of imaging devices may lead to more precise

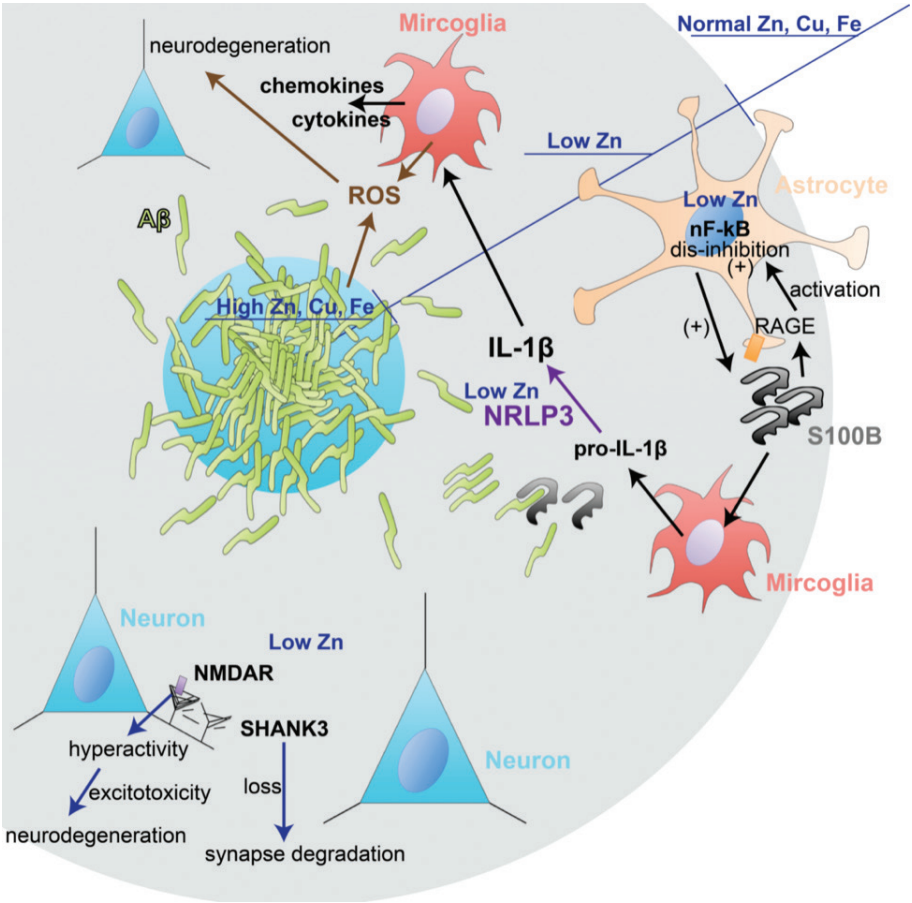

Figure 3 **Interaction between Aβ plaques, glial cells and trace metals.** Senile plaques sequester large amounts of Zn, Cu, and Fe, creating a zone of metal depletion, especially Zn depletion, in their vicinity. While high levels of Cu and Fe at the center of plaques may contribute to the generation of ROS and damage neurons through oxidative stress, Zn deficiency in a zone surrounding plaques will lead to further effects: Accumulation of Aβ aggregates leads to the release of S100B from astrocytes. S100B as DAMP can initially prevent Aβ aggregation. However, S100B signals back to astrocytes via RAGE receptor activation that will, in turn, activate NF-κB. Active NF-κB is dis-inhibited by low levels of Zn and thus results in further production of S100B, which enters a positive feedback cycle. High levels of S100B further deplete Zn through Zn binding. In response to high DAMP levels (S100B), microglia cells will produce pro-IL-1β. This will be cleaved by Casp-1-dependent processes through the NLRP3 inflammasome. NLRP3 activity is further increased by low Zn levels. Production of IL-1β leads to further generation of ROS and release of cytokines and chemokines from microglia cells that facilitate NFT formation and neurodegeneration. Further, low Zn levels facilitate excitotoxicity through dis-inhibition of NMDAR signaling, and low levels of Zn destabilize the postsynaptic Shank3 scaffold resulting in synapse loss.

diagnosis and monitoring of progression and therapeutic effects based on the role of trace metals in AD in the future (124).

METALS HOMEOSTASIS AS A THERAPEUTIC STRATEGY FOR AD

Based on the interactions of metals with several key proteins of the AD pathology, different therapeutic approaches aimed at restoring or manipulating metal homeostasis and, thereby, regulating oxidative stress, tau phosphorylation, Aβ aggregation, and inflammation have been developed in the last decade.

For example, metallothionein 3 (MT-3), a key regulator of metal homeostasis in neural tissue, has been found down-regulated by up to 30% in AD brains. Given that MT-3 contributes, among others, to detoxification and storage of heavy metals, regulation of Cu and Zn metabolism, and modulation of Aβ endocytosis of astrocytes (125), increasing MT-3 levels in AD has been explored as therapeutic strategy. In vivo studies demonstrated that effects of Zn-loaded MT-3 treatment in a mouse model for AD (Tg2576 mice) are inconsistent if MT-3 is injected subcutaneously. However, MT-3 injected intracerebroventricularly is able to ameliorate behavioral deficits and hippocampal impairments in APP/PS1 mice. In these mice, MT-3 treatment was also able to restore metal homeostasis, inhibit Aβ aggregation, and reduce oxidative stress and neurodegeneration (125).

Another interesting treatment strategy is based on metal protein attenuating compounds (MPACs): Clioquinol (CQ) represents the prototypic MPAC. It is a small hydrophobic molecule that can cross the BBB and that has moderate affinity for metal ions. When administered to Tg2576 mice, a 49% decrease of Aβ in the brain of AD model mice compared to control mice was shown (126). In humans, oral CQ treatment for 36 weeks of severely affected AD patients was able to significantly prevent cognitive deterioration. Subsequent clinical studies of this compound were not pursued. However, PBT2, a highly soluble derivate of CQ (a second-generation MPAC), has been used first in APP/PS1 mice and then in human clinical trials (phase I and II). The results showed improved cognitive performance and reduced Aβ load in the mouse model. A 12-week-long treatment of 78 patients with early AD showed that PBT2 is safe (127). Although the effects of PBT2 were inconsistent, executive dysfunction was significantly reduced in the patients. Several other metal chelators were engineered over the last years, and most of them are currently investigated for use in AD. Some of them have been shown to be effective at inhibiting Aβ–metal interactions both in vitro and in vivo. For example, it has been demonstrated that the normally insoluble Aβ deposits of postmortem brain tissue from AD patients can be solubilized in aqueous media in the presence of specific Cu chelators (128).

Another promising approach is the delivery of metals directly to the brain using nanotechnological approaches. Polymeric g7-poly-lactide-co-glycolide (PLGA) nanoparticles (NPs) are able to cross the BBB and release metals within the brain. This system has been considered as a Trojan horse strategy to effectively deliver Zn to the brain with a low-toxicity profile (129). Three hours after ip injection of NPs, an increase of Zn levels in the brain and the increase of zinc-sensitive

genes such as MT and Zn transporters were seen (130). The same pharmacological approach applied to APP23 mice, an animal model of AD, showed promising effects such as Aβ dis-aggregation, a reduction of inflammation, and synapse stabilization (129). Thus, both redistribution of metals bound to Aβ through MPACs and increase in metal levels that has dropped through trapping of metals in Aβ deposits have beneficial effects. However, additional research is necessary to redefine time point of application, duration, and concentration of NP-based metal delivery.

CONCLUSIONS AND FUTURE PERSPECTIVES

In general, impaired biometal homeostasis and/or the accumulation of non-essential trace metals have significant effects, most prominently on proteotoxic stress, synapse function, oxidative stress, and inflammatory processes. Building on the metal-binding abilities of key proteins in AD, brain imaging-based methods for the diagnosis of AD in humans have been, and are, currently developed. However, despite improving techniques for the detection of trace metals in the brain, re-establishing metal balances remains a difficult task. Initial studies using Zn ionophores have been promising and showed that targeting metal homeostasis in AD may be one of the most auspicious therapeutic strategies. However, new targeted and improved approaches are needed in the future.

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REFERENCES

1. Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. J Proteome Res. 2006;5(1):196–201. http://dx.doi.org/10.1021/pr050361j
2. Maret W. Metallomics: The science of biometals and biometalloids. In: Arruda MAZ, editor. Metallomics: The science of biometals. New York: Springer; 2018. p. 1–20.
3. Bitanihirwe BK, Cunningham MG. Zinc: The brain's dark horse. Synapse. 2009;63(11):1029–49. http://dx.doi.org/10.1002/syn.20683
4. Grochowski C, Blicharska E, Krukow P, Jonak K, Maciejewski M, Szczepanek D, et al. Analysis of trace elements in human brain: Its aim, methods, and concentration levels. Front Chem. 2019;7:115. http://dx.doi.org/10.3389/fchem.2019.00115
5. Krebs N, Langkammer C, Goessler W, Ropele S, Fazekas F, Yen K, et al. Assessment of trace elements in human brain using inductively coupled plasma mass spectrometry. J Trace Elem Med Biol. 2014;28(1):1–7. http://dx.doi.org/10.1016/j.jtemb.2013.09.006

6. Grabrucker AM, Ruozi B, Belletti D, Pederzoli F, Forni F, Vandelli MA, et al. Nanoparticle transport across the blood brain barrier. Tissue Barriers. 2016;4(1):e1153568. http://dx.doi.org/10.1080/21688370.2016.1153568
7. Baltaci AK, Yuce K. Zinc transporter proteins. Neurochem Res. 2018;43(3):517–30. http://dx.doi.org/10.1007/s11064-017-2454-y
8. Sensi SL, Granzotto A, Siotto M, Squitti R. Copper and zinc dysregulation in Alzheimer's disease. Trends Pharmacol Sci. 2018;39(12):1049–63. http://dx.doi.org/10.1016/j.tips.2018.10.001
9. Pfaender S, Grabrucker AM. Characterization of biometal profiles in neurological disorders. Metallomics. 2014;6(5):960–77. http://dx.doi.org/10.1039/C4MT00008K
10. Ziller A, Fraissinet-Tachet L. Metallothionein diversity and distribution in the tree of life: A multifunctional protein. Metallomics. 2018;10(11):1549–59. http://dx.doi.org/10.1039/C8MT00165K
11. Brown DR. α-Synuclein as a ferrireductase. Biochem Soc Trans. 2013;41(6):1513–17. http://dx.doi.org/10.1042/BST20130130
12. Roberts BR, Ryan TM, Bush AI, Masters CL, Duce JA. The role of metallobiology and amyloid-β peptides in Alzheimer's disease. J Neurochem. 2012;120(Suppl. 1):149–166. http://dx.doi.org/10.1111/j.1471-4159.2011.07500.x
13. Grabrucker AM, Schmeisser MJ, Udvardi PT, Arons M, Schoen M, Woodling NS, et al. Amyloid beta protein-induced zinc sequestration leads to synaptic loss via dysregulation of the ProSAP2/Shank3 scaffold. Mol Neurodegener. 2011;6:65. http://dx.doi.org/10.1186/1750-1326-6-65
14. Strozyk D, Launer LJ, Adlard PA, Cherny RA, Tsatsanis A, Volitakis I, et al. Zinc and copper modulate Alzheimer Abeta levels in human cerebrospinal fluid. Neurobiol Aging. 2009;30(7):1069–77. http://dx.doi.org/10.1016/j.neurobiolaging.2007.10.012
15. Cornett CR, Markesbery WR, Ehmann WD. Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. Neurotoxicology. 1998;19(3):339–45.
16. Perry G, Cash AD, Smith MA. Alzheimer disease and oxidative stress. J Biomed Biotechnol. 2002;2(3):120–3. http://dx.doi.org/10.1155/S1110724302203010
17. Huang WJ, Zhang X, Chen WW. Role of oxidative stress in Alzheimer's disease. Biomed Rep. 2016;4(5):519–22. http://dx.doi.org/10.3892/br.2016.630
18. Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem. 1992;59(5):1609–23. http://dx.doi.org/10.1111/j.1471-4159.1992.tb10990.x
19. Maret W. The redox biology of redox-inert zinc ions. Free Radic Biol Med. 2019;134:311–26. http://dx.doi.org/10.1016/j.freeradbiomed.2019.01.006
20. Cabrera ÁJ. Zinc, aging, and immunosenescence: An overview. Pathobiol Aging Age Relat Dis. 2015;5:25592. http://dx.doi.org/10.3402/pba.v5.25592
21. Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F, Dardenne M. Zinc deficiency in elderly patients. Nutrition. 1993;9(3):218–24.
22. Haase H, Rink L. The immune system and the impact of zinc during aging. Immun Ageing. 2009;6:9. http://dx.doi.org/10.1186/1742-4933-6-9
23. Hagmeyer S, Haderspeck JC, Grabrucker AM. Behavioral impairments in animal models for zinc deficiency. Front Behav Neurosci. 2014;8:443. http://dx.doi.org/10.3389/fnbeh.2014.00443
24. Greenough MA, Volitakis I, Li QX, Laughton K, Evin G, Ho M, et al. Presenilins promote the cellular uptake of copper and zinc and maintain copper chaperone of SOD1-dependent copper/zinc superoxide dismutase activity. J Biol Chem. 2011;286(11):9776–86. http://dx.doi.org/10.1074/jbc.M110.163964
25. Walsh DM, Minogue AM, Sala Frigerio C, Fadeeva JV, Wasco W, Selkoe DJ. The APP family of proteins: Similarities and differences. Biochem Soc Trans. 2007;35(Pt 2):416–20. http://dx.doi.org/10.1042/BST0350416
26. Bonda DJ, Lee HG, Blair JA, Zhu X, Perry G, Smith MA. Role of metal dyshomeostasis in Alzheimer's disease. Metallomics. 2011;3(3):267–70. http://dx.doi.org/10.1039/c0mt00074d
27. Kepp KP. Bioinorganic chemistry of Alzheimer's disease. Chem Rev. 2012;112(10):5193–239. http://dx.doi.org/10.1021/cr300009x
28. Dahms SO, Könnig I, Roeser D, Gührs KH, Mayer MC, Kaden D, et al. Metal binding dictates conformation and function of the amyloid precursor protein (APP) E2 domain. J Mol Biol. 2012;416(3):438–52. http://dx.doi.org/10.1016/j.jmb.2011.12.057

29. Barnham KJ, McKinstry WJ, Multhaup G, Galatis D, Morton CJ, Curtain CC, et al. Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis. J Biol Chem. 2003;278(19):17401–7. http://dx.doi.org/10.1074/jbc.M300629200
30. Spoerri L, Vella LJ, Pham CL, Barnham KJ, Cappai R. The amyloid precursor protein copper binding domain histidine residues 149 and 151 mediate APP stability and metabolism. J Biol Chem. 2012;287(32):26840–53. http://dx.doi.org/10.1074/jbc.M112.355743
31. Acevedo KM, Hung YH, Dalziel AH, Li QX, Laughton K, Wikhe K, et al. Copper promotes the trafficking of the amyloid precursor protein. J Biol Chem. 2011;286(10):8252–62. http://dx.doi.org/10.1074/jbc.M110.128512
32. Bellingham SA, Coleman LA, Masters CL, Camakaris J, Hill AF. Regulation of prion gene expression by transcription factors SP1 and metal transcription factor-1. J Biol Chem. 2009;284(2):1291–301. http://dx.doi.org/10.1074/jbc.M804755200
33. Bush AI, Multhaup G, Moir RD, Williamson TG, Small DH, Rumble B, et al. A novel zinc(II) binding site modulates the function of the beta A4 amyloid protein precursor of Alzheimer's disease. J Biol Chem. 1993;268(22):16109–12.
34. Bush AI, Pettingell WH, Multhaup G, d Paradis M, Vonsattel JP, Gusella JF, et al. Rapid induction of Alzheimer A beta amyloid formation by zinc. Science. 1994;265(5177):1464–7. http://dx.doi.org/10.1126/science.8073293
35. Scheuermann S, Hambsch B, Hesse L, Stumm J, Schmidt C, Beher D, et al. Homodimerization of amyloid precursor protein and its implication in the amyloidogenic pathway of Alzheimer's disease. J Biol Chem. 2001;276(36):33923–9. http://dx.doi.org/10.1074/jbc.M105410200
36. Rogers JT, Randall JD, Cahill CM, Eder PS, Huang X, Gunshin H, et al. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem. 2002;277(47):45518–28. http://dx.doi.org/10.1074/jbc.M207435200
37. Venti A, Giordano T, Eder P, Bush AI, Lahiri DK, Greig NH, et al. The integrated role of desferrioxamine and phenserine targeted to an iron-responsive element in the APP-mRNA 5'-untranslated region. Ann N Y Acad Sci. 2004;1035:34–48. http://dx.doi.org/10.1196/annals.1332.003
38. Duce JA, Tsatsanis A, Cater MA, James SA, Robb E, Wikhe K, et al. Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. Cell. 2010;142(6):857–67. http://dx.doi.org/10.1016/j.cell.2010.08.014
39. Cross JB, Duca JS, Kaminski JJ, Madison VS. The active site of a zinc-dependent metalloproteinase influences the computed pK(a) of ligands coordinated to the catalytic zinc ion. J Am Chem Soc. 2002;124(37):11004–7. http://dx.doi.org/10.1021/ja0201810
40. Angeletti B, Waldron KJ, Freeman KB, Bawagan H, Hussain I, Miller CC, et al. BACE1 cytoplasmic domain interacts with the copper chaperone for superoxide dismutase-1 and binds copper. J Biol Chem. 2005;280(18):17930–7. http://dx.doi.org/10.1074/jbc.M412034200
41. Park IH, Jung MW, Mori H, Mook-Jung I. Zinc enhances synthesis of presenilin 1 in mouse primary cortical culture. Biochem Biophys Res Commun. 2001;285(3):680–8. http://dx.doi.org/10.1006/bbrc.2001.5243
42. Savelieff MG, Lee S, Liu Y, Lim MH. Untangling amyloid-β, tau, and metals in Alzheimer's disease. ACS Chem Biol. 2013;8(5):856–65. http://dx.doi.org/10.1021/cb400080f
43. Johnstone EM, Chaney MO, Norris FH, Pascual R, Little SP. Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis. Brain Res Mol Brain Res. 1991;10(4):299–305. http://dx.doi.org/10.1016/0169-328X(91)90088-F
44. Gaggelli E, Grzonka Z, Kozłowski H, Migliorini C, Molteni E, Valensin D, et al. Structural features of the Cu(II) complex with the rat Abeta(1–28) fragment. Chem Commun (Camb). 2008;(3):341–3. http://dx.doi.org/10.1039/B713453C
45. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci. 1998;158(1):47–52. http://dx.doi.org/10.1016/S0022-510X(98)00092-6
46. Cuajungco MP, Lees GJ. Zinc and Alzheimer's disease: Is there a direct link? Brain Res Brain Res Rev. 1997;23(3):219–36. http://dx.doi.org/10.1016/S0165-0173(97)00002-7

47. Huang X, Atwood CS, Moir RD, Hartshorn MA, Vonsattel JP, Tanzi RE, et al. Zinc-induced Alzheimer's Abeta1–40 aggregation is mediated by conformational factors. J Biol Chem. 1997;272(42):26464–70. http://dx.doi.org/10.1074/jbc.272.42.26464
48. Kim AC, Lim S, Kim YK. Metal ion effects on Aβ and tau aggregation. Int J Mol Sci. 2018;19(1):128. http://dx.doi.org/10.3390/ijms19010128
49. Minicozzi V, Stellato F, Comai M, Dalla Serra M, Potrich C, Meyer-Klaucke W, et al. Identifying the minimal copper- and zinc-binding site sequence in amyloid-beta peptides. J Biol Chem. 2008;283(16):10784–92. http://dx.doi.org/10.1074/jbc.M707109200
50. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, et al. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron. 2001;30(3):665–76. http://dx.doi.org/10.1016/S0896-6273(01)00317-8
51. Bush AI. Metal complexing agents as therapies for Alzheimer's disease. Neurobiol Aging. 2002;23(6):1031–8. http://dx.doi.org/10.1016/S0197-4580(02)00120-3
52. Telling ND, Everett J, Collingwood JF, Dobson J, van der Laan G, Gallagher JJ, et al. Iron biochemistry is correlated with amyloid plaque morphology in an established mouse model of Alzheimer's disease. Cell Chem Biol. 2017;24(10):1205–1215.e3. http://dx.doi.org/10.1016/j.chembiol.2017.07.014
53. van Duijn S, Bulk M, van Duinen SG, Nabuurs RJA, van Buchem MA, van der Weerd L, et al. Cortical iron reflects severity of Alzheimer's disease. J Alzheimers Dis. 2017;60(4):1533–45.
54. Tong Y, Yang H, Tian X, Wang H, Zhou T, Zhang S, et al. High manganese, a risk for Alzheimer's disease: High manganese induces amyloid-β related cognitive impairment. J Alzheimers Dis. 2014;42(3):865–78. http://dx.doi.org/10.3233/JAD-140534
55. Neumann M, Diekmann S, Bertsch U, Vanmassenhove B, Bogerts B, Kretzschmar HA. Novel G335V mutation in the tau gene associated with early onset familial frontotemporal dementia. Neurogenetics. 2005;6(2):91–5. http://dx.doi.org/10.1007/s10048-005-0210-y
56. Bunker JM. Modulation of microtubule dynamics by tau in living cells: Implications for development and neurodegeneration. Mol Biol Cell. 2004;15(6):2720–8. http://dx.doi.org/10.1091/mbc.e04-01-0062
57. Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Götz J. Dendritic function of tau mediates amyloid-β toxicity in Alzheimer's disease mouse models. Cell. 2010;142(3):387–97. http://dx.doi.org/10.1016/j.cell.2010.06.036
58. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. Proc Natl Acad Sci U S A. 1975;72(5):1858–62. http://dx.doi.org/10.1073/pnas.72.5.1858
59. Fontaine SN, Sabbagh JJ, Baker J, Martinez-Licha AD, Chad AD. Cellular factors modulating the mechanism of tau protein aggregation. Cell Mol Life Sci. 2015;72(10):1863–79. http://dx.doi.org/10.1007/s00018-015-1839-9
60. Cleveland DW, Hwo SY, Kirschner MW. Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin. J Mol Biol. 1977;116(2):207–225. http://dx.doi.org/10.1016/0022-2836(77)90213-3
61. Mandelkow EM, Mandelkow E. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. Cold Spring Harb Perspect Med. 2012;2(7):a006247. http://dx.doi.org/10.1101/cshperspect.a006247
62. Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, Biernat J, Griesinger C, Zweckstetter M. Structural polymorphism of 441-residue Tau at single residue resolution. PLoS Biol. 2009;7(2):e34. http://dx.doi.org/10.1371/journal.pbio.1000034
63. Hirokawa N, Shiomura Y, Okabe S. Tau proteins: The molecular structure and mode of binding on microtubules. J Cell Biol. 1988;107(4):1449–59. http://dx.doi.org/10.1083/jcb.107.4.1449
64. Brandt R, Léger J, Lee G. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. J Cell Biol. 1995;131(5):1327–40. http://dx.doi.org/10.1083/jcb.131.5.1327
65. Sergeant N, Bretteville A, Hamdane M, Caillet-Boudin ML, Grognet P, Bombois S, Buée L. Biochemistry of Tau in Alzheimer's disease and related neurological disorders. Expert Rev Proteomics. 2008;5(2):207–24. http://dx.doi.org/10.1586/14789450.5.2.207

66. Sergeant N, Delacourte A, Buée L. Tau protein as a differential biomarker of tauopathies. Biochim Biophys Acta. 2005;1739(2–3):179–97. http://dx.doi.org/10.1016/j.bbadis.2004.06.020
67. Cox K, Combs B, Abdelmesih B, Morfini G, Brady ST, Kanaan NM. Analysis of isoform-specific tau aggregates suggests a common toxic mechanism involving similar pathological conformations and axonal transport inhibition. Neurobiol Aging. 2016;47:113–26. http://dx.doi.org/10.1016/j.neurobiolaging.2016.07.015
68. Sayre LM, Perry G, Harris PLR, Liu Y, Schubert KA, Smith MA. In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: A central role for bound transition metals. J Neurochem. 2000;74(1):270–279. http://dx.doi.org/10.1046/j.1471-4159.2000.0740270.x
69. Mo ZY, Zhu YZ, Zhu HL, Fan JB, Chen J, Liang Y. Low micromolar zinc accelerates the fibrillization of human Tau via bridging of Cys-291 and Cys-322. J Biol Chem. 2009;284(50):34648–57. http://dx.doi.org/10.1074/jbc.M109.058883
70. Kim I, Park EJ, Seo J, Ko SJ, Lee J, Kim CH. Zinc stimulates tau S214 phosphorylation by the activation of Raf/mitogen-activated protein kinase-kinase/extracellular signal-regulated kinase pathway. Neuro Rep. 2011;22(16):839–44. http://dx.doi.org/10.1097/WNR.0b013e32834c0a2d
71. Xiong Y, Jing XP, Zhou XW, Wang XL, Yang Y, Sun XY, Wang JZ. Zinc induces protein phosphatase 2A inactivation and tau hyperphosphorylation through Src dependent PP2A (tyrosine 307) phosphorylation. Neurobiol Aging. 2013;34(3):745–56. http://dx.doi.org/10.1016/j.neurobiolaging.2012.07.003
72. Huang Y, Wu Z, Cao Y, Lang M., Lu B, Zhou B. Zinc binding directly regulates tau toxicity independent of tau hyperphosphorylation. Cell Rep. 2014;8(3):831–42. http://dx.doi.org/10.1016/j.celrep.2014.06.047
73. Li X, Du X, Ni J. Zn^{2+} aggravates tau aggregation and neurotoxicity. Int J Mol Sci. 2019;20(3):pii: E487. http://dx.doi.org/10.3390/ijms20030487
74. Shafiei SS, Guerrero-Muñoz MJ, Castillo-Carranza DL. Tau oligomers: Cytotoxicity, propagation, and mitochondrial damage. Front Aging Neurosci. 2017;9:83. http://dx.doi.org/10.3389/fnagi.2017.00083
75. Cristóvão JS, Santos R, Gomes CM. Metals and neuronal metal binding proteins implicated in Alzheimer's disease. Oxid Med Cell Longevity. 2016;(1):1–13. http://dx.doi.org/10.1155/2016/9812178
76. Crouch PJ, Hung LW, Adlard PA, Cortes M, Lal V, Filiz G, et al. Increasing Cu bioavailability inhibits abetaoligomers and tau phosphorylation. Proc Natl Acad Sci. 2009;106(2):381–6. http://dx.doi.org/10.1073/pnas.0809057106
77. Voss K, Harris C, Ralle M, Duffy M, Murchison C, Quinn JF. Modulation of tau phosphorylation by environmental copper. Trans Neurodegener. 2014;3(1):24. http://dx.doi.org/10.1186/2047-9158-3-24
78. Kitazawa M, Cheng D, Laferla FM. Chronic copper exposure exacerbates both amyloid and tau pathology and selectively dysregulates cdk5 in a mouse model of AD. J Neurochem. 2009;108(6):1550–60. http://dx.doi.org/10.1111/j.1471-4159.2009.05901.x
79. Soragni A, Zambelli B, Mukrasch MD, Biernat J, Jeganathan S, Griesinger C, et al. Structural characterization of binding of Cu (II) to tau protein. Biochemistry. 2008;47(41):10841–51. http://dx.doi.org/10.1021/bi8008856
80. Ma Q, Li Y, Du J, Liu H, Kanazawa K, Nemoto T, et al. Copper binding properties of a tau peptide associated with Alzheimer's disease studied by CD, NMR, and MALDI-TOF MS. Peptides. 2006;27(4):841–9. http://dx.doi.org/10.1016/j.peptides.2005.09.002
81. Zhou LX, Du JT, Zeng ZY, Wu WH, Zhao YF, Kanazawa K, et al. Copper (II) modulates in vitro aggregation of a tau peptide. Peptides. 2007;28(11):2229–34. http://dx.doi.org/10.1016/j.peptides.2007.08.022
82. Su XY, Wu WH, Huang ZP, Hu J, Lei P, Yu CH, et al. Hydrogen peroxide can be generated by tau in the presence of Cu(II). Biochem Biophys Res Commun. 2007;358(2):661–5. http://dx.doi.org/10.1016/j.bbrc.2007.04.191
83. Ma Q, Li Y, Du J, Kanazawa K, Nemoto T, Nakanishi H, et al. Binding of copper (II) ion to an Alzheimer's tau peptide as revealed by MALDI-TOF MS, CD, and NMR. Biopolymers. 2005;79(2):74–85. http://dx.doi.org/10.1002/bip.20335

84. Andrási, E, Farkas É, Scheibler H, Réffy A, Bezúr L. Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. Arch Gerontol Geriatr. 1995;21(1):89–97. http://dx.doi.org/10.1016/0167-4943(95)00643-Y
85. Smith MA, Harris PLR, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A. 1997;94(18):9866–8. http://dx.doi.org/10.1073/pnas.94.18.9866
86. Ahmadi S, Zhu S, Sharma R, Wilson DJ, Kraatz H. Interaction of metal ions with tau protein. The case for a metal-mediated tau aggregation. J Inorganic Biochem. 2018;194:44–51. http://dx.doi.org/10.1016/j.jinorgbio.2019.02.007
87. Rao SS, Adlard PA. Untangling tau and iron: Exploring the interaction between iron and tau in neurodegeneration. Front Mol Neurosci. 2018;11:276. http://dx.doi.org/10.3389/fnmol.2018.00276
88. Guo C, Wang P, Zhong ML, Wang T, Huang XS, Li JY, Wang ZY. Deferoxamine inhibits iron induced hippocampal tau phosphorylation in the Alzheimer transgenic mouse brain. Neurochem Int. 2013;62(2):165–72. http://dx.doi.org/10.1016/j.neuint.2012.12.005
89. Andrási E, Páli N, Molnár Z, Kösel S. Brain aluminum, magnesium and phosphorus contents of control and Alzheimer-diseased patients. J Alzheimers Dis. 2005;7(4):273–84. http://dx.doi.org/10.3233/JAD-2005-7402
90. Xu ZP, Li L, Bao J, Wang ZH, Zeng J, Liu EJ, Wang JZ. Magnesium protects cognitive functions and synaptic plasticity in streptozotocin-induced sporadic Alzheimer's model. PLoS One. 2014;9(9):e108645. http://dx.doi.org/10.1371/journal.pone.0108645
91. Ramos P, Santos A, Pinto NR, Mendes R, Magalhães T, Almeida A. Iron levels in the human brain: A post-mortem study of anatomical region differences and age-related changes. J Trace Elements Med Biol. 2014;28(1):13–17. http://dx.doi.org/10.1016/j.jtemb.2013.08.001
92. Cai T, Che H, Yao T, Chen Y, Huang C, Zhang LW. Manganese induces Tau hyperphosphorylation through the activation of ERK MAPK pathway in PC12 cells. Toxicol Sci. 2011;119(1):169–77. http://dx.doi.org/10.1093/toxsci/kfq308
93. Rankin CA, Sun Q, Gamblin TC. Tau phosphorylation by GSK-3ß promotes tangle-like filament morphology. Mol Neurodegener. 2007;2:12. http://dx.doi.org/10.1186/1750-1326-2-12
94. Liu F, Li B, Tung EJ, Grundke-Iqbal I, Iqbal K, Gong C. X. Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation. Eur J Neurosci. 2007;26(12):3429–36. http://dx.doi.org/10.1111/j.1460-9568.2007.05955.x
95. Nübling G, Bader B, Levin J, Hildebrandt J, Kretzschmar H, Giese A. Synergistic influence of phosphorylation and metal ions on tau oligomer formation and coaggregation with α -synuclein at the single molecule level. Mol Neurodegener. 2012;7:35. http://dx.doi.org/10.1186/1750-1326-7-35
96. Klatzo I, Wisniewski H, Streicher E. Experimental production of neurofibrillary degeneration: I. Light microscopic observations. J Neuropathol Exp Neurol. 1965;24(2):187–99. http://dx.doi.org/10.1097/00005072-196504000-00002
97. Walton JR. An aluminum-based rat model for Alzheimer's disease exhibits oxidative damage, inhibition of PP2A activity, hyperphosphorylated tau, and granulovacuolar degeneration. J Inorganic Biochem. 2007;101(9):1275–84. http://dx.doi.org/10.1016/j.jinorgbio.2007.06.001
98. Budimir A. Metal ions, Alzheimer's disease and chelation therapy. Acta Pharm. 2011;61:1–14. http://dx.doi.org/10.2478/v10007-011-0006-6
99. Panayi AE, Spyrou NM, Iversen BS, White MA, Part P. Determination of cadmium and zinc in Alzheimer's brain tissue using inductively coupled plasma mass spectrometry. J Neurol Sci. 2002;195(1):1–10. http://dx.doi.org/10.1016/S0022-510X(01)00672-4
100. Notarachille G, Arnesano F, Calò V, Meleleo D. Heavy metals toxicity: Effect of cadmium ions on amyloid beta protein 1-42. Possible implications for Alzheimer's disease. Bio Metals. 2014;27(2):371–88. http://dx.doi.org/10.1007/s10534-014-9719-6
101. Jiang LF, Yao TM, Zhu ZL, Wang C, Ji LN. Impacts of Cd (II) on the conformation and self-aggregation of Alzheimer's tau fragment corresponding to the third repeat of microtubule-binding domain. Biochim Biophys Acta. 2007;1774(11):1414–21. http://dx.doi.org/10.1016/j.bbapap.2007.08.014
102. del Pino J, Zeballos G, Anadón MJ, Moyano P, Díaz MJ, García JM, et al. Cadmium-induced cell death of basal forebrain cholinergic neurons mediated by muscarinic M1 receptor blockade, increase in GSK-3β enzyme, β-amyloid and tau protein levels. Arch Toxicol. 2016;90:1081–92. http://dx.doi.org/10.1007/s00204-015-1540-7

103. Bihaqi SW, Bahmani A, Adem A, Zawia NH. Infantile postnatal exposure to lead (Pb) enhances tau expression in the cerebral cortex of aged mice: Relevance to AD. Neuro Toxicol. 2014;44:114–20. http://dx.doi.org/10.1016/j.neuro.2014.06.008
104. Cedrola S, Guzzi GP, Ferrari D, Gritti A, Vescovi AL, Pendergrass JC, et al. Inorganic mercury changes the fate of murine CNS stem cells. FASEB J. 2003;17(8):869–71. http://dx.doi.org/10.1096/fj.02-0491fje
105. Mutter J, Naumann J, Sadaghiani C, Schneider R, Walach H. Alzheimer disease: Mercury as pathogenetic factor and apolipoprotein E as a moderator. Neuro Endocrinol Lett. 2004;25(5):331–9.
106. Busciglio J, Lorenzo A, Yeh J, Yankner BA. β-Amyloid fibrils induce tau phosphorylation and loss of microtubule binding. Neuron. 1995;14(4):879–88. http://dx.doi.org/10.1016/0896-6273(95)90232-5
107. Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Mol Cell Biol. 1990;10(5):2327–34. http://dx.doi.org/10.1128/MCB.10.5.2327
108. Liu MJ, Bao S, Gálvez-Peralta M, Pyle CJ, Rudawsky AC, Pavlovicz RE, et al. ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB. Cell Rep. 2013;3(2):386–400. http://dx.doi.org/10.1016/j.celrep.2013.01.009
109. Saco T, Parthasarathy PT, Cho Y, Lockey RF, Kolliputi N. Inflammasome: A new trigger of Alzheimer's disease. Front Aging Neurosci. 2014;6:80. http://dx.doi.org/10.3389/fnagi.2014.00080
110. Prinz M, Priller J, Sisodia SS, Ransohoff RM. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. Nat Neurosci. 2011;14(10):1227–35. http://dx.doi.org/10.1038/nn.2923
111. Deigendesch N, Zychlinsky A, Meissner F. Copper regulates the canonical NLRP3 inflammasome. J Immunol. 2018;200(5):1607–17. http://dx.doi.org/10.4049/jimmunol.1700712
112. Summersgill H, England H, Lopez-Castejon G, Lawrence CB, Luheshi NM, Pahle J, et al. Zinc depletion regulates the processing and secretion of IL-1β. Cell Death Dis. 2014;5:e1040. http://dx.doi.org/10.1038/cddis.2013.547
113. Cristóvão JS, Morris VK, Cardoso I, Leal SS, Martínez J, Botelho HM, et al. The neuronal S100B protein is a calcium-tuned suppressor of amyloid-β aggregation. Sci Adv. 2018;4(6):eaaq1702. http://dx.doi.org/10.1126/sciadv.aaq1702
114. Hagmeyer S, Cristóvão JS, Mulvihill JJE, Boeckers TM, Gomes CM, Grabrucker AM. Zinc binding to S100B affords regulation of trace metal homeostasis and excitotoxicity in the brain. Front Mol Neurosci. 2017;10:456. http://dx.doi.org/10.3389/fnmol.2017.00456
115. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, et al. Functions of S100 proteins. Curr Mol Med. 2013;13(1):24–57. http://dx.doi.org/10.2174/156652413804486214
116. Swindell WR, Johnston A, Xing X, Little A, Robichaud P, Voorhees JJ, et al. Robust shifts in S100a9 expression with aging: A novel mechanism for chronic inflammation. Sci Rep. 2013;3:1215. http://dx.doi.org/10.1038/srep01215
117. Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, et al. Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation. EMBO J. 2011;30(3):569–81. http://dx.doi.org/10.1038/emboj.2010.336
118. Roselli F, Hutzler P, Wegerich Y, Livrea P, Almeida OF. Disassembly of shank and homer synaptic clusters is driven by soluble beta-amyloid(1-40) through divergent NMDAR-dependent signalling pathways. PLoS One. 2009;4(6):e6011. http://dx.doi.org/10.1371/journal.pone.0006011
119. Gong Y, Lippa CF, Zhu J, Lin Q, Rosso AL. Disruption of glutamate receptors at Shank-postsynaptic platform in Alzheimer's disease. Brain Res. 2009;1292:191–8. http://dx.doi.org/10.1016/j.brainres.2009.07.056
120. Adlard PA, Parncutt JM, Finkelstein DI, Bush AI. Cognitive loss in zinc transporter-3 knockout mice: A phenocopy for the synaptic and memory deficits of Alzheimer's disease? J Neurosci. 2010;30(5):1631–6. http://dx.doi.org/10.1523/JNEUROSCI.5255-09.2010
121. Paoletti P, Ascher P, Neyton J. High-affinity zinc inhibition of NMDA NR1-NR2A receptors. J Neurosci. 1997;17(15):5711–25. http://dx.doi.org/10.1523/JNEUROSCI.17-15-05711.1997
122. Johanssen T, Suphantarida N, Donnelly PS, Liu XM, Petrou S, Hill AF, et al. PBT2 inhibits glutamate-induced excitotoxicity in neurons through metal-mediated preconditioning. Neurobiol Dis. 2015;81:176–85. http://dx.doi.org/10.1016/j.nbd.2015.02.008
123. Braidy N, Poljak A, Marjo C, Rutlidge H, Rich A, Jayasena T, et al. Metal and complementary molecular bioimaging in Alzheimer's disease. Front Aging Neurosci. 2014;6:138. http://dx.doi.org/10.3389/fnagi.2014.00138

124. Bourassa MW, Miller LM. Metal imaging in neurodegenerative diseases. Metallomics. 2012;4(8): 721–38. http://dx.doi.org/10.1039/c2mt20052j
125. Xu W, Xu Q, Cheng H, Tan X. The efficacy and pharmacological mechanism of Zn7MT3 to protect against Alzheimer's disease. Sci Rep. 2017;7(1):13763. http://dx.doi.org/10.1038/s41598-017-12800-x
126. Bush AI. The metallobiology of Alzheimer's disease. Trends Neurosci. 2003;26(4):207–14. http://dx.doi.org/10.1016/S0166-2236(03)00067-5
127. Sampson EL, Jenagaratnam L, McShane R. Metal protein attenuating compounds for the treatment of Alzheimer's dementia. Cochrane Database Syst Rev. 2012;(5):CD005380. http://dx.doi.org/10.1002/14651858.CD005380.pub4
128. Cherny RA, Legg JT, McLean CA, Fairlie DP, Huang X, Atwood CS, et al. Aqueous dissolution of Alzheimer's disease Abeta amyloid deposits by biometal depletion. J Biol Chem. 1999; 274(33): 23223–8. http://dx.doi.org/10.1074/jbc.274.33.23223
129. Vilella A, Belletti D, Sauer AK, Hagmeyer S, Sarowar T, Masoni M, et al. Reduced plaque size and inflammation in the APP23 mouse model for Alzheimer's disease after chronic application of polymeric nanoparticles for CNS targeted zinc delivery. J Trace Elem Med Biol. 2018;49:210–21. http://dx.doi.org/10.1016/j.jtemb.2017.12.006
130. Chhabra R, Ruozi B, Vilella A, Belletti D, Mangus K, Pfaender S, et al. Application of polymeric nanoparticles for CNS targeted zinc delivery in vivo. CNS Neurol Disord Drug Targets. 2015;14(8):1041–53. http://dx.doi.org/10.2174/1871527314666150821111455

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N-Terminally Truncated Aβ Peptide Variants in Alzheimer's Disease

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Abstract: The accumulation and aggregation of amyloid-β (Aβ) peptides in the brain is believed to be the initial trigger in the molecular pathology of Alzheimer's disease (AD). In addition to the widely studied full-length Aβ peptides (mainly $Aβ_{1–40}$ and $Aβ_{1–42}$), a variety of amino-terminally truncated (N-truncated) peptides, such as $Aβ_{pE3-x}$ and $Aβ_{4-x}$, have been detected in high abundance in autopsy samples from sporadic and familial AD patients. N-truncated Aβ species adopt specific physicochemical properties resulting in a higher aggregation propensity and increased peptide stability, which likely account for their neurotoxic potential. The presence of N-truncated Aβ peptides in transgenic mouse models of AD and the selective overexpression of specific N-truncated variants in the murine brain have facilitated their investigation in relevant in vivo settings. In this chapter, we address the pathological relevance of N-truncated Aβ peptide species and summarize the current knowledge about the enzymatic activities that might be involved in their generation.

Keywords: ADAMTS4; Alzheimer's disease; amyloid; N-truncation; protease

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INTRODUCTION

The deposition of extracellular plaques consisting of amyloid-β (Aβ) peptides in the brain parenchyma is one of the neuropathological hallmarks of Alzheimer's disease (AD). Although these deposits have also been found in non-demented control individuals, they are believed to play an important role in the disease process, and their presence and abundance is an obligatory criterion for a diagnosis of AD. Full-length Aβ peptides composed of 40 ($Aβ_{1–40}$) or 42 ($Aβ_{1–42}$) amino acids constitute the main components of extracellular amyloid plaques, together with other proteins such as ubiquitin and different proteoglycans. These peptides are generated by sequential proteolytic cleavage of the amyloid precursor protein (APP), a large type-I transmembrane protein that in rare families was found to carry mutations causative of inherited cases of AD. After an initial cleavage by either α- or β-secretase, which facilitates shedding of the APP ectodomain, the remaining membrane-bound β- or α-C-terminal fragments (CTFs) are cleaved by γ-secretase within their transmembrane domains. In the latter case, a small peptide fragment named p3 is released, while the cleavage of β-CTFs results in the generation of Aβ peptides (1) (Figure 1).

The analysis of brain samples from non-demented control cases, pathological aging (which is being regarded as a prodromal phase of AD), and AD revealed that, apart from full-length $Aβ_{1–40}$ and $Aβ_{1–42}$, N-truncated $Aβ_{x-42}$ species were the most abundant in AD with considerable overlap in pathological aging samples (2). This is interesting from a pathological point of view as full-length Aβ peptides are normal metabolites generated under physiological conditions. The exact physiological function of these peptides remains unresolved; however, it has been hypothesized that modulation of endogenous Aβ production might play an important role in the regulation of neuronal activity via a feedback loop mechanism (3). Other possible physiological functions include promoting recovery from traumatic brain injury, sealing leaks in the blood–brain barrier, or antimicrobial activities (4). While full-length Aβ peptides starting with an aspartic acid (Asp) residue at position 1 of the Aβ sequence are generated by an enzymatic activity called β-site APP cleaving enzyme 1 (BACE1) (5, 6), much less is known about the proteases responsible for the production of N-truncated Aβ peptides.

Aβ peptides with varying N-termini were described more than 30 years ago. In 1984, the identification of full-length Aβ peptides starting with an Asp residue in position 1 purified from cerebrovascular amyloid deposits was reported (7). The following year, N-terminal sequencing of Aβ peptides purified from amyloid plaque cores from AD cases demonstrated the presence of peptides starting with phenylalanine (Phe) in position four ($Aβ_{4-x}$), as well as with serine (Ser) or glycine (Gly) in position eight ($Aβ_{8-x}$) or nine ($Aβ_{9-x}$) (8, 9). By means of immunohistochemistry, N-truncated Aβ species with post-translational modifications such as pyroglutamylation at position 3 ($Aβ_{pE3-x}$) and 11($Aβ_{pE11-x}$) were subsequently described in human AD brains (10, 11). The loss of charged amino acids at the N-terminus changes the biophysical properties of the Aβ peptides, thus influencing their aggregation propensity and toxicity. As a consequence, efforts to understand the relevance of N-truncated Aβ species in the pathogenesis of AD, as well as the mechanisms responsible for their generation, have recently increased.

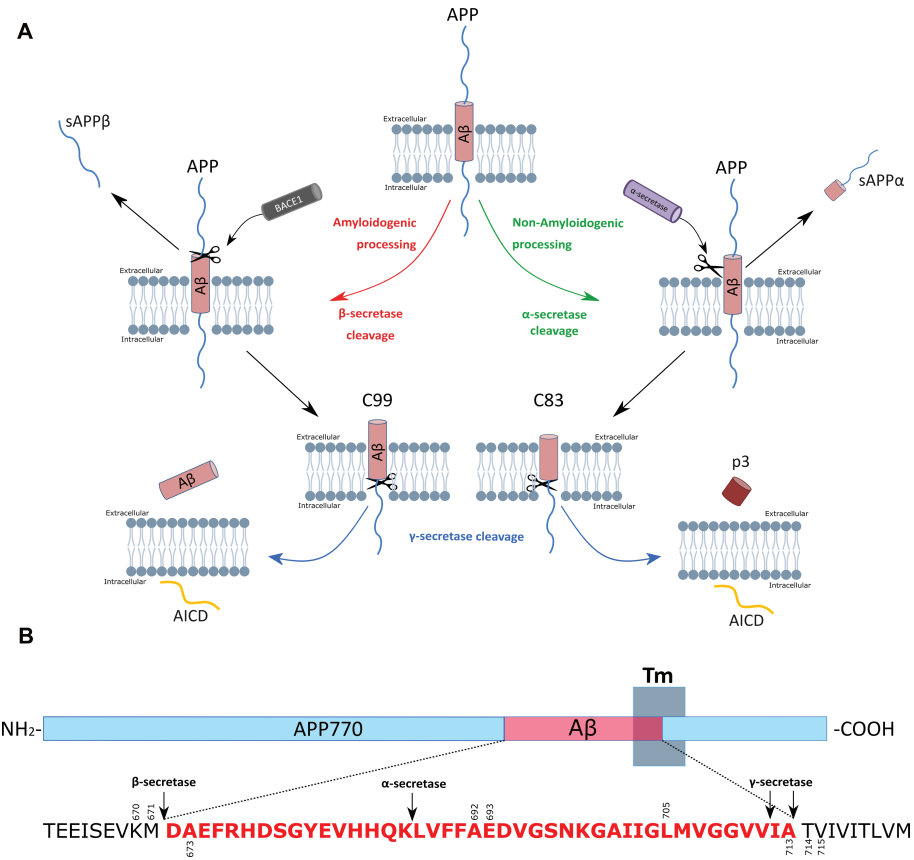

Figure 1 **APP processing pathways. A**) The non-amyloidogenic processing pathway (depicted on the right) is initiated through cleavage by α-secretase, which cleaves within the Aβ domain and generates the soluble ectodomain sAPPα. Subsequent cleavage of the membrane-bound C-terminal APP fragment C83 by the γ-secretase complex releases the soluble fragments p3 and the APP intracellular domain (AICD). Amyloidogenic APP processing (left panel) is initiated by β-secretase cleavage with the liberation of the soluble sAPPβ fragment. The remaining C-terminal fragment C99 is then cleaved by γ-secretase generating Aβ peptides as well as AICD. **B**) APP is a large transmembrane protein containing up to 770 amino acids. The Aβ peptide sequence (in red) starts within the ectodomain and ends within the transmembrane (TM) domain.

HETEROGENEITY OF N-TRUNCATED Aβ SPECIES IN AD BRAIN

Several studies employing mass spectrometry (MS) that intended to analyze the full spectrum of Aβ peptides in postmortem brain samples of AD patients have been published. In the earliest of these studies, purified amyloid core and cerebrovascular amyloid peptides were sequenced using matrix-assisted laser-desorption-time-of-flight (MALDI-TOF) mass spectrometry. While the amino acid composition of cerebrovascular Aβ peptides consisted mainly of species

starting with residues 1 or 2, the preparations from amyloid cores were more heterogeneous, corresponding to peptides beginning with every residue between Asp-1 and Glu-11(Figure 2), with major signals for peptides starting with Phe-4, Ser-8, and Glu-11 (12). In good agreement, using surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry, $A\beta_{4\text{-}42}$ was also identified as the major N-truncated species in postmortem brain samples from aged controls, patients with vascular dementia, and AD patients (13). This suggested that N-truncated species account for a substantial proportion of total Aβ in the aged human brain, a finding that was corroborated in subsequent studies. The entire spectrum of Aβ peptides ranging from $A\beta_{1\text{-}x}$ to $A\beta_{pE11\text{-}x}$was detected in frontal cortex samples of a sporadic AD case and of an individual affected by the FAD-associated presenilin (*PSEN1)* V261I mutation. This mutation is associated with the deposition of the so-called cotton wool plaques, which are lesions lacking a central amyloid core (14). By investigating non-demented individuals with incipient amyloid pathology as well as AD patients, it was further demonstrated that initial insoluble Aβ aggregates are largely composed of N-truncated Aβ42 variants such as peptides starting at positions 4-, 5-, 8-, or 9–42 (15). Portelius et al. also studied the Aβ isoform pattern in the hippocampus, cortex, and cerebellum of non-demented controls, sporadic AD cases, and patients suffering from familial AD (FAD). In all groups, $A\beta_{1\text{–}42}$, $A\beta_{1\text{–}40}$, $A\beta_{pE3\text{–}42}$, and $A\beta_{4\text{–}42}$ were identified as the dominant isoforms (16), which is in good agreement with the most recent studies from other investigators (2, 17, 18).

N-TERMINALLY TRUNCATED Aβ SPECIES IN TRANSGENIC MOUSE MODELS OF AD

Transgenic mouse models overexpressing mutant forms of human APP, either alone or in combination with mutant forms of *PSEN1* or *PSEN2*, are valuable and

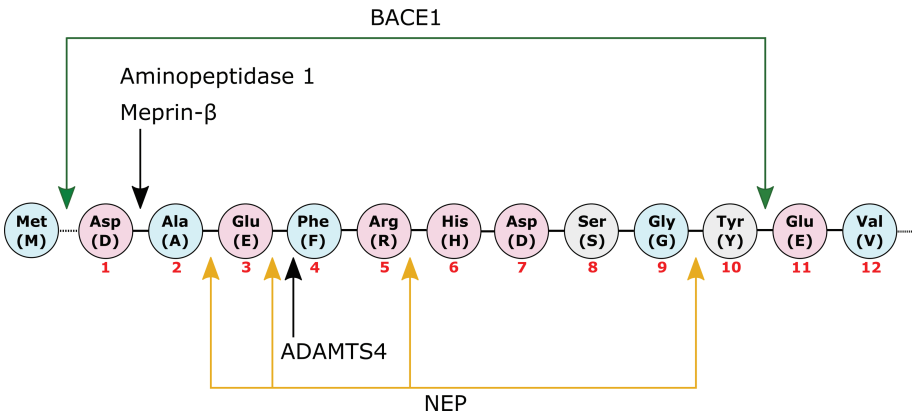

Figure 2 Sequence of the Aβ N-terminus with indicated cleavage sites and enzymes involved in the generation of N-truncated Aβ species. Amino acids (AA) are color-coded according to their properties (red: charged AA; grey: uncharged AA; blue: nonpolar hydrophobic AA).

widely used model systems to study AD-associated pathological alterations such as extracellular amyloid deposition, inflammatory responses, and cognitive deficits (19–21). The analysis of Aβ peptide species in brain samples using mass spectrometry revealed that most transgenic AD mouse models only partially reflect the Aβ spectrum in human sporadic AD. While the overall heterogeneity of N-terminal truncated Aβ species could be reproduced in mouse models such as APP/PS1KI (22) or 5XFAD (23, 24), the ratio of full-length Aβ peptides to N-truncated variants is much different in human brain samples. While N-truncated variants such as $A\beta_{pE3\text{-}x}$ or $A\beta_{4\text{-}x}$ might be present in comparable quantities compared to full-length $A\beta_{1–40}$ or $A\beta_{1–42}$ species in human samples (16), full-length peptides comprise by far the majority of all Aβ peptides in transgenic AD models (23, 25, 26). This is likely explained by the fact that most of these models (e.g., Tg2576 (27), APP23 (28), APP/PS1KI (22), 5XFAD (29), or APPswe/PSEN1dE9 (30)) utilize the Swedish APP mutation. Cell lines transfected with the Swedish APP670/671 mutation have been shown to release three to six times more Aβ peptides than wild-type cells (31, 32). Due to the location of the double mutation in the immediate vicinity of the β-secretase cleavage site (Figure 1), the Swedish mutation increases the affinity of the substrate APP for BACE1, thus favoring the generation of full-length Aβ peptides starting with Asp in position 1 (33).

Using two-dimensional gel electrophoresis with subsequent mass spectrometry analysis, a variety of N-truncated Aβ species have been detected in APP/PS1KI mice. While full-length $A\beta_{1–42}$ peptides were already detectable in young mice at 2.5 months of age, other Aβ variants such as $A\beta_{2/3–42}$, $A\beta_{pE3–42}$, and $A\beta_{4/5–42}$ became apparent only at later time points (22). Mass spectrometry analyses have also supported that N-truncated species represent only a small percentage of the total Aβ peptide amount in mouse models such as 5XFAD or APP23, although variable ionization efficiencies for the different Aβ species might contribute to a distorted image of the Aβ peptide composition in both mouse and human brains (23, 34). In conclusion, N-truncated Aβ species are substantially underrepresented in transgenic mouse models compared to human AD brain samples (34, 35).

MAJOR N-TERMINAL TRUNCATED Aβ SPECIES DETECTED IN HUMAN BRAIN

As pointed out above, a huge variety of different N-terminal truncated Aβ species has been identified by either MS or immunohistochemical staining methods in brain samples from human AD patients. In this section, we discuss the current knowledge on the most important variants in more detail.

$A\beta_{2\text{-}x}$

In AD patients, a consistent elevation of Aβ peptides lacking the N-terminal Asp residue have been observed in the detergent-soluble pool of brain extracts, as well as in cerebrospinal fluid (CSF) samples (36). Using SELDI-MS, several Aβ peptides including those starting with Ala-2 were found in extractions from senile plaques (13). Immunohistochemical analysis of postmortem brain samples using

an $A\beta_{2\text{-}x}$-specific polyclonal antibody confirmed the presence of $A\beta_{2\text{-}x}$ peptides in both parenchymal and vascular deposits of sporadic AD cases as well as transgenic mouse models such as APP/PS1KI or 5XFAD (37). As the sequence of full-length Aβ starts with an Asp residue in position 1, it has been suggested that proteolysis of $A\beta_{1\text{-}x}$ peptides by the exopeptidase aminopeptidase A, which releases Glu and Asp residues from the N-termini of proteins, could result in the generation of $A\beta_{2\text{-}x}$ species (38). However, the evidence in this study was limited to showing that Western blot immunoreactivity with an $A\beta_{1\text{-}x}$-specific antibody was reduced after the co-incubation of purified aminopeptidase A with recombinant full-length $A\beta_{1–40}$ peptides (Table 1). The identity of specific degradation products and, in particular, the generation of $A\beta_{2\text{-}x}$ species, was not confirmed by mass spectrometry or other methodology (38). In contrast, it has been convincingly demonstrated in cell-free and cell-based assays that cleavage of APP or Aβ by the metalloprotease meprin-β can result in the generation of $A\beta_{2\text{-}x}$ species (39, 40). In both HEK293T and CHO cells, co-expression of human APP and meprin-β facilitated the secretion of $A\beta_{2–40}$ peptides, whose identity was confirmed by mass spectrometry, and this was blocked by treatment with a γ-secretase but not a β-secretase inhibitor, indicating that $A\beta_{2–40}$ peptides were produced through a BACE1-independent mechanism. Later, these results were partially confirmed by another group (41). Still missing is in vivo proof that meprin-β is responsible for the brain production of $A\beta_{2\text{-}x}$ peptides in AD mouse models. However, this experiment is complicated by the fact that meprin-β does not generate $A\beta_{2\text{-}x}$ peptides with Swedish mutant APP as a substrate, which excludes most of the commonly used APP-transgenic strains as in vivo model systems (40).

$A\beta_{pE3\text{-}x}$

Pyroglutamate-modified $A\beta_{pE3\text{-}x}$ represents a major Aβ species identified in human AD brains (16, 50). In 1995, Saido et al. reported the identification of these

TABLE 1 **List of proteases involved in the generation of N-truncated Aβ species**

Protease	Levels/activity in human AD brain versus control	Cleavage site	Potential Aβ peptides	References
BACE1	Increased (42)	Met(-1) ↓ Asp(1) Tyr(10) ↓ Glu(11)	$A\beta_{1\text{-}x}$ $A\beta_{11\text{-}x}$, $A\beta_{pE11\text{-}x}$	(6)
Aminopeptidase A	Reduced (43)	Asp(1) ↓ Ala(2)	$A\beta_{2\text{-}x}$	(38)
Meprin-β	Unknown	Asp(1) ↓ Ala(2)	$A\beta_{2\text{-}x}$	(39, 40)
Neprilysin (NEP)	Increased (44) Reduced activity (45)	Asp(2) ↓ Ala(3) Ala(3) ↓ Phe(4) Arg(5) ↓ His(6) Gly(9) ↓ Tyr(10)	$A\beta_{3\text{-}x}$, $A\beta_{pE3\text{-}x}$ $A\beta_{4\text{-}x}$ $A\beta_{6\text{-}x}$ $A\beta_{10\text{-}x}$	(46, 47) (48, 49)
ADAMTS4	Unknown	Ala(3) ↓ Phe(4)	$A\beta_{4\text{-}x}$	(24)

post-translationally modified peptides in which the glutamate at position three becomes converted to pyroglutamate through intramolecular dehydration (51). This cyclization alters the physicochemical properties of Aβ and results in increased hydrophobicity due to the loss of a negative charge, faster aggregation kinetics compared to full-length Aβ peptides in in vitro assays (52–54), and increased insolubility and stability (55). Importantly, higher abundance of these peptides in AD as compared to age-matched non-demented control patients has been demonstrated (56–58). With regard to their toxic properties, increased neurotoxicity compared to full-length Aβ peptides (59) has been reported; however, some studies found full-length and $Aβ_{pE3-x}$ peptides to be equally toxic (60, 61), while others suggested that $Aβ/Aβ_{pE}$ hetero-oligomers constitute the main neurotoxic Aβ fraction (62). Interestingly, related properties have also been reported for pyroglutamylated ABri and ADan peptides, representing the major peptide species accumulating in the neurodegenerative disorders familial British dementia and familial Danish dementia (63, 64).

The formation of $Aβ_{pE3-x}$ peptides appears to be at least a two-step process, with removal of the first two amino acid residues from full-length Aβ followed by cyclization. Recently, it has been suggested that meprin-β might not only generate $Aβ_{2-x}$ but also $Aβ_{3-x}$ peptides as substrates for cyclization (41). However, $Aβ_{3-x}$ peptides were not detected in an earlier study by mass spectrometry (39), and whether genetic deletion of meprin-β would reduce $Aβ_{pE3-x}$ peptide formation in vivo is unknown. In contrast, solid evidence supports that glutaminyl cyclase (QC) is at least one of the enzymes capable of catalyzing the second step of $Aβ_{pE3-x}$ formation (65). Treatment using an orally available QC inhibitor resulted in a reduction of the $Aβ_{pE3–42}$ burden in transgenic mouse models of AD (66). The same was also seen in 5XFAD mice on a QC knock-out background and was accompanied by a rescue of behavioral deficits (67). The observation of a significant age-dependent increase of the $Aβ_{pE3-x}$ parenchymal plaque burden at the expense of $Aβ_{1-x}$ full-length peptides suggested that $Aβ_{pE3-x}$ formation might occur late in the process of amyloidosis and could involve the remodeling of existing extracellular amyloid deposits (68). On the other hand, the presence of $Aβ_{pE3-x}$ peptides has been also described within neurons both in mouse models (22, 69) and human AD samples (53, 70), raising the question of whether the localization is important for toxicity. In order to address such questions, transgenic mouse models have been developed with constructs that only encode the $Aβ_{3-x}$ peptide, with a glutamate to glutamine substitution at the initial position to facilitate cyclization (71–73). This construct is expressed under the control of the murine neuron-specific Thy1-promotor and contains the thyrothropin-releasing hormone (TRH) signal peptide sequence to ensure liberation of the peptide preferentially in the secretory pathway (74). In contrast to other models, these mice do not express human full-length APP or any FAD-associated mutations, but impress with a rapid onset of behavioral deficits, neuron loss, and microgliosis (71, 72).

$Aβ_{4-x}$

$Aβ_{4–42}$ was one of the first Aβ peptide species that was detected in the amyloid plaque cores of human AD brains (9). More recently, novel $Aβ_{4-x}$ specific antibodies have been described, and the localization of $Aβ_{4-x}$ to amyloid plaque cores has been confirmed in immunohistochemical studies in both human AD and

transgenic AD mouse models (75, 76). In addition, $A\beta_{4\text{-}x}$ peptides were also found within blood vessels in the majority of the analyzed AD cases (76). Similar to $A\beta_{pE3\text{-}x}$ peptides, $A\beta_{4–42}$ peptides lacking another charged amino acid residue have also been described to quickly aggregate into soluble oligomers and fibrillar, high-molecular weight aggregates (61, 75, 76). Quantitatively, $A\beta_{4–42}$ peptides seem to be among the most abundant Aβ species in human AD brain with equal or even higher amounts compared to $A\beta_{1–42}$. It should be noted again, however, that in studies using mass spectrometry to assess Aβ peptide patterns, the ratios between the respective peptide variants cannot be regarded as a direct reflection of their abundance (16, 77). With regard to their neurotoxicity, $A\beta_{4–42}$ and $A\beta_{4–40}$ demonstrated equal toxicity as $A\beta_{1–42}$ or $A\beta_{pE3–42}$ using in vitro assays with primary neuronal cultures. This was also observed in an in vivo setting in which freshly prepared Aβ peptides were applied by intraventricular injection followed by an analysis of working memory using a Y-maze task after 5 days (61).

The metalloprotease neprilysin (NEP) has been proposed as a candidate enzyme responsible for the generation of $A\beta_{4\text{-}x}$ peptides by cleaving between Glu-3 and Phe-4 among other sites, with full-length $A\beta_{1\text{-}x}$ peptides acting as the immediate substrate. This has been shown by high-performance liquid chromatography analysis yielding several product peaks after incubation of $A\beta_{1–40}$ with either recombinant soluble NEP produced in Sf9 cells or NEP purified from rabbit kidney cortex (46). More recent studies using synthetic Aβ peptides and recombinant human NEP confirmed the generation of Aβ peptide fragments starting with Phe-4 (such as $A\beta_{4–9}$ or $A\beta_{4–16}$ but also the existence of several other cleavage sites, at least under the given in vitro conditions (48, 49). Therefore, it is currently unclear whether NEP might contribute to the generation of longer Aβ peptides such as $A\beta_{4–40}$ and $A\beta_{4–42}$. However, we regard this possibility as unlikely as in vivo studies have demonstrated that the rate-limiting step in the proteolysis of Aβ by NEP is cleavage of the Gly-9–Tyr-10 bond, which would rule out the generation of full-length $A\beta_{4–40}$ and $A\beta_{4–42}$ peptides (78).

Most recently, it was shown that APP contains a cleavage site for the metalloprotease ADAMTS4 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif) between Glu-3 and Phe-4 of the Aβ peptide sequence (24). ADAMTS proteases constitute a family of secreted Zn^{2+}-metalloproteases that degrade or modify major components of the extracellular matrix (79). ADAMTS4 participates in the proteolytic degradation of proteoglycans like aggrecan, brevican, and versican (80). Aggrecan is a hyaluronan-binding proteoglycan, which is present in large amounts in the articular cartilage. In an important pathological process leading to osteoarthritis and rheumatoid arthritis, aggrecan is degraded by ADAMTS4 and the homologous family member ADAMTS5, leading to the exposure and subsequent degradation of collagen fibrils by collagenases (81). Co-expression of ADAMTS4 and APP in HEK293 cells resulted in the secretion of $A\beta_{4–40}$ peptides as measured by mass spectrometry and ELISA, while several species of $A\beta_{1\text{-}x}$ peptides were not affected (24). $A\beta_{4–40}$ secretion was not blocked by treatment of the cells with a potent β-secretase inhibitor indicating that $A\beta_{4\text{-}x}$ peptides were generated in a BACE1-independent fashion. IHC analysis of ADAMTS4 reporter mice showed that ADAMTS4 was exclusively expressed in oligodendrocytes in the adult murine brain. Consistently, the culture of murine oligodendrocytes demonstrated that these primary cells secrete $A\beta_{4–40}$ peptides among a spectrum of other Aβ species very similar to established cell lines. However, $A\beta_{4–40}$ peptides were

undetectable in primary oligodendrocytes derived from ADAMTS4 knockout (KO) mice, providing genetic proof that ADAMTS4 is responsible for $A\beta_{4–40}$ peptide generation in this cell type. In vivo, the crossing of 5XFAD mice to ADAMTS4 knockout mice reduced $A\beta_{4–40}$ levels by 50%, but the overall amyloid plaque load and the distribution of $A\beta_{4\text{-}x}$ peptides in amyloid plaque cores appeared to be unchanged, clearly suggesting that other mechanisms for $A\beta_{4\text{-}x}$ generation beside ADAMTS4 must exist. Compellingly, abundant $A\beta_{4\text{-}x}$ immunoreactivity was observed in white matter structures of 5XFAD mice, and this signal was entirely abolished in the ADAMTS4 knockout background (24). This could be of pathological relevance as numerous neuropathological, biochemical, and imaging studies have reported white matter abnormalities and oligodendrocyte dysfunction in AD patients (82, 83). However, further studies are required to define a potential detrimental role of $A\beta_{4\text{-}x}$ peptides in white matter structures. In any case, the recent link of ADAMTS4 to AD risk as well as single-cell transcriptomic data supporting that many oligodendroglia-specific and myelination-associated genes are dysregulated in human AD brains should provide new urgency to consider the role of oligodendrocytes in AD (84, 85).

As a tool to investigate the in vivo role of $A\beta_{4\text{-}x}$ peptides, a transgenic mouse model has been generated that only expresses $A\beta_{4–42}$ peptides under the control of the murine Thy1-promotor. These mice develop age-dependent behavioral deficits with spatial or working memory impairments, which are detectable in paradigms such as Morris water maze or novel object recognition task, as well as motor deficits. These mice do not develop amyloid plaque pathology but show a robust hippocampal CA1 neuron loss correlating with the transgene expression pattern in a gene-dose dependent manner (61, 86). Interestingly, altered basal excitatory synaptic transmission with $A\beta_{4–42}$-dependent neuronal hyperexcitability is already obvious in young Tg4–42 mice preceding neuron loss and behavioral deficits (87).

$A\beta_{5\text{-}x}$

Aβ peptides starting with an Arg residue at position 5 have been detected in brains of transgenic mice such as APP/PS1KI (22) or 5XFAD (23), as well as in human AD brains (15–17) by mass spectrometry. Conditions of BACE1 inhibition resulted in strongly increased levels of $A\beta_{5\text{-}x}$ species in cellular models (88–90). This clearly suggests that $A\beta_{5\text{-}x}$ peptides are produced through a BACE1-independent pathway, with some evidence supporting α-secretase-like proteases (e.g., ADAM family proteases such as TACE or ADAM10) as potential candidate enzymes (88).

In vivo studies with several BACE1 inhibitors in beagle dogs confirmed the absolute signal reduction of all Aβ isoforms in the CSF except for $A\beta_{5–40}$ peptides, and an analysis of relative levels demonstrated a clear increase of $A\beta_{5–40}$ (90). This was further corroborated in a placebo-controlled study in healthy human subjects in which dose-dependent increases in $A\beta_{5\text{-}x}$ levels were measured in the CSF upon treatment with the BACE1 inhibitor LY2811376 (91). Immunohistochemical analyses using $A\beta_{5\text{-}x}$ selective antibodies confirmed the presence of $A\beta_{5\text{-}x}$ peptides in brain tissues samples from sporadic AD patients showing immunoreactivity primarily in vascular deposits (88, 92). In cases from individuals harboring FAD-associated *APP* or *PSEN1* mutations, both vascular

and parenchymal deposits were detected, while in mouse models such as 5XFAD, APP/PS1KI, or 3xTg $A\beta_{5\text{-}x}$, immunoreactivity was confined to extracellular plaques (92).

$A\beta_{11\text{-}x}/A\beta_{pE11\text{-}x}$

In addition to the cleavage site between methionine and aspartate in position 1 (Asp-1) generating β-CTFs, BACE1 has also been shown to cleave APP between tyrosine and glutamate in position 11 (Glu-11) of the Aβ sequence resulting in N-truncated $A\beta_{11\text{-}x}$ species (β′-cleavage) (6). There is evidence that the BACE1 cleavage preference depends on the intracellular localization, with β′-cleavage being favored in the trans-Golgi network (93). $A\beta_{11\text{-}x}$ peptides have been detected in brains from AD and Down's syndrome patients (94) and have been shown to accumulate within neurons in cellular models upon BACE1 overexpression (95). Similar to Glu-3, the free Glu residue in position 11 can also undergo cyclization and modification to an N-terminal pyroglutamate ($A\beta_{pE11\text{-}x}$). In contrast to $A\beta_{pE3\text{–}42}$, which is mainly confined to mature plaque cores in AD patients, unmodified $A\beta_{11\text{–}40}$ and $A\beta_{pE11\text{–}40}$ peptides have been detected in the vasculature using selective antibodies (95). Within amyloid plaques cores, $A\beta_{pE11\text{-}x}$ has been found to co-localize with full-length Aβ peptides but also with $A\beta_{pE3\text{-}x}$ (96).

CONCLUSION

There is substantial evidence that N-truncated Aβ species, in addition to the extensively studied full-length Aβ peptides, might play an important role in the molecular pathology of AD. In recent years, new candidate proteases and nonneuronal cell types have been linked to the generation of N-truncated Aβ species. Novel antibodies specific for some N-truncated Aβ peptides have been developed, and this should allow the development of quantitative detection assays to better define their abundance in relation to full-length Aβ peptides. To advance the functional analysis of N-truncated Aβ peptides, novel animal models might be needed as N-truncated Aβ species are underrepresented in the available AD models. These efforts should improve our understanding of the pathological role of N-truncated Aβ peptides. They could provide novel insights into currently unexplained aspects of AD pathology, and they might be crucial to develop novel therapeutic approaches.

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REFERENCES

1. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. J Biol Chem. 2008;283(44):29615–19. http://dx.doi.org/10.1074/jbc.R800019200
2. Moore BD, Chakrabarty P, Levites Y, Kukar TL, Baine AM, Moroni T, et al. Overlapping profiles of abeta peptides in the Alzheimer's disease and pathological aging brains. Alzheimers Res Ther. 2012;4(3):18. http://dx.doi.org/10.1186/alzrt121
3. Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, et al. APP processing and synaptic function. Neuron. 2003;37(6):925–37. http://dx.doi.org/10.1016/S0896-6273(03)00124-7
4. Brothers HM, Gosztyla ML, Robinson SR. The physiological roles of amyloid-β peptide hint at new ways to treat Alzheimer's disease. Front Aging Neurosci. 2018;10:118. http://dx.doi.org/10.3389/fnagi.2018.00118
5. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, et al. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature. 1999;402(6761):537–40. http://dx.doi.org/10.1038/990114
6. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science. 1999;286(5440):735–41. http://dx.doi.org/10.1126/science.286.5440.735
7. Glenner GG, Wong CW. Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 1984;120:885–90. http://dx.doi.org/10.1016/S0006-291X(84)80190-4
8. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K. Neuronal origin of a cerebral amyloid: Neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J. 1985;4(11):2757–63. http://dx.doi.org/10.1002/j.1460-2075.1985.tb04000.x
9. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A. 1985;82(12):4245–9. http://dx.doi.org/10.1073/pnas.82.12.4245
10. Saido TC, Yamao-Harigaya W, Iwatsubo T, Kawashima S. Amino- and carboxyl-terminal heterogeneity of beta-amyloid peptides deposited in human brain. Neurosci Lett. 1996;215(3):173–6. http://dx.doi.org/10.1016/0304-3940(96)12970-0
11. Iwatsubo T, Saido TC, Mann DM, Lee VM, Trojanowski JQ. Full-length amyloid-beta (1-42(43)) and amino-terminally modified and truncated amyloid-beta 42(43) deposit in diffuse plaques. Am J Pathol. 1996;149(6):1823–30.
12. Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, et al. Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. Arch Biochem Biophys. 1993;301(1):41–52. http://dx.doi.org/10.1006/abbi.1993.1112
13. Lewis H, Beher D, Cookson N, Oakley A, Piggott M, Morris CM, et al. Quantification of Alzheimer pathology in ageing and dementia: Age-related accumulation of amyloid-beta(42) peptide in vascular dementia. Neuropathol Appl Neurobiol. 2006;32(2):103–18. http://dx.doi.org/10.1111/j.1365-2990.2006.00696.x
14. Miravalle L, Calero M, Takao M, Roher AE, Ghetti B, Vidal R. Amino-terminally truncated Abeta peptide species are the main component of cotton wool plaques. Biochemistry. 2005;44(32):10810–21. http://dx.doi.org/10.1021/bi0508237
15. Sergeant N, Bombois S, Ghestem A, Drobecq H, Kostanjevecki V, Missiaen C, et al. Truncated beta-amyloid peptide species in pre-clinical Alzheimer's disease as new targets for the vaccination approach. J Neurochem. 2003;85(6):1581–91. http://dx.doi.org/10.1046/j.1471-4159.2003.01818.x
16. Portelius E, Bogdanovic N, Gustavsson MK, Volkmann I, Brinkmalm G, Zetterberg H, et al. Mass spectrometric characterization of brain amyloid beta isoform signatures in familial and sporadic Alzheimer's disease. Acta Neuropathol. 2010;120(2):185–93. http://dx.doi.org/10.1007/s00401-010-0690-1
17. Gkanatsiou E, Portelius E, Toomey CE, Blennow K, Zetterberg H, Lashley T, et al. A distinct brain beta amyloid signature in cerebral amyloid angiopathy compared to Alzheimer's disease. Neurosci Lett. 2019;701:125–31. http://dx.doi.org/10.1016/j.neulet.2019.02.033

18. Wildburger NC, Esparza TJ, LeDuc RD, Fellers RT, Thomas PM, Cairns NJ, et al. Diversity of amyloid-beta proteoforms in the Alzheimer's disease brain. Sci Rep. 2017;7(1):9520. http://dx.doi.org/10.1038/s41598-017-10422-x
19. LaFerla FM, Green KN. Animal models of Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(11):a006320. http://dx.doi.org/10.1101/cshperspect.a006320
20. Morrissette DA, Parachikova A, Green KN, LaFerla FM. Relevance of transgenic mouse models to human Alzheimer disease. J Biol Chem. 2009 Mar 6;284(10):6033–7. http://dx.doi.org/10.1074/jbc.R800030200
21. Duyckaerts C, Potier MC, Delatour B. Alzheimer disease models and human neuropathology: Similarities and differences. Acta Neuropathol. 2008;115(1):5–38. http://dx.doi.org/10.1007/s00401-007-0312-8
22. Casas C, Sergeant N, Itier JM, Blanchard V, Wirths O, van der Kolk N, et al. Massive CA1/2 neuronal loss with intraneuronal and N-terminal truncated Abeta42 accumulation in a novel Alzheimer transgenic model. Am J Pathol. 2004;165(4):1289–300. http://dx.doi.org/10.1016/S0002-9440(10)63388-3
23. Reinert J, Richard BC, Klafki HW, Friedrich B, Bayer TA, Wiltfang J, et al. Deposition of C-terminally truncated Aβ species Aβ37 and Aβ39 in Alzheimer's disease and transgenic mouse models. Acta Neuropathol Comm. 2016;4:24. http://dx.doi.org/10.1186/s40478-016-0294-7
24. Walter S, Jumpertz T, Hüttenrauch M, Ogorek I, Gerber H, Storck SE, et al. The metalloprotease ADAMTS4 generates N-truncated Aβ4–x species and marks oligodendrocytes as a source of amyloidogenic peptides in Alzheimer's disease. Acta Neuropathol. 2019;137(2):239–57. http://dx.doi.org/10.1007/s00401-018-1929-5
25. Kalback W, Watson MD, Kokjohn TA, Kuo YM, Weiss N, Luehrs DC, et al. APP transgenic mice Tg2576 accumulate Abeta peptides that are distinct from the chemically modified and insoluble peptides deposited in Alzheimer's disease senile plaques. Biochemistry. 2002;41(3):922–8. http://dx.doi.org/10.1021/bi015685+
26. Kuo YM, Kokjohn TA, Beach TG, Sue LI, Brune D, Lopez JC, et al. Comparative analysis of amyloid-beta chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. J Biol Chem. 2001;276(16):12991–8. http://dx.doi.org/10.1074/jbc.M007859200
27. Hsiao KK, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, et al. Correlative memory deficits, Abeta elevation and amyloid plaques in transgenic mice. Science. 1996;274:99–102. http://dx.doi.org/10.1126/science.274.5284.99
28. Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci U S A. 1997;94(24):13287–92. http://dx.doi.org/10.1073/pnas.94.24.13287
29. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. J Neurosci. 2006;26(40):10129–40. http://dx.doi.org/10.1523/JNEUROSCI.1202-06.2006
30. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, et al. Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: Evidence for augmentation of a 42-specific γ secretase. Human Molecular Genetics. 2003;13(2):159–70. http://dx.doi.org/10.1093/hmg/ddh019
31. Cai X, Golde T, Younkin S. Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science. 1993;259(5094):514–16. http://dx.doi.org/10.1126/science.8424174
32. Citron M, Vigo-Pelfrey C, Teplow DB, Miller C, Schenk D, Johnston J, et al. Excessive production of amyloid beta-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer disease mutation. Proc Natl Acad Sci U S A. 1994;91(25):11993–7. http://dx.doi.org/10.1073/pnas.91.25.11993
33. Weggen S, Beher D. Molecular consequences of amyloid precursor protein and presenilin mutations causing autosomal-dominant Alzheimer's disease. Alzheimers Res Ther. 2012;4(2):9. http://dx.doi.org/10.1186/alzrt107
34. Schieb H, Kratzin H, Jahn O, Mobius W, Rabe S, Staufenbiel M, et al. Beta-amyloid peptide variants in brains and cerebrospinal fluid from amyloid precursor protein (APP) transgenic mice: Comparison

with human Alzheimer amyloid. J Biol Chem. 2011;286(39):33747–58. http://dx.doi.org/10.1074/jbc.M111.246561
35. Rüfenacht P, Güntert A, Bohrmann B, Ducret A, Döbeli H. Quantification of the Aβ peptide in Alzheimer's plaques by laser dissection microscopy combined with mass spectrometry. J Mass Spectrom. 2005;40(2):193–201. http://dx.doi.org/10.1002/jms.739
36. Wiltfang J, Esselmann H, Cupers P, Neumann M, Kretzschmar H, Beyermann M, et al. Elevation of beta-amyloid peptide 2–42 in sporadic and familial Alzheimer's disease and its generation in PS1 knockout cells. J Biol Chem. 2001;276(46):42645–57. http://dx.doi.org/10.1074/jbc.M102790200
37. Savastano A, Klafki H, Haussmann U, Oberstein TJ, Muller P, Wirths O, et al. N-Truncated abeta 2-X starting with position two in sporadic Alzheimer's disease cases and two Alzheimer mouse models. J Alzheimers Dis. 2016;49(1):101–10. http://dx.doi.org/10.3233/JAD-150394
38. Sevalle J, Amoyel A, Robert P, Fournie-Zaluski MC, Roques B, Checler F. Aminopeptidase A contributes to the N-terminal truncation of amyloid beta-peptide. J Neurochem. 2009;109(1):248–56. http://dx.doi.org/10.1111/j.1471-4159.2009.05950.x
39. Bien J, Jefferson T, Čaušević M, Jumpertz T, Munter L, Multhaup G, et al. The metalloprotease meprin β generates amino terminal-truncated amyloid β peptide species. J Biol Chem. 2012;287(40):33304–13. http://dx.doi.org/10.1074/jbc.M112.395608
40. Schönherr C, Bien J, Isbert S, Wichert R, Prox J, Altmeppen H, et al. Generation of aggregation prone N-terminally truncated amyloid β peptides by meprin β depends on the sequence specificity at the cleavage site. Mol Neurodegen. 2016;11:19. http://dx.doi.org/10.1186/s13024-016-0084-5
41. Schlenzig D, Cynis H, Hartlage-Rubsamen M, Zeitschel U, Menge K, Fothe A, et al. Dipeptidyl-peptidase activity of meprin beta links N-truncation of abeta with glutaminyl cyclase-catalyzed pGlu-abeta formation. J Alzheimers Dis. 2018;66(1):359–75. http://dx.doi.org/10.3233/JAD-171183
42. Ahmed RR, Holler CJ, Webb RL, Li F, Beckett TL, Murphy MP. BACE1 and BACE2 enzymatic activities in Alzheimer's disease. J Neurochem. 2010;112(4):1045–53. http://dx.doi.org/10.1111/j.1471-4159.2009.06528.x
43. Kehoe PG, Hibbs E, Palmer LE, Miners JS. Angiotensin-III is increased in Alzheimer's disease in association with amyloid-beta and tau pathology. J Alzheimers Dis. 2017;58(1):203–14. http://dx.doi.org/10.3233/JAD-161265
44. Miners JS, Baig S, Tayler H, Kehoe PG, Love S. Neprilysin and insulin-degrading enzyme levels are increased in Alzheimer disease in relation to disease severity. J Neuropathol Exp Neurol. 2009;68(8):902–14. http://dx.doi.org/10.1097/NEN.0b013e3181afe475
45. Miners JS, Van Helmond Z, Chalmers K, Wilcock G, Love S, Kehoe PG. Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. J Neuropathol Exp Neurol. 2006;65(10):1012–21. http://dx.doi.org/10.1097/01.jnen.0000240463.87886.9a
46. Howell S, Nalbantoglu J, Crine P. Neutral endopeptidase can hydrolyze β-amyloid(1–40) but shows no effect on β-amyloid precursor protein metabolism. Peptides. 1995;16(4):647–52. http://dx.doi.org/10.1016/0196-9781(95)00021-B
47. Leissring MA, Lu A, Condron MM, Teplow DB, Stein RL, Farris W, et al. Kinetics of amyloid β-protein degradation determined by novel fluorescence- and fluorescence polarization-based assays. J Biol Chem. 2003;278(39):37314–20. http://dx.doi.org/10.1074/jbc.M305627200
48. Hornung K, Zampar S, Engel N, Klafki H, Liepold T, Bayer TA, et al. N-terminal truncated Abeta4-42 is a substrate for neprilysin degradation in vitro and in vivo. J Alzheimers Dis. 2019;67(3):849–58. http://dx.doi.org/10.3233/JAD-181134
49. Mital M, Bal W, Frączyk T, Drew SC. Interplay between copper, neprilysin, and N-truncation of β-amyloid. Inorg Chem. 2018;57(11):6193–7. http://dx.doi.org/10.1021/acs.inorgchem.8b00391
50. Harigaya Y, Saido TC, Eckman CB, Prada CM, Shoji M, Younkin SG. Amyloid beta protein starting pyroglutamate at position 3 is a major component of the amyloid deposits in the Alzheimer's disease brain. Biochem Biophys Res Commun. 2000;276(2):422–7. http://dx.doi.org/10.1006/bbrc.2000.3490
51. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. Neuron. 1995;14(2):457–66. http://dx.doi.org/10.1016/0896-6273(95)90301-1

52. Schilling S, Lauber T, Schaupp M, Manhart S, Scheel E, Bohm G, et al. On the seeding and oligomerization of pGlu-amyloid peptides (in vitro). Biochemistry. 2006;45(41):12393–9. http://dx.doi.org/10.1021/bi0612667
53. Wirths O, Erck C, Martens H, Harmeier A, Geumann C, Jawhar S, et al. Identification of low molecular weight pyroglutamate abeta oligomers in Alzheimer disease: A novel tool for therapy and diagnosis. J Biol Chem. 2010;285(53):41517–24. http://dx.doi.org/10.1074/jbc.M110.178707
54. Dammers C, Schwarten M, Buell AK, Willbold D. Pyroglutamate-modified Abeta3-42 affects aggregation kinetics of Abeta1-42 by accelerating primary and secondary pathways. Chem Sci. 2017;8(7):4996–5004. http://dx.doi.org/10.1039/C6SC04797A
55. Kuo YM, Webster S, Emmerling MR, De Lima N, Roher AE. Irreversible dimerization/tetramerization and post-translational modifications inhibit proteolytic degradation of A beta peptides of Alzheimer's disease. Biochim Biophys Acta. 1998;1406(3):291–8. http://dx.doi.org/10.1016/S0925-4439(98)00014-3
56. Piccini A, Russo C, Gliozzi A, Relini A, Vitali A, Borghi R, et al. Beta-amyloid is different in normal aging and in Alzheimer disease. J Biol Chem. 2005;280(40):34186–92. http://dx.doi.org/10.1074/jbc.M501694200
57. Frost JL, Le KX, Cynis H, Ekpo E, Kleinschmidt M, Palmour RM, et al. Pyroglutamate-3 amyloid-β deposition in the brains of humans, non-human primates, canines, and Alzheimer disease–like transgenic mouse models. Am J Pathol. 2013;183(2):369–81. http://dx.doi.org/10.1016/j.ajpath.2013.05.005
58. Mandler M, Walker L, Santic R, Hanson P, Upadhaya AR, Colloby SJ, et al. Pyroglutamylated amyloid-beta is associated with hyperphosphorylated tau and severity of Alzheimer's disease. Acta Neuropathol. 2014;128(1):67–79. http://dx.doi.org/10.1007/s00401-014-1296-9
59. Galante D, Corsaro A, Florio T, Vella S, Pagano A, Sbrana F, et al. Differential toxicity, conformation and morphology of typical initial aggregation states of Abeta1-42 and Abetapy3-42 beta-amyloids. Int J Biochem Cell Biol. 2012;44(11):2085–93. http://dx.doi.org/10.1016/j.biocel.2012.08.010
60. Tekirian TL, Yang AY, Glabe C, Geddes JW. Toxicity of pyroglutaminated amyloid beta-peptides 3(pE)-40 and -42 is similar to that of A beta1-40 and -42. J Neurochem. 1999;73(4):1584–9. http://dx.doi.org/10.1046/j.1471-4159.1999.0731584.x
61. Bouter Y, Dietrich K, Wittnam JL, Rezaei-Ghaleh N, Pillot T, Papot-Couturier S, et al. N-truncated amyloid beta (Abeta) 4-42 forms stable aggregates and induces acute and long-lasting behavioral deficits. Acta Neuropathol. 2013;126(2):189–205. http://dx.doi.org/10.1007/s00401-013-1129-2
62. Goldblatt G, Cilenti L, Matos JO, Lee B, Ciaffone N, Wang QX, et al. Unmodified and pyroglutamylated amyloid β peptides form hypertoxic hetero-oligomers of unique secondary structure. FEBS J. 2017;284(9):1355–69. http://dx.doi.org/10.1111/febs.14058
63. Ghiso J, Rostagno A, Tomidokoro Y, Lashley T, Bojsen-Moller M, Braendgaard H, et al. Genetic alterations of the BRI2 gene: Familial British and Danish dementias. Brain Pathol. 2006;16(1):71–9. http://dx.doi.org/10.1111/j.1750-3639.2006.tb00563.x
64. Saul A, Lashley T, Revesz T, Holton J, Ghiso JA, Coomaraswamy J, et al. Abundant pyroglutamate-modified ABri and ADan peptides in extracellular and vascular amyloid deposits in familial British and Danish dementias. Neurobiol Aging. 2013;34:1416–25. http://dx.doi.org/10.1016/j.neurobiolaging.2012.11.014
65. Schilling S, Hoffmann T, Manhart S, Hoffmann M, Demuth HU. Glutaminyl cyclases unfold glutamyl cyclase activity under mild acid conditions. FEBS Lett. 2004;563(1–3):191–6. http://dx.doi.org/10.1016/S0014-5793(04)00300-X
66. Schilling S, Zeitschel U, Hoffmann T, Heiser U, Francke M, Kehlen A, et al. Glutaminyl cyclase inhibition attenuates pyroglutamate Abeta and Alzheimer's disease-like pathology. Nat Med. 2008;14(10):1106–11. http://dx.doi.org/10.1038/nm.1872
67. Jawhar S, Wirths O, Schilling S, Graubner S, Demuth HU, Bayer TA. Overexpression of glutaminyl cyclase, the enzyme responsible for pyroglutamate abeta formation, induces behavioral deficits, and glutaminyl cyclase knock-out rescues the behavioral phenotype in 5XFAD mice. J Biol Chem. 2011;286(6):4454–60. http://dx.doi.org/10.1074/jbc.M110.185819
68. Wirths O, Bethge T, Marcello A, Harmeier A, Jawhar S, Lucassen PJ, et al. Pyroglutamate abeta pathology in APP/PS1KI mice, sporadic and familial Alzheimer's disease cases. J Neural Transm. 2010;117(1):85–96. http://dx.doi.org/10.1007/s00702-009-0314-x

69. Breyhan H, Wirths O, Duan K, Marcello A, Rettig J, Bayer TA. APP/PS1KI bigenic mice develop early synaptic deficits and hippocampus atrophy. Acta Neuropathol. 2009;117(6):677–85. http://dx.doi.org/10.1007/s00401-009-0539-7
70. Moro ML, Phillips AS, Gaimster K, Paul C, Mudher A, Nicoll JAR, et al. Pyroglutamate and isoaspartate modified amyloid-beta in ageing and Alzheimer's disease. Acta Neuropathol Comm. 2018;6(1):3. http://dx.doi.org/10.1186/s40478-017-0505-x
71. Wirths O, Breyhan H, Cynis H, Schilling S, Demuth HU, Bayer TA. Intraneuronal pyroglutamate-abeta 3-42 triggers neurodegeneration and lethal neurological deficits in a transgenic mouse model. Acta Neuropathol. 2009;118(4):487–96. http://dx.doi.org/10.1007/s00401-009-0557-5
72. Alexandru A, Jagla W, Graubner S, Becker A, Bauscher C, Kohlmann S, et al. Selective hippocampal neurodegeneration in transgenic mice expressing small amounts of truncated abeta is induced by pyroglutamate-abeta formation. J Neurosci. 2011;31(36):12790–801. http://dx.doi.org/10.1523/JNEUROSCI.1794-11.2011
73. Wittnam JL, Portelius E, Zetterberg H, Gustavsson MK, Schilling S, Koch B, et al. Pyroglutamate amyloid β (Aβ) aggravates behavioral deficits in transgenic amyloid mouse model for Alzheimer disease. J Biol Chem. 2012;287(11):8154–62. http://dx.doi.org/10.1074/jbc.M111.308601
74. Cynis H, Schilling S, Bodnar M, Hoffmann T, Heiser U, Saido TC, et al. Inhibition of glutaminyl cyclase alters pyroglutamate formation in mammalian cells. Biochim Biophys Acta. 2006;1764(10):1618–25. http://dx.doi.org/10.1016/j.bbapap.2006.08.003
75. Cabrera E, Mathews P, Mezhericher E, Beach TG, Deng J, Neubert TA, et al. Aβ truncated species: Implications for brain clearance mechanisms and amyloid plaque deposition. Biochim Biophys Acta. 2018;1864(1):208–25. http://dx.doi.org/10.1016/j.bbadis.2017.07.005
76. Wirths O, Walter S, Kraus I, Klafki HW, Stazi M, Oberstein TJ, et al. N-truncated Aβ4–x peptides in sporadic Alzheimer's disease cases and transgenic Alzheimer mouse models. Alzheimers Res Ther. 2017;9:80. http://dx.doi.org/10.1186/s13195-017-0309-z
77. Portelius E, Lashley T, Westerlund A, Persson R, Fox NC, Blennow K, et al. Brain amyloid-beta fragment signatures in pathological ageing and Alzheimer's disease by hybrid immunoprecipitation mass spectrometry. Neurodegen Dis. 2015;15(1):50–7. http://dx.doi.org/10.1159/000369465
78. Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, et al. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: Suppression leads to biochemical and pathological deposition. Nat Med. 2000;6(2):143–50. http://dx.doi.org/10.1038/72237
79. Apte SS. A disintegrin-like and metalloprotease (reprolysin-type) with thrombospondin type 1 motif (ADAMTS) superfamily: Functions and mechanisms. J Biol Chem. 2009;284(46):31493–7. http://dx.doi.org/10.1074/jbc.R109.052340
80. Tortorella MD, Pratta M, Liu R-Q, Austin J, Ross OH, Abbaszade I, et al. Sites of aggrecan cleavage by recombinant human aggrecanase-1 (ADAMTS-4). J Biol Chem. 2000;275(24):18566–73. http://dx.doi.org/10.1074/jbc.M909383199
81. Tortorella MD, Burn TC, Pratta MA, Abbaszade I, Hollis JM, Liu R, et al. Purification and cloning of aggrecanase-1: A member of the ADAMTS family of proteins. Science. 1999;284(5420):1664–6. http://dx.doi.org/10.1126/science.284.5420.1664
82. Nasrabady SE, Rizvi B, Goldman JE, Brickman AM. White matter changes in Alzheimer's disease: A focus on myelin and oligodendrocytes. Acta Neuropathol Comm. 2018;6(1):22. http://dx.doi.org/10.1186/s40478-018-0515-3
83. Ringman JM, O'Neill J, Geschwind D, Medina L, Apostolova LG, Rodriguez Y, et al. Diffusion tensor imaging in preclinical and presymptomatic carriers of familial Alzheimer's disease mutations. Brain. 2007;130(Pt 7):1767–76. http://dx.doi.org/10.1093/brain/awm102
84. Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, et al. Single-cell transcriptomic analysis of Alzheimer's disease. Nature. 2019;570(7761):332–7. http://dx.doi.org/10.1038/s41586-019-1195-2
85. Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. Nat Genet. 2019;51(3):404–13. http://dx.doi.org/10.1038/s41588-018-0311-9
86. Hüttenrauch M, Brauss A, Kurdakova A, Borgers H, Klinker F, Liebetanz D, et al. Physical activity delays hippocampal neurodegeneration and rescues memory deficits in an Alzheimer disease mouse model. Transl Psych. 2016;6:e800. http://dx.doi.org/10.1038/tp.2016.65

87. Dietrich K, Bouter Y, Muller M, Bayer TA. Synaptic alterations in mouse models for Alzheimer disease – A special focus on N-truncated Abeta 4-42. Molecules (Basel, Switzerland). 2018;23:718. http://dx.doi.org/10.3390/molecules23040718
88. Takeda K, Araki W, Akiyama H, Tabira T. Amino-truncated amyloid beta-peptide (Abeta5-40/42) produced from caspase-cleaved amyloid precursor protein is deposited in Alzheimer's disease brain. FASEB J. 2004;18(14):1755–7. http://dx.doi.org/10.1096/fj.03-1070fje
89. Haußmann U, Jahn O, Linning P, Janßen C, Liepold T, Portelius E, et al. Analysis of amino-terminal variants of amyloid-β peptides by capillary isoelectric focusing immunoassay. Anal Chem. 2013;85(17):8142–9. http://dx.doi.org/10.1021/ac401055y
90. Mattsson N, Rajendran L, Zetterberg H, Gustavsson M, Andreasson U, Olsson M, et al. BACE1 inhibition induces a specific cerebrospinal fluid β-amyloid pattern that identifies drug effects in the central nervous system. PLoS One. 2012;7(2):e31084. http://dx.doi.org/10.1371/journal.pone.0031084
91. Portelius E, Dean RA, Andreasson U, Mattsson N, Westerlund A, Olsson M, et al. Beta-site amyloid precursor protein-cleaving enzyme 1(BACE1) inhibitor treatment induces Abeta5-X peptides through alternative amyloid precursor protein cleavage. Alzheimers Res Ther. 2014;6:75. http://dx.doi.org/10.1186/s13195-014-0075-0
92. Guzman EA, Bouter Y, Richard BC, Lannfelt L, Ingelsson M, Paetau A, et al. Abundance of Abeta5-x like immunoreactivity in transgenic 5XFAD, APP/PS1KI and 3xTG mice, sporadic and familial Alzheimer's disease. Mol Neurodegen. 2014;9(1):13. http://dx.doi.org/10.1186/1750-1326-9-13
93. Huse JT, Liu K, Pijak DS, Carlin D, Lee VM-Y, Doms RW. β-Secretase processing in the Trans-Golgi Network preferentially generates truncated amyloid species that accumulate in Alzheimer's disease brain. J Biol Chem. 2002;277(18):16278–84. http://dx.doi.org/10.1074/jbc.M111141200
94. Liu K, Solano I, Mann D, Lemere C, Mercken M, Trojanowski JQ, et al. Characterization of Abeta 11-40/42 peptide deposition in Alzheimer's disease and young Down's syndrome brains: Implication of N-terminally truncated Abeta species in the pathogenesis of Alzheimer's disease. Acta Neuropathol. 2006;112(2):163–74. http://dx.doi.org/10.1007/s00401-006-0077-5
95. Lee EB, Skovronsky DM, Abtahian F, Doms RW, Lee VM. Secretion and intracellular generation of truncated Abeta in beta-site amyloid-beta precursor protein-cleaving enzyme expressing human neurons. J Biol Chem. 2003;278(7):4458–66. http://dx.doi.org/10.1074/jbc.M210105200
96. Sullivan CP, Berg EA, Elliott-Bryant R, Fishman JB, McKee AC, Morin PJ, et al. Pyroglutamate-Abeta 3 and 11 colocalize in amyloid plaques in Alzheimer's disease cerebral cortex with pyroglutamate-Abeta 11 forming the central core. Neurosci Lett. 2011;505(2):109–12. http://dx.doi.org/10.1016/j.neulet.2011.09.071

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Insulin Resistance and Oligodendrocyte/Microvascular Endothelial Cell Dysfunction as Mediators of White Matter Degeneration in Alzheimer's Disease

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Abstract: In Alzheimer's disease (AD), white matter (WM) degeneration begins early, increases with disease progression, and contributes to cognitive impairment, yet the mechanisms are poorly understood. This article reviews the roles of myelin loss, oligodendrocyte dysfunction, and microvasculopathy in relation to AD WM degeneration. Myelin loss impairs axonal function and its breakdown promotes oxidative stress, inflammation, and lipid peroxidation, further compromising the structure and function of axons. Oligodendrocyte dysfunction impairs homeostatic mechanisms needed to maintain myelin. Microvascular disease with endothelial cell pathology leads to thrombin activation and pro-inflammatory cytokine release, oxidative stress, and increased vascular permeability. Progressive fibrotic

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replacement of smooth muscle cells reduces vaso-responsiveness to metabolic demands. Fibrotic thickening of vessel walls narrows the lumens, rendering them more susceptible to occlusion, endothelial cell injury, and thrombin activation. Since normal physiological functions of oligodendrocytes and microvascular endothelial cells rely on intact insulin/insulin-like growth factor (IGF) signaling through cell survival, metabolic and anti-inflammatory pathways, conceivably, WM degeneration in AD is mediated by insulin and IGF resistance with attendant pathogenic targeting of oligodendroglia and endothelial cells. The apolipoprotein E-ε4 genotype may serve as a co-factor in AD-associated glial-vascular WM degeneration due to its role as a mediator of insulin resistance.

Keywords: Alzheimer's disease; Insulin resistance; oligodendrocytes; thrombin; white matter

INTRODUCTION

Alzheimer's disease (AD) is the 6th leading cause of death and the most prevalent aging-associated dementia, afflicting over 5 million people in the United States. Despite intense and comprehensive research efforts over the past 4–5 decades, we still lack effective disease-modifying therapies, and thus, the annual economic burden of over $170 billion continues to grow (1). Perhaps one of the main obstacles to success has been the failure to appreciate the full spectrum of disease which extends well beyond cerebral accumulations of amyloid-beta (Aβ) and neuronal structural pathologies caused by abnormally phosphorylated tau. AD is mechanistically linked to: (i) insulin resistance; (ii) neuroinflammation; (iii) white matter (WM) atrophy with myelin loss and axonal degeneration; (iv) vasculopathy; (v) leukoaraiosis; (vi) blood–brain barrier disruption; (vii) oxidative stress; (viii) mitochondrial dysfunction; (ix) loss of neuronal plasticity; and (x) synaptic disconnection. Furthermore, consideration should be given to the concept that different mediators of neurodegeneration may emerge at various time points and could be inter-dependent. These points are not addressed by the present-day diagnostic and therapeutic approaches.

In light of the varied and complex nature of AD-associated pathologies, it is not surprising that mono-therapeutic strategies have failed to remediate this disease. The development of a more rational and effective therapeutic design requires that we attain a greater understanding of how various pathogenic processes contribute to the onset and progression of AD. Furthermore, additional information about systemic and central nervous system (CNS) forces that drive the cascade of neurodegeneration could lead to preventive strategies. For example, a better understanding of how co-factors such as vascular disease, head trauma, and lifestyle exposures modify risk and the phenotypic features of AD could ultimately help refine and personalize diagnostics and therapeutics. In this regard, the role of vascular disease in AD has been strongly suggested by the finding that at least 40% of people with clinically diagnosed AD have significant cerebrovascular disease yet neither disease process would be regarded as sufficient to cause dementia (2). Correspondingly, the relatively recent incident decline in AD severity in the United States has been attributed to improved vascular protective care (3).

Now it is time for investigators to re-focus their efforts by capturing a better understanding of the protean pathogenic factors that drive progressive neurodegeneration. Evidence- and mechanism-based approaches are needed to develop multi-pronged therapeutics, utilizing strategies that already have been successful for cancers and other chronic diseases.

AD-ASSOCIATED WM PATHOLOGY

White matter degeneration is a major and consistent but vastly under-studied abnormality in AD (Figure 1). Its occurrence was initially characterized in 1986 by Brun and Englund (4, 5) and subsequently shown to be an early pre-clinical abnormality (6). WM atrophy in AD is most pronounced in the parietal and temporal lobes, followed by the frontal lobes, whereas the occipital lobes tend to be spared (6). Consequently, the severities of WM atrophy correspond with the distribution and degree of cerebral cortical pathology. WM degeneration in AD is associated with loss of myelin and myelinated axons, together with dysfunction or loss of oligodendrocytes, increased activation of astrocytes, that is gliosis, and microvascular disease (7–9). Leukoaraiosis, an extreme form of WM degeneration in which the loss of myelinated axons is extensive and associated with WM hyperintensities by magnetic resonance imaging (MRI) (7–9), is most prominently distributed in periventricular and central compared with subcortical WM (5, 6, 10–15).

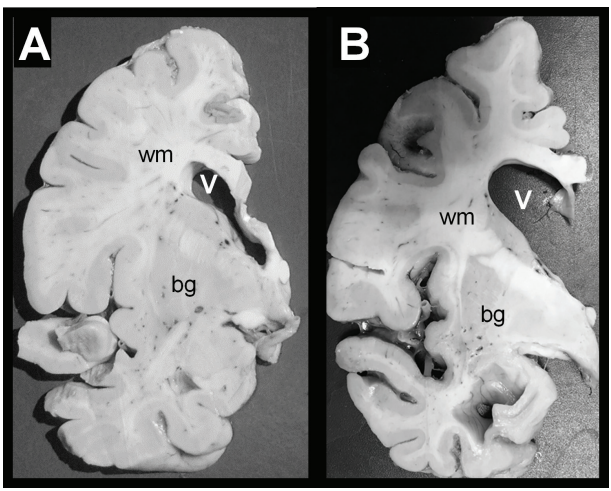

Figure 1 White matter atrophy in AD. Postmortem coronal slices of the left cerebral hemisphere from patients with (A) normal aging or (B) advanced AD. Panels A and B show approximately the same coronal slice levels depicting the cingulate gyrus, corpus callosum, basal ganglia (bg), central and periventricular posterior frontal white matter (wm) and lateral ventricle. Note the markedly atrophic white matter and associated ex vacuo enlargement of ventricles (V) in (B) AD relative to (A) control.

The histopathologic correlates of myelin degeneration in AD have been characterized by assessing relative reductions in Luxol fast blue (LFB) staining intensity and uniformity. LFB reacts with phospholipids and lipoproteins and is suitable for detecting myelin pathology in formalin-fixed, paraffin-embedded tissue. However, additional approaches are required to evaluate axonal degeneration and attrition. Traditional histochemical stains, such as Bielschowsky, utilize silver impregnation to label cytoskeletal proteins in axons, dendrites, and degenerated or dystrophic fibers. Current, more specific approaches employ immunohistochemical staining with antibodies to phosphorylated neuronal cytoskeletal proteins such as neurofilament and tau. In AD, myelin loss and axonal degeneration, respectively, marked by pallor of LFB staining and fragmentation, irregular swelling, and rarefaction of fibers, tend to be more pronounced in central and periventricular compared with subcortical WM, and they increase with severity of AD.

ASTROCYTES, MICROGLIA, AND OLIGODENDROCYTES

Astrocyte activation or gliosis is a conspicuous feature of AD. Gliosis marks responses to cellular and tissue degeneration. One potential outcome of gliosis is tissue repair, but an alternative outcome is the elaboration of pro-inflammatory cytokines that promote oxidative stress and tissue injury, thereby worsening neurodegeneration. WM gliosis is marked by increased glial fibrillary acidic protein (GFAP) immunoreactivity in enlarged (hypertrophic) astrocytes and fibrillary deposits within the extracellular matrix (Figure 2). Dense fibrillary gliosis, which reflects severe degeneration, is most prominently distributed in periventricular and subcortical U-fiber regions. In contrast, central WM gliosis is generally less pronounced and associated with increased reactive hypertrophic astrocytes and variable densities of GFAP-positive fibrillary deposits.

WM gliosis, particularly in the early and intermediate stages of AD, is accompanied by microglial activation. Microglia have rod-shaped, curved, or twisted nuclei and can be detected by immunohistochemical staining with antibodies to common leukocyte antigen (CD45) or ionized calcium-binding adaptor protein-1 (IBA-1) (16). In AD, activated microglia together with reactive astrocytes promote neuroinflammation via increased elaboration of pro-inflammatory cytokines and chemokines and suppression of anti-inflammatory molecules (17). Neuroinflammation causes injury and degeneration of myelin and axons. Although the underlying causes of WM neuroinflammation have yet to be determined, plausible etiologies include insulin/insulin-like growth factor (IGF) resistance and microvascular ischemic injury since both have been demonstrated in AD and are well-documented mediators of inflammation, oxidative stress, and metabolic dysfunction.

Oligodendrocytes synthesize and maintain myelin sheaths needed to support axonal integrity and function. Oligodendrocytes, like neurons, are highly vulnerable to both insulin and IGF-1 resistance and ischemic injury. Loss or dysfunction of oligodendrocytes impairs myelin maintenance, axonal function, and ultimately axonal structure. To better understand the mechanisms of WM degeneration

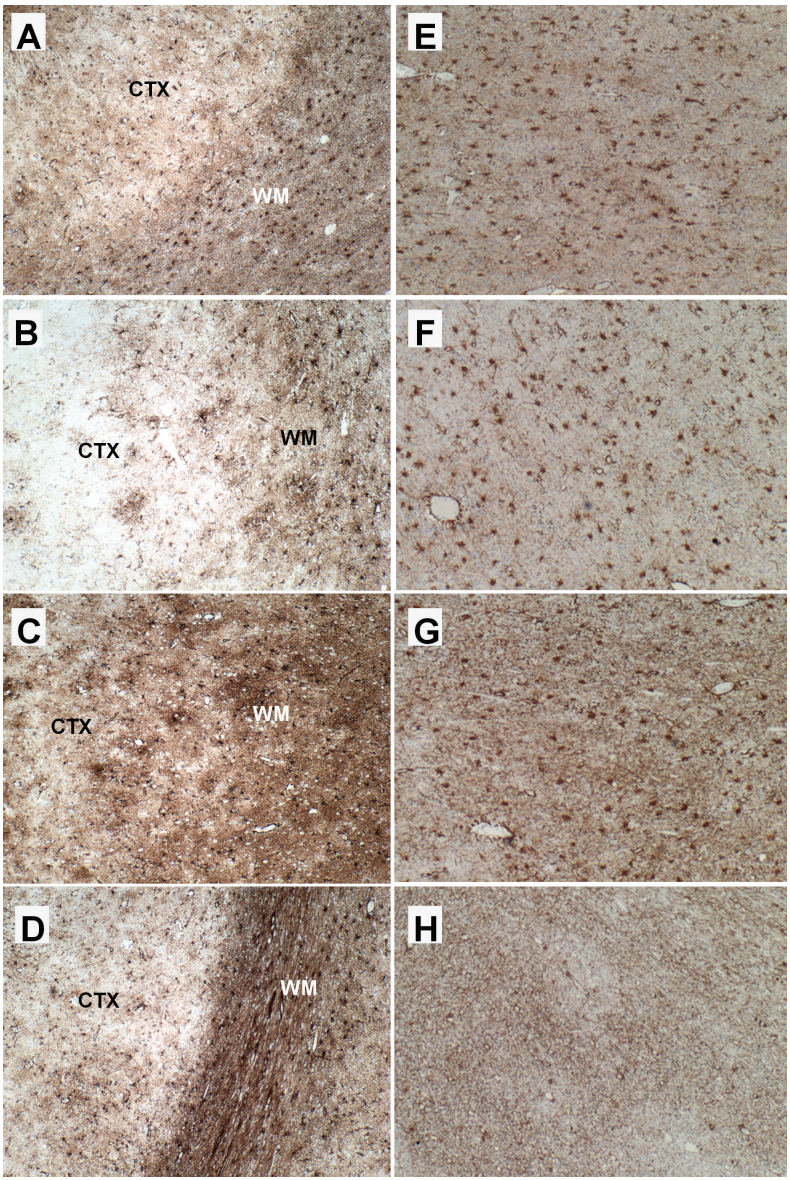

Figure 2 WM gliosis exhibits regional variation in AD. Immunohistochemical staining was used to detect glial fibrillary acidic protein (GFAP) in the(A, E) anterior frontal, (B, F) posterior frontal, (C, G) parietal, and (D, H) occipital cortex (CTX) and underlying white matter (WM) in formalin-fixed paraffin-embedded human postmortem brain tissue. Immunoreactivity was detected with biotinylated secondary antibodies, horseradish peroxidase-conjugated avidin-biotin complexes, and diaminobenzidine (brown precipitant). Panels A–D (100x original magnification) show intense GFAP immunoreactivity in white matter (wm) and variable labeling of the cortex (ctx). Panels E–G (200x original magnification) show abundant GFAP-positive hypertrophic reactive astrocytes (dot-like structures) in a background of diffuse fibrillar labeling, whereas Panel H shows predominantly fibrillar labeling of central occipital WM.

in AD, more information is needed about the biochemical and molecular nature of oligodendrocyte injury and dysfunction leading to myelin and axonal loss. However, recent advances in adult brain WM cell isolation techniques (18), lipidomics mass spectrometry (19, 20), and targeted gene array analysis now provide practical methodologic approaches for characterizing WM oligodendrocyte and myelin lipid pathologies.

BIOCHEMICAL AND CELLULAR BASIS OF WM PATHOLOGY IN AD

White matter is largely composed of myelinated axons. Traditionally, the integrities of myelin and axons are studied by histochemical or immunohistochemical staining. However, overlapping responses to various types of injury and degeneration limit the utility of these approaches for characterizing disease-specific pathologies and responses to treatment. To better understand the nature of AD-associated myelin pathology, biochemical approaches that assess reproducible alterations in lipid composition are needed.

CNS myelin, a specialized membrane synthesized by oligodendrocytes, has a much higher dry mass of lipids (70–85%) compared with proteins (15–30%) and plays a major role in insulating axons to support conductivity. Myelin lipids primarily include cholesterol, glycosphingolipids, sulfatides, gangliosides, phospholipids, and sphingomyelin (21). Many diseases that impair the structural and functional integrity of WM are associated with abnormalities in the expression and metabolism of phospholipids and sulfatides (20, 22–28). Membrane phospholipids have important roles in regulating lipid rafts and receptor functions. Sulfatides, located on extracellular leaflets of plasma membranes (29) and generated via sulfonation of galactocerebroside, regulate neuronal plasticity, memory, myelin maintenance, protein trafficking, adhesion, glial-axonal signaling, insulin secretion, and oligodendrocyte survival (30). Degradation of sulfatide via galactosylceramidase and sulfatidase yields ceramide (29, 31), which promotes neuroinflammation, apoptosis, and production of reactive oxygen species (ROS), and impairs signaling through survival and metabolic pathways (32). Furthermore, deficiencies in membrane sulfatide disrupt myelin's structure, function, and capacity to support neuronal conductivity (32). Thus, imbalances in sphingolipid composition that reduce sulfatide and increase ceramide are potentially important mediators of WM degeneration and attendant cognitive impairment.

Potential role of oligodendrocyte dysfunction as a mediator of WM degeneration

Oligodendrocytes generate and maintain CNS myelin by controlling the expression and activity of enzymes that modulate its biosynthesis, turnover, and degradation (33–36). Loss or damage to myelin impairs neuronal conductivity and

compromises axonal integrity, releasing neurofilament and myelin sulfatides (37). Increases in lipid peroxidation after myelin breakdown exacerbate oxidative damage, neuroinflammation, and astrocyte activation (gliosis). Therefore, the presence of WM atrophy and degeneration early in the course of AD, including in its preclinical stages (6), could be due to pathogenic processes that impair function and survival of mature oligodendrocytes and promote secondary reactive injury via increased oxidative stress, inflammation, and lipid peroxidative. In light of the known importance of insulin and IGF signaling for maintaining a broad array of homeostatic functions in both neurons and oligodendrocytes (38–40), and strong evidence for brain insulin and IGF deficiencies and resistances beginning early in the course of AD (41, 42), it is plausible to hypothesize that impaired signaling through the insulin and IGF receptors also mediates oligodendrocyte dysfunction in AD.

Many critical functions of oligodendrocytes, including cell survival, myelin synthesis, and myelin maintenance, are supported by insulin and IGF-1 signaling (43–46). Consequently, disruption of related networks decreases oligodendrocyte viability, increases oxidative stress, and impairs myelin maintenance and maturation. Likewise, experimental models of chronically impaired brain insulin and IGF signaling exhibit WM atrophy and degeneration (47, 48) together with oligodendrocyte dysfunction (49), all of which can be partly reversed or prevented by early treatment with insulin sensitizers (47, 49). Another consequence of impaired insulin/IGF signaling is dysregulated sphingolipid metabolism resulting in decreased sulfatide and increased ceramide levels (23, 26–28, 50–52). Increases in ceramide can cause WM degeneration via several mechanisms, including inhibition of insulin/IGF signaling through pathways needed for oligodendrocyte survival and metabolic functions, and stimulation of pro-inflammatory and oxidative stress responses (28).

Besides lipids, oligodendrocytes synthesize integral membrane proteins whose expressions are differentially modulated at each stage of myelin maturation as well as in response to injury. As immature oligodendrocyte precursor cells (OPC) pass through phases of differentiation to eventually become mature myelin-producing oligodendrocytes, the proteins needed to support the structure and function of myelin also change. Mature oligodendrocytes express myelin basic protein (MBP), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP) (53), and adenoma polyposis coli (APC) (54), as well as O4 sulfatide (54). PLP is the most abundant protein in CNS myelin (55, 56). Olig 1–3 transcription factors are expressed at various stages of oligodendroglial maturation (54). Injury and degeneration of myelin cause the populations of intact mature functional oligodendrocytes to decline. That effect can lead to the proliferation of immature oligodendrocytes that express different myelin glycoproteins, transcription factors, and myelin glyco- and phospholipids which may not support optimum conductivity and CNS function. The conspicuous abnormalities in oligodendrocyte myelin-associated gene and lipid expression observed in human brains with AD and relevant experimental models including those linked to brain insulin and IGF resistance (19, 22–24, 57), lend strong support the hypothesis that oligodendrocytes are targets of WM atrophy and neurodegeneration in AD.

VASCULOPATHY, VASCULAR DEGENERATION, AND ISCHEMIC INJURY IN AD

Small vessel disease is a recognized component of WM degeneration in AD, but its pathogenesis and contributions to neurodegeneration are poorly understood. The nature of vasculopathy and its progression to vascular degeneration and attendant ischemic injury require mechanistic understanding to guide preventive and therapeutic measures. Important initial steps include drawing distinctions between amyloid and non-amyloid associated vasculopathy and degeneration and characterizing the mediators and consequences of non-amyloid microvasculopathy, which is a feature of WM degeneration in AD.

The well-established AD-associated progressive declines in cerebral blood flow, glucose metabolism, and oxygen utilization suggest that impairments in brain perfusion are important components of AD (58, 59). However, the extent to which vascular disease causes AD or represents an integral component of neurodegeneration remains controversial. Postmortem studies demonstrated cerebral vascular pathology in over 80% of brains with AD (60). In a separate postmortem study, substantial overlap was observed between AD and vascular-mediated injury, but very few cases of dementia could be attributed to vascular disease alone (2). The Gothenburg study reported that mental slowness and deficits in executive function were linked to WM vascular dysfunction and pathology but not cortical vasculopathy (37). Together, these studies suggest that although CNS vasculopathy contributes to AD, it is seldom sufficient to cause dementia on its own (37).

In AD, there are two major types of microvascular pathology: amyloid angiopathy and non-amyloid vasculopathy (Figure 3). Amyloid angiopathy affects vessels in the cerebral cortex and leptomeninges, but not WM (61–63). In AD, non-amyloid vascular degeneration occurs in microvessels, including capillaries, arterioles, and venules in the cerebral cortex, WM, and subcortical nuclei. Non-amyloid microvascular disease is characterized by fibrotic thickening of vessel walls (sclerosis), loss of endothelial cells, thickening of basement membranes, attrition of perivascular tissue (64), reduced vascular density (micro-vasculopenia), and increased vessel coiling (65). Mural sclerosis leads to extreme narrowing of the lumens and reduced vaso-responsiveness, restricting perfusion, particularly in times of increased metabolic demand (66). Chronic hypoperfusion of WM causes ischemic injury ranging from myelin loss to fiber attrition, and in extreme cases, leukoaraiosis and micro-infarcts (5, 13). Another consequence of microvascular pathology is weakening and increased permeability of vessel walls as that occurs in diabetic nephropathy (67). Leakiness of microvessels enables toxins and inflammatory mediators from the peripheral circulation to enter the brain and cause perivascular tissue injury and attrition (58, 68–74).

Potential role of nitric oxide in cerebral microvascular dysfunction and pathology

Nitric oxide (NO) is an important physiological modulator of vascular smooth muscle function and blood flow. However, NO in high concentrations can be cytotoxic due to the activation of stress and inflammatory responses.

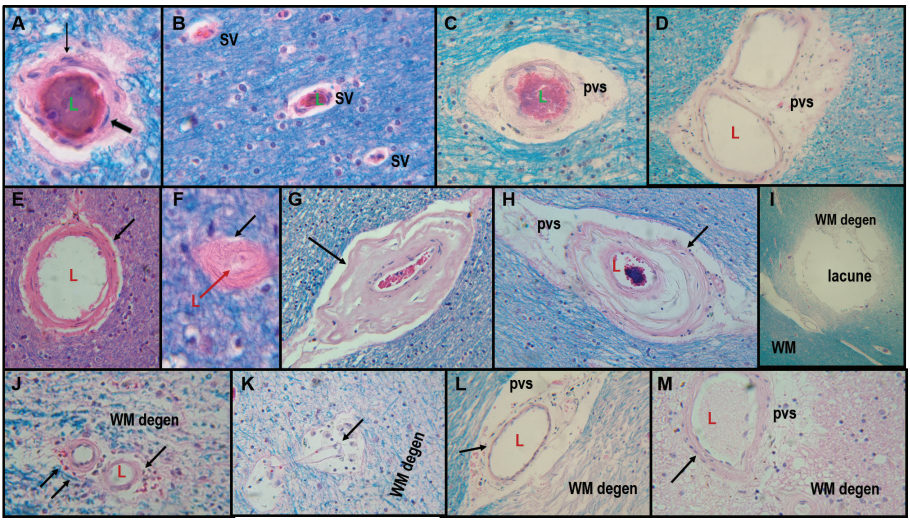

Figure 3 White matter (WM) vascular pathology in AD. Human postmortem parietal lobe samples were fixed in formalin, embedded in paraffin, and stained with Luxol fast blue (LFB), hematoxylin and eosin (LHE). (A, B) Control white matter (A) medium size and (B) small vessels (sv). Fine arrow in A shows normal smooth muscle cells and the broad arrow shows a normal endothelial cell. L = lumen. Panels C–M depict vascular pathology in AD WM. (C) Medium size vessel with reduced smooth muscle and a widened perivascular space (pvs) reflecting to perivascular tissue attrition. (D) Vascular fibrosis with enlarged pvs containing lipid-laden macrophages and hemosiderin deposits. (E) Vascular fibrosis (arrow) and myelin loss (markedly reduced LFB staining compared with A–D, and F–I). (F) Severe microvascular disease with fibrotic thickening (black arrow) and an extremely narrow lumen (red arrow; L). (G, H) Severe arteriosclerosis with degeneration and splitting of vessel walls, narrowing of lumens and in (H), widened pvs. (I) WM lacune (micro-infarct) associated with severe vascular degeneration and circumscribed area of WM degeneration. (J–M) Progressive WM degeneration associated with vasculopathy. WM degeneration is associated with loss of myelin staining, perivascular tissue attrition, and fiber loss. (M) Absent LFB staining and cystic degeneration of WM.

Microvascular degeneration is ultimately mediated by oxidative stress and inflammation. The potential role of NO as a mediator of vascular degeneration in AD was suggested by the findings that high levels of nitric oxide synthase (NOS) activity were co-localized with nuclear p53 in cerebral vessels (75) and cells with increased expression of pro-inflammatory and immune signaling genes (76). A later independent study of postmortem human brains demonstrated increased endothelial NOS (NOS3) immunoreactivity co-localized with nuclear p53 in AD microvascular smooth muscle and endothelial cells, confirming a role for aberrant NOS expression in cerebrovascular cells with increased proneness to apoptosis (77). Together, these findings suggest that non-amyloid vascular degeneration in AD is mediated by increased NO production and activation of inflammatory mechanisms.

However, the simultaneous detection of many sclerotic vessels with increased nuclear p53 but no detectable NOS3 immunoreactivity in either smooth muscle or endothelial cells, that is NOS3 expression was aberrantly down-regulated, suggests additional mechanisms mediate vascular dysfunction in AD (77).

For example, degenerative vascular sclerosis compromises vaso-responsiveness and flow, resulting in chronic ischemic injury that can be exacerbated by repeated and multifocal thrombotic microvascular occlusions. Furthermore, fibrotic degeneration disrupts vessel wall integrity, rendering them more permeable to toxic-inflammatory factors from the peripheral circulation. This phenomenon could account for the perivascular tissue attrition that accompanies microvascular degeneration in AD (Figure 3).

Thrombin activation, inflammation, microvascular occlusion, and ischemic injury in AD

In AD, the cerebral microcirculation is pathophysiologically activated due to endothelial cell overexpression of bioactive, neurotoxic, and inflammatory proteins including thrombin. Endothelial-derived thrombin is a multifunctional protease which in AD, besides promoting vascular occlusion, functions as a stress-activated neurotoxin (78, 79). Mechanistically, thrombin initiates neuronal apoptosis via activation of glial and microglial cells, leading to increased oxidative stress and neuroinflammation (80). Thrombin also stimulates Aβ precursor protein production and cleavage, mediates proteolytic processing of tau, and causes tau hyperphosphorylation and aggregation (80, 81). In essence, vessel-derived thrombin could represent a critical modulator of AD pathology via its regulation of inflammatory and bioactive protein expression. In this regard, thrombin activation of endothelial cells enhances expression and release of many pro-inflammatory proteins including monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), IL-1, IL-6, and IL-8 (80, 81). Thus, microvascular disease could initiate and propagate neuroinflammation in AD.

Pro-inflammatory cytokine activation in endothelial cells leads to oxidative stress and thrombin release, with attendant thrombotic luminal occlusion or vessel wall injury causing increased permeability (82). However, the same responses can be mediated by up-regulation of the thrombin receptor protease-activated receptor 1 (PAR-1) or down-regulation of the brain thrombin inhibitor, protease nexin-1 near blood vessels (80). Since brain endothelial cells produce thrombin and also express functionally active PAR-1 and PAR-3 (79, 83), thrombin may initiate autocrine stimulation of a noxious feed-forward cycle. In addition, the intimate proximity of microvascular endothelial cells to microglia, astrocytes, and oligodendrocytes enables secretory products, including thrombin to influence cellular responses via a paracrine-type stimulation. For example, treatment of human microglia with thrombin induces TNF-α/TNR-dependent up-regulation of NF-κB (84). In astrocytes, thrombin activation of PAR-1 leads to increased MMP-9 expression through the regulation of several signaling pathways including PKC, JNK, and MAPK (85).

Microvascular endothelial cells elaborate trophic factors that positively impact oligodendroglia, but under conditions of stress, injury, or inflammation, endothelial cell dysfunction can adversely affect oligodendrocytes. An important role for endothelial-derived factors in oligodendroglial health was suggested by studies showing that endothelial cell-conditioned media enhances survival of OPCs (86, 87). On the other hand, in an experimental animal model of cerebral small vessel disease, early development of endothelial cell dysfunction was found to promote

secretion of heat shock protein 90alpha and subsequently block oligodendroglial differentiation and production of mature myelin (88). Mechanistically, oxidative stress impairs the capacity of endothelial cells to generate trophic factors (89) needed to support critical functions of mature oligodendrocytes, including myelin maintenance.

In AD, WM degeneration could stem from both endothelial and oligodendrocyte dysfunction. Endothelial cell dysfunction leading to excess thrombin release could cause micro-ischemic injury due to thrombosis or perivascular toxic injury mediated by increased vessel wall degeneration and permeability. At the same time, increased thrombin release would compromise the functional integrity of oligodendroglia, impairing survival and maintenance of mature myelin (79, 90–92). The concept that increased thrombin release could impair oligodendrocyte function is supported by the findings that the PAR1 thrombin receptor is a critical extracellular switch that controls myelination and that PAR1 deletion increases myelination (93), survival, maturation, and myelin maintenance. Altogether, the findings suggest that increased thrombin signaling by any one of several mechanisms can lead to micro-vessel-related WM injury in AD. However, the missing link is that we still do not understand the underlying causes of brain microvascular degeneration and endothelial cell dysfunction that lead to thrombin activation. One potential etiopathic candidate is insulin resistance since type 2 diabetes mellitus and other insulin resistance diseases are typically associated with microvascular disease and increased thrombin activation, accompanied by oxidative stress, platelet aggregation, vascular occlusions, and ischemic injury (94, 95). Correspondingly, insulin inhibits thrombin-induced endothelial dysfunction and mitigates microvascular permeability by decreasing thrombin-mediated vascular endothelial-cadherin translocation to the cytoskeleton/nuclear compartment (96).

INSULIN AND INSULIN-IGF SIGNALING IMPAIRMENTS AS MEDIATORS OF WM GLIAL-VASCULAR DEGENERATION IN AD

Considerable research had already demonstrated roles for impaired insulin and IGF-1 signaling in AD cortical and subcortical gray matter structures. However, little information is available regarding alterations of these same signaling pathways in AD WM degeneration, despite evidence that oligodendrocyte survival and function are dependent upon intact insulin and IGF networks. Therefore, additional research on the nature, mechanisms, and effects of impaired insulin and IGF signaling in relation to brain WM degeneration could generate a solid foundation for enhancing a broader understanding of the spectrum of brain pathology in AD.

In AD, deficits in brain energy metabolism, particularly concerning glucose utilization have been recognized for years (97–100). Positron emission tomography (PET) imaging with (18) F-fluoro-deoxyglucose (FDG) is a standard approach for detecting early impairments in brain glucose uptake and utilization (101–103). Insulin and IGF are major regulators of energy metabolism in the

brain, and they have critical roles in maintaining broad neuronal and oligodendrocyte functions (38, 40). Impairments in brain insulin/IGF signaling due to insulin/IGF deficiencies or receptor resistances cause deficits in learning and memory (104).

Postmortem human studies demonstrated that AD is associated with significantly reduced expression of brain insulin and IGF polypeptides and receptors, insulin and IGF receptor tyrosine phosphorylation and receptor binding, activation of downstream pathways that promote cell survival, metabolism, neuronal plasticity, and myelin maintenance, and inhibition of signaling mechanisms that promote oxidative stress, neuroinflammation, cell death, and lipid peroxidation (42, 105, 106). Insulin/IGF deficiencies and resistances increase with Braak stage severity of AD (41, 105) and therefore correlate with accumulations of Aβ and pTau pathologies. The finding that cerebrospinal fluid (CSF) insulin levels decline in the early or intermediate stages of AD (107), and overlap with progressive accumulations of Aβ and advanced glycation end-products (AGEs) (Figure 4A) (52, 107, 108) which cause oxidative stress and neuroinflammation, suggests that insulin deficiency contributes to progressive neurodegeneration in AD.

The human studies linking AD pathogenesis and progression to impairments in brain insulin/IGF signaling are supported by data from experimental models of sporadic AD produced by intracerebral (i.c.) administration of streptozotocin (STZ). STZ, a pro-diabetes toxin, injected into the cerebral hemispheres and ventricles, causes selective insulin deficiency and resistance in the brain with deficits in learning and memory, elevated levels of pTau, Aβ, and ubiquitin, loss of neurons, gliosis, oxidative stress, neuroinflammation, WM atrophy, and microvascular disease (47–49, 104, 109). Importantly, data from these models support the concept that sustained and progressive deficits in brain insulin/IGF signaling cause nearly all of the known structural, functional, biochemical, molecular, and neurobehavioral abnormalities identified in AD. Correspondingly, insulin administration improves working memory and cognition (110–113) and enhances Aβ clearance (114). Moreover, CNS-appropriate insulin sensitizer drugs have been shown to prevent or reduce AD-associated abnormalities in experimental animals (49, 109, 115).

Until now, the adverse effects of brain insulin/IGF deficiencies and resistances have been focused on neurodegeneration and the functional impairments in gray matter structures due to the interest in linking them to standard neuropathological processes. Additional research is needed to determine how insulin/IGF-1 metabolic dysfunction mediates other aspects of AD. In this regard, recent preliminary studies showed that with the increasing severity of AD, WM atrophy and degeneration are associated with corresponding impairments in the expression of Akt pathway proteins and phosphoproteins (de la Monte, S.M. and Tong, M, Unpublished). Compromised signaling along these pathways could lead to loss of structural and functional integrities of oligodendrocytes and myelin. Although the steps leading from brain insulin and IGF deficiencies and resistances to WM degeneration have not yet been delineated, clues may be harnessed from data generated via unrelated experiments. For example, several studies have shown that WM atrophy and degeneration in other models of brain insulin and IGF resistances were associated with significant oligodendrocyte dysfunction. For example, in a rat model of i.c. STZ, WM degeneration was associated with reduced expression of mature myelin-associated genes and increased expression of

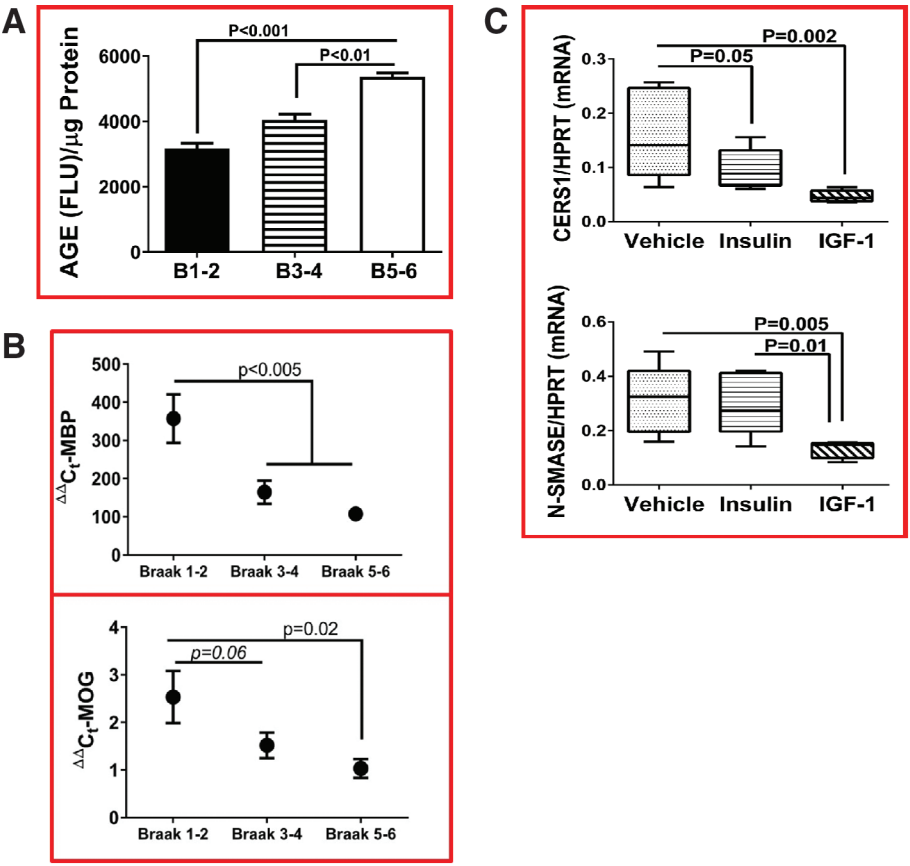

Figure 4 Cellular signaling abnormalities mediating WM degeneration. (A) Advanced glycation end-product (AGE) immunoreactivity was measured in postmortem frontal WM homogenates from humans with normal aging (Braak 0–2; B0–2), moderate AD (B3–4),or severe AD (B5–6) AD using a direct binding ELISA (107). Immunoreactivity was detected with horseradish peroxidase-conjugated secondary antibody and Amplex Red fluorophor. Fluorescence light units (FLU) were measured (Ex 579 nm/Em 595 nm) in a Spectromax M5, and results were normalized to protein content. (B) AD WM atrophy and degeneration are associated with reduced expression of mature MAGs. Quantitative RT-PCR was used to measure mRNA levels of MAG 1 and myelin oligodendroglial glycoprotein (MOG). PCR primer pairs were designed with Primer 3 (http://primer3.sourceforge.net/) software. PCR reactions were performed in a Roche Lightcycler 480 System (116). Gene expression was analyzed using the $^{\Delta\Delta}C_t$ method with results normalized to hypoxanthine-guanine phosphoribosyl transferase, HPRT. (C) Insulin and IGF-1 suppress expression of genes encoding enzymes that produce ceramides or break down sphingomyelin. Frontal lobe WM slice cultures from an i.c. STZ adult Long Evans rat model of sporadic AD (109) were stimulated for 24 h with 10 nM insulin, 10 nM IGF-1, or vehicle (control). Graphs depict the mean ± S.E.M. for each group. Intergroup comparisons were made by one-way ANOVA with the post hoc Tukey's test. Significant P-values and trends are indicated.

immature myelin-associated genes (116), corresponding with deficits in myelin maturation and striking alterations in WM structure (117). Similar observations have been made in human postmortem brains with different severities of AD and WM atrophy (Figure 4B).

An additional feature of experimental brain insulin resistance with WM atrophy is that oligodendrocyte dysfunction is associated with altered expression of sphingolipid metabolizing enzymes such that ceramide accumulation and sulfatide depletion would be favored (Figure 4C) (118, 119). In humans and experimental animals, reductions in brain sulfatide and increases in ceramide correlate with cognitive impairment, oxidative stress, lipid peroxidation, and neuroinflammation (22–24, 120). Ceramides inhibit insulin signaling through PI3K-Akt (25, 121) and increase oxidative stress, Aβ, pTau, and pro-apoptosis activation (122). Furthermore, preliminary studies suggest that insulin and IGF-1 stimulation suppress expression of enzymes that generate ceramides via degradation of sphingomyelin (Figure 4C). Therefore, it is likely that impairments in insulin/IGF-1 signaling in oligodendrocytes are important in the pathogenesis of WM atrophy and degeneration and mediate disease progression in AD.

Proposed role of APOE-ε4

Apolipoprotein E is the major lipid transport protein in the CNS. This 34 kDa protein has three major isoforms (APOE-***ε2***, APOE-***ε3***, and APOE-***ε4***) that differ by single amino acid substitutions at residues 112 and 158 (123). The ***ε4*** allele is the strongest genetic risk factor for late-onset sporadic AD (124–127) in that carriers account for over 50% of all AD cases (128); however, the APOE-***ε4*** risk assessments vary across different countries and ethnicities (129). AD risk is increased by three- or fourfold among APOE-***ε4*** carriers, and 15-fold in APOE-***ε4*** homozygotes. APOE-***ε4*** confers increased risk for AD by reducing brain glucose metabolism in the preclinical stages of disease (130), and ultimately impairing signal transduction through the insulin receptor, reducing Aβ clearance, and increasing Aβ aggregation (131). APOE-***ε4*** may also have a role in mediating AD-associated WM degeneration via insulin resistance (131) and attendant microvascular endothelial cell and oligodendrocyte dysfunction.

Insulin/IGF signaling impairments and glial-vascular WM pathologies in AD

Our overarching hypothesis is that in AD, WM degeneration is mediated by impairments in insulin and IGF signaling that cast a wide net of pathophysiological responses including oxidative stress, inflammation, and dysregulated glucose and lipid metabolism (Figure 5). In WM, the targets of degeneration are oligodendrocytes and microvessels. Reduced signaling through insulin/IGF receptors, IRS and downstream Akt pathways compromises oligodendrocyte survival, myelin maintenance and integrity, and sphingolipid homeostasis, favoring sulfatide depletion and ceramide accumulation. Ceramide-mediated neurotoxicity, inflammation, oxidative stress, lipid peroxidation, and further impairment of insulin signaling reinforce the cascade of WM degeneration.

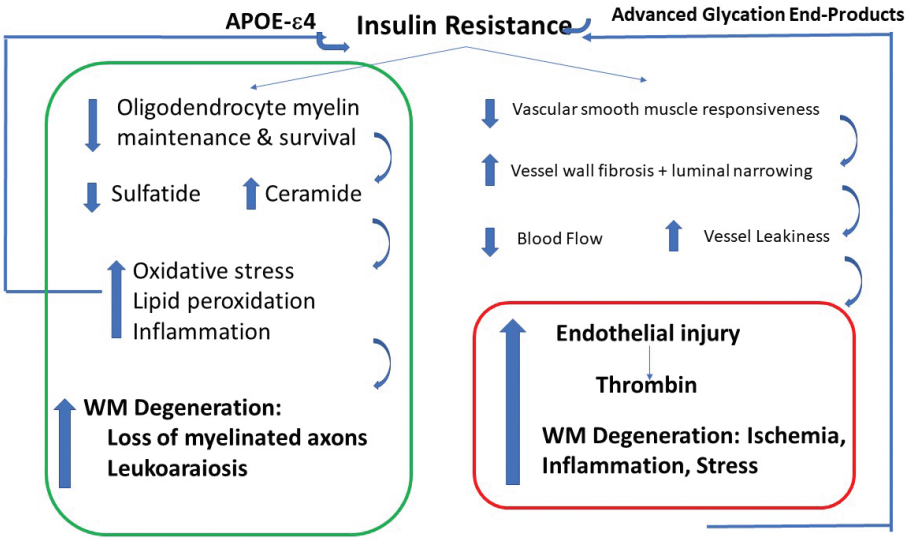

Figure 5 Hypothesis: White matter degeneration in AD is mediated by insulin and IGF resistances that target oligodendrocytes and microvessels. Reduced myelin and vascular integrity promote neuroinflammation, stress, and ischemic injury. APOE-ε4 genotype, obesogenic diets, and poor lifestyle choices have cofactor roles in mediating WM degeneration due to exacerbation of insulin resistance.

Microvascular disease is also driven by insulin deficient and resistant states such as in types 1 and 2 diabetes mellitus. Initially, microvascular disease is mediated by combined effects of hyperglycemia, increased levels of AGE (Figure 4A), up-regulation of receptors for AGE (RAGE), and reduced responsiveness to NOS/NO (67, 132). Therefore, insulin and IGF resistances negatively impact microvascular structural integrity, vaso-responsiveness, and endothelial function. In later stages, microvascular disease is associated with the replacement of smooth muscle by collagen (sclerosis) leading to degenerative mural fibrosis and luminal narrowing, restricted blood flow, reduced vessel wall integrity marked by increased leakiness, and endothelial damage with attendant up-regulation and release of thrombin. Thrombin activation drives inflammation (cytokines), oxidative stress, microvascular occlusions. Microvascular occlusions cause ischemia which can injure oligodendrocytes, myelin, axons, and vessels. Also, vessel wall leakiness exposes perivascular tissue to toxins from the peripheral circulation. Late and probably permanent microvascular-associated WM pathologies in AD include leukoaraiosis with loss of myelin and degeneration of axons, microinfarcts, perivascular tissue attrition, and vasculopenia (vessels can be destroyed by ischemic necrosis). Finally, microvascular disease can drive WM degeneration by worsening insulin resistance, oxidative stress, and inflammation.

Inflammation and oxidative stress are recognized mediators of neurodegeneration in AD (26, 39, 133). Potential sources of stress and inflammation include increased levels of AGE and RAGE expression (107, 108, 132, 134–137), impaired insulin/IGF signaling through Akt pathways, lipid peroxidation linked to myelin

breakdown, and ceramide accumulation. Neuroinflammation in AD is associated with increased pro-inflammatory cytokine expression in astrocytes and microglia (133, 138, 139). Although TNF-α, IFN-γ and IL-1β are key players, preliminary data suggest that neuroinflammatory responses are broader and include activation of pro-inflammatory and inhibition of neuroprotective cytokines/chemokines (17, 140).

CONCLUSION

Combined effects of oligodendrocyte and microvascular dysfunction interact to cause WM degeneration, including leukoaraiosis in AD. Insulin resistance exacerbation by APOE-*ε4* may accelerate AD-associated WM molecular, biochemical, and structural pathologies linked to impaired function of oligodendrocytes and microvascular endothelial cells. Therefore, WM degeneration and cognitive impairment may be preventable or reversible by lifestyle measures that restore insulin responsiveness in the CNS.

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REFERENCES

1. Alzheimer's Association. New Alzheimer's Association report reveals sharp increases in Alzheimer's prevalence, deaths, costs of care. May, 2018. Available from: https://www.alz.org/news/2018/new_alzheimer_s_association_report_reveals_sharp_i
2. Etiene D, Kraft J, Ganju N, Gomez-Isla T, Gemelli B, Hyman BT, et al. Cerebrovascular pathology contributes to the heterogeneity of Alzheimer's disease. J Alzheimers Dis. 1998;1(2):119–34. http://dx.doi.org/10.3233/JAD-1998-1205
3. Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, et al. Alzheimer's disease. Lancet. 2016;388(10043):505–17. http://dx.doi.org/10.1016/S0140-6736(15)01124-1
4. Brun A, Englund E. A white matter disorder in dementia of the Alzheimer type: A pathoanatomical study. Ann Neurol. 1986;19(3):253–62. http://dx.doi.org/10.1002/ana.410190306
5. Brun A, Englund E. Brain changes in dementia of Alzheimer's type relevant to new imaging diagnostic methods. Prog Neuropsychopharmacol Biol Psychiatry. 1986;10(3–5):297–308. http://dx.doi.org/10.1016/0278-5846(86)90009-6
6. de la Monte SM. Quantitation of cerebral atrophy in preclinical and end-stage Alzheimer's disease. Ann Neurol. 1989;25(5):450–9. http://dx.doi.org/10.1002/ana.410250506
7. Scheltens P. Imaging in Alzheimer's disease. Dialogues Clin Neurosci. 2009;11(2):191–9.
8. Scheltens P, Barkhof F, Leys D, Wolters EC, Ravid R, Kamphorst W. Histopathologic correlates of white matter changes on MRI in Alzheimer's disease and normal aging. Neurology. 1995;45(5):883–8. http://dx.doi.org/10.1212/WNL.45.5.883

9. Scheltens PH. Structural neuroimaging of Alzheimer's disease and other dementias. Aging (Milano). 2001;13(3):203–9. http://dx.doi.org/10.1007/BF03351478
10. Brickman AM, Honig LS, Scarmeas N, Tatarina O, Sanders L, Albert MS, et al. Measuring cerebral atrophy and white matter hyperintensity burden to predict the rate of cognitive decline in Alzheimer disease. Arch Neurol. 2008;65(9):1202–8. http://dx.doi.org/10.1001/archneur.65.9.1202
11. Burns JM, Church JA, Johnson DK, Xiong C, Marcus D, Fotenos AF, et al. White matter lesions are prevalent but differentially related with cognition in aging and early Alzheimer disease. Arch Neurol. 2005;62(12):1870–6. http://dx.doi.org/10.1001/archneur.62.12.1870
12. Brilliant M, Hughes L, Anderson D, Ghobrial M, Elble R. Rarefied white matter in patients with Alzheimer disease. Alzheimer Dis Assoc Disord. 1995;9(1):39–46. http://dx.doi.org/10.1097/00002093-199505000-00008
13. Englund E. Neuropathology of white matter changes in Alzheimer's disease and vascular dementia. Dement Geriatr Cogn Disord. 1998;9(Suppl 1):6–12. http://dx.doi.org/10.1159/000051183
14. Sjobeck M, Englund E. Glial levels determine severity of white matter disease in Alzheimer's disease: A neuropathological study of glial changes. Neuropathol Appl Neurobiol. 2003;29(2):159–69. http://dx.doi.org/10.1046/j.1365-2990.2003.00456.x
15. Sjobeck M, Haglund M, Englund E. Decreasing myelin density reflected increasing white matter pathology in Alzheimer's disease – A neuropathological study. Int J Geriatr Psychiatry. 2005;20(10):919–26. http://dx.doi.org/10.1002/gps.1384
16. Hovens I, Nyakas C, Schoemaker R. A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: Cell body to cell size ratio. Neuroimmunol Neuroinflamm. 2014;1(2):82–8. http://dx.doi.org/10.4103/2347-8659.139719
17. de la Monte SM, Daiello LA, Hapel AJ, Tong M, Ott BR. Altered serum and cerebrospinal fluid inflammatory cascades in mild cognitive impairment and Alzheimer's disease. J Neuroinflam Neurodegen. 2017;1(1):100004, 1–24.
18. Flores-Obando RE, Freidin MM, Abrams CK. Rapid and specific immunomagnetic isolation of mouse primary oligodendrocytes. J Vis Exp. 2018;135(135):357543. http://dx.doi.org/10.3791/57543
19. Tong M, Leao R, Vimbela GV, Yalcin EB, Kay J, Krotow A, et al. Altered temporal lobe white matter lipid ion profiles in an experimental model of sporadic Alzheimer's disease. Mol Cell Neurosci. 2017;82:23–34. http://dx.doi.org/10.1016/j.mcn.2017.04.010
20. Han X. Multi-dimensional mass spectrometry-based shotgun lipidomics and the altered lipids at the mild cognitive impairment stage of Alzheimer's disease. Biochim Biophys Acta. 2010;1801(8):774–83. http://dx.doi.org/10.1016/j.bbalip.2010.01.010
21. Quarles RH, Macklin WB, Morell P. Myelin formation, structure and biochemistry. 6th ed. Philadelphia, PA: Elsevier; 2006.
22. Han X. Lipid alterations in the earliest clinically recognizable stage of Alzheimer's disease: Implication of the role of lipids in the pathogenesis of Alzheimer's disease. Curr Alzheimer Res. 2005;2(1):65–77. http://dx.doi.org/10.2174/1567205052772786
23. Han X, D MH, McKeel DW, Jr., Kelley J, Morris JC. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: Potential role in disease pathogenesis. J Neurochem. 2002;82(4):809–18. http://dx.doi.org/10.1046/j.1471-4159.2002.00997.x
24. Irizarry MC. A turn of the sulfatide in Alzheimer's disease. Ann Neurol. 2003;54(1):7–8. http://dx.doi.org/10.1002/ana.10642
25. Czubowicz K, Strosznajder R. Ceramide in the molecular mechanisms of neuronal cell death. The role of sphingosine-1-phosphate. Mol Neurobiol. 2014;50(1):26–37. http://dx.doi.org/10.1007/s12035-013-8606-4
26. de la Monte SM. Triangulated mal-signaling in Alzheimer's disease: Roles of neurotoxic ceramides, ER stress, and insulin resistance reviewed. J Alzheimers Dis. 2012;30(Suppl 2):S231–49. http://dx.doi.org/10.3233/JAD-2012-111727
27. de la Monte SM, Re E, Longato L, Tong M. Dysfunctional pro-ceramide, ER stress, and insulin/IGF signaling networks with progression of Alzheimer's disease. J Alzheimers Dis. 2012;30(Suppl 2):S217–29. http://dx.doi.org/10.3233/JAD-2012-111728
28. de la Monte SM, Tong M, Nguyen V, Setshedi M, Longato L, Wands JR. Ceramide-mediated insulin resistance and impairment of cognitive-motor functions. J Alzheimers Dis. 2010;21(3):967–84. http://dx.doi.org/10.3233/JAD-2010-091726

29. Vos JP, Lopes-Cardozo M, Gadella BM. Metabolic and functional aspects of sulfogalactolipids. Biochim Biophys Acta. 1994;1211(2):125–49. http://dx.doi.org/10.1016/0005-2760(94)90262-3
30. Takahashi T, Suzuki T. Role of sulfatide in normal and pathological cells and tissues. J Lipid Res. 2012;53(8):1437–50. http://dx.doi.org/10.1194/jlr.R026682
31. Eckhardt M. The role and metabolism of sulfatide in the nervous system. Mol Neurobiol. 2008; 37(2–3):93–103. http://dx.doi.org/10.1007/s12035-008-8022-3
32. Kolesnick RN, Kronke M. Regulation of ceramide production and apoptosis. Ann Rev Physiol. 1998;60:643–65. http://dx.doi.org/10.1146/annurev.physiol.60.1.643
33. Campagnoni AT, Macklin WB. Cellular and molecular aspects of myelin protein gene expression. Mol Neurobiol. 1988;2(1):41–89. http://dx.doi.org/10.1007/BF02935632
34. Nave KA, Milner RJ. Proteolipid proteins: Structure and genetic expression in normal and myelin-deficient mutant mice. Crit Rev Neurobiol. 1989;5(1):65–91.
35. Gordon MN, Kumar S, Espinosa de los Monteros A, Scully S, Zhang MS, Huber J, et al. Developmental regulation of myelin-associated genes in the normal and the myelin deficient mutant rat. Adv Exp Med Biol. 1990;265:11–22. http://dx.doi.org/10.1007/978-1-4757-5876-4_2
36. Collarini EJ, Pringle N, Mudhar H, Stevens G, Kuhn R, Monuki ES, et al. Growth factors and transcription factors in oligodendrocyte development. J Cell Sci Suppl. 1991;15:117–23. http://dx.doi.org/10.1242/jcs.1991.Supplement_15.16
37. Wallin A, Nordlund A, Jonsson M, Blennow K, Zetterberg H, Ohrfelt A, et al. Alzheimer's disease – Subcortical vascular disease spectrum in a hospital-based setting: Overview of results from the Gothenburg MCI and dementia studies. J Cereb Blood Flow Metab. 2016;36(1):95–113. http://dx.doi.org/10.1038/jcbfm.2015.148
38. de la Monte SM. Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's disease. Drugs. 2012;72(1):49–66. http://dx.doi.org/10.2165/11597760-000000000-00000
39. de la Monte SM. Insulin resistance and neurodegeneration: Progress towards the development of new therapeutics for Alzheimer's disease. Drugs. 2017;77(1):47–65. http://dx.doi.org/10.1007/s40265-016-0674-0
40. de la Monte SM, Wands JR. Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: Relevance to Alzheimer's disease. J Alzheimers Dis. 2005;7(1):45–61. http://dx.doi.org/10.3233/JAD-2005-7106
41. Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: Link to brain reductions in acetylcholine. J Alzheimers Dis. 2005;8(3):247–68. http://dx.doi.org/10.3233/JAD-2005-8304
42. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? J Alzheimers Dis. 2005;7(1):63–80. http://dx.doi.org/10.3233/JAD-2005-7107
43. Chesik D, De Keyser J, Wilczak N. Insulin-like growth factor system regulates oligodendroglial cell behavior: Therapeutic potential in CNS. J Mol Neurosci. 2008;35(1):81–90. http://dx.doi.org/10.1007/s12031-008-9041-2
44. Freude S, Leeser U, Muller M, Hettich MM, Udelhoven M, Schilbach K, et al. IRS-2 branch of IGF-1 receptor signaling is essential for appropriate timing of myelination. J Neurochem. 2008;107(4): 907–17. http://dx.doi.org/10.1111/j.1471-4159.2008.05631.x
45. Gong X, Xie Z, Zuo H. Invivo insulin deficiency as a potential etiology for demyelinating disease. Med Hypotheses. 2008;71(3):399–403. http://dx.doi.org/10.1016/j.mehy.2008.04.006
46. Barres BA, Schmid R, Sendnter M, Raff MC. Multiple extracellular signals are required for long-term oligodendrocyte survival. Development (Cambridge, England). 1993;118(1):283–95.
47. de la Monte SM, Tong M, Lester-Coll N, Plater M Jr., Wands JR. Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: Relevance to Alzheimer's disease. J Alzheimers Dis. 2006;10(1):89–109. http://dx.doi.org/10.3233/JAD-2006-10113
48. Lester-Coll N, Rivera EJ, Soscia SJ, Doiron K, Wands JR, de la Monte SM. Intracerebral streptozotocin model of type 3 diabetes: Relevance to sporadic Alzheimer's disease. J Alzheimers Dis. 2006;9(1): 13–33. http://dx.doi.org/10.3233/JAD-2006-9102

49. de la Monte SM, Tong M, Schiano I, Didsbury J. Improved brain insulin/IGF signaling and reduced neuroinflammation with T3D-959 in an experimental model of sporadic Alzheimer's disease. J Alzheimers Dis. 2017;55(2):849–64. http://dx.doi.org/10.3233/JAD-160656
50. de la Monte SM. Metabolic derangements mediate cognitive impairment and Alzheimer's disease: Role of peripheral insulin-resistance diseases. Panminerva Med. 2012;54(3):171–8.
51. de la Monte SM, Longato L, Tong M, Wands JR. Insulin resistance and neurodegeneration: Roles of obesity, type 2 diabetes mellitus and non-alcoholic steatohepatitis. Curr Opin Investig Drugs. 2009;10(10):1049–60.
52. de la Monte SM, Tong M. Brain metabolic dysfunction at the core of Alzheimer's disease. Biochem Pharmacol. 2014;88(4):548–59. http://dx.doi.org/10.1016/j.bcp.2013.12.012
53. Bordner KA, George ED, Carlyle BC, Duque A, Kitchen RR, Lam TT, et al. Functional genomic and proteomic analysis reveals disruption of myelin-related genes and translation in a mouse model of early life neglect. Front Psychiatry. 2011;2:18. http://dx.doi.org/10.3389/fpsyt.2011.00018
54. Back SA. White matter injury in the preterm infant: Pathology and mechanisms. Acta Neuropathol. 2017;134(3):331–49. http://dx.doi.org/10.1007/s00401-017-1718-6
55. Nicklay JJ, Harris GA, Schey KL, Caprioli RM. MALDI imaging and in situ identification of integral membrane proteins from rat brain tissue sections. Anal Chem. 2013;85(15):7191–6. http://dx.doi.org/10.1021/ac400902h
56. Groseclose MR, Andersson M, Hardesty WM, Caprioli RM. Identification of proteins directly from tissue: In situ tryptic digestions coupled with imaging mass spectrometry. J Mass Spectrom. 2007;42(2):254–62. http://dx.doi.org/10.1002/jms.1177
57. Cheng H, Xu J, McKeel DW Jr., Han X. Specificity and potential mechanism of sulfatide deficiency in Alzheimer's disease: An electrospray ionization mass spectrometric study. Cell Mol Biol (Noisy-le-grand). 2003;49(5):809–18.
58. de la Torre JC. Cerebral hypoperfusion, capillary degeneration, and development of Alzheimer disease. Alzheimer Dis Assoc Disord. 2000;14(Suppl 1):S72–81. http://dx.doi.org/10.1097/00002093-200000001-00012
59. de la Torre JC, Mussivand T. Can disturbed brain microcirculation cause Alzheimer's disease? Neurol Res. 1993;15(3):146–53. http://dx.doi.org/10.1080/01616412.1993.11740127
60. Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, et al. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. Brain. 2013;136(Pt 9):2697–706. http://dx.doi.org/10.1093/brain/awt188
61. Joachim CL, Selkoe DJ. The seminal role of beta-amyloid in the pathogenesis of Alzheimer disease. Alzheimer Dis Assoc Disord. 1992;6(1):7–34. http://dx.doi.org/10.1097/00002093-199205000-00003
62. Vinters HV. Emerging concepts in Alzheimer's disease. Annu Rev Pathol. 2015;10:291–319. http://dx.doi.org/10.1146/annurev-pathol-020712-163927
63. Vinters HV, Wang ZZ, Secor DL. Brain parenchymal and microvascular amyloid in Alzheimer's disease. Brain Pathol. 1996;6(2):179–95. http://dx.doi.org/10.1111/j.1750-3639.1996.tb00799.x
64. Scheibel AB, Duong TH, Jacobs R. Alzheimer's disease as a capillary dementia. Ann Med. 1989;21(2):103–7. http://dx.doi.org/10.3109/07853898909149194
65. Buee L, Hof PR, Delacourte A. Brain microvascular changes in Alzheimer's disease and other dementias. Ann N Y Acad Sci. 1997;826:7–24. http://dx.doi.org/10.1111/j.1749-6632.1997.tb48457.x
66. Farkas E, De Jong GI, Apro E, De Vos RA, Steur EN, Luiten PG. Similar ultrastructural breakdown of cerebrocortical capillaries in Alzheimer's disease, Parkinson's disease, and experimental hypertension. What is the functional link? Ann N Y Acad Sci. 2000;903:72–82. http://dx.doi.org/10.1111/j.1749-6632.2000.tb06352.x
67. Perlmutter LS, Chui HC. Microangiopathy, the vascular basement membrane and Alzheimer's disease: A review. Brain Res Bull. 1990;24(5):677–86. http://dx.doi.org/10.1016/0361-9230(90)90007-M
68. Chalmers K, Wilcock G, Love S. Contributors to white matter damage in the frontal lobe in Alzheimer's disease. Neuropathol Appl Neurobiol. 2005;31(6):623–31. http://dx.doi.org/10.1111/j.1365-2990.2005.00678.x

69. Jellinger KA. The pathology of ischemic-vascular dementia: An update. J Neurol Sci. 2002;203–204:153–7. http://dx.doi.org/10.1016/S0022-510X(02)00282-4
70. Thal LJ. Therapeutics and mild cognitive impairment: Current status and future directions. Alzheimer Dis Assoc Disord. 2003;17(Suppl 2):S69–71. http://dx.doi.org/10.1097/00002093-200304002-00012
71. Verny M, Duyckaerts C, Pierot L, Hauw JJ. Leuko-araiosis. Dev Neurosci. 1991;13(4–5):245–50. http://dx.doi.org/10.1159/000112168
72. Neltner JH, Abner EL, Baker S, Schmitt FA, Kryscio RJ, Jicha GA, et al. Arteriolosclerosis that affects multiple brain regions is linked to hippocampal sclerosis of ageing. Brain. 2014;137(Pt 1):255–67. http://dx.doi.org/10.1093/brain/awt318
73. Kalaria RN, Akinyemi R, Ihara M. Does vascular pathology contribute to Alzheimer changes? J Neurol Sci. 2012;322(1–2):141–7. http://dx.doi.org/10.1016/j.jns.2012.07.032
74. Ferrer I, Bella R, Serrano MT, Marti E, Guionnet N. Arteriolosclerotic leucoencephalopathy in the elderly and its relation to white matter lesions in Binswanger's disease, multi-infarct encephalopathy and Alzheimer's disease. J Neurol Sci. 1990;98(1):37–50. http://dx.doi.org/10.1016/0022-510X(90)90180-U
75. Dorheim MA, Tracey WR, Pollock JS, Grammas P. Nitric oxide synthase activity is elevated in brain microvessels in Alzheimer's disease. Biochem Biophys Res Commun. 1994;205(1):659–65. http://dx.doi.org/10.1006/bbrc.1994.2716
76. Wang S, Qaisar U, Yin X, Grammas P. Gene expression profiling in Alzheimer's disease brain microvessels. J Alzheimers Dis. 2012;31(1):193–205. http://dx.doi.org/10.3233/JAD-2012-120454
77. de la Monte SM, Sohn YK, Etienne D, Kraft J, Wands JR. Role of aberrant nitric oxide synthase-3 expression in cerebrovascular degeneration and vascular-mediated injury in Alzheimer's disease. Ann N Y Acad Sci. 2000;903:61–71. http://dx.doi.org/10.1111/j.1749-6632.2000.tb06351.x
78. Grammas P. A damaged microcirculation contributes to neuronal cell death in Alzheimer's disease. Neurobiol Aging. 2000;21(2):199–205. http://dx.doi.org/10.1016/S0197-4580(00)00102-0
79. Yin X, Wright J, Wall T, Grammas P. Brain endothelial cells synthesize neurotoxic thrombin in Alzheimer's disease. Am J Pathol. 2010;176(4):1600–6. http://dx.doi.org/10.2353/ajpath.2010.090406
80. Grammas P. Neurovascular dysfunction, inflammation and endothelial activation: Implications for the pathogenesis of Alzheimer's disease. J Neuroinflamm. 2011;8:26. http://dx.doi.org/10.1186/1742-2094-8-26
81. Grammas P, Yamada M, Zlokovic B. The cerebromicrovasculature: A key player in the pathogenesis of Alzheimer's disease. J Alzheimers Dis. 2002;4(3):217–23. http://dx.doi.org/10.3233/JAD-2002-4311
82. van Hinsbergh VW. Endothelium – Role in regulation of coagulation and inflammation. Semin Immunopathol. 2012;34(1):93–106. http://dx.doi.org/10.1007/s00281-011-0285-5
83. Bartha K, Domotor E, Lanza F, Adam-Vizi V, Machovich R. Identification of thrombin receptors in rat brain capillary endothelial cells. J Cereb Blood Flow Metab. 2000;20(1):175–82. http://dx.doi.org/10.1097/00004647-200001000-00022
84. Yin M, Chen Z, Ouyang Y, Zhang H, Wan Z, Wang H, et al. Thrombin-induced, TNFR-dependent miR-181c downregulation promotes MLL1 and NF-kappaB target gene expression in human microglia. J Neuroinflammation. 2017;14(1):132. http://dx.doi.org/10.1186/s12974-017-0887-5
85. Choi MS, Kim YE, Lee WJ, Choi JW, Park GH, Kim SD, et al. Activation of protease-activated receptor1 mediates induction of matrix metalloproteinase-9 by thrombin in rat primary astrocytes. Brain Res Bull. 2008;76(4):368–75. http://dx.doi.org/10.1016/j.brainresbull.2008.02.031
86. Hayakawa K, Seo JH, Pham LD, Miyamoto N, Som AT, Guo S, et al. Cerebral endothelial derived vascular endothelial growth factor promotes the migration but not the proliferation of oligodendrocyte precursor cells in vitro. Neurosci Lett. 2012;513(1):42–6. http://dx.doi.org/10.1016/j.neulet.2012.02.004
87. Kurachi M, Mikuni M, Ishizaki Y. Extracellular vesicles from vascular endothelial cells promote survival, proliferation and motility of oligodendrocyte precursor cells. PLoS One. 2016;11(7):e0159158. http://dx.doi.org/10.1371/journal.pone.0159158
88. Rajani RM, Quick S, Ruigrok SR, Graham D, Harris SE, Verhaaren BFJ, et al. Reversal of endothelial dysfunction reduces white matter vulnerability in cerebral small vessel disease in rats. Sci Transl Med. 2018;10:448. http://dx.doi.org/10.1126/scitranslmed.aam9507
89. Sanchez A, Tripathy D, Luo J, Yin X, Martinez J, Grammas P. Neurovascular unit and the effects of dosage in VEGF toxicity: Role for oxidative stress and thrombin. J Alzheimers Dis. 2013;34(1):281–91. http://dx.doi.org/10.3233/JAD-121636

90. Grammas P, Samany PG, Thirumangalakudi L. Thrombin and inflammatory proteins are elevated in Alzheimer's disease microvessels: Implications for disease pathogenesis. J Alzheimers Dis. 2006;9(1):51–8. http://dx.doi.org/10.3233/JAD-2006-9105
91. Luo J, Grammas P. Endothelin-1 is elevated in Alzheimer's disease brain microvessels and is neuroprotective. J Alzheimers Dis. 2010;21(3):887–96. http://dx.doi.org/10.3233/JAD-2010-091486
92. Grammas P, Martinez JM. Targeting thrombin: An inflammatory neurotoxin in Alzheimer's disease. J Alzheimers Dis. 2014;42(Suppl 4):S537–44. http://dx.doi.org/10.3233/JAD-141557
93. Yoon H, Radulovic M, Drucker KL, Wu J, Scarisbrick IA. The thrombin receptor is a critical extracellular switch controlling myelination. Glia. 2015;63(5):846–59. http://dx.doi.org/10.1002/glia.22788
94. Undas A, Wiek I, Stepien E, Zmudka K, Tracz W. Hyperglycemia is associated with enhanced thrombin formation, platelet activation, and fibrin clot resistance to lysis in patients with acute coronary syndrome. Diabetes Care. 2008;31(8):1590–5. http://dx.doi.org/10.2337/dc08-0282
95. Ceriello A, Giacomello R, Stel G, Motz E, Taboga C, Tonutti L, et al. Hyperglycemia-induced thrombin formation in diabetes. The possible role of oxidative stress. Diabetes. 1995;44(8):924–8. http://dx.doi.org/10.2337/diab.44.8.924
96. Liu Y, Chen XL, Wang L, Martins-Green M. Insulin antagonizes thrombin-induced microvessel leakage. J Vasc Res. 2017;54(3):143–55. http://dx.doi.org/10.1159/000470844
97. de Leon MJ, George AE, Ferris SH, Rosenbloom S, Christman DR, Gentes CI, et al. Regional correlation of PET and CT in senile dementia of the Alzheimer type. AJNR Am J Neuroradiol. 1983;4(3):553–6.
98. Faulstich ME. Brain imaging in dementia of the Alzheimer type. Int J Neurosci. 1991;57(1–2):39–49. http://dx.doi.org/10.3109/00207459109150345
99. Waldron AM, Wintmolders C, Bottelbergs A, Kelley JB, Schmidt ME, Stroobants S, et al. In vivo molecular neuroimaging of glucose utilization and its association with fibrillar amyloid-beta load in aged APPPS1-21 mice. Alzheimers Res Ther. 2015;7(1):76. http://dx.doi.org/10.1186/s13195-015-0158-6
100. Wurtman R. Biomarkers in the diagnosis and management of Alzheimer's disease. Metabolism. 2015;64(3 Suppl 1):S47–50. http://dx.doi.org/10.1016/j.metabol.2014.10.034
101. Daulatzai MA. Quintessential risk factors: Their role in promoting cognitive dysfunction and Alzheimer's disease. Neurochem Res. 2012;37(12):2627–58. http://dx.doi.org/10.1007/s11064-012-0854-6
102. Daulatzai MA. Cerebral hypoperfusion and glucose hypometabolism: Key pathophysiological modulators promote neurodegeneration, cognitive impairment, and Alzheimer's disease. J Neurosci Res. 2017;95(4):943–72. http://dx.doi.org/10.1002/jnr.23777
103. Schaffer C, Sarad N, DeCrumpe A, Goswami D, Herrmann S, Morales J, et al. Biomarkers in the diagnosis and prognosis of Alzheimer's disease. J Lab Autom. 2015;20(5):589–600. http://dx.doi.org/10.1177/2211068214559979
104. de la Monte SM, Tong M, Bowling N, Moskal P. si-RNA inhibition of brain insulin or insulin-like growth factor receptors causes developmental cerebellar abnormalities: Relevance to fetal alcohol spectrum disorder. Mol Brain. 2011;4:13. http://dx.doi.org/10.1186/1756-6606-4-13
105. de la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. J Diabetes Sci Technol. 2008;2(6):1101–13. http://dx.doi.org/10.1177/193229680800200619
106. Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest. 2012;122(4):1316–38. http://dx.doi.org/10.1172/JCI59903
107. Lee S, Tong M, Hang S, Deochand C, de la Monte S. CSF and brain indices of insulin resistance, oxidative stress and neuro-inflammation in early versus late Alzheimer's disease. J Alzheimers Dis Parkinsonism. 2013;3:128.
108. Shuvaev VV, Laffont I, Serot JM, Fujii J, Taniguchi N, Siest G. Increased protein glycation in cerebrospinal fluid of Alzheimer's disease. Neurobiol Aging. 2001;22(3):397–402. http://dx.doi.org/10.1016/S0197-4580(00)00253-0
109. Tong M, Deochand C, Didsbury J, de la Monte SM. T3D-959: A multi-faceted disease remedial drug candidate for the treatment of Alzheimer's disease. J Alzheimers Dis. 2016;51(1):123–38. http://dx.doi.org/10.3233/JAD-151013
110. Benedict C, Frey WH, 2nd, Schioth HB, Schultes B, Born J, Hallschmid M. Intranasal insulin as a therapeutic option in the treatment of cognitive impairments. Exp Gerontol. 2011;46(2–3):112–15. http://dx.doi.org/10.1016/j.exger.2010.08.026

111. de la Monte SM. Intranasal insulin therapy for cognitive impairment and neurodegeneration: Current state of the art. Expert Opin Drug Deliv. 2013;10(12):1699–709. http://dx.doi.org/10.1517/17425247.2013.856877
112. Kidd PM. Alzheimer's disease, amnestic mild cognitive impairment, and age-associated memory impairment: Current understanding and progress toward integrative prevention. Altern Med Rev. 2008;13(2):85–115.
113. Reger MA, Watson GS, Green PS, Baker LD, Cholerton B, Fishel MA, et al. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. J Alzheimers Dis. 2008;13(3):323–31. http://dx.doi.org/10.3233/JAD-2008-13309
114. Reger MA, Watson GS, Green PS, Wilkinson CW, Baker LD, Cholerton B, et al. Intranasal insulin improves cognition and modulates {beta}-amyloid in early AD. Neurology. 2008;70(6):440–8. http://dx.doi.org/10.1212/01.WNL.0000265401.62434.36
115. Tong M, Dominguez C, Didsbury J, de la Monte SM. Targeting Alzheimer's disease neuro-metabolic dysfunction with a small molecule nuclear receptor agonist (T3D-959) reverses disease pathologies. J Alzheimers Dis Parkinsonism. 2016;6(3):238–44. http://dx.doi.org/10.4172/2161-0460.1000238
116. Tong M, Yu R, Silbermann E, Zabala V, Deochand C, de la Monte SM. Differential contributions of alcohol and nicotine-derived nitrosamine ketone (NNK) to white matter pathology in the adolescent rat brain. Alcohol Alcohol. 2015;50(6):680–9. http://dx.doi.org/10.1093/alcalc/agv102
117. Papp-Peka A, Tong M, Kril JJ, De La Monte SM, Sutherland GT. The differential effects of alcohol and nicotine-specific nitrosamine ketone on white matter ultrastructure. Alcohol Alcohol. 2017;52(2):165–71. http://dx.doi.org/10.1093/alcalc/agw067
118. Lyn-Cook LE, Jr., Lawton M, Tong M, Silbermann E, Longato L, Jiao P, et al. Hepatic ceramide may mediate brain insulin resistance and neurodegeneration in type 2 diabetes and non-alcoholic steatohepatitis. J Alzheimers Dis. 2009;16(4):715–29. http://dx.doi.org/10.3233/JAD-2009-0984
119. Yalcin EB, Nunez K, Tong M, de la Monte SM. Differential sphingolipid and phospholipid profiles in alcohol and nicotine-derived nitrosamine ketone-associated white matter degeneration. Alcohol Clin Exp Res. 2015;39(12):2324–33. http://dx.doi.org/10.1111/acer.12909
120. Gottfries CG, Karlsson I, Svennerholm L. Membrane components separate early-onset Alzheimer's disease from senile dementia of the Alzheimer type. Int Psychogeriatr. 1996;8(3):365–72. http://dx.doi.org/10.1017/S1041610296002736
121. Zinda MJ, Vlahos CJ, Lai MT. Ceramide induces the dephosphorylation and inhibition of constitutively activated Akt in PTEN negative U87mg cells. Biochem Biophys Res Commun. 2001;280(4):1107–15. http://dx.doi.org/10.1006/bbrc.2000.4248
122. Tong M, de la Monte SM. Mechanisms of ceramide-mediated neurodegeneration. J Alzheimers Dis. 2009;16(4):705–14. http://dx.doi.org/10.3233/JAD-2009-0983
123. Cedazo-Minguez A. Apolipoprotein E and Alzheimer's disease: Molecular mechanisms and therapeutic opportunities. J Cell Mol Med. 2007;11(6):1227–38. http://dx.doi.org/10.1111/j.1582-4934.2007.00130.x
124. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science. 1993;261(5123):921–3. http://dx.doi.org/10.1126/science.8346443
125. Myers RH, Schaefer EJ, Wilson PW, D'Agostino R, Ordovas JM, Espino A, et al. Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study. Neurology. 1996;46(3):673–7. http://dx.doi.org/10.1212/WNL.46.3.673
126. Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, van Harskamp F, et al. Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam study. Lancet. 1997;349(9046):151–4. http://dx.doi.org/10.1016/S0140-6736(96)09328-2
127. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, et al. Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A. 1993;90(5):1977–81. http://dx.doi.org/10.1073/pnas.90.5.1977
128. Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. Nat Rev Neurol. 2013;9(2):106–18. http://dx.doi.org/10.1038/nrneurol.2012.263

129. Crean S, Ward A, Mercaldi CJ, Collins JM, Cook MN, Baker NL, et al. Apolipoprotein E epsilon4 prevalence in Alzheimer's disease patients varies across global populations: A systematic literature review and meta-analysis. Dement Geriatr Cogn Disord. 2011;31(1):20–30. http://dx.doi.org/10.1159/000321984
130. Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minoshima S, et al. Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. N Engl J Med. 1996;334(12):752–8. http://dx.doi.org/10.1056/NEJM199603213341202
131. Zhao N, Liu CC, Van Ingelgom AJ, Martens YA, Linares C, Knight JA, et al. Apolipoprotein E4 Impairs neuronal insulin signaling by trapping insulin receptor in the endosomes. Neuron. 2017;96(1):115–29.e5. http://dx.doi.org/10.1016/j.neuron.2017.09.003
132. Yamagishi S, Nakamura N, Suematsu M, Kaseda K, Matsui T. Advanced glycation end products: A molecular target for vascular complications in diabetes. Mol Med. 2015;21(Suppl 1):S32–40. http://dx.doi.org/10.2119/molmed.2015.00067
133. Agostinho P, Cunha RA, Oliveira C. Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Des. 2010;16(25):2766–78. http://dx.doi.org/10.2174/138161210793176572
134. Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, et al. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med. 2003;9(7):907–13. http://dx.doi.org/10.1038/nm890
135. Donahue JE, Flaherty SL, Johanson CE, Duncan JA, 3rd, Silverberg GD, Miller MC, et al. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. Acta Neuropathol. 2006;112(4):405–15. http://dx.doi.org/10.1007/s00401-006-0115-3
136. Lovestone S, Smith U. Advanced glycation end products, dementia, and diabetes. Proc Natl Acad Sci U S A. 2014;111(13):4743–4. http://dx.doi.org/10.1073/pnas.1402277111
137. Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, et al. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature. 1996;382(6593):685–91. http://dx.doi.org/10.1038/382685a0
138. Piro JR, Benjamin DI, Duerr JM, Pi Y, Gonzales C, Wood KM, et al. A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. Cell Rep. 2012;1(6):617–23. http://dx.doi.org/10.1016/j.celrep.2012.05.001
139. Singhal G, Jaehne EJ, Corrigan F, Toben C, Baune BT. Inflammasomes in neuroinflammation and changes in brain function: A focused review. Front Neurosci. 2014;8:315. http://dx.doi.org/10.3389/fnins.2014.00315
140. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481–7. http://dx.doi.org/10.1038/nature21029

9

Relationship between Alzheimer's Disease and the Human Microbiome

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by memory and language disorders, and the accumulation of amyloid-β and tau protein in the brain has been considered a feature of AD. The accumulation of amyloid-β has been reported to be observed 15 to 20 years before the onset by image analysis-based diagnostic methods. In addition, it has been reported that AD is associated with various diseases such as type 2 diabetes, periodontal disease, and obesity. It is conceivable that these diseases trigger the onset of AD. The human gut and brain form a network called "brain–gut–microbiota axis," and it is suggested that the gut microbiota is involved in brain diseases. Recently, the microbiota has also been reported to be involved in diseases such as depression and Parkinson's disease, and so attention is being paid to the relationship between AD and gut microbiota. This chapter outlines the relationship between AD and the human microbiome.

Keywords: amyloid-β; behavior; brain–gut–microbiota axis; gut microbiota; metabolome

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INTRODUCTION

The cause of Alzheimer's disease (AD) is presently unknown, but its onset has been shown to involve mainly genetic and environmental factors. About 700 risk genes (as of April 2019) are registered in Alzforum (https://www.alzforum.org/) as the genetic factors of AD. Other factors found to be involved include lifestyle habits such as sleep, exercise, diet, and educational history. Recently, it has been reported that sleep is related to the accumulation of amyloid-β, which is a characteristic of AD. In healthy middle-aged men, sleep reduced amyloid-β 42 in cerebrospinal fluid by 6%, while lack of sleep abolished that reduction (1). In a study investigating the relationship between the Mediterranean diet and dementia, it was noted that the traditional Mediterranean diet that consists of a large amount of fruits, vegetables, and cereals reduces the risk of developing dementia and AD (2). In addition, Ozawa, upon following more than 1000 subjects over 17 years, has reported that the incidence of AD decreased significantly with increased intake of milk and dairy products (3). Lifestyle plays an important role in the prevention of AD, and dysregulation of lifestyle leads not only to AD but also to various other diseases. This chapter first outlines the relationship between lifestyle diseases and AD, and then the relationship between gut microbiota and AD.

AD AND DIABETES

Association of AD to diabetes led to the classification of a new category of diabetes called type 3 diabetes (4). Ott et al. (5) examined the association between diabetes and dementia in 6330 people aged 55–99 years, and the results suggest an association between diabetes mellitus and dementia. In addition, in a prospective population-based cohort study among 6370 elderly subjects, diabetes mellitus reportedly doubled the risk of dementia and AD. The study also reported that patients treated with insulin had four times higher the risk of dementia. A cohort study of 2574 men reported that patients with type 2 diabetes are associated with dementia, AD, and vascular dementia (6). The same study concluded that these associations are stronger in patients carrying the APOE ε4 allele (7). Furthermore, borderline diabetes is also associated with the increased risk of dementia and AD (8). Conversely, Michal et al. (9) reported that in the hippocampus of AD patients, diabetics had significantly lower plaque ratings than the non-diabetics. In addition, inflammation in the brain by the intake of a high-fat diet promotes accumulation of amyloid in diabetes model mice, regardless of the decrease in insulin (10). Thus, prior studies suggest that the factors like eating habits, mild glucose intolerance, and onset of type 2 diabetes are involved in the onset of AD. On the other hand, amyloidosis is a key pathological feature of both AD and type 2 diabetes (11). Bacterial endotoxin lipopolysaccharide and bacterial cell wall peptidoglycan are involved in amyloidosis, suggesting that chronic bacterial inflammation may link the two diseases. In addition, recent advances in gene analysis technology have revealed the relationship between intestinal bacteria and diabetes. Adachi et al. (12) have reported the relationship between type 2 diabetes and short-chain fatty acids (SCFAs), the metabolites of the microbiota, in the

Japanese population. From these studies, it can be considered that changes in the microbiota affect the production of SCFAs, thereby promoting the onset of type 2 diabetes as well as AD.

AD AND PERIODONTAL DISEASE

Periodontitis is considered as a risk factor for dementia and AD. Periodontal disease is a chronic disease caused by gram-negative bacteria such as *Porphyromonas gingivalis, Treponema denticola,* and *Tannerella forsythia*. It has been clarified that this chronic inflammation is related to the accumulation of amyloid-β and cognitive impairment that are characteristic of AD (13). In addition, tumor necrosis factor-α and antibodies against periodontitis in plasma have been reported to be biomarkers of AD (14, 15), and periodontal disease has been suggested to be a probable trigger for AD. Recently, gingipain, a protease produced by *P. gingivalis*, has been detected in the brains of patients with AD (16). The concentration of gingipain is high in the brain of patients with AD, and the accumulation of tau protein is promoted, whereas the accumulation of amyloid-β is suppressed by the gingipain inhibitor. Furthermore, oral administration of *P. gingivalis* to mice promotes the accumulation of amyloid-β (17). Gingipain reportedly activates microglia and causes inflammation in the brain (18). These activated microglia cause accumulation of amyloid-β and cognitive decline (19). However, Noble et al. (20) have reported that, in a cohort study of 219 subjects (consisting of 110 patients with AD and 109 healthy volunteers), subjects with high serum IgG against *Actinomyces naeslundii* (which is associated with periodontal disease) were at a high risk of developing AD. Thus, the periodontitis bacteria have been linked to AD through the microbial toxins, inflammatory substances, and serum antibodies. Chronic inflammation developed by these bacteria is a predisposing factor for AD.

AD AND OBESITY

Obesity is considered as one of the risk factors associated with AD. Recently, the relationship between obesity and AD has been studied extensively. Animal studies have shown that mice fed with high-fat diet significantly increases the accumulation of amyloid-β in the hippocampus and are involved in cognitive decline (21–23). Another clinical study characterized by magnetic resonance imaging (MRI) scan of the brains of 700 patients having mild cognitive impairment (MCI) inferred that higher body mass index (BMI) is associated with brain volume deficits (24). Gustafson reported the relationship between BMI and risk of dementia as investigated in an 18-year follow-up of 392 Swedish adults (aged 70–88 years) without dementia. Higher body weight was observed in women who developed AD compared to women without dementia (70, 75, and 79 years). In particular, at the age of 70 years, every 1.0 increase in BMI showed a 36% increase in AD risk (25). In addition, Luchsinger's study has shown that the waist to hip ratio is related to a higher risk of AD (26). In the

Swedish, 8534 twin individuals over the age of 65 were assessed to detect cases of dementia. Overweight (BMI 25–30) and obesity (BMI over 30) at midlife were related to dementia with odds ratios of 1.71 and 3.88, respectively (27). However, the risk for dementia associated with obesity gradually reduced with increasing age (28). Obesity has been implicated as a risk factor for AD in middle age, whereas its associated risk decreases with increasing age. Conversely, weight loss and low BMI have been found to be associated with increased risk of AD in older adults (29).

MICROBIOTA AND AMYLOID ACCUMULATION

The relationship between microbiota and amyloid-β accumulation has been studied by Harach et al. (30). APPPS1, an AD mouse model, presents accumulation of amyloid-β in the brain in an age-dependent manner. The generation of germ-free APPPS1 mice was inhibited by the accumulation of amyloid-β. In addition, the microbiota of this mouse model is different from that of the wild type, and it has been reported that accumulation of amyloid-β increases in mice transplanted with the microbiota of an AD mouse model. Furthermore, Ho et al. (31) found that valeric acid and butyric acid, SCFAs produced by the microbiota, strongly inhibit the aggregation of amyloid-β in an in vitro test. Furthermore, bacterial endotoxin may be involved in the inflammations associated with amyloidosis and AD (32). Although some bacteria such as *Escherichia coli* produce amyloids (33), the relationship between the amyloid that is caused by neurodegenerative diseases such as AD and bacterial amyloids has not been clarified (34). However, bacterial amyloid has been shown to activate signaling pathways that play a role in the pathogenesis of neurodegenerative diseases and AD, and microbiota is a noted key player that enhances inflammation associated with the accumulation of amyloid-β (35). Furthermore, the lipopolysaccharide of gram-negative bacteria promotes accumulation of amyloid-β in mouse brain and induces cognitive dysfunction (36, 37). Hence, it has been suggested that microbiota is involved in the accumulation of amyloids, which is known to be a pathological feature of AD, via metabolites such as extracellular components and SCFAs. In addition, bacteria that produce amyloids are also present in the enteric bacterial groups, but it is thought that further research is necessary to clarify whether amyloids derived from the bacteria are involved in AD progression.

EFFECTS OF DAIRY PRODUCTS AND PROBIOTICS IN AD

Acute and chronic inflammation is associated with neurodegenerative diseases such as AD and Parkinson's disease (38–41). Probiotics such as lactic acid bacteria and *Bifidobacterium* have attracted attention as tools to suppress this inflammation. In the Bonfili study, administration of the probiotic cocktail SLAB 51 (*Streptococcus thermophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum, L. paracasei*, and *L. delbrueckii* subsp. *bulgaricus*, *L. brevis*) in AD model mice (3xTg-ADmouse) affected changes in the microbiota, thus

affecting the content of metabolites of enteric bacteria such as SCFAs and cognitive function (42). Kobayashi et al. (31) also reported that oral administration of *B. breve* A1 led to behavior impairment to the same level as donepezil, a centrally acting cholinesterase inhibitor, in AD model mice injected with amyloid-β into the ventricles. Acetic acid, which is a metabolite of *Bifidobacterium*, is known to play an important role in the improvement of AD. However, there have been cases where improvement in memory due to probiotics was not observed. Benton et al. (43) confirmed the cognitive function by following 3 weeks of probiotic milk drink or placebo control consumption in 124 healthy volunteers (mean age 61.8 years), and the cognitive function was higher in the placebo consuming group than the probiotic drinking group upon 20 days of consumption. Furthermore, recent research has shown that consumption of not only these probiotics but also yogurt and cheese has been linked to AD and dementia. David et al. (44) reviewed that bioactive peptides in dairy products improve cognitive function. Ano et al. (45, 46) have also shown that in vivo experiments the peptides present in Camembert cheese improve the decline in memory and cognitive function. On the contrary, Rahman et al. (47), in an epidemiological study of 1056 subjects, reported that dietary intake of cheese is associated with a lower prevalence of cognitive impairment. Also, a study conducted on a total of 1006 community-dwelling Japanese subjects without dementia, aged 60–79 years (followed up for a median of 15 years), reported that high intake of milk and milk products reduced the risk of dementia (48). Although many model animals for AD and dementia are produced and their application to this field is advanced, further studies are needed to establish the influence of probiotics and dairy products on brain function.

AD AND GUT MICROBIOTA

Recently, the development of next-generation sequencing technology has made it possible to estimate the gut microbiota rapidly at a low cost, and the relationship between various diseases and the gut microbiota has been studied extensively. The Vogt study compared the microbiota in 50 subjects (Healthy control HC: n = 25 and AD: n = 25) and noted decreased microbial diversity in the AD subjects. It also reported a decrease in *Firmicutes* and increase in *Bacteroidetes* percentage abundance (49). Saji et al. (50) compared the microbiota of non-demented patients (n = 49) with demented patients (n = 34) among 128 Japanese subjects and found that *Bacteroides* decreased in demented patients compared to non-demented patients (Figure 1). Furthermore, Nguyen et al. (51) reported that butyrate-producing bacteria involved in cognitive function have been isolated from the microbiota of patients with AD. Liu et al. (52) reported that in a study of 97 subjects (AD: n = 33, MCI: n = 32, and HC: n = 32), the fecal microbial diversity was decreased in AD patients compared with MCI patients and healthy volunteers. In addition, it also reported a decrease in *Firmicutes* and increase in *Proteobacteria* abundance. There are similar reports on the relationship between gut microbiota and AD as the studies stated above. Therefore, further research is needed to clarify the difference. With the growing research in this field, future boom in AD cure research might as well be directed toward microbiome research.

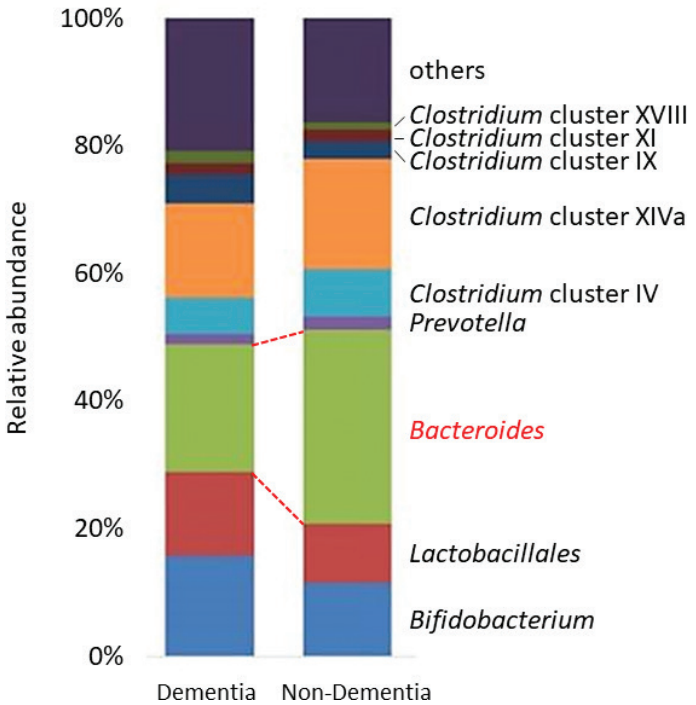

Figure 1 Relative bacterial abundance in the gut microbiota of dementia and non-dementia patients. It was suggested that a lower prevalence of *Bacteroides* is seen in the gut of dementia patients than non-dementia patients (50).

GUT MICROBIOTA AND BEHAVIOR

Human intestine and brain form a network called the "brain–gut–microbiota axis" through physiologically active substances. The gut microbiota has been shown to play an important role in this network. Researchers have transplanted mice with different microbiota and compared their response to stress with adrenocorticotropic hormone and corticosterone as indicators (53). Specifically, as compared with specific pathogen-free mice, adrenocorticotropic hormone and corticosterone levels have been reported to significantly increase in germ-free mice due to restraint stress. In addition, the effect is also seen in mice transplanted with *B. infantis*, suggesting that the effect differs depending on the microbe transplanted and that the microbiota also affects neurotransmitters in the brain (54, 55). Kim et al. (56) reported that pregnant mice colonized with the human commensal bacteria (a mix of 20 human bacterial strains), which induce intestinal Th17 cells due to poly (I:C)-induced inflammation, produced offspring that were found to have increased anxiety behaviors such as increased ultrasonic vocalization, enhanced repetitive behavior with marble burying test, and shortened time in the center of the open-field arena. In contrast, these anxiety behaviors were not observed if the mothers were pre-treated with interleukin-17a blocking antibody, since interleukin-17 production of intestinal Th17 cells induced by human

commensal bacteria contributes to the development of anxiety behaviors in mouse offspring. Thus, it was revealed that the gut microbiota of the mother mouse is involved in the behavior of the offspring mouse. In addition, the authors previously reported cognitive behavior decline in germ-free mice transplanted with the microbiota of AD patients (57). Cognitive behavior was assessed by Object Location Test (OLT) and Novel Object Recognition Test (ORT). A significant deterioration of cognitive function was observed through both OLT (70 and 75 weeks of age vs. 10 weeks of age; 55, 70, and 75 weeks of age vs. 15 weeks of age) and ORT (70 weeks of age vs. 10 weeks of age; 35, 55, 65, 70, and 75 weeks of age vs. 15 weeks of age) in mice transplanted with microbiota from affected patients. Moreover, significant reduction of cognitive function of these mice was confirmed by both OLT (55 and 70 weeks of age) and ORT (55, 60, 65, and 70 weeks of age) in comparison with cognitive function of mice transplanted with microbiota of healthy volunteers (Figure 2). In this article, these data were re-analyzed by linear regression analysis (Figure 3). A significant decrease in cognitive function was confirmed in mice transplanted with microbiota from affected donors in relation

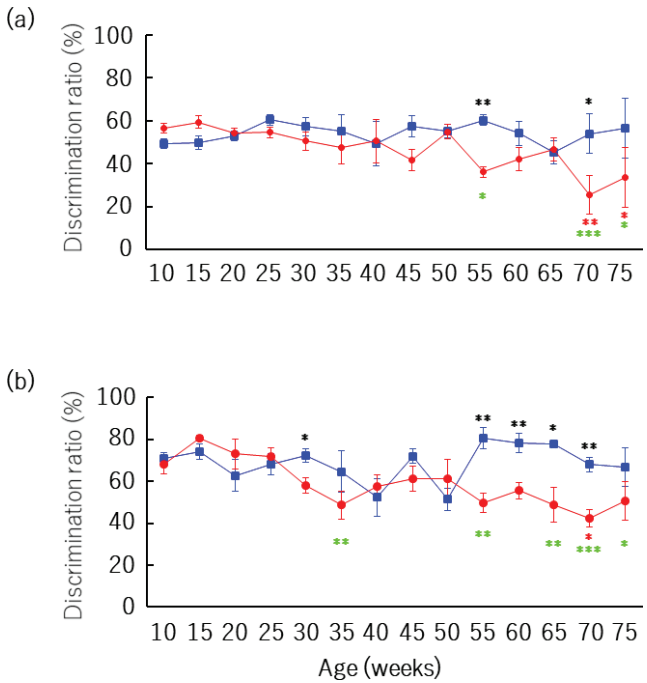

Figure 2 Novel object recognition test in mice transplanted with microbiota. (A) Ratio of time spent exploring a familiar object in a new location to time spent exploring a familiar object in an old location. (B) Ratio of time spent exploring a novel object to time spent exploring a familiar object. Blue and red lines indicate the ratio of time spent by mice transplanted with microbiota from a healthy donor and a patient with Alzheimer's disease, respectively. Black, * and ** indicate comparison between groups; red,* and ** indicate mice transplanted with microbiota from a patient with Alzheimer's disease had significantly altered cognitive function at respectively weeks of age compared with that at 10 weeks of age; green, *, **, and *** indicate mice transplanted with microbiota from a patient with Alzheimer's disease had significantly altered cognitive function at respectively weeks of age compared with that at 15 weeks of age. Data are mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (57).

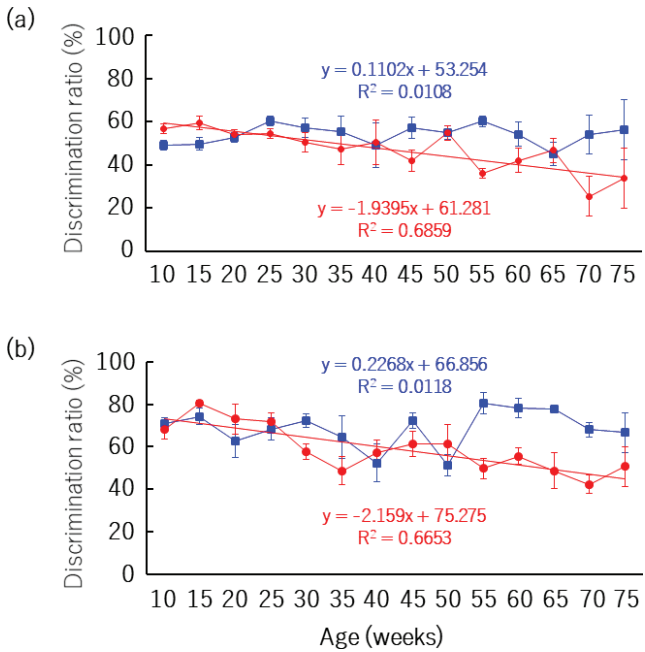

Figure 3 Regression analysis of two behavior tests. (A) Ratio of exploring novel objects in the 5 min of OLT. Blue square and red circle indicate healthy control (HC) group and Alzheimer's disease (AD) group, respectively. (B) Ratio of exploring for novel objects in the 5 min of object recognition test. In both panels means ± SEM are shown (57).

with age. The regression analysis results showed association between cognitive decline and age in mice transplanted with microbiota from affected patients, but not in mice transplanted with microbiota of healthy volunteers. Therefore, it was clear that mice transplanted with microbiota from affected patients had reduced cognitive function. This was further investigated by fecal metabolome analysis. The principal component analysis of the metabolites from individual mice exhibits separate clusters representing each of the mice categories (Figure 4). And the different metabolites from affected donors to mice transplanted with microbiota included gamma-aminobutyric acid, taurine, and valine, all of which are involved in central nervous system function. In addition, a difference in the concentration of other amino acids such as tryptophan, tyrosine, propionic acid, and SCFAs was also reported. Thus, it was suggested that the microbiota influences host behavior through its metabolites.

CONCLUSION

AD is known to cause deposition of amyloid-β, which is the main component of senile plaques in the brain. This deposition of amyloid-β is caused by the accumulation of amyloid generated from the amyloid precursor protein (APP) by the action of two enzymes β-secretase and γ-secretase in the cerebral cortex of the brain.

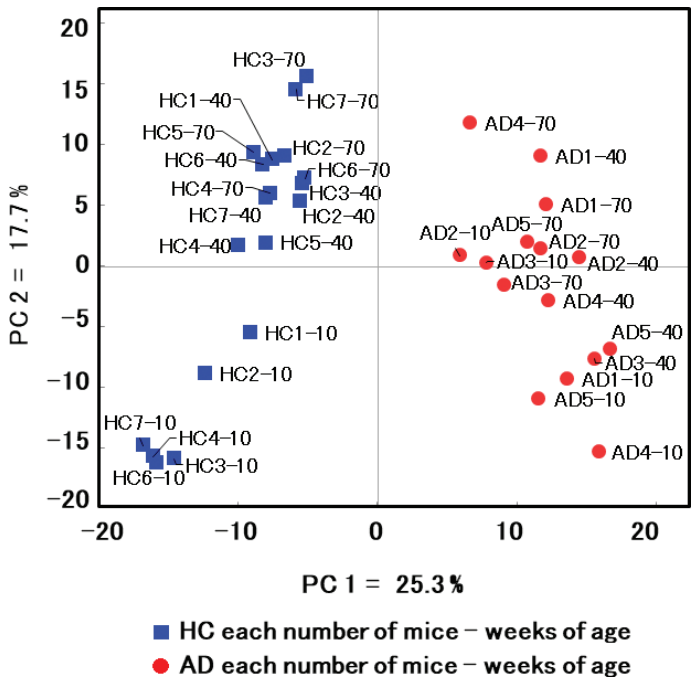

Figure 4 Comparison of metabolites in HC and AD mice feces. Principal component analysis of fecal metabolites in mice transplanted with microbiotas from a healthy volunteer (blue) and a patient with Alzheimer's disease (red) (57).

It has been clarified that this accumulation of amyloid-β starts 15–20 years before the onset of AD. At the same time, after a decrease in cognitive function is observed, there is not much change in the accumulation of amyloid-β, and no effect is seen in a drug targeting amyloid-β. Hence, some investigators have questioned the involvement of amyloid-β in AD. However, further studies are needed to investigate whether AD caused changes in the gut microbiota or vice versa.

Conflict of interest: The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this chapter.

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REFERENCES

1. Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JAHR. Effect of 1 night of total sleep deprivation on cerebrospinal fluid β-amyloid 42 in healthy middle-aged men: A randomized clinical trial. JAMA Neurol. 2014 Aug 1;71(8):971–7. http://dx.doi.org/10.1001/jamaneurol.2014.1173

2. Lourida I, Soni M, Thompson-Coon J, Purandare N, Lang IA, Ukoumunne OC, et al. Mediterranean diet, cognitive function, and dementia: A systematic review. Epidemiology. 2013 Jul;24(4):479–89. http://dx.doi.org/10.1097/EDE.0b013e3182944410
3. Ozawa M, Ohara T, Ninomiya T, Hata J, Yoshida D, Mukai N, et al. Milk and dairy consumption and risk of dementia in an elderly Japanese population: The Hisayama Study. J Am Geriatr Soc. 2014 Jul;62(7):1224–30. http://dx.doi.org/10.1111/jgs.12887
4. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease – Is this type 3 diabetes? J Alzheimers Dis. 2005 Jan 1;7(1):63–80. http://dx.doi.org/10.3233/JAD-2005-7107
5. Ott A, Stolk RP, Hofman A, van Harskamp F, Grobbee D, Breteler MM. Association of diabetes mellitus and dementia: The Rotterdam Study. Diabetologia. 1996 Nov;39(11):1392–7. http://dx.doi.org/10.1007/s001250050588
6. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology. 1999 Dec;53(9):1937–42. http://dx.doi.org/10.1212/WNL.53.9.1937
7. Peila R, Rodriguez B, Launer L. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. Diabetes. 2002 Apr;51(4):1256–62. http://dx.doi.org/10.2337/diabetes.51.4.1256
8. Xu W, Qiu C, Winblad B, Fratiglioni L. The effect of borderline diabetes on the risk of dementia and Alzheimer's disease. Diabetes. 2007 Jan;56(1):211–16. http://dx.doi.org/10.2337/db06-0879
9. Michal SB, Jeremy MS, Kenneth LD, Deborah M, Hillel ZG, James S, et al. Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. J Gerontol A Biol Sci Med Sci. 2005 Apr;60(4):471–5.
10. Wakabayashi T, Yamaguchi K, Matsui K, Hashimoto T, Mano A, Yamada K. Differential effects of diet- and genetically-induced brain insulin resistance on amyloid pathology in a mouse model of Alzheimer's disease. Mol Neurodegener. 2019 Apr;14:15. http://dx.doi.org/10.1186/s13024-019-0315-7
11. Judith M, Patrick LM. Common mechanisms involved in Alzheimer's disease and type 2 diabetes: A key role of chronic bacterial infection and inflammation. Aging. 2016 Apr;8(4):575–88. http://dx.doi.org/10.18632/aging.100921
12. Adachi K, Sugiyama T, Yamaguchi Y, Tamura Y, Izaw S, Hijikata Y, et al. Gut microbiota disorders cause type 2 diabetes mellitus and homeostatic disturbances in gut-related metabolism in Japanese subjects. J Clin Biochem Nutr. 2019 May;63(3):231–23. http://dx.doi.org/10.3164/jcbn.18-101
13. Kamer AR, Pirraglia E, Tsui W, Rusinek H, Vallabhajosula S, Mosconi L, et al. Periodontal disease associates with higher brain amyloid load in normal elderly. Neurobiol Aging. 2015 Feb;36(2):627–33. http://dx.doi.org/10.1016/j.neurobiolaging.2014.10.038
14. Farhad SZ, Amini S, Khalilian A, Barekatain M, Mafi M, Barekatain M, et al. The effect of chronic periodontitis on serum levels of tumor necrosis factor-alpha in Alzheimer disease. Dent Res J (Isfahan). 2014 Sep;11(5):549–52.
15. Kamer AR, Craig RG, Pirraglia E, Dasanayake AP, Norman RG, Boylan RJ, et al. TNF-α and antibodies to periodontal bacteria discriminate between Alzheimer's disease patients and normal subjects. J Neuroimmunol. 2009 Nov 30;216(1–2):92–7. http://dx.doi.org/10.1016/j.jneuroim.2009.08.013
16. Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, et al. *Porphyromonas gingivalis* in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv. 2019 Jan 1;5(1):eaau3333. http://dx.doi.org/10.1126/sciadv.aau3333
17. Ilievski V, Zuchowska PK, Green SJ, Toth PT, Ragozzino ME, Le K, et al. Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice. PLoS One. 2018 Oct 3;13(10):e0204941. http://dx.doi.org/10.1371/journal.pone.0204941
18. Liu Y, Wu Z, Zhang X, Ni J, Yu W, Zhou Y, et al. Leptomeningeal cells transduce peripheral macrophages inflammatory signal to microglia in reponse to *Porphyromonas gingivalis* LPS. Mediators Inflamm. 2013 Dec;2013:407562. http://dx.doi.org/10.1155/2013/407562
19. Wu Z, Ni J, Liu Y, Teeling JL, Takayama F, Collcutt A, et al. Cathepsin B plays a critical role in inducing Alzheimer's disease-like phenotypes following chronic systemic exposure to lipopolysaccharide

from *Porphyromonas gingivalis* in mice. Brain Behav Immun. 2017 Oct;65:350–61. http://dx.doi.org/10.1016/j.bbi.2017.06.002
20. Noble JM, Scarmeas N, Celenti RS, Elkind MSV, Wright CB, Schupf N, et al. Serum IgG antibody levels to periodontal microbiota are associated with incident Alzheimer disease. PLoS One. 2014 Dec;9(12):e114959. http://dx.doi.org/10.1371/journal.pone.0114959
21. Barron AM, Rosario ER, Elteriefi R, Pike CJ. Sex-specific effects of high fat diet on indices of metabolic syndrome in 3xTg-AD mice: Implications for Alzheimer's disease. PLoS One. 2013 Oct;8:10. http://dx.doi.org/10.1371/journal.pone.0078554
22. Julien C, Tremblay C, Phivilay A, Berthiaume L, Emond V, Julien P, et al. High-fat diet aggravates amyloid-beta and tau pathologies in the 3xTg-AD mouse model. Neurobiol Aging. 2010 Sep;31(9):1516–31. http://dx.doi.org/10.1016/j.neurobiolaging.2008.08.022
23. Knight EM, Martins IVA, Gümüsgöz S, Allan SM, Lawrence CB. High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. Neurobiol Aging. 2014 Aug;35(8):1821–32. http://dx.doi.org/10.1016/j.neurobiolaging.2014.02.010
24. Ho AJ, Raji CA, Becker JT, Lopez OL, Kuller LH, Hua X, et al. Obesity is linked with lower brain volume in 700 AD and MCI patients. Neurobiol Aging. 2010 Aug;31(8):1326–39. http://dx.doi.org/10.1016/j.neurobiolaging.2010.04.006
25. Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I. An 18-year follow-up of overweight and risk of Alzheimer disease. Arch Intern Med. 2003 Jul;163(13):1524–8. http://dx.doi.org/10.1001/archinte.163.13.1524
26. Luchsinger J, Cheng D, Tang M, Schupf N, Mayeux R. Central obesity in the elderly is related to late onset Alzheimer's disease. Alzheimer Dis Assoc Disord. 2012 Apr;26(2):101–5. http://dx.doi.org/10.1097/WAD.0b013e318222f0d4
27. Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Midlife overweight and obesity increase late-life dementia risk: A population-based twin study. Neurology. 2011 May;76(18):1568–74. http://dx.doi.org/10.1212/WNL.0b013e3182190d09
28. Wotton CJ, Goldacre MJ. Age at obesity and association with subsequent dementia: Record linkage study. Postgrad Med J. 2014 Oct;90(1068):547–51. http://dx.doi.org/10.1136/postgradmedj-2014-132571
29. Moser VA, Pike CJ. Obesity and sex interact in the regulation of Alzheimer's disease. Neurosci Biobehav Rev. 2016 Aug;67:102–18. http://dx.doi.org/10.1016/j.neubiorev.2015.08.021
30. Harach T, Marungruang N, Duthilleul N, Cheatham V, Mc Coy KD, Frisoni G, et al. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci Rep. 2017 Feb 8;7:41802. http://dx.doi.org/10.1038/srep41802
31. Ho L, Ono K, Tsuji M, Mazzola P, Singh R, Pasinetti GM. Protective roles of intestinal microbiota derived short chain fatty acids in Alzheimer's disease-type beta-amyloid neuropathological mechanisms. Expert Rev Neurother. 2018 Jan;18(1):83–90. http://dx.doi.org/10.1080/14737175.2018.1400909
32. Asti A, Gioglio L. Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? J Alzheimers Dis. 2014;39(1):169–79. http://dx.doi.org/10.3233/JAD-131394
33. Syed AK, Boles BR. Fold modulating function: Bacterial toxins to functional amyloids. Front Microbiol. 2014 Aug 1;5:401. http://dx.doi.org/10.3389/fmicb.2014.00401
34. Bhattacharjee S, Lukiw WJ. Alzheimer's disease and the microbiome. Front Cell Neurosci. 2013 Sep 17;7:153. http://dx.doi.org/10.3389/fncel.2013.00153
35. Pistollato F, Sumalla Cano S, Elio I, Masias Vergara M, Giampieri F, Battino M. Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. Nutr Rev. 2016 Oct 1;74(10):624–34. http://dx.doi.org/10.1093/nutrit/nuw023
36. Zhan X, Stamova B, Jin L-W, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology. 2016 Nov;87(22):2324–32. http://dx.doi.org/10.1212/WNL.0000000000003391
37. Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, et al. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflamm. 2008 Aug;5:37. http://dx.doi.org/10.1186/1742-2094-5-37
38. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, et al. Systemic inflammation and disease progression in Alzheimer disease. Neurology. 2009 Sep 8;73(10):768–74. http://dx.doi.org/10.1212/WNL.0b013e3181b6bb95

39. Tan ZS, Beiser AS, Vasan RS, Roubenoff R, Dinarello CA, Harris TB, et al. Inflammatory markers and the risk of Alzheimer disease: The Framingham study. Neurology. 2007 May;68(22):1902–8. http://dx.doi.org/10.1212/01.wnl.0000263217.36439.da
40. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry. 2010 Nov;68(10):930–41. http://dx.doi.org/10.1016/j.biopsych.2010.06.012
41. Chen H, O'Reilly EJ, Schwarzschild MA, Ascherio A. Peripheral inflammatory biomarkers and risk of Parkinson's disease. Am J Epidemiol. 2008 Jan;167(1):90–5. http://dx.doi.org/10.1093/aje/kwm260
42. Bonfili L, Cecarini V, Berardi S, Scarpona S, Suchodolski JS, Nasuti C, et al. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. Sci Rep. 2017 May 25;7:2426. http://dx.doi.org/10.1038/s41598-017-02587-2
43. Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. Eur J Clin Nutr. 2007 Mar;61(3):355–61. http://dx.doi.org/10.1038/sj.ejcn.1602546
44. Camfield DA, Owen L, Scholey AB, Pipingas A, Stough C. Dairy constituents and neurocognitive health in ageing. Br J Nutr. 2011 Jul;106(2):159–74. http://dx.doi.org/10.1017/S0007114511000158
45. Ano Y, Yoshino Y, Kutsukake T, Ohya R, Fukuda T, Uchida K, et al. Tryptophan-related dipeptides in fermented dairy products suppress microglial activation and prevent cognitive decline. Aging (Albany NY). 2019 May 23;11(10):2949–67. http://dx.doi.org/10.18632/aging.101909
46. Ano Y, Ayabe T, Kutsukake T, Ohya R, Takaichi Y, Uchida S, et al. Novel lactopeptides in fermented dairy products improve memory function and cognitive decline. Neurobiol Aging. 2018 Dec;72:23–31. http://dx.doi.org/10.1016/j.neurobiolaging.2018.07.016
47. Rahman A, Sawyer Baker P, Allman RM, Zamrini E. Dietary factors and cognitive impairment in community-dwelling elderly. J Nutr Health Aging. 2007 Feb;11(1):49–54.
48. Ozawa M, Ninomiya T, Ohara T, Doi Y, Uchida K, Shirota T, et al. Dietary patterns and risk of dementia in an elderly Japanese population: The Hisayama Study. Am J Clin Nutr. 2013 May;97(5):1076–82. http://dx.doi.org/10.3945/ajcn.112.045575
49. Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. Gut microbiome alterations in Alzheimer's disease. Sci Rep. 2017 Oct 19;7(1):13537. http://dx.doi.org/10.1038/s41598-017-13601-y
50. Saji N, Niida S, Murotani K, Hisada T, Tsuduki T, Sugimoto T, et al. Analysis of the relationship between the gut microbiome and dementia: A cross-sectional study conducted in Japan. Sci Rep. 2019 Jan;9(1):1008. http://dx.doi.org/10.1038/s41598-018-38218-7
51. Nguyen TTT, Fujimura Y, Mimura I, Fujii Y, Nguyen NL, Arakawa K, et al. Cultivable butyrate-producing bacteria of elderly Japanese diagnosed with Alzheimer's disease. J Microbiol. 2018 Oct 1;56(10):760–71. http://dx.doi.org/10.1007/s12275-018-8297-7
52. Liu P, Wu L, Peng G, Han Y, Tang R, Ge J, et al. Altered microbiomes distinguish Alzheimer's disease from amnestic mild cognitive impairment and health in a Chinese cohort. Brain Behav Immun. 2019 May;80:633–43. http://dx.doi.org/10.1016/j.bbi.2019.05.008
53. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu X-N, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol (Lond). 2004 Jul;558(Pt 1):263–75. http://dx.doi.org/10.1113/jphysiol.2004.063388
54. Liang S, Wu X, Jin F. Gut-brain psychology: Rethinking psychology from the microbiota–gut–brain axis. Front Integr Neurosci. 2018 Sep;12:33. http://dx.doi.org/10.3389/fnint.2018.00033
55. Sudo N. Stress and gut microbiota: Does postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response? Int Congr Ser. 2006 Apr;1287:350–4. http://dx.doi.org/10.1016/j.ics.2005.12.019
56. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse off spring. Nature. 2017 Sep 28;549(7673):528–532. http://dx.doi.org/10.1038/nature23910
57. Fujii Y, Nguyen TTT, Fujimura Y, Kameya N, Nakamura Y, Arakawa K, et al. Fecal metabolite of a gnotobiotic mouse transplanted with gut microbiota from a patient with Alzheimer's disease. Biosci Biotechnol Biochem. 2019 Nov;83(11):2144–2152. http://dx.doi.org/10.1080/09168451.2019.1644149

10

Ocular Exploration in the Diagnosis and Follow-Up of the Alzheimer's Dementia

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Abstract: The retina is part of the central nervous system (CNS), and therefore, in Alzheimer's disease (AD), retinal and optic nerve degeneration could take place. This degeneration leads to neurofunctional changes that can be detected early and followed up throughout the evolution of the disease. As opposed to other CNS structures, the eye is easily accessible for in vivo observation. Retinal organization allows for the identification of its different neurons, and in consequence, detection of minimal changes taking place during neurodegeneration is possible. Functional vision studies performed on AD patients in recent years have shown how visual acuity, contrast sensitivity, color vision, and visual integration vary with the progression of neurodegeneration. The development of optical coherence tomography in ophthalmology has meant a breakthrough in retinal exploratory techniques, allowing the obtention of high-resolution images using light. This technique enables retinal analysis in the earliest stages of AD, being considered as a biomarker of neuronal damage. Given AD's high prevalence and its expected increase, it is important to

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perform easy tests that cause minimal discomfort to the patients at a low cost while offering abundant information on the stage of the disease.

Keywords: Alzheimer's disease; biomarker; neurodegeneration; retina; visual system

INTRODUCTION

Alzheimer's disease (AD) is recognized by the World Health Organization as a global public health priority. AD is the single principal cause of dementia, between 50 and 75%, and is primarily a condition of aging, roughly doubling in prevalence every 5 years after age 65 (1). The incidence of AD increases with age, and the prevalence is growing as a result of the aging of the population (2); however, there are no disease-modifying therapies currently available, and none have been successful in late-stage clinical trials (3).

Late-onset AD is likely to be driven by a complex interplay between genetic and environmental factors, implicating inflammatory, cholesterol metabolism and endosomal-vesicle recycling pathway (4) and the presence of the APOE+4 allele (5). In addition, AD is frequently associated with vascular dysfunctions and inflammation (6). In particular, it is now recognized to play a key role in AD pathogenesis the microglial activation in response to amyloid deposition (7).

The basis of AD has not been fully elucidated. However, the progressive accumulation of β-amyloid (Aβ) plaques and abnormal forms of phosphorylated tau (tau tangles) within and outside of neurons and neuroinflammation, both of which could lead to neuronal loss and synaptic dysfunction (8), are considered to be the neuropathological hallmarks (9–11).

The "amyloid cascade hypothesis" (12) is based on the progressive deposition of fibrillar Aβ as diffuse plaques, which activates an inflammatory response, altered ion homeostasis, oxidative stress, and altered kinase/phosphatase activity, leading to the formation of NFTs and widespread synaptic dysfunction and neuronal death (13). Recently, it has been demonstrated that an Aβ plaque environment can accelerate the templated spread of tau pathology (14, 15).

Hyperphosphorylation of tau has numerous pathogenic effects. It reduces tau's affinity for microtubules and increases its possibility to aggregate and fibrillize (16). This impact leads to weakening of microtubules with consequent axonal transport failure and neurodegeneration (15).

In the past decade, remarkable advances have been made in disease-specific biomarkers based on the detection of amyloid or neurodegeneration. With the knowledge that the pathological changes occur years previous to symptoms, the arrival of biomarkers of Aβ and tau pathology, and nuclear imaging measures of atrophy, diagnostic criteria have evolved to allow for the diagnosis to be made both earlier and with increased molecular specificity.

These biomarkers not only enable the diagnosis of AD in the stage of dementia but also beforehand, in the prodromal stages of AD. However, these biomarkers are not applicable as population-wide screening tools because they are invasive, not easily applicable and expensive.

EYE AND BRAIN: SYMBIOTIC RELATIONSHIP

Over the last few decades, in neurodegenerative diseases of the central nervous system (CNS), the importance of ophthalmic examination has reportedly increased. It is not surprising that the retina, as an extension of the CNS, is impaired in patients with CNS degeneration (17). The eye has unique physical structures and is host to specialized immune responses similar to those in the brain and spinal cord (18–20). In fact, abnormal results were found in AD patients in test exploring visual processing/visual pathways and also in those examining the retina (17).

The neuroinflammatory changes could be detected using a routinely diagnostic technique used in ophthalmology, the optical coherence tomography (OCT). OCT allows to see the anatomic detail of pathological changes in the retina and optic nerve. Changes in OCT measurements have been used to study the course of neurodegenerative diseases such AD (21–25), suggesting that the data compiled may be useful as a biomarker in diagnosing and treating neurodegenerative disease.

The retina is made up of specialized neuron layers that are interconnected via synapses (photoreceptors, bipolar cells, horizontal cells, amacrine cells, interplexiform cells and ganglion cells) (18, 26). In the eye, the light that enters is captured by the photoreceptor cells in the outer retina, initiating a cascade of neural signals that finally reach the retinal ganglion cells (RGCs), whose axons form the optic nerve. These axons project to the lateral geniculate nucleus in the thalamus and to the superior colliculus in the midbrain, whose information is then transmitted to specialized visual processing centers in the brain that provide a perception of the world.

The first study, showing postmortem anomalies in the optic nerve of patients with AD, demonstrated not only widespread axonal degeneration but also a reduction in the number of RGC and the thickness of nerve fiber layer (NFL), with a 25% decrease of ganglion cell layer (GCL) (27–29). More recent OCT studies also found a decrease in the thickness of inner retinal layers (NFL and GCL) (30–41).

The presence of Aβ plaques in GCL could explain the RGC degeneration in the AD course (19, 27, 42). In fact, it has been demonstrated that most of the Aβ plaques deposited in the retina are located in the GCL (43, 44). Deposits of Aβ trigger a neurotoxic effect in the RGC, inducing apoptosis (45). This apoptosis is dose- and time-dependent (45). Some pieces of evidence showed that Aβ expression is greater in the central retina than in the periphery of the eye of an AD mouse model (46). As in the brain, Aβ deposits in the retina have the classical plaque structure, forming clusters along the blood vessels (47). Aβ accumulations were located inside and around melanopsin retinal ganglion cells (mRGC) and more evident in the superior quadrant of the retina (47).

In the last few years, it was found that mRGCs also showed a significant loss in postmortem AD retinas (47). These cells represent the 1–2% subpopulation of RGC that are intrinsically photosensitive (47, 48). The mRGC send ambient light information to the hypothalamus nucleus via the retinohypothalamic tract (48), regulating circadian rhythms, pupil size, sleep alertness, and pineal melatonin synthesis (49–51). This mRGC loss could contribute to circadian dysfunction in AD (47). Indeed, its presence in the early stages of AD of circadian dysfunction was postulated as the worst prognostic value in AD (47).

All these retinal changes could be responsible, in part, for the visual deficit that occurs in AD patients. The acetylcholine decrease is also characteristic of this disease, and therefore contributes to the visual deficit that occurs in AD patients because acetylcholine is essential for the correct visual process of healthy retinas (52).

VISUAL FUNCTIONAL TESTS IN THE EXPLORATION OF AD

Aging affects visual function because light transmission diminishes inside the eye, whereas the scattering of light increases. With age, there is not only a decrease in the density of photoreceptors in the retina, but there is also less efficiency in phototransduction and photopigment regeneration (53). In addition to aging, visual processing is affected in AD patients. The brain's visual areas are involved in AD pathology (in the dorsal and ventral regions), worsening the perception of movement; angular and color discrimination; and form and face identification (54–60). There are several tests such as the visual acuity test, and the contrast sensitivity and color vision test to explore this visual processing in the ophthalmology practice.

Visual acuity

Visual acuity (VA) is a measure of the spatial resolution of the visual system to detect and discriminate an object. In patients with AD, it is very important to choose the correct VA test. It was demonstrated that VA tests present better values if the letters are isolated (61).

Contrast sensitivity and color vision

The contrast sensitivity (CS) test assesses the capacity of the visual system to distinguish an object from the background in which it is placed. The CS test allows us to ascertain the integration of the information of the ganglion cells receptor field and their cortical processes. CS is measured by a threshold curve in which the spatial frequencies examined are depicted. Color vision is an illusion created by the interactions of the neurons in our brain. It is intimately linked to the perception of form where color facilitates detecting borders of objects (62). Parvo- and magnocellular ganglion cells are located in the GCL and lead to two different visual pathways that identify color and contrast (63). Parvocellular ganglion cells are smaller and more numerous than other retina ganglion cells, with smaller receptor fields located in the macular retinal area. They give rise to the parvocellular visual pathway, specialized in pattern identification and color; and it is most sensitive to high spatial frequency (51). The magnocellular pathway originates in magnocellular retina ganglion cells, which are larger and more numerous, and have larger receptor fields that are more sensitive to low spatial frequencies (63). There is a third type of ganglion cell that is called koniocellular, which receives information from short wave cones. Koniocellular cells are also sensitive to blue–yellow tones (64, 65). CS is a really important visual function. Even several studies showed that a CS loss is the best predictor of the ability of elders to perform daily life activities (66, 67).

Visual fields

The visual field (VF) refers to the total space in which objects can be seen in the side (peripheral) vision as your eyes are focused on a central point. The fovea, where the cone photoreceptor density is at its highest, is the area of greatest sensitivity. The visual sensitivity comes down further from the fovea. Traditional perimetry is carried out under photopic conditions, and therefore, rod photoreceptors do not contribute to the visual field (68). The normal visual field extends to approximately 60° nasally, 90° temporally, 60° superiorly and 70° inferiorly. In the area of the optic nerve head, temporal part of the VF, exists a blind spot that indicates an area with no photoreceptors (69).

Visual integration

Identifying a visual stimulus requires not only physical input analysis but also the contact between the neuronal representations of the stimulus and the memories that the perceivers have accumulated through their life experiences with the objects. Object identification arises from the dynamic interaction between a sensorial/physical process (upstream processing) and a cognitive process (downstream processing). Spatial frequency is an important physical property of the image. The extraction of visual sensory characteristics follows a course to a fine processing scheme where the low spatial frequency represents the overall information about the shape and orientation of the stimulus, while the high spatial frequency corresponds to the configuration information and fine details (70–75).

OPHTHALMOLOGICAL METHODS FOR RETINAL ANALYSIS

Over the past decade OCT has evolved as one of the most important tests in ophthalmic practice. It is a non-invasive imaging technique that provides high-resolution, cross-sectional images of the retina.

Optical coherence tomography

OCT was first demonstrated for cross-sectional retinal imaging in 1991 by a Massachusetts Institute of Technology (MIT) team (76). OCT synthesizes cross-sectional images from a series of laterally adjacent depth-scans giving a non-invasive clinical tool to evaluate the structural anatomy and the evaluation of the integrity of the retina.

Optical coherence tomography angiography

Optical coherence tomography angiography (OCTA) is a promising new method for visualizing the retinal vasculature and choroidal vascular layers. A key advantage of OCTA over traditional fluorescein angiography is that it provides depth-resolved information without contrast. The basis of OCTA is to repeatedly scan a region and then examine the resultant images for changes. Stationary tissue

structures will show little change, whereas moving structures, such as blood flowing through vessels, can show changes between images. Contrast is generated based on the difference between moving cells in the vasculature and the static surrounding tissue. This imaging technique can be performed in patients for whom fluorescein angiography or indocyanine green angiography may not be indicated (77). OCTA is clinically used as an *en face* imaging modality, which is generated by summarizing the flow information within the depth range encompassed by the current scheme. This scheme subdivides the retinal circulation into two plexuses and choroidal circulation into two slabs. Angiograms, which are similar to fluorescein angiography or indocyanine green angiography, are also produced (78).

FUNCTIONAL CHANGES IN AD

Nowadays, it is known that, in AD, in addition to altering brain structures, the involvement of the different regions of the visual system also occurs, with a manifestation of distinct symptoms and signs that can be detected by clinical history and ophthalmological studies.

VA has proven to be a controversial test in AD. Studies have not found an alteration in AD patients (79–86), and others have found VA loss and linked them to visual hallucinations (87, 88) (Table 1). Moreover, these alterations of VA are

TABLE 1 **Eye changes in AD patients**

		References
Visual alterations		
	Visual acuity	87, 88
	Contrast sensitivity	82, 84, 85, 90–102, 103, 104
	Visual field	105–109
	Color vision	58, 84, 93, 110, 113–116
	Visual integration	93, 117
Structural alterations		
	Retinal Aβ deposition	19, 27, 42–47
	Optic nerve	27–29
	Macular thickness	
	Inner retinal layers	30–41, 138, 140–145
	Outer retinal layers	135
	Peripapillary thickness	21, 24, 30–38, 120–127
	Retinal vascularization	148–150
	Choroid thickness	125, 148, 151–153

AD: Alzheimer's disease; Aβ: beta-amyloid

related to difficulties in writing and reading (89). On the other hand, recent studies have found that CS testing is a more sensitive tool than VA testing to identify the subclinical impairment of visual function (90, 91). CS precedes the development of dementia at 10 years of the longitudinal follow-up in a well-phenotyped, prospective, community-based cohort (90, 91). It has been shown that the CS function is affected in AD patients. The impairment ranges from a reduction in all spatial frequencies (85, 92–99) to a greater decline in high (92, 93, 98, 100) or low spatial frequencies (82, 84, 101, 102). Such discrepancies in the affected frequencies could be due to differences among the CS test used as well as the patients included in the studies (17, 66). Recent works show that CS is the main manifestation during the initial disease stage. There is a progressive impairment throughout the disease course (93, 103, 104) (Table 1). CS impairment in AD has consequences for cognitive abilities and daily functions, given that the most affected spatial frequencies are the higher frequencies corresponding to macular function (17). The presence of reduced CS years before the clinical onset of dementia suggests that this association is not simply a consequence of later stage dementia. Furthermore, reduced CS can precede the clinical onset of cortical or subcortical dementia neurodegeneration (90).

Visual field test requires significant cooperation from the patient. Therefore, the reports of VF and AD are scarce, and most are case reports (17, 68). However, it has been observed that decreased VF sensitivity correlated with cognitive impairment. A large prospective study of threshold VF perimetry in patients with probable AD demonstrated that the most common VF abnormality was bilateral inferior constriction of the VF in an arcuate-like pattern (105, 106). AD patients underwent a diffuse sensitivity loss and defects that involved the central field. In 39% of AD patients, the density of plaques and tangles was greater in the cuneal compared with lingual gyri, supporting the theory that cortical disease is responsible for the VF loss (105). Recent findings show that the side of the homonymous defect is predicted by lateralized occipital atrophy (107–109) (Table 1).

Another manifestation of AD is the fluctuations in color perception, which are mainly errors in color recognition due to the involvement of the parvocellular pathway (110).

In the color perception, some studies using the Farnsworth test and Ishihara test found no differences between AD patients and control group (96, 111, 112). On the other hand, some tritan-axis defects were found, showing a correlation with the cognitive decline (58, 84, 93, 113, 114). The discrepancy in the results of both studies may be due to the fact that each study used a different color vision method. A recent investigation showed that the Ishihara color vision test could discriminate between AD and vascular dementia (115). The Ishihara test may involve dorsal cortical pathways that extend from the occipital to the parietal lobes. In the Ishihara test, the patients have to identify a number occulted in a pattern made up of small color forms with different tones. AD patients usually present simultagnosia caused by an occipitoparietal dysfunction, and therefore, they cannot recognize the pattern that is presented in the Ishihara test. The problem does not lie in the color sense, but in the inability to reconstruct the pattern (115). Using the Farnsworth-Munsell 100 hue test, a significantly decreased color discrimination was found in AD. In addition, the number of color discrimination errors was inversely related to Mini-Mental State Examination scores (MMSE) (110). Some studies using the Farnsworth color testing methods, not influenced

by dorsal stream dysfunction, suggest that AD patients tend to have tritan color defects (58, 93) while others have found a protanomaly (116) (Table 1).

The perception digital test (PDT) is a sensitive method in mild AD patients developed for evaluating their visual-perception disorders (117). The test is designed to assess the visual recognition of familiar situations. PDT has a significant correlation with the cognitive decline of the AD patient, indicating that patients with mild AD have significantly more failures in PDT than controls (93, 117) (Table 1).

STRUCTURAL CHANGES IN AD

The retinal nerve fiber layer (RNFL), RGC and inner retinal layers are considered indirect biomarkers of the CNS, allowing the prediction of brain pathology in patients suffering from different neurological diseases (118, 119). Many studies focus on the thickness of segmented peripapillary RNFL (superior, inferior, nasal, and temporal) in patients with AD comparing them with controls. Some works showed a decrease in the peripapillary RNFL thickness in all areas (30, 31, 34, 36–38). However, others authors found that the peripapillary RNFL thinning occurred in the inferior and superior regions (35, 39, 40), while other works demonstrated that peripapillary RNFL thinning appeared only in the superior region (120–124). Some studies reported thinning in the RNFL associated with a progressive cognitive decline (21, 24, 123, 125, 126) (Table 1). The variance in peripapillary RNFL thickness reported in AD might be due to differences in disease progression among patients studied since patients with greater peripapillary region alteration were those with a more advanced stage of AD. In any case, thinner peripapillary RNFL indicates fewer RGCs in AD, which confirmed the differences in OCT measurements in AD patients (127). The loss of RGCs is matched with the pathologic cascade hypothesis in AD, which affects both the cerebral neuron and the RGCs in the retina (36). This whole peripapillary RNFL controversy is the result of studies based on small size samples and important methodological heterogeneity (37, 128–130). In line with this hypothesis, pattern electroretinography showed a decrease in their wave response, suggesting that RGCs are directly involved in AD (38, 92, 131–133).

Some authors did not show any statistical significance with respect to the macular outer retinal thickness analysis between the neurodegenerative disease and control groups (134). However, other studies in the context of early AD observed a loss in the outer nuclear layer that could suggest retrograde transsynaptic degeneration (135). In AD, most of the studies have been done with OCT, and they have focused on the inner retinal layers, whereas less attention has been devoted to the outer retinal layers. The discrepancy in results could be due to technical variability, examination time and OCT interpretation (129, 136, 137). By using human *postmortem* tissue in the eyes of severe AD patients with confirmed neuropathology, different patterns of thinning in the superior-nasal and superior-temporal regions of the retina relative to the optic nerve have been found. Also, they found a gradient of thickness reduction whereby thinning was greatest for the inner layers of the retina, followed by the outer layers of the retina (138). This thickness profile matches the distribution of the retinal Aβ deposits in the mid- and

far-periphery of the superior quadrants of these tissues as previously demonstrated (19, 28, 29, 47, 139).

In the last few years, some studies focusing on the analysis of patients with mild cognitive impairment (MCI) found a thinning in the macular inner layers (140). By contrast, a macular volume increase was found in MCI compared with controls in others works (141). This finding could be explained as a possible inflammation and gliosis prior to neurodegeneration.

CHANGES IN THE EARLY AD AND THEIR PROGNOSTIC VALUE IN THE DETECTION AND FOLLOW-UP

In the most incipient AD stages, the macular RNFL thickness and total macular volume measured by OCT have better prognostic values in mild AD patients than in healthy subjects. The thickness of the inner superior macula seems to have the highest diagnostic value in early AD neurodegeneration. Possibly, the macular area is the first affected area of the retina, which may be due to the large number of ganglion cells in this retinal area (21, 24). Other studies have primarily assessed retinal thickness changes in the macula to explain the visual symptoms experienced by AD patients (138). The earliest detectable structural retinal change associated with AD is suggested to be a decrease in macular RNFL volume, and it is related to neocortical Aβ accumulation in the very early AD (135). In healthy eyes, the macular region of the retina is physiologically very active, and this hyperexcitation might be diminishing in the preclinical stage of AD (28). In support of this theory, *postmortem* histological studies have found pathological alteration of RGC in the macular region in AD patients (28, 47). In a meta-analysis of 17 studies comparing AD patients with healthy controls and in five studies comparing individuals with MCI with controls, there were significant decreases in the thickness of the macular region in all four quadrants compared to controls, thus suggesting that the degenerative process affects the entire macular region (130). Another work, using a multivariate regression model show the existence of specific areas of thickening, interspersed with areas of thinning in the macula of AD and MCI patients. This finding supports the idea that inner retinal layers may be suffering dynamic changes during the course of AD progression (142). The retinal thickening in MCI was attributed to gliosis preceding neuronal loss and atrophy of the axonal projections in the RNFL (143). This theory has been supported by histopathology work, suggesting that gliosis precedes human AD pathology in the brain (144, 145). However, other studies in OCT suggested that the outer retinal thickness did not show any statistical significance between the neurodegenerative disease groups and controls (134). Other authors consider that many other findings have been described such as a reduction in macular volume, RGC layer thickness, choroid thickness and some vascular alteration. These results might be promising biomarkers for dementia staging and AD progression (146, 147).

In recent years, thanks to the development of the OCTA, several studies analyzed the retinal vascularization and the choroid. Most of the studies, published in moderate AD, have found a loss of the retinal vascular density in the macular area with slower blood flow and an increase in the foveal avascular

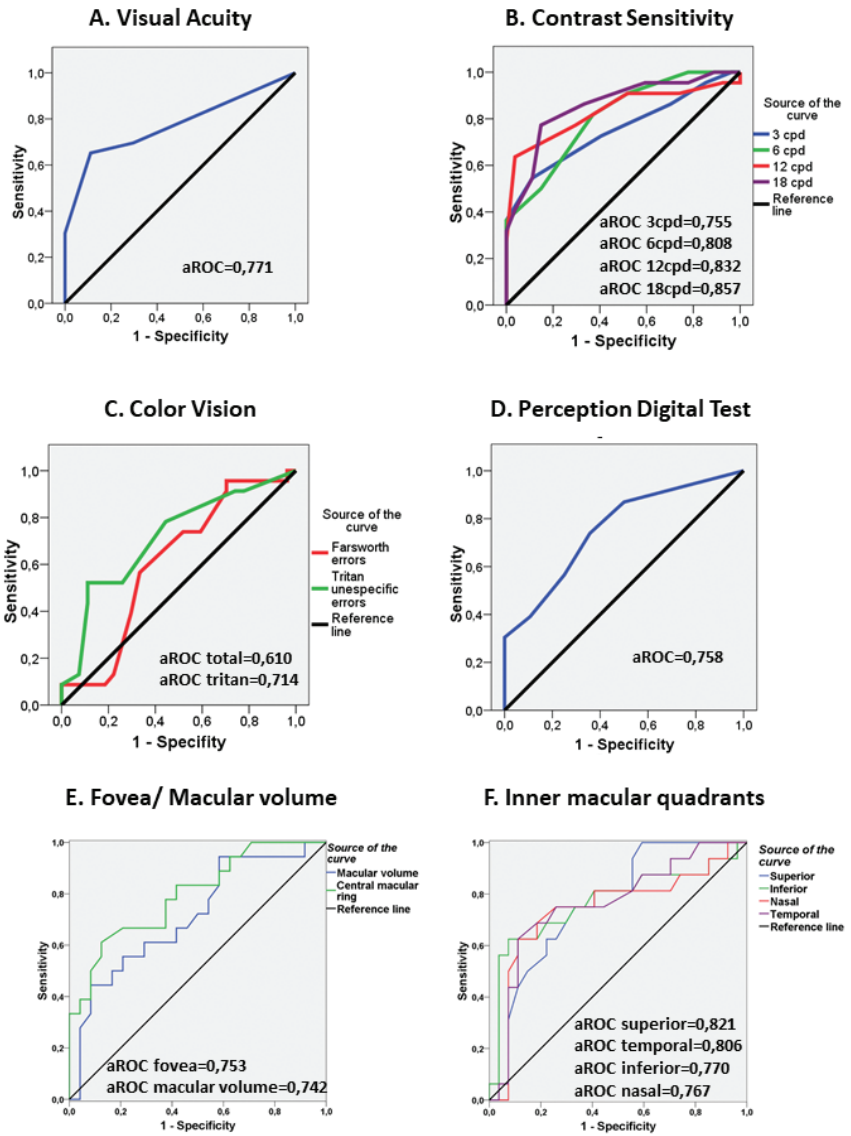

Figure 1 Areas under the ROC curves of the psychophysical tests (A–D) and macular OCT (E–F) in discriminating between mild AD patients and control subjects. (A) Visual acuity (dec), (B) contrast sensitivity, (C) Rue 28-hue color test, (D) perception digital test, (E) fovea and macular volume, and (F) inner macular quadrants. Modified from (A–D) Salobrar-Garcia et al., 2015 (93) and (E–F) Garcia-Martin et al., 2014 (21).

zone (148–150). All these parameters presented a correlation with the disease stage (148). In the same way, a thinning of the choroid, measured by OCT, was also found (125, 148, 151–153) (Table 1).

All these changes could be explained as a consequence of the amyloid angiopathy, which occurs in AD, in which amyloid deposits formed in the walls of the blood vessels. This process resulted in an ocular vascular occlusion and the diminishing of blood flow (120, 148, 149, 154).

It is possible that retinal AD biomarkers can only be obtained after having integrated various of the already cited biomarkers, which include both neuroretinal (such as RFNL, GCL, macular thickness) and retinovascular parameters (vessel morphology among others), in a composite biomarker (128).

The analysis of the ophthalmological tests prognostic value of AD showed that VA, CS, color perception, and visual integration (93) have a significant predictive value in early AD disease (Figure 1). The CS is the best predictive test in the diagnosis of the AD with an aROC between 0.857 and 0.755 (93), while the aROC curves of the OCT showed the best prognostic value is found in the macular area with values of r = 0.821 (21) (Figure 1). The focus must be centered on these tests to see the visual changes in the AD disease.

CONCLUSION

In conclusion, several alterations have been shown in the visual perception and the retinal structure in the eyes of AD patients, even in the earliest stages. The VA, CS, color perception, and visual integration tests, as well as macular OCT, have been altered in the early stages. When the disease progresses in the eyes of moderate AD patients, retina alteration reaches the peripapillary area, showing the progression of neurodegeneration in the eye.

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REFERENCES

1. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2017 Jan;25(1):56–67. http://dx.doi.org/10.1111/ene.13439
2. Alzheimer's Association. 2017 Alzheimer's disease facts and figures. Alzheimers Dementia. 2017;13(4):325–73. http://dx.doi.org/10.1016/j.jalz.2017.02.001
3. Bachurin SO, Bovina EV, Ustyugov AA. Drugs in clinical trials for Alzheimer's disease: The major trends. Med Res Rev. 2017 Sep;37(5):1186–225. http://dx.doi.org/10.1002/med.21434
4. Karch C, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry. 2015 Jan 1;77(1):43–51. http://dx.doi.org/10.1016/j.biopsych.2014.05.006

5. Martínez-Lazcano JC, Boll-Woehrlen MC, Hernández-Melesio MA, Rubio-Osornio M, Sánchez-Mendoza MA, Ríos C. Radicales libres y estrés oxidativo en las enfermedades neurodegenerativas. Mensaje Bioquim. 2010;34:43–59.
6. Dudvarski Stankovic N, Teodorczyk M, Ploen R, Zipp F, Schmidt MHH. Microglia–blood vessel interactions: A double-edged sword in brain pathologies. Acta Neuropathol. 2016 Mar 28;131(3):347–63. http://dx.doi.org/10.1007/s00401-015-1524-y
7. Escott-Price V, Sims R, Bannister C, Harold D, Vronskaya M, Majounie E, et al. Common polygenic variation enhances risk prediction for Alzheimer's disease. Brain. 2015 Dec;138(Pt 12):3673–84. http://dx.doi.org/10.1093/brain/awv268
8. García-Ospina G, Jímenez-Del MR, Lopera F, Vélez-Pardo C. Neuronal DNA damage correlates with a positive detection of c-Jun, nuclear factor kB, p53 and Par-4 transcription factors in Alzheimer's disease. Rev Neurol. 2003;36(11):1004–10. http://dx.doi.org/10.33588/rn.3611.2002533
9. Jan AT, Azam M, Rahman S, Almigeiti AMS, Choi DH, Lee EJ, et al. Perspective insights into disease progression, diagnostics, and therapeutic approaches in Alzheimer's disease: A judicious update. Front Aging Neurosci. 2017;9:356. http://dx.doi.org/10.3389/fnagi.2017.00356
10. Jaunmuktane Z, Mead S, Ellis M, Wadsworth JDF, Nicoll AJ, Kenny J, et al. Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. Nature. 2015 Sep 10; 525(7568):247–50. http://dx.doi.org/10.1038/nature15369
11. Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature. 2013 Sep 5;501(7465):45–51. http://dx.doi.org/10.1038/nature12481
12. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. Science. 1992 Apr 10; 256(5054):184–5. http://dx.doi.org/10.1126/science.1566067
13. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016 Jun;8(6):595–608. http://dx.doi.org/10.15252/emmm.201606210
14. He Z, Guo JL, McBride JD, Narasimhan S, Kim H, Changolkar L, et al. Amyloid-β plaques enhance Alzheimer's brain tau-seeded pathologies by facilitating neuritic plaque tau aggregation. Nat Med. 2018 Jan 4;24(1):29–38. http://dx.doi.org/10.1038/nm.4443
15. Molinuevo JL, Ayton S, Batrla R, Bednar MM, Bittner T, Cummings J, et al. Current state of Alzheimer's fluid biomarkers. Acta Neuropathol. 2018 Dec 28;136(6):821–53. http://dx.doi.org/10.1007/s00401-018-1932-x
16. Sanabria-Castro A, Alvarado-Echeverría I, Monge-Bonilla C. Molecular pathogenesis of Alzheimer's disease: An update. Ann Neurosci. 2017 May;24(1):46–54. http://dx.doi.org/10.1159/000464422
17. Salobrar-García E, Ramírez AI, de Hoz R, Rojas P, Salazar JJ, Rojas B, et al. Chapter 15: The impact of the eye in dementia: The eye and its role in diagnosis and follow up. In: Update on dementia. In Tech; 2016. ISBN 978-953-51-4833-3. http://dx.doi.org/10.5772/64490. Available from: http://www.intechopen.com/books/update-on-dementia/the-impact-of-the-eye-in-dementia-the-eye-and-its-role-in-diagnosis-and-follow-up
18. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to CNS disorders. Nat Rev. 2013;9(1):44–53. http://dx.doi.org/10.1038/nrneurol.2012.227
19. Ramirez AIAI, de Hoz R, Salobrar-Garcia E, Salazar JJJJ, Rojas B, Ajoy D, et al. The role of microglia in retinal neurodegeneration: Alzheimer's disease, Parkinson, and glaucoma. Front Aging Neurosci. 2017 Jul 6;9:214. http://dx.doi.org/10.3389/fnagi.2017.00214
20. MacCormick IJ, Czanner G, Faragher B. Developing retinal biomarkers of neurological disease: An analytical perspective. Biomark Med. 2015;9(7):691–701. http://dx.doi.org/10.2217/bmm.15.17
21. Garcia-Martin ES, Rojas B, Ramirez AI, de Hoz R, Salazar JJ, Yubero R, et al. Macular thickness as a potential biomarker of mild Alzheimer's disease. Ophthalmology. 2014;121(5):1149–51. http://dx.doi.org/10.1016/j.ophtha.2013.12.023
22. Maldonado RSRS, Mettu P, El-Dairi M, Bhatti MTT. The application of optical coherence tomography in neurologic diseases. Neurol Clin Pract. 2015;5(5):460–9. http://dx.doi.org/10.1212/CPJ.0000000000000187
23. Salobrar-Garcia E, Garcia Y, Lostao C, Jañez L, de Hoz R, Rojas B, et al. Maculopapillary analysis in the posterior pole in patients with mild Alzheimer's disease. Acta Ophthalmol. 2016;94:S256. http://dx.doi.org/10.1111/j.1755-3768.2016.0372

24. Salobrar-Garcia E, Hoyas I, Leal M, de Hoz R, Rojas B, Ramirez AI, et al. Analysis of retinal peripapillary segmentation in early Alzheimer's disease patients. Biomed Res Int. 2015 Oct 18;2015:1–8. http://dx.doi.org/10.1155/2015/636548
25. Salobrar-Garcia E, Leal M, Hoyas I, Salazar JJ, Ramirez AI, de Hoz R, et al. Early changes in mild Alzheimer's disease in the neuroretinal rim segmentation. Acta Ophthalmol. 2016;94:S256. http://dx.doi.org/10.1111/j.1755-3768.2016.0449
26. Ramachandran VS. editor. Encyclopedia of the human brain. San Diego, CA: Academic Press; 2002;3:183–200.
27. Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-nerve degeneration in Alzheimer's disease. N Engl J Med. 1986 Aug 21;315(8):485–7. http://dx.doi.org/10.1056/NEJM198608213150804
28. Blanks JC, Torigoe Y, Hinton DR, Blanks RHI. Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. Neurobiol Aging. 1996;17(3):377–84. http://dx.doi.org/10.1016/0197-4580(96)00010-3
29. Blanks JC, Schmidt SY, Torigoe Y, Porrello K V, Hinton DR, Blanks RHI. Retinal pathology in Alzheimer's disease. II. Regional neuron loss and glial changes in GCL. Neurobiol Aging. 1996;17(3):385–95. http://dx.doi.org/10.1016/0197-4580(96)00009-7
30. Paquet C, Boissonnot M, Roger F, Dighiero P, Gil R, Hugon J. Abnormal retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Neurosci Lett. 2007;420(2):97–9. http://dx.doi.org/10.1016/j.neulet.2007.02.090
31. Iseri PK, Altinas Ö, Tokay T, Yüksel N. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in Alzheimer disease. J Neuro Ophthalmol. 2006;26(1):18. http://dx.doi.org/10.1097/01.wno.0000204645.56873.26
32. Ferrari L, Huang SC, Magnani G, Ambrosi A, Comi G, Leocani L. Optical coherence tomography reveals retinal neuroaxonal thinning in frontotemporal dementia as in Alzheimer's disease. J Alzheimers Dis. 2017;56(3):1101–7. http://dx.doi.org/10.3233/JAD-160886
33. Ko F, Muthy ZA, Gallacher J, Sudlow C, Rees G, Yang Q, et al. Association of retinal nerve fiber layer thinning with current and future cognitive decline: A study using optical coherence tomography. JAMA Neurol. 2018 Oct 1;75(10):1198–205. http://dx.doi.org/10.1001/jamaneurol.2018.1578
34. Valenti DA. Neuroimaging of retinal nerve fiber layer in AD using optical coherence tomography. Neurology. 2007;69(10):1060. http://dx.doi.org/10.1212/01.wnl.0000280584.64363.83
35. Kesler A, Vakhapova V, Korczyn AD, Naftaliev E, Neudorfer M. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Clin Neurol Neurosurg. 2011;113(7):523–6. http://dx.doi.org/10.1016/j.clineuro.2011.02.014
36. Moreno-Ramos T, Benito-León J, Villarejo A, Bermejo-Pareja F. Retinal nerve fiber layer thinning in dementia associated with Parkinson's disease, dementia with lewy bodies, and Alzheimer's disease. J Alzheimer's Dis. 2013;34(3):659–64. http://dx.doi.org/10.3233/JAD-121975
37. He XF, Liu YT, Peng C, Zhang F, Zhuang S, Zhang JS. Optical coherence tomography assessed retinal nerve fiber layer thickness in patients with Alzheimer's disease: A meta-analysis. Int J Ophthalmol. 2012;5(3):401–5.
38. Parisi V, Restuccia R, Fattapposta F, Mina C, Bucci MG, Pierelli F. Morphological and functional retinal impairment in Alzheimer's disease patients. Clin Neurophysiol. 2001;112(10):1860–7. http://dx.doi.org/10.1016/S1388-2457(01)00620-4
39. Lu Y, Li Z, Zhang X, Ming B, Jia J, Wang R, et al. Retinal nerve fiber layer structure abnormalities in early Alzheimer's disease: Evidence in optical coherence tomography. Neurosci Lett. 2010;480(1):69–72. http://dx.doi.org/10.1016/j.neulet.2010.06.006
40. Moschos M, Markopoulos I, Chatziralli I, Rouvas A, Papageorgiou SG, Ladas I, et al. Structural and functional impairment of the retina and optic nerve in Alzheimer's disease. Curr Alzheimer Res. 2012;9(7):782–8. http://dx.doi.org/10.2174/156720512802455340
41. Cunha LP, Lopes LC, Costa-Cunha LVFVF, Costa CF, Pires LAA, Almeida ALM, et al. Macular thickness measurements with frequency domain-OCT for quantification of retinal neural loss and its correlation with cognitive impairment in Alzheimer's disease. PLoS One. 2016;11(4):e0153830. http://dx.doi.org/10.1371/journal.pone.0153830

42. Williams PA, Thirgood RA, Oliphant H, Frizzati A, Littlewood E, Votruba M, et al. Retinal ganglion cell dendritic degeneration in a mouse model of Alzheimer's disease. Neurobiol Aging. 2013;34(7): 1799–806. http://dx.doi.org/10.1016/j.neurobiolaging.2013.01.006
43. Liu B, Rasool S, Yang Z, Glabe CG, Schreiber SS, Ge J, et al. Amyloid-peptide vaccinations reduce β-amyloid plaques but exacerbate vascular deposition and inflammation in the retina of Alzheimer's transgenic mice. Am J Pathol. 2009;175(5):2099–110. http://dx.doi.org/10.2353/ajpath.2009.090159
44. Koronyo Y, Biggs D, Barron E, Boyer DS, Pearlman JA, Au WJ, et al. Retinal amyloid pathology and proof-of-concept imaging trial in Alzheimer's disease. JCI Insight. 2017;2(16):pii: 93621. http://dx.doi.org/10.1172/jci.insight.93621
45. Guo L, Salt TE, Luong V, Wood N, Cheung W, Maass A, et al. Targeting amyloid-beta in glaucoma treatment. Proc Natl Acad Sci. 2007 Aug 14;104(33):13444–9. http://dx.doi.org/10.1073/pnas.0703707104
46. Du LY, Chang LYL, Ardiles AO, Tapia-Rojas C, Araya J, Inestrosa NC, et al. Alzheimer's disease-related protein expression in the retina of Octodon degus. PLoS One. 2015;10(8):1–17. http://dx.doi.org/10.1371/journal.pone.0135499
47. La Morgia C, Ross-Cisneros FN, Koronyo Y, Hannibal J, Gallassi R, Cantalupo G, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease. Ann Neurol. 2016;79(1):90–109. http://dx.doi.org/10.1002/ana.24548
48. Sadun AA, Schaechter JD, Smith LE. A retinohypothalamic pathway in man: Light mediation of circadian rhythms. Brain Res. 1984;302(2):371–7. http://dx.doi.org/10.1016/0006-8993(84)90252-X
49. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. Science. 2002 Feb 8;295(5557):1065–70. http://dx.doi.org/10.1126/science.1069609
50. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. Science. 2002 Feb 8;295(5557):1070–3. http://dx.doi.org/10.1126/science.1067262
51. Sand A, Schmidt TM, Kofuji P. Diverse types of ganglion cell photoreceptors in the mammalian retina. Prog Retin Eye Res. 2012 Jul;31(4):287–302. http://dx.doi.org/10.1016/j.preteyeres.2012.03.003
52. Oliveira-Souza FG, DeRamus ML, van Groen T, Lambert AE, Bolding MS, Strang CE. Retinal changes in the Tg-SwDI mouse model of Alzheimer's disease. Neuroscience. 2017;354:43–53. http://dx.doi.org/10.1016/j.neuroscience.2017.04.021
53. Dagnelie G. Age-related psychophysical changes and low vision. Invest Ophthalmol Vis Sci. 2013;54(14):ORSF88–93. http://dx.doi.org/10.1167/iovs.13-12934
54. Mapstone M, Dickerson K, Duffy CJ. Distinct mechanisms of impairment in cognitive ageing and Alzheimer's disease. Brain. 2008;131(Pt 6):1618–29. http://dx.doi.org/10.1093/brain/awn064
55. Prvulovic D, Hubl D, Sack AT, Melillo L, Maurer K, Frölich L, et al. Functional imaging of visuospatial processing in Alzheimer's disease. Neuroimage. 2002;17(3):1403–14. http://dx.doi.org/10.1006/nimg.2002.1271
56. Rizzo M, Anderson SW, Dawson J, Nawrot M. Vision and cognition in Alzheimer's disease. Neuropsychologia. 2000;38(8):1157–69. http://dx.doi.org/10.1016/S0028-3932(00)00023-3
57. Tippett WJ, Black SE. Regional cerebral blood flow correlates of visuospatial tasks in Alzheimer's disease. J Int Neuropsychol Soc. 2008;14(06):1034–45. http://dx.doi.org/10.1017/S1355617708081241
58. Cronin-Golomb A, Sugiura R, Corkin S, Growdon JH. Incomplete achromatopsia in Alzheimer's disease. Neurobiol Aging. 1993;14(5):471–7. http://dx.doi.org/10.1016/0197-4580(93)90105-K
59. Kurylo DD, Corkin S, Dolan RP, Rizzo JF, Parker SW, Growdon JH. Broad-band visual capacities are not selectively impaired in Alzheimer's disease. Neurobiol Aging. 1994;15(3):305–11. http://dx.doi.org/10.1016/0197-4580(94)90025-6
60. Rizzo M, Anderson SW, Dawson J, Myers R, Ball K. Visual attention impairments in Alzheimer's disease. Neurology. 2000;54(10):1954–9. http://dx.doi.org/10.1212/WNL.54.10.1954
61. Vighetto A. Towards an earlier diagnosis of Alzheimer's disease presenting with visuospatial disorders (posterior cortical atrophy). Rev Neurol (Paris). 2013 Oct 1;169(10):687–94. http://dx.doi.org/10.1016/j.neurol.2013.08.001
62. Gouras P. Color vision [Internet]. Webvision: The Organization of the Retina and Visual System. University of Utah Health Sciences Center; 1995.

63. Laycock R, Crewther SG, Crewther DP. A role for the "magnocellular advantage" in visual impairments in neurodevelopmental and psychiatric disorders. Neurosci Biobehav Rev. 2007;31(3):363–76. http://dx.doi.org/10.1016/j.neubiorev.2006.10.003
64. Martin PR, White AJR, Goodchild AK, Wilder HD, Sefton AE. Evidence that blue-on cells are part of the third geniculocortical pathway in primates. Eur J Neurosci. 1997;9(7):1536–41. http://dx.doi.org/10.1111/j.1460-9568.1997.tb01509.x
65. Solomon SG, Lennie P. The machinery of colour vision. Nat Rev Neurosci. 2007;8(4):276–86. http://dx.doi.org/10.1038/nrn2094
66. Neargarder SA, Stone ER, Cronin-Golomb A, Oross S. The impact of acuity on performance of four clinical measures of contrast sensitivity in Alzheimer's disease. J Gerontol B Psychol Sci Soc Sci. 2003;58(1):P54–62. http://dx.doi.org/10.1093/geronb/58.1.P54
67. Dargent-Molina P, Hays M, Breart G. Sensory impairments and physical disability in aged women living at home. Int J Epidemiol. 1996;25(3):621–9. http://dx.doi.org/10.1093/ije/25.3.621
68. Wong SH, Plant GT. How to interpret visual fields. Pract Neurol. 2015;15(5). http://dx.doi.org/10.1136/practneurol-2015-001155
69. Cooper SA, Metcalfe RA. Assess and interpret the visual fields at the bedside. Pract Neurol. 2009 Dec;9(6):324–34. http://dx.doi.org/10.1136/jnnp.2009.193920
70. Viggiano MP, Gori G, Zaccara G, Righi S, Vannucci M, Giovannelli F. Category-specific visual identification of filtered objects in Alzheimer's disease. Arch Gerontol Geriatr. 2007;44(2):125–39. http://dx.doi.org/10.1016/j.archger.2006.04.003
71. Hayes T, Morrone MC, Burr DC. Recognition of positive and negative bandpass-filtered images. Perception. 1986 Oct 25;15(5):595–602. http://dx.doi.org/10.1068/p150595
72. Braje WL, Tjan BS, Legge GE. Human efficiency for recognizing and detecting low-pass filtered objects. Vision Res. 1995 Nov;35(21):2955–66. http://dx.doi.org/10.1016/0042-6989(95)00071-7
73. Hughes HC, Nozawa G, Kitterle F. Global precedence, spatial frequency channels, and the statistics of natural images. J Cogn Neurosci. 1996 Jul;8(3):197–230. http://dx.doi.org/10.1162/jocn.1996.8.3.197
74. Parker DM, Lishman JR, Hughes J. Role of coarse and fine spatial information in face and object processing. J Exp Psychol Hum Percept Perform. 1996 Dec;22(6):1448–66. http://dx.doi.org/10.1037//0096-1523.22.6.1448
75. Olds ES, Engel SA. Linearity across spatial frequency in object recognition. Vision Res. 1998 Jul;38(14):2109–18. http://dx.doi.org/10.1016/S0042-6989(97)00393-3
76. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence tomography. Opt Coher Tomogr. 1991 Nov 22;254(5035):1178–81. http://dx.doi.org/10.1126/science.1957169
77. Bandello F, Souied E, Querques G. OCT angiography in retinal and macular diseases. Bandello F, Souied E, Querques G, editors. New York: Karger Medical and Scientific Publishers; 2016. http://dx.doi.org/10.1159/isbn.978-3-318-05830-7
78. Huang D, Lumbroso B, Jia Y, Waheed N. Optical coherence tomography angiography of the eye. USA: SLACK Incorporated; 2018.
79. Lakshminarayanan V, Lagrave J, Kean ML, Dick M, Shankle R. Vision in dementia: Contrast effects. Neurol Res. 1996;18(1):9–15. http://dx.doi.org/10.1080/01616412.1996.11740369
80. Schlotterer G, Moscovitch M, Crapper-McLachlan D. Visual processing deficits as assessed by spatial frequency contrast sensitivity and backward masking in normal ageing and Alzheimer's disease. Brain. 1984;107(1):309–25. http://dx.doi.org/10.1093/brain/107.1.309
81. Wright CE, Drasdo N, Harding GFAA. Pathology of the optic nerve and visual association areas information given by the flash and pattern visual evoked potential, and the temporal and spatial contrast sensitivity function. Brain. 1987 Feb;110(1):107–20. http://dx.doi.org/10.1093/brain/110.1.107
82. Levine DN, Lee JM, Fisher CM. The visual variant of Alzheimer's disease A clinicopathologic case study. Neurology. 1993;43(2):305. http://dx.doi.org/10.1212/WNL.43.2.305
83. Martinelli V, Locatelli T, Comi G, Lia C, Alberoni M, Bressi S, et al. Pattern Visual Evoked Potential Mapping in Alzheimers Disease Correlations with Visuospatial Impairment. Dement Geriatr Cogn Disord. 1996;7(2):63–8. http://dx.doi.org/10.1159/000106855

84. Cronin-Golomb A, Corkin S, Rizzo JF, Cohen J, Growdon JH, Banks KS. Visual dysfunction in Alzheimer's disease: Relation to normal aging. Ann Neurol. 1991;29(1):41–52. http://dx.doi.org/10.1002/ana.410290110
85. Mendez MF, Tomsak RL, Remler B. Disorders of the visual system in Alzheimer's disease. J Neuro Ophthalmol. 1990;10(1):62–9.
86. Rizzo M, Nawrot M. Perception of movement and shape in Alzheimer's disease. Brain. 1998;121(12):2259–70. http://dx.doi.org/10.1093/brain/121.12.2259
87. Murgatroyd C, Prettyman R. An investigation of visual hallucinosis and visual sensory status in dementia. Int J Geriatr Psychiatry. 2001;16(7):709–13. http://dx.doi.org/10.1002/gps.426
88. Chapman FM, Dickinson J, McKeith I, Ballard C. Association among visual hallucinations, visual acuity, and specific eye pathologies in Alzheimer's disease: Treatment implications. Am J Psychiatry. 1999;156(12):1983–5.
89. Kaeser P-F, Ghika J, Borruat F-X. Visual signs and symptoms in patients with the visual variant of Alzheimer disease. BMC Ophthalmol. 2015 Dec 30;15(1):65. http://dx.doi.org/10.1186/s12886-015-0060-9
90. Ward ME, Gelfand JM, Lui L-Y, Ou Y, Green AJ, Stone K, et al. Reduced contrast sensitivity among older women is associated with increased risk of cognitive impairment. Ann Neurol. 2018 Apr;83(4):730–8. http://dx.doi.org/10.1002/ana.25196
91. Marmor MF. Contrast sensitivity versus visual acuity in retinal disease. Br J Ophthalmol. 1986 Jul;70(7):553–9. http://dx.doi.org/10.1136/bjo.70.7.553
92. Trick GL, Barris MC, Bickler-Bluth M. Abnormal PERG's in patients with senile dementia of the Alzheimer type. Ann Neurol. 1989;26:226–31. http://dx.doi.org/10.1002/ana.410260208
93. Salobrar-Garcia E, De Hoz R, Rojas B, Ramirez AIAI, Salazar JJJJ, Yubero R, et al. Ophthalmologic psychophysical tests support OCT findings in mild Alzheimer's disease. J Ophthalmol. 2015;2015:736949, 1–10. http://dx.doi.org/10.1155/2015/736949
94. Cronin-Golomb A, Rizzo JF, Corkin S, Growdon JH. Visual function in Alzheimer's disease and normal aging. Ann N Y Acad Sci. 1991;640:28–35. http://dx.doi.org/10.1111/j.1749-6632.1991.tb00186.x
95. Sadun AA, Borchert M, DeVita E, Hinton DR, Bassi CJ. Assessment of visual impairment in patients with Alzheimer's disease. Am J Ophthalmol. 1987;104(15):113–20. http://dx.doi.org/10.1016/0002-9394(87)90001-8
96. Bassi CJ, Solomon K, Young D. Vision in aging and dementia. Optom Vis Sci. 1993;70(10):809–13. http://dx.doi.org/10.1097/00006324-199310000-00005
97. Gilmore GC, Levy JA. Spatial contrast sensitivity in Alzheimer's disease: A comparison of two methods. Optom Vis Sci. 1991;68(10):790–4. http://dx.doi.org/10.1097/00006324-199110000-00006
98. Gilmore GC, Whitehouse PJ. Contrast sensitivity in Alzheimer's disease: A 1-year longitudinal analysis. Optom Vis Sci. 1995;72(2):83–91. http://dx.doi.org/10.1097/00006324-199502000-00007
99. Crow RW, Levin LB, LaBree L, Rubin R, Feldon SE. Sweep visual evoked potential evaluation of contrast sensitivity in Alzheimer's dementia. Invest Ophthalmol Vis Sci. 2003;44(2):875–8. http://dx.doi.org/10.1167/iovs.01-1101
100. Hutton JT, Morris JL, Elias JW, Poston JN. Contrast sensitivity dysfunction in Alzheimer's disease. Neurology. 1993;43(11):2328. http://dx.doi.org/10.1212/WNL.43.11.2328
101. Baker DR, Mendez MF, Townsend JC, Ilsen PF, Bright DC. Optometric management of patients with Alzheimer's disease. J Am Optom Assoc. 1997;68(8):483–94.
102. Cronin-Golomb A, Gilmore GC, Neargarder S, Morrison SR, Laudate TM. Enhanced stimulus strength improves visual cognition in aging and Alzheimer's disease. Cortex. 2007;43(7):952–66. http://dx.doi.org/10.1016/S0010-9452(08)70693-2
103. Risacher SL, WuDunn D, Pepin SM, MaGee TR, McDonald BC, Flashman LA, et al. Visual contrast sensitivity in Alzheimer's disease, mild cognitive impairment, and older adults with cognitive complaints. Neurobiol Aging. 2013;34(4):1133–44. http://dx.doi.org/10.1016/j.neurobiolaging.2012.08.007
104. Cerquera-Jaramillo MA, Nava-Mesa MO, González-Reyes RE, Tellez-Conti C, de-la-Torre A. Visual features in Alzheimer's disease: From basic mechanisms to clinical overview. Neural Plast. 2018 Oct 14; 2018:1–21. http://dx.doi.org/10.1155/2018/2941783
105. Trick GL, Trick LR, Morris P, Wolf M. Visual field loss in senile dementia of the Alzheimer's type. Neurology. 1995;45(1):68–74. http://dx.doi.org/10.1212/WNL.45.1.68

106. Armstrong RA. Visual field defects in Alzheimer's disease patients may reflect differential pathology in the primary visual cortex. Optom Vis Sci. 1996;73(11):677. http://dx.doi.org/10.1097/00006324-199611000-00001
107. Millington R, James-Galton M, Maia Da Silva MN, Plant GT, Bridge H. Lateralized occipital degeneration in posterior cortical atrophy predicts visual field deficits. Neuroimage Clin. 2017 Jan 17;14:242–9. http://dx.doi.org/10.1016/j.nicl.2017.01.012
108. Shuren J, Heilman KM. Visual field loss in Alzheimer's disease. J Am Geriatr Soc. 1993;41(10):1114–15. http://dx.doi.org/10.1111/j.1532-5415.1993.tb06461.x
109. Whittaker KW, Burdon MA, Shah P. Visual field loss and Alzheimer's disease. Eye. 2002;16(2):206–8. http://dx.doi.org/10.1038/sj/eye/6700037
110. Salamone G, Di Lorenzo C, Mosti S, Lupo F, Cravello L, Palmer K, et al. Color discrimination performance in patients with Alzheimer's disease. Dement Geriatr Cogn Disord. 2009;27(6):501–7. http://dx.doi.org/10.1159/000218366
111. Wood S, Mortel KF, Hiscock M, Breitmeyer BG, Caroselli JS. Adaptive and maladaptive utilization of color cues by patients with mild to moderate Alzheimer's disease. Arch Clin Neuropsychol. 1997;12(5):483–9. http://dx.doi.org/10.1093/arclin/12.5.483
112. Massoud F, Chertkow H, Whitehead V, Overbury O, Bergman H. Word-reading thresholds in Alzheimer disease and mild memory loss: A pilot study. Alzheimer Dis Assoc Disord. 2002;16(1):31–9. http://dx.doi.org/10.1097/00002093-200201000-00005
113. Cronin-Golomb A, Corkin S, Growdon JH. Visual dysfunction predicts cognitive deficits in Alzheimer's disease. Optom Vis Sci. 1995;72(3):168–76. http://dx.doi.org/10.1097/00006324-199503000-00004
114. Pache M, Smeets CHW, Gasio PF, Savaskan E, Flammer J, Wirz-Justice A, et al. Colour vision deficiencies in Alzheimer's disease. Age Ageing. 2003;32(4):422–6. http://dx.doi.org/10.1093/ageing/32.4.422
115. Pelak VS, Hills W. Vision in Alzheimer's disease: A focus on the anterior afferent pathway. Neurodegener Dis Manag. 2018 Feb 23;8(1):49–67. http://dx.doi.org/10.2217/nmt-2017-0030
116. Polo V, Rodrigo MJ, Garcia-Martin E, Otin S, Larrosa JM, Fuertes MI, et al. Visual dysfunction and its correlation with retinal changes in patients with Alzheimer's disease. Eye (Lond). 2017;31(7):1034–41. http://dx.doi.org/10.1038/eye.2017.23
117. Rami L, Serradell M, Bosch B, Villar A, Molinuevo JL. Perception digital test (PDT) for the assessment of incipient visual disorder in initial Alzheimer's disease. Neurologia. 2007;22(6):342–7.
118. Ong Y-L, Ong Y-T, Ikram MK, Chen CLH, Wong TY, Cheung CY. Potential applications of spectral-domain optical coherence tomography (SD-OCT) in the study of Alzheimer's disease. Proc Singapore Heal Vol. 2014;23(1):74. http://dx.doi.org/10.1177/201010581402300112
119. Simao LM. The contribution of optical coherence tomography in neurodegenerative diseases. Curr Opin Ophthalmol. 2013;24(6):521–7. http://dx.doi.org/10.1097/ICU.0000000000000000
120. Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. Invest Ophthalmol Vis Sci. 2007;48(5):2285–9. http://dx.doi.org/10.1167/iovs.06-1029
121. Kromer R, Serbecic N, Hausner L, Aboul-enein F, Froelich L, Beutelspacher S. Detection of retinal nerve fiber layer defects in Alzheimer's disease using SD-OCT. Name Front Psychiatry. 2014;5:22. http://dx.doi.org/10.3389/fpsyt.2014.00022
122. Chi Y, Wang YH, Yang L. The investigation of retinal nerve fiber loss in Alzheimer's disease. Zhonghua Yan Ke Za Zhi. 2010;46(2):134–9.
123. Shen Y, Shi Z, Jia R, Zhu Y, Cheng Y, Feng W, et al. The attenuation of retinal nerve fiber layer thickness and cognitive deterioration. Front Cell Neurosci. 2013;7(September):1–7. http://dx.doi.org/10.3389/fncel.2013.00142
124. Kirbas S, Turkyilmaz K, Anlar O, Tufekci A, Durmus M. Retinal nerve fiber layer thickness in patients with Alzheimer disease. J Neuro Ophthalmol. 2013;33(1):58–61. http://dx.doi.org/10.1097/WNO.0b013e318267fd5f
125. Bayhan HA, Aslan Bayhan S, Celikbilek A, Tanık N, Gürdal C, Tanik N, et al. Evaluation of the chorioretinal thickness changes in Alzheimer's disease using spectral-domain optical coherence tomography. Clin Experiment Ophthalmol. 2014;43(2):145–51. http://dx.doi.org/10.1111/ceo.12386
126. Oktem EO, Derle E, Kibaroglu S, Oktem C, Akkoyun I, Can U. The relationship between the degree of cognitive impairment and retinal nerve fiber layer thickness. Neurol Sci. 2015;36(7):1141–6. http://dx.doi.org/10.1007/s10072-014-2055-3. http://dx.doi.org/10.1007/s10072-014-2055-3

127. Chan VTT, Sun Z, Tang S, Chen LJ, Wong A, Tham CC, et al. Spectral-domain OCT measurements in Alzheimer's disease. Ophthalmology. 2019 Apr;126(4):497–510. http://dx.doi.org/10.1016/j.ophtha.2018.08.009
128. Sánchez D, Castilla-Marti M, Rodríguez-Gómez O, Valero S, Piferrer A, Martínez G, et al. Usefulness of peripapillary nerve fiber layer thickness assessed by optical coherence tomography as a biomarker for Alzheimer's disease. Sci Rep. 2018 Dec 5;8(1):16345. http://dx.doi.org/10.1038/s41598-018-34577-3
129. Coppola G, Di Renzo A, Ziccardi L, Martelli F, Fadda A, Manni G, et al. Optical coherence tomography in Alzheimer's disease: A meta-analysis. PLoS One. 2015;10(8):1–14. http://dx.doi.org/10.1371/journal.pone.0134750
130. Thomson KL, Yeo JM, Waddell B, Cameron JR, Pal S. A systematic review and meta-analysis of retinal nerve fiber layer change in dementia, using optical coherence tomography. Alzheimers Dement. 2015;1(2):136–43. http://dx.doi.org/10.1016/j.dadm.2015.03.001
131. Krasodomska K, Lubiński W, Potemkowski A, Honczarenko K. Pattern electroretinogram (PERG) and pattern visual evoked potential (PVEP) in the early stages of Alzheimer's disease. Doc Ophthalmol. 2010;121(2):111–21. http://dx.doi.org/10.1016/j.dadm.2015.03.001
132. Justino L, Kergoat M-J, Bergman H, Chertkow H, Robillard A, Kergoat H. Neuroretinal function is normal in early dementia of the Alzheimer type. Neurobiol Aging. 2001;22(4):691–5. http://dx.doi.org/10.1016/S0197-4580(01)00234-2
133. Parisi V. Correlation between morphological and functional retinal impairment in patients affected by ocular hypertension, glaucoma, demyelinating optic neuritis and Alzheimer's disease. Semin Ophthalmol. 2003 Jan 2;18(2):50–7. http://dx.doi.org/10.1076/soph.18.2.50.15855
134. Uchida A, Pillai JA, Bermel R, Bonner-Jackson A, Rae-Grant A, Fernandez H, et al. Outer retinal assessment using spectral-domain optical coherence tomography in patients with Alzheimer's and Parkinson's disease. Invest Ophthalmol Vis Sci. 2018 Jun 1;59(7):2768–77. http://dx.doi.org/10.1167/iovs.17-23240
135. Santos CY, Johnson LN, Sinoff SE, Festa EK, Heindel WC, Snyder PJ. Change in retinal structural anatomy during the preclinical stage of Alzheimer's disease. Alzheimer's Dement (Amsterdam, Netherlands). 2018;10:196–209. http://dx.doi.org/10.1016/j.dadm.2018.01.003
136. Spaide RF, Curcio CA. Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: Literature review and model. Retina. 2011 Sep;31(8):1609–19. http://dx.doi.org/10.1097/IAE.0b013e3182247535
137. Staurenghi G, Sadda S, Chakravarthy U, Spaide RF, International Nomenclature for Optical Coherence Tomography (IN•OCT) Panel. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: The IN•OCT consensus. Ophthalmology. 2014 Aug;121(8):1572–8. http://dx.doi.org/10.1016/j.ophtha.2014.02.023
138. Asanad S, Ross-Cisneros FN, Nassisi M, Barron E, Karanjia R, Sadun AA. The retina in Alzheimer's disease: Histomorphometric analysis of an ophthalmologic biomarker. Invest Ophthalmol Vis Sci. 2019 Apr 1;60(5):1491–500. http://dx.doi.org/10.1167/iovs.18-25966
139. Blanks JC, Hinton DR, Sadun AA, Miller CA. Retinal ganglion cell degeneration in Alzheimer's disease. Brain Res. 1989;501(2):364–72. http://dx.doi.org/10.1016/0006-8993(89)90653-7
140. Cheung CY, Ong YTLT, Hilal S, Ikram MK, Low S, Ong YTLT, et al. Retinal ganglion cell analysis using high-definition optical coherence tomography in patients with mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis Retin Ganglion Cell Anal MCI AD. 2015;45(1):45–56. http://dx.doi.org/10.3233/JAD-141659
141. Ascaso FJ, Cruz N, Modrego PJ, Lopez-Anton R, Santabárbara J, Pascual LF, et al. Retinal alterations in mild cognitive impairment and Alzheimer's disease: An optical coherence tomography study. J Neurol. 2014;261(8):1522–30. http://dx.doi.org/10.1007/s00415-014-7374-z
142. Lad EM, Mukherjee D, Stinnett SS, Cousins SW, Potter GG, Burke JR, et al. Evaluation of inner retinal layers as biomarkers in mild cognitive impairment to moderate Alzheimer's disease. Paul F, editor. PLoS One. 2018 Feb 8;13(2):e0192646. http://dx.doi.org/10.1371/journal.pone.0192646
143. Knoll B, Simonett J, Volpe NJ, Farsiu S, Ward M, Rademaker A, et al. Retinal nerve fiber layer thickness in amnestic mild cognitive impairment: Case-control study and meta-analysis. Alzheimers Dement. 2016;4:85–93. http://dx.doi.org/10.1016/j.dadm.2016.07.004
144. Mandybur TI. Cerebral amyloid angiopathy and astrocytic gliosis in Alzheimer's disease. Acta Neuropathol. 1989;78(3):329–31. http://dx.doi.org/10.1007/BF00687764

145. Bates KA, Fonte J, Robertson TA, Martins RN, Harvey AR. Chronic gliosis triggers Alzheimer's disease-like processing of amyloid precursor protein. Neuroscience. 2002;113(4):785–96. http://dx.doi.org/10.1016/S0306-4522(02)00230-0
146. de Jong FJ, Schrijvers EMC, Ikram MK, Koudstaal PJ, de Jong PTVM, Hofman A, et al. Retinal vascular caliber and risk of dementia: The Rotterdam study. Neurology. 2011 Mar 1;76(9):816–21. http://dx.doi.org/10.1212/WNL.0b013e31820e7baa
147. O'Bryhim BE, Apte RS, Kung N, Coble D, Van Stavern GP. Association of preclinical Alzheimer disease with optical coherence tomographic angiography findings. JAMA Ophthalmol. 2018 Nov 1;136(11):1242–8. http://dx.doi.org/10.1001/jamaophthalmol.2018.3556
148. Bulut M, Kurtulus F, Gozkaya O, Erol MK, Cengiz A, Akidan M, et al. Evaluation of optical coherence tomography angiographic findings in Alzheimer's type dementia. Br J Ophthalmol. 2018;102(2):233–7. http://dx.doi.org/10.1136/bjophthalmol-2017-310476
149. Jiang H, Wei Y, Shi Y, Wright CB, Sun X, Gregori G, et al. Altered macular microvasculature in mild cognitive impairment and Alzheimer disease. J Neuroophthalmol. 2018 Sep;38(3):292–8. http://dx.doi.org/10.1097/WNO.0000000000000580
150. Yoon SP, Grewal DS, Thompson AC, Polascik BW, Dunn C, Burke JR, et al. Retinal microvascular and neurodegenerative changes in Alzheimer's disease and mild cognitive impairment compared with control participants. Ophthalmol Retina. 2019 Jun;3(6):489–99. http://dx.doi.org/10.1016/j.oret.2019.02.002
151. Gharbiya M, Trebbastoni A, Parisi F, Manganiello S, Cruciani F, D'Antonio F, et al. Choroidal thinning as a new finding in Alzheimer's disease: Evidence from enhanced depth imaging spectral domain optical coherence tomography. J Alzheimer's Dis. 2014;40(4):907–17. http://dx.doi.org/10.3233/JAD-132039
152. Cunha JPJP, Proença R, Dias-Santos A, Melancia D, Almeida R, Águas H, et al. Choroidal thinning: Alzheimer's disease and aging. Alzheimers Dement. 2017;8:11–17. http://dx.doi.org/10.1016/j.dadm.2017.03.004
153. Trebbastoni A, Marcelli M, Mallone F, D'Antonio F, Imbriano L, Campanelli A, et al. Attenuation of choroidal thickness in patients with Alzheimer disease: Evidence from an Italian prospective study. Alzheimer Dis Assoc Disord. 2017;31(2):128–34. http://dx.doi.org/10.1097/WAD.0000000000000176
154. Dorr A, Sahota B, Chinta LV, Brown ME, Lai AY, Ma K, et al. Amyloid-β-dependent compromise of microvascular structure and function in a model of Alzheimer's disease. Brain. 2012 Oct;135(10):3039–50. http://dx.doi.org/10.1093/brain/aws243

11

The Deterioration of Semantic Networks in Alzheimer's Disease

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Abstract: Language impairments in Alzheimer's disease may appear at the prodromal stage. The most significant impairment is found at the lexical-semantic process level, which is explained either by a degradation of the areas that store the semantic network or by a failure at retrieving the information from that network. Regardless of the retrieval failure happening, there is evidence of the degradation of the semantic network at some levels. Several studies support the bottom-up breakdown, according to which the loss starts at the specific concept attribute level, along with the link with its coordinates, while superordinates are preserved. Some characteristics can affect this loss such as familiarity, age of acquisition, frequency, or affective features. While classic studies have focused on concrete neutral nouns, recent research is exploring the role of emotion. Since emotional processes strengthen the semantic relationship between concepts, it could be a relevant dimension for the preservation of the semantic network.

Keywords: conceptual knowledge; concreteness; emotion; language impairments; lexical-semantic access; semantic memory; semantic network

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INTRODUCTION

Language impairments appear in most Alzheimer's disease (AD) patients early in the course of the disease, and by the end of it, language becomes completely absent. Although memory impairment is thought to be the first and more evident symptom, an increasing interest in the progression of the deterioration of language throughout the disease shows that language impairments are also present since its prodromal stage (1). An important aspect of language impaired in AD is the ability to access meaning, the representation of knowledge that allows us to understand the world.

Language is strongly based on semantic memory, a storage of concepts linked to linguistic labels. These linkages allow speakers to process the meaning of a linguistic input and to elaborate an appropriate output in response. Language as a cognitive function is sustained by semantic networks, that is, a framework of concepts linked through verbal associations. This network system would be compromised in AD, as the most remarkable impairments in the early stages of AD are related to the lexical-semantic processes. Such difficulties are most commonly thought to be caused either by a progressive deterioration of the associative structures of a patient's semantic memory, or by a degradation of their content itself (2). In either way, this would cause a loss of concept representation or a severe disorganization of semantic knowledge, which would lead the patients to present with difficulties to find some words and to access to their meaning or attributes, along with a rising number of comprehension errors, and a reduction of the production of semantic features (3). In contrast to that theory, some researches state that the semantic network is preserved, and the difficulties may be due to an impairment in executive processes that causes a failure to retrieve information (4). Consequently, the failure to modulate semantic memory would cause errors and slowness in the search of words.

This chapter intends to summarize the course and characteristics of the semantic deterioration due to Alzheimer's disease and to shed light on the ongoing debate about the underlying impairments. It will start with a brief overview of language impairments, continuing with the course and characteristics of the semantic impairment.

LANGUAGE IMPAIRMENTS IN ALZHEIMER'S DISEASE

Language suffers several changes throughout the course of AD. First impairment to become evident is anomia and, as a consequence, a deficit in verbal fluency tasks (5). In the mild stages, patients show comprehension as well as production problems, their anomia worsens and their language is characterized by paraphasias, circumlocutions, and lack of content. Verbal production becomes unintelligible when other symptoms such as dysarthria, echolalia, palilalia, and lack of coherence appear in the later stages of the disease. Lastly, the final stage is characterized by mutism and a severe comprehension deficit, thus impeding any kind of social interaction (6).

Lexical-semantic access appears to be first impaired. This process involves searching for a concept in the mind, activating potential phonological candidates and selecting the appropriate one. Thus, the impairments on this regard are

evidenced by longer response time in lexical decision tasks, and patients face word-finding difficulties along with damage in the semantic processing (7). For this reason, verbal fluency and naming tasks are widely used for cognitive assessment in AD. In verbal fluency tasks, patients must produce as many words as possible in a given time; semantic categories - semantic fluency - or letters - phonemic fluency - may be used as cues. These tasks demand a significant involvement of executive processes, as they require the subject to search for and organize proper responses, monitor their previous ones, and inhibit inappropriate responses. Semantic (SF) and phonemic fluency (PF) differ in the processes involved, and they show divergent declines: while PF relies on lexical representations, SF does so on meaning associations with a superordinate. Moreover, SF tasks in patients with AD are more impaired than PF ones (8), which is explained by the distinct involvement of semantic memory. Other tasks affected by the semantic memory breakdown in AD are naming tests, in which the patients produce semantic paraphasias and, as the disease progresses, an increase in the number of non-response errors, reflecting a pure anomia (9).

In a linguistic level, language production is impaired at early stages, in both written and spoken. Using a description task, Croisile et al. (10) provided evidence of deterioration on both modalities. Overall, written performance was worse than oral, and this effect was found on healthy subjects as well. Regarding the speech of AD patients, they produced shorter responses with fewer information units than healthy older people. Written responses tended to be shorter than oral ones, but they were equally informative. Additionally, it was observed a significant reduction of word categories and an increase of semantic errors in both tasks in AD. Syntax is relatively preserved in the early stages (11), but it worsens through the course of the disease (12). In the beginning, syntax is correct and coherent, but not long afterwards patients start to produce syntactically simplified statements, using fewer subordinate clauses. When maintaining a conversation, AD patients may encounter difficulties responding to open questions and providing new information (13). Most of them are caused by their impairments in comprehension and lexical-semantic access, although damaged verbal pragmatics seems to be also affecting.

In recent years, new techniques of voice and speech analysis have allowed researchers to explore oral production in AD patients. These studies aim to explore if the changes in language processes have behavioral consequences in the vocal execution. In this regard, speech in AD is characterized by changes in different temporal and acoustic voice parameters, such as a greater number of voice breaks and hesitations, more pauses when speaking, lower rate of expressive articulation, longer phonation time, and higher mean of the fundamental frequency. All these changes that can be used for early diagnosis of AD (14, 15) are not only due to difficulties in finding the proper word but also caused by some impairments in the processes involved in planning language and how the words relate to each other semantically and syntactically.

SEMANTIC IMPAIRMENTS: PRECLINICAL CHANGES

Iris Murdoch was a renowned British writer and philosopher. After a prolific career with over 40 published works that had been applauded, critics found her

last novel, *Jackson's Dilemma,* to be disappointing. A couple of years later, Murdoch was diagnosed with Alzheimer's disease. Presumably, she had written her last work while the cognitive impairment was already present, in the years leading to the diagnosis. This finding led Garrard et al. (16) to analyze *Jackson's Dilemma* and two of her other works, written in different periods of her life, in search of early impaired language parameters as a consequence of AD. Among other subtle differences, they noted that content words (nouns, words, and descriptors) had an overall higher mean word frequency in *Jackson's Dilemma* than in her previous works.

Although it may seem anecdotal, the story of Iris Murdoch is supported by other studies that look for preclinical language markers of the disease by comparing AD, mild cognitive impairment (MCI) and healthy control patients, MCI involves a cognitive decline that it is not explained by normal aging and does not interfere with everyday life. It is expected that about 18% of the people with MCI will develop AD (17) and that is the reason why MCI is often considered a preclinical stage of the disease. In a study from Mueller et al. (18), early MCI patients had their speech recorded while describing the "Cookie Theft" picture from the Boston Diagnostic Aphasia Examination. The results showed that there was no decline in syntax and lexical processes; however, an interaction between age and cognitive status was found in semantic and fluency processes, showing a faster decline for patients in a preclinical phase of dementia. Therefore, semantics were affected, producing proportionally less nouns and more pronouns and verbs, which adds evidence to the notion that language may become semantically impoverished early on the continuum of cognitive decline. Another study used this same method to explore potential changes in healthy carriers of the E280A autosomal dominant mutation in the *presenilin-1* gene in chromosome 14, which is related to early onset Alzheimer's disease. These participants did not present clinical symptoms or cognitive problems at the time of the evaluation. Nevertheless, it was found that carriers produced a lower number of semantic units, used simpler sentences, and expressed less semantic information than their non-carriers counterparts, although the number of words employed was similar in both cases. Thus, it can be concluded that a deterioration of the conceptual system is present since the preclinical phase of AD (19). This has been confirmed through classical neuropsychological measures such as the Isaacs test, a semantic verbal fluency task, in which participants show a low performance up to 9 years prior to diagnosis of AD (20, 21).

Given the high rate of progression to AD, it is a current challenge to differentiate those subjects with MCI who will develop AD from those who will not. There is evidence that semantic verbal fluency tasks could be used for that purpose. A study comparing AD, MCI, and healthy control patients on several language measures found that, while the AD group showed widespread impairments on traditional semantic memory measures of naming, the MCI group did not differ significantly from controls, except on semantic fluency (22). In longitudinal studies, it has been observed that MCI subjects that will eventually develop AD present with a different pattern in verbal fluency tasks, showing a faster decline in semantic compared to phonemic verbal fluency tasks (23–25). However, these results are not such a promising tool to predict MCI conversion into AD, as MCI patients' patterns are still very similar to those of healthy controls (26, 27).

CHARACTERISTICS OF THE DETERIORATION OF THE SEMANTIC NETWORK IN AD

Semantic impairments in AD patients have been well documented through several cognitive tasks. Confrontation naming tasks consist on presenting images of items, animals, or famous person in order for them to provide the target name. As mentioned before, AD patients progress from subtle difficulties to find words to pure anomia. The pattern of errors produced by AD patients in confrontation naming tasks is characterized by a tendency to produce semantic or visuo-perceptive errors. Semantic errors usually result from producing the name of the correspondent category instead of that of the target, from mentioning another word from the same category as the target, or from committing circumlocutory errors by giving correct information about the target but not its proper name. As the disease progresses, first type of error become more prevalent, while the opposite occurs to circumlocutory errors. This suggest that less and more inaccurate information is available (28). Overall, this pattern seems to suggest the existence of a disruption in semantic knowledge. However, this might not be the case. Several studies suggest that the pattern is similar for healthy, MCI, and AD groups and that they only differ quantitatively in the number of errors and non-response errors, but not qualitatively. This, added to the fact that AD patients can improve their performance by using phonological clues, suggests that the semantic network might be preserved longer than thought as the information can still be accessed, and the disruption only occurs at later stages, when the non-response errors are more common (29, 30).

Another impaired task that gives clues about the state of the semantic associative network is verbal fluency, in which the participant must produce as many words as possible when given a cue. These tasks are widely used for assessing dementia of the Alzheimer's type due to the consistency of the impairment results found (31, 32). AD patients produce fewer exemplars per category than healthy controls and tend to produce more general category labels. Although the most common measure is the number of words produced, some other data from this task can be useful to explore the semantic network in AD. For instance, clustering (producing words within subcategories) and switching (shifting between subcategories) are two components that predict performance on verbal fluency tasks. Clustering would be related to the state of the semantic storage with an implication of the temporal lobe, while switching would be related to control, laying on the frontal lobe. According to Troyer et al. (33), AD patients produce smaller clusters than healthy controls, for both semantic and phonemic fluency tasks, but only in the semantic task they show significantly less number of switchings. Therefore, they conclude that the impairment found in fluency tasks is due to an impoverished semantic memory.

The organization of semantic knowledge seems to be compromised as well. In a series of studies using multidimensional scales and pathfinder analysis, Salmon et al. (34) checked the semantic network of AD patients. These techniques can be used to elaborate cognitive maps representing the distant and relationships between concepts by using different semantic tasks measures. In this kind of map, individuals who have never developed a degree of knowledge on an item or who have lost it get a chaotic representation with many unnecessary nodes and that is the case for AD patients.

For instance, these studies showed how healthy participants tended to produce clearly differentiated clusters of wild and domestic animals, while AD participants tended to mix them. Assuming that verbal fluency represents the activation spreading through the semantic network, this would mean that for AD, that differentiation is not clear and the network is disorganized (35).

Concepts are the basic units of semantic memory. They are constituted by attributes or characteristics and they appear to be structured in a hierarchical way on multiple levels based upon their relationships: super ordinates, coordinates, and subordinates. In AD, these levels of semantic associative relationships show a distinct deterioration. The bottom-up theory states that attributes and coordinates are earlier impaired while the higher level of super ordinates connections remain longer intact. As knowledge of attributes allow individuals to perceive and distinguish an object, the loss of this knowledge would affect different processes such as naming, comprehension, and encoding (36). Semantic priming tasks provide evidence of this theory. When pairing concepts with their attributes, AD patients show a significant slower response time. However, this degradation is not homogeneous as the more salient and significant the attributes are, the longer they are preserved. AD patients are still able to identify the core attributes, that is, those with a higher relative importance for the meaning of the concept (37, 38).

On the other hand, semantic priming tasks with pairs of words with a coordinate relationship show the opposite effect. A significant facilitatory effect for coordinate concepts appears in AD patients (39, 40). Semantically close concepts are defined by the number of attributes that they share and by those distinctive features that belong to only one of them. For example, both tiger and lion have fur, but only the tiger has stripes. According to this, the semantic priming effect should be related to the overlap of features between the prime and the target. In fact, it has been found that when a pair of words share many attributes and have few distinctive features, the priming effect is larger (41). These results of hyperpriming support the idea that semantic memory is suffering a progressive deterioration that starts with the loss of specific attribute information, and as a consequence, AD patients are no longer capable of distinguishing between two coordinate concepts. Lasney et al. (42) conducted an experiment based on the semantic priming paradigm in which words were paired either by a category coordinate or by an attribute relationship. In addition, they distinguished between close or distant relationships for coordinates, and between shared or distinctive attributes. They found the hyperpriming effect in both close and distant coordinates. On the attributes condition, an impaired priming effect was observed for the distinctive attributes at the beginning of the disease. Only at later stages, shared attributes were affected and showed a weak prime effect. Therefore, as mentioned earlier in the chapter, features shared by many concepts are more resilient to the damage caused by AD, while the distinctive features are more vulnerable. In this sense, the confusion between close concepts that causes the hyperpriming effect would be explained by a loss of distinctive attributes.

DEGRADED NETWORK OR FAILURE TO RETRIEVE?

Up to this point, we have described the characteristic deterioration of the semantic associative network of AD patients. Now, the challenge is to explain why

this happens. There are two possible explanations for the impairments described. The first theory states that the impairment is linked to a breakdown in the organization and structure of the semantic network. The degradation of the neocortical association areas, that are assumed to store the representation of concepts and their attributes, would cause an actual loss of this knowledge. Contrarily, the other theory defends that the cause is a failure to retrieve information from a preserved semantic network, due to impairments in the executive processes involved in accessing such representations (43). While many researchers think that the consistency of semantic impairments through different tasks proves the deterioration of the network, others argue that the fact that AD patients still can do most of the tasks—although slower than healthy controls—or that they can benefit from phonological cues supports the idea of troubled retrieval mechanisms.

Such controversy has been explored through tasks that imply different cognitive demands. AD patients' distinct performance on phonemic and semantic fluency tasks supports the notion that they may be experiencing a loss or a breakdown in the organization of the semantic memory rather than suffering from difficulties in the retrieval of semantic knowledge. Both phonemic and semantic modalities imply similar executive control demands, and therefore, the main difference is the implication of the semantic memory. This would suggest a degradation of the semantic store (31, 44). Rohrer et al. (45) conducted a study asking AD patients to produce words within small categories subsets, and it was found that they produced items faster than controls. This finding could be explained therefore by a loss of associations between concepts within the semantic memory. The degradation of the network would cause a reduction on the potential items that could be activated. Hence, as less items would be available, less time would be needed to reach to them.

The studies by Chan et al. (46) about the organization of semantic network also seem to support the hypothesis of a degraded storage. By analyzing the clusters in verbal fluency tasks, they concluded that AD patients tend to rely more on the size or other perceptual dimensions rather than on abstract features such as wilderness, compared to healthy controls. The distinct difficulties in accessing words through perceptual or through abstract features seem quite laborious to be explained by the retrieval deficit theory. However, this kind of breakdown can be explained by a disorganized network, the loss of associative relationships and the establishment of new atypical ones.

Errors in naming tasks and their relationship with the integrity of the semantic network has also been explored. There is a relationship between the ability of an AD patient to name an object and their knowledge of that same object. When they are asked to define an object whose name they cannot access, their descriptions are impoverished, providing less attributes, and even losing core features (8, 47).

On the other hand, it has been argued that the impairment found in explicit semantic memory tasks would be caused by the implication of attentional and executive control systems. Therefore, tasks that allow researchers to assess the integrity of the semantic memory while minimizing the influence of those systems can shed light on the matter. That is the case of implicit semantic priming paradigm, which is based on the idea that spreading activation throughout the semantic network requires intact connections within the system. If the network disappears, it should not be possible to prime the target at all. The evidence provided by this task, therefore, strongly supports the retrieval mechanisms

impairment, since different studies using it consistently found an intact semantic priming in AD patients (48–50). Nevertheless, it seems that this intact network is limited to superordinates, as AD patients show a decline in priming for coordinates and attributes. In this sense, there would be at least a partial disruption in the semantic storage (43).

As can be seen, there is evidence that support both perspectives and therefore there is not a clear conclusion for the debate. One way to address the question would be to demonstrate the complete loss of a concept, which would imply that the person cannot access to the word consistently in different tasks and times, they cannot benefit from semantic cueing and they lose knowledge about the item. Hodges et al. (2) made an attempt to explore that, in which they assessed the semantic memory of AD patients through several tests. They found that when patients were not able to use an item in one task, it was likely to be absent in other task using that same item as a target, thus evidencing a storage degradation. Nonetheless, the aforementioned evidence of an intact semantic network cannot be ignored. Therefore, it seems likely that both impairments occur and have consequences on the performance of AD patients. Neuroimaging seems to support this notion, as an abnormal functioning in the semantic control network and its connections with several areas involved in the semantic processing has been found (51). A conciliatory approach that has been proposed states that, at early stages, the retrieval deficit could be causing the difficulties to which later the degradation is added (52) causing, in the end, a total breakdown.

THE ORGANIZATION OF THE SEMANTIC NETWORK

There is an intense debate about the existence of amodal or modality-specific domains in the representation of semantic knowledge, with certain deficits providing evidence for both of them. Although it is not the objective of this chapter to discuss the principles by which semantic network is organized, it is noteworthy the amount of research questioning whether it exists category specific deficits or other features that determine that organization. Neurological pathologies with a localized lesion strongly support the notion that separated neural systems process different semantic domains, but the fact that in AD the deterioration affects many cortical regions and patients still seem to show specific domain impairments has created controversy.

It has been commonly reported a differential deficit for living and non-living things in which living things show a better performance (53–55). This difference is usually explained by a sensory-functional view, according to which the semantic representations of living things are identified by sensory properties while non-living things are so by functional properties. On the other hand, some authors have not found such effect or, having done it, they have attributed it to methodological issues (56–58). Moreover, they argued that the diffuse pattern of deterioration affecting many cortical regions would not justify a differential loss. Furthermore, the fact that some studies find an advantage of non-living things, or no difference at all, may be explained by the election of the tasks or the heterogeneity of the impairments of people with AD.

There are several factors that could be influencing the categorical deficits, such as word frequency, familiarity, imageability or age of acquisition (59, 60). All those variables are related to performance in several language tasks like naming, reading, or priming. Age of acquisition has been studied as a relevant variable due to the possible similarity to the impairment observed in autobiographical memory. That is, the same way that episodic memory for recent events deteriorates faster than for distant memories, concepts acquired later in life could be more easily affected by neural degeneration while early learned words would be more resilient (61, 62). In this regard, the patterns of deterioration would mirror the acquisition of semantic associative networks in life-span development.

The role of emotion in the processing of concepts is a fairly unexplored factor. Most studies have been conducted with neutral words. However, some recent studies have focused on the affective information of concepts. Although controversial, the idea of the emotional connotation of words having a role on semantic processes is interesting due to its implications. As emotional processes are relatively preserved in the early stages of AD compared with other domains, the affective value of concepts could support the preservation of semantic information and, therefore, it could be useful for communication with the patient. In AD patients, the affective information of concepts is longer preserved than other features (63), which would allow AD patients to retain links between close emotional concepts.

Concreteness is another ignored factor. The previous idea leads to the study of concreteness of concepts because there is an interaction between emotional and abstract ones, as the latter usually refer to internal states of the body. In healthy participants, the concreteness effect refers to a faster and more accurate processing of concrete concepts than abstract ones. In AD, as well as in other neurodegenerative diseases, this effect has been noted to suffer a reversal. Giffard et al. (64) conducted a study in which they compared the processing of different concrete and abstract words that could either have a positive, neutral or negative emotional valence. Their result supports the concreteness effect for neutral concepts, while there is no effect for emotional concepts. This suggest that the emotional component of words is the most relevant feature that binds abstract concepts and influences the reversal of the concreteness effect.

CONCLUSION

Further research on the impairments found in the semantic network of people with AD is required, as most questions concerning the mechanisms that store and retrieve meaning still have to be solved. For instance, the debate about the underlying processes that cause such impairments remains still open. It seems to be a growing agreement that both deterioration of the network and difficulties in retrieval have a role to some extent. It is noteworthy that in recent years, the discussion has moved from the reason why the semantic network becomes disrupted to how it does it. This is the result of an increasing interest in the way the brain processes meaning and stores semantic information. In this regard, Alzheimer's disease can teach us a lot about the pathological and, by extension, normal functioning of the brain, and contribute to increase the understanding of

cognitive processes. At the same time, the priority is to help those who suffer from AD or other neurodegenerative diseases by finding ways to support their abilities and preserve their communication.

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REFERENCES

1. Bastin C, Salmon E. Early neuropsychological detection of Alzheimer's disease. Eur J Clin Nutr. 2014 Nov;68(11):1192–9. http://dx.doi.org/10.1038/ejcn.2014.176
2. Hodges JR, Salmon DP, Butters N. Semantic memory impairment in Alzheimer's disease: Failure of access or degraded knowledge? Neuropsychologia. 1992 Apr;30(4):301–14. http://dx.doi.org/10.1016/0028-3932(92)90104-T
3. Chertkow H, Bub D. Semantic memory loss in dementia of Alzheimer's type. What do various measures measure? Brain J Neurol. 1990 Apr;113(Pt 2):397–417. http://dx.doi.org/10.1093/brain/113.2.397
4. Nebes RD, Martin DC, Horn LC. Sparing of semantic memory in Alzheimer's disease. J Abnorm Psychol. 1984;93(3):321–30. http://dx.doi.org/10.1037/0021-843X.93.3.321
5. Weiner MF, Neubecker KE, Bret ME, Hynan LS. Language in Alzheimer's disease. J Clin Psychiatry. 2008 Aug;69(8):1223–7. http://dx.doi.org/10.4088/JCP.v69n0804
6. Kempler D. Language changes in dementia of the Alzheimer type. Dement Commun. 1995;98–114.
7. Szatloczki G, Hoffmann I, Vincze V, Kalman J, Pakaski M. Speaking in Alzheimer's disease, is that an early sign? Importance of changes in language abilities in Alzheimer's disease. Front Aging Neurosci. 2015;7:195. http://dx.doi.org/10.3389/fnagi.2015.00195
8. Hodges JR, Patterson K, Graham N, Dawson K. Naming and knowing in dementia of Alzheimer's type. Brain Lang. 1996;54(2):302–25. http://dx.doi.org/10.1006/brln.1996.0077
9. Silagi ML, Bertolucci PHF, Ortiz KZ. Naming ability in patients with mild to moderate Alzheimer's disease: What changes occur with the evolution of the disease? Clinics. 2015;70(6):423–8. http://dx.doi.org/10.6061/clinics/2015(06)07
10. Croisile B, Ska B, Brabant M-J, Duchene A, Lepage Y, Aimard G, et al. Comparative study of oral and written picture description in patients with Alzheimer's disease. Brain Lang. 1996;53(1):1–19. http://dx.doi.org/10.1006/brln.1996.0033
11. Mueller KD, Koscik RL, Turkstra LS, Riedeman SK, LaRue A, Clark LR, et al. Connected language in late middle-aged adults at risk for Alzheimer's disease. J Alzheimers Dis JAD. 2016 18;54(4):1539–50. http://dx.doi.org/10.3233/JAD-160252
12. Kemper S, LaBarge E, Ferraro FR, Cheung H, Cheung H, Storandt M. On the preservation of syntax in Alzheimer's disease. Evidence from written sentences. Arch Neurol. 1993 Jan;50(1):81–6. http://dx.doi.org/10.1001/archneur.1993.00540010075021
13. Rousseaux M, Sève A, Vallet M, Pasquier F, Mackowiak-Cordoliani MA. An analysis of communication in conversation in patients with dementia. Neuropsychologia. 2010;48(13):3884–90. http://dx.doi.org/10.1016/j.neuropsychologia.2010.09.026
14. König A, Satt A, Sorin A, Hoory R, Toledo-Ronen O, Derreumaux A, et al. Automatic speech analysis for the assessment of patients with predementia and Alzheimer's disease. Alzheimers Dement Diagn Assess Dis Monit. 2015;1(1):112–24. http://dx.doi.org/10.1016/j.dadm.2014.11.012
15. Martínez-Sánchez F, Meilán JJG, Carro J, Ivanova O. A prototype for the voice analysis diagnosis of Alzheimer's disease. J Alzheimers Dis. 2018;(Preprint):1–9.

16. Garrard P, Maloney LM, Hodges JR, Patterson K. The effects of very early Alzheimer's disease on the characteristics of writing by a renowned author. Brain J Neurol. 2005 Feb;128(Pt 2):250–60. http://dx.doi.org/10.1093/brain/awh341
17. Aretouli E, Okonkwo OC, Samek J, Brandt J. The fate of the 0.5s: Predictors of 2-year outcome in mild cognitive impairment. J Int Neuropsychol Soc JINS. 2011 Mar;17(2):277–88. http://dx.doi.org/10.1017/S1355617710001621
18. Mueller KD, Koscik RL, Hermann BP, Johnson SC, Turkstra LS. Declines in connected language are associated with very early mild cognitive impairment: Results from the Wisconsin registry for Alzheimer's prevention. Front Aging Neurosci. 2018;9:437. http://dx.doi.org/10.3389/fnagi.2017.00437
19. Cuetos F, Arango-Lasprilla JC, Uribe C, Valencia C, Lopera F. Linguistic changes in verbal expression: A preclinical marker of Alzheimer's disease. J Int Neuropsychol Soc. 2007;13(3):433–9. http://dx.doi.org/10.1017/S1355617707070609
20. Amieva H, Jacqmin-Gadda H, Orgogozo J-M, Le Carret N, Helmer C, Letenneur L, et al. The 9 year cognitive decline before dementia of the Alzheimer type: A prospective population-based study. Brain. 2005 May 1;128(5):1093–101. http://dx.doi.org/10.1093/brain/awh451
21. Jones S, Laukka EJ, Bäckman L. Differential verbal fluency deficits in the preclinical stages of Alzheimer's disease and vascular dementia. Cortex. 2006 Jan 1;42(3):347–55. http://dx.doi.org/10.1016/S0010-9452(08)70361-7
22. Adlam A-LR, Bozeat S, Arnold R, Watson P, Hodges JR. Semantic knowledge in mild cognitive impairment and mild Alzheimer's disease. Cortex. 2006 Jan 1;42(5):675–84. http://dx.doi.org/10.1016/S0010-9452(08)70404-0
23. Clark LJ, Gatz M, Zheng L, Chen Y-L, McCleary C, Mack WJ. Longitudinal verbal fluency in normal aging, preclinical, and prevalent Alzheimer's disease. Am J Alzheimers Dis Dementias®. 2009;24(6):461–8. http://dx.doi.org/10.1177/1533317509345154
24. Murphy KJ, Rich JB, Troyer AK. Verbal fluency patterns in amnestic mild cognitive impairment are characteristic of Alzheimer's type dementia. J Int Neuropsychol Soc. 2006;12(4):570–4. http://dx.doi.org/10.1017/S1355617706060590
25. Vaughan RM, Coen RF, Kenny R, Lawlor BA. Semantic and phonemic verbal fluency discrepancy in mild cognitive impairment: Potential predictor of progression to Alzheimer's disease. J Am Geriatr Soc. 2018;66(4):755–9. http://dx.doi.org/10.1111/jgs.15294
26. Rinehardt E, Eichstaedt K, Schinka JA, Loewenstein DA, Mattingly M, Fils J, et al. Verbal fluency patterns in mild cognitive impairment and Alzheimer's disease. Dement Geriatr Cogn Disord. 2014;38(1–2):1–9. http://dx.doi.org/10.1159/000355558
27. Teng E, Leone-Friedman J, Lee GJ, Stephanie W, Apostolova LG, Harrell S, et al. Similar verbal fluency patterns in amnestic mild cognitive impairment and Alzheimer's disease. Arch Clin Neuropsychol. 2013;28(5):400–10. http://dx.doi.org/10.1093/arclin/act039
28. Hodges JR, Salmon DP, Butters N. The nature of the naming deficit in Alzheimer's and Huntington's disease. Brain. 1991;114(4):1547–58. http://dx.doi.org/10.1093/brain/114.4.1547
29. Balthazar MLF, Cendes F, Damasceno BP. Semantic error patterns on the Boston Naming Test in normal aging, amnestic mild cognitive impairment, and mild Alzheimer's disease: Is there semantic disruption? Neuropsychology. 2008 Nov;22(6):703–9. http://dx.doi.org/10.1037/a0012919
30. Lin C-Y, Chen T-B, Lin K-N, Yeh Y-C, Chen W-T, Wang K-S, et al. Confrontation naming errors in Alzheimer's disease. Dement Geriatr Cogn Disord. 2014;37(1–2):86–94. http://dx.doi.org/10.1159/000354359
31. Henry JD, Crawford JR, Phillips LH. Verbal fluency performance in dementia of the Alzheimer's type: A meta-analysis. Neuropsychologia. 2004;42(9):1212–22. http://dx.doi.org/10.1016/j.neuropsychologia.2004.02.001
32. Gomez RG, White DA. Using verbal fluency to detect very mild dementia of the Alzheimer type. Arch Clin Neuropsychol. 2006;21(8):771–5. http://dx.doi.org/10.1016/j.acn.2006.06.012
33. Troyer AK, Moscovitch M, Winocur G, Leach L, Freedman M. Clustering and switching on verbal fluency tests in Alzheimer's and Parkinson's disease. J Int Neuropsychol Soc. 1998;4(2):137–43. http://dx.doi.org/10.1017/S1355617798001374
34. Salmon DP, Butters N, Chan AS. The deterioration of semantic memory in Alzheimer's disease. Can J Exp Psychol Rev Can Psychol Exp. 1999 Mar;53(1):108–17. http://dx.doi.org/10.1037/h0087303

35. Chan AS, Salmon DP, De La Pena J. Abnormal semantic network for "animals" but not "tools" in patients with Alzheimer's disease. Cortex. 2001 Jan 1;37(2):197–217. http://dx.doi.org/10.1016/S0010-9452(08)70568-9
36. Cox DM, Bayles KA, Trosset MW. Category and attribute knowledge deterioration in Alzheimer's disease. Brain Lang. 1996;52(3):536–50. http://dx.doi.org/10.1006/brln.1996.0028
37. Nebes RD, Brady CB. Preserved organization of semantic attributes in Alzheimer's disease. Psychol Aging. 1990;5(4):574–9. http://dx.doi.org/10.1037/0882-7974.5.4.574
38. Perri R, Carlesimo GA, Monaco M, Caltagirone C, Zannino GD. The attribute priming effect in patients with Alzheimer's disease. J Neuropsychol. 2019;13(3):485–502. http://dx.doi.org/10.1111/jnp.12168
39. Giffard B, Desgranges B, Nore-Mary F, Lalevée C, Beaunieux H, De La Sayette V, et al. The dynamic time course of semantic memory impairment in Alzheimer's disease: Clues from hyperpriming and hypopriming effects. Brain. 2002;125(9):2044–57. http://dx.doi.org/10.1093/brain/awf209
40. Giffard B, Desgranges B, Nore-Mary F, Lalevée C, De La Sayette V, Pasquier F, et al. The nature of semantic memory deficits in Alzheimer's disease: New insights from hyperpriming effects. Brain. 2001;124(8):1522–32. http://dx.doi.org/10.1093/brain/124.8.1522
41. Perri R, Zannino GD, Caltagirone C, Carlesimo GA. Semantic priming for coordinate distant concepts in Alzheimer's disease patients. Neuropsychologia. 2011 Apr;49(5):839–47. http://dx.doi.org/10.1016/j.neuropsychologia.2011.02.035
42. Laisney M, Giffard B, Belliard S, de la Sayette V, Desgranges B, Eustache F. When the zebra loses its stripes: Semantic priming in early Alzheimer's disease and semantic dementia. Cortex. 2011;47(1):35–46. http://dx.doi.org/10.1016/j.cortex.2009.11.001
43. Rogers SL, Friedman RB. The underlying mechanisms of semantic memory loss in Alzheimer's disease and semantic dementia. Neuropsychologia. 2008 Jan 15;46(1):12–21. http://dx.doi.org/10.1016/j.neuropsychologia.2007.08.010
44. Haugrud N, Crossley M, Vrbancic M. Clustering and switching strategies during verbal fluency performance differentiate Alzheimer's disease and healthy aging. J Int Neuropsychol Soc. 2011;17(6):1153–7. http://dx.doi.org/10.1017/S1355617711001196
45. Rohrer D, Wixted JT, Salmon DP, Butters N. Retrieval from semantic memory and its implications for Alzheimer's disease. J Exp Psychol Learn Mem Cogn. 1995 Sep;21(5):1127–39. http://dx.doi.org/10.1037//0278-7393.21.5.1127
46. Chan AS, Butters N, Salmon DP, McGuire KA. Dimensionality and clustering in the semantic network of patients with Alzheimer's disease. Psychol Aging. 1993;8(3):411–19. http://dx.doi.org/10.1037/0882-7974.8.3.411
47. Garrard P, Lambon Ralph MA, Patterson K, Pratt KH, Hodges JR. Semantic feature knowledge and picture naming in dementia of Alzheimer's type: A new approach. Brain Lang. 2005 Apr 1;93(1):79–94. http://dx.doi.org/10.1016/j.bandl.2004.08.003
48. Chertkow H, Bub D, Bergman H, Bruemmer A, Merling A, Rothfleisch J. Increased semantic priming in patients with dementia of the Alzheimer's type. J Clin Exp Neuropsychol. 1994 Aug;16(4):608–22. http://dx.doi.org/10.1080/01688639408402672
49. Nakamura H, Nakanishi M, Hamanaka T, Nakaaki S, Yoshida S. Semantic priming in patients with Alzheimer and semantic dementia. Cortex. 2000;36(2):151–62. http://dx.doi.org/10.1016/S0010-9452(08)70521-5
50. Bell EE, Chenery HJ, Ingram JCL. Semantic priming in Alzheimer's dementia: Evidence for dissociation of automatic and attentional processes. Brain Lang. 2001;76(2):130–44. http://dx.doi.org/10.1006/brln.2000.2416
51. Mascali D, DiNuzzo M, Serra L, Mangia S, Maraviglia B, Bozzali M, et al. Disruption of semantic network in mild Alzheimer's disease revealed by resting-state fMRI. Neuroscience. 2018 Feb 10;371:38–48. http://dx.doi.org/10.1016/j.neuroscience.2017.11.030
52. Salehi M, Reisi M, Ghasisin L. Lexical retrieval or semantic knowledge? Which one causes naming errors in patients with mild and moderate Alzheimer's disease? Dement Geriatr Cogn Disord Extra. 2017 Dec;7(3):419–29. http://dx.doi.org/10.1159/000484137
53. Silveri MC, Daniele A, Giustolisi L, Gainotti G. Dissociation between knowledge of living and nonliving things in dementia of the Alzheimer type. Neurology. 1991;41(4):545. http://dx.doi.org/10.1212/WNL.41.4.545

54. Passafiume D, Federicis LSD, Carbone G, Giacomo DD. Loss of semantic associative categories in patients with Alzheimer's disease. Appl Neuropsychol Adult. 2012 Oct 1;19(4):305–11. http://dx.doi.org/10.1080/09084282.2012.670160
55. Peraita H, Díaz C, Anllo-Vento L. Processing of semantic relations in normal aging and Alzheimer's disease. Arch Clin Neuropsychol. 2008 Jan 1;23(1):33–46. http://dx.doi.org/10.1016/j.acn.2007.09.001
56. Laws KR, Gale TM, Leeson VC, Crawford JR. When is category specific in Alzheimer's disease? Cortex. 2005 Jan 1;41(4):452–63. http://dx.doi.org/10.1016/S0010-9452(08)70186-2
57. Moreno-Martínez FJ, Goñi-Imízcoz M, Spitznagel MB. Domain or not domain? That is the question: Longitudinal semantic deterioration in Alzheimer's disease. Brain Cogn. 2011 Oct 1;77(1):89–95. http://dx.doi.org/10.1016/j.bandc.2011.05.006
58. Tippett LJ, Meier SL, Blackwood K, Diaz-Asper C. Category specific deficits in Alzheimer's disease: Fact or artefact? Cortex. 2007 Jan 1;43(7):907–20. http://dx.doi.org/10.1016/S0010-9452(08)70690-7
59. Gainotti G, Di Betta AM, Silveri MC. The production of specific and generic associates of living and nonliving, high- and low-familiarity stimuli in Alzheimer's disease. Brain Lang. 1996 Aug 1; 54(2):262–74. http://dx.doi.org/10.1006/brln.1996.0075
60. Thompson-Schill SL, Gabrieli JDE, Fleischman DA. Effects of structural similarity and name frequency on picture naming in Alzheimer's disease. J Int Neuropsychol Soc. 1999;5(7):659–67. http://dx.doi.org/10.1017/S1355617799577084
61. Holmes S, Jane Fitch F, Ellis A. Age of acquisition affects object recognition and naming in patients with Alzheimer's disease. J Clin Exp Neuropsychol. 2006;28(6):1010–22. http://dx.doi.org/10.1080/13803390591004392
62. Silveri MC, Cappa A, Mariotti P, Puopolo M. Naming in patients with Alzheimer's disease: Influence of age of acquisition and categorical effects. J Clin Exp Neuropsychol. 2002;24(6):755–64. http://dx.doi.org/10.1076/jcen.24.6.755.8407
63. Giffard B, Laisney M, Eustache F, Desgranges B. Can the emotional connotation of concepts modulate the lexico-semantic deficits in Alzheimer's disease? Neuropsychologia. 2009 Jan 1;47(1):258–67. http://dx.doi.org/10.1016/j.neuropsychologia.2008.07.013
64. Giffard B, Laisney M, Desgranges B, Eustache F. An exploration of the semantic network in Alzheimer's disease: Influence of emotion and concreteness of concepts. Cortex. 2015;69:201–11. http://dx.doi.org/10.1016/j.cortex.2015.05.020

12

Alzheimer's Disease: Memory Interference and the Role of Exercise

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Abstract: Alzheimer's disease is an irreversible, progressive brain disorder that damages memory, behavioral, and cognitive skills. This condition causes brain cells to degenerate and die leading to many cognitive issues. Although the exact cause is unknown, it is thought to be due to a combination of genetic, lifestyle, and environmental factors. Due to its progressive nature, symptoms can vary from mild memory loss to complete lack of ability to respond to one's surroundings. The memory impairments brought on by this disease can lead to specific problems with memory interference, which may be caused by dysfunction in working and semantic memory. When conducting experiments on Alzheimer's patients, there is also the added difficulty of the individual having trouble remembering the instructions and needing external cues to complete memory tasks. This chapter outlines the disease, its symptoms, risk factors, how it affects memory, and how exercise may be a prevention and treatment option.

Keywords: Alzheimer's disease; memory; memory interference; proactive interference; retroactive interference

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INTRODUCTION

Alzheimer's disease is an irreversible, progressive brain disorder which damages memory, behavioral, and cognitive skills (1–4). This condition causes brain cells to degenerate and die, leading to many cognitive issues (4). It is the most common cause of dementia, accounting for 60 to 80% of dementia cases (2). Although the exact cause is unknown, it is thought to be due to a combination of genetic, lifestyle, and environmental factors (2). Due to its progressiveness, there are different signs and symptoms throughout the stages (1, 5). Typically, Alzheimer's disease progresses through stages, including mild (early stage), moderate (middle stage), and severe (late stage) (6). Since the condition affects people in different ways, individuals may have a unique experience to each stage. In the early stage, an individual may experience mild memory loss or difficulty remembering newly learned information, whereas in the late stage, individuals may lose the ability to function independently or respond to their surroundings (2, 5). Early-stage signs include forgetting names, appointments, recent events, trouble following instructions, keeping track of responsibilities (e.g., remembering to pay the bills), or difficulty using familiar items in their home (e.g., how to operate the thermostat) (1, 2, 4–7). The middle stage is usually the longest stage in the disease progression and involves more serious signs of the first stage (6). An individual may have greater difficulty performing certain tasks and be more likely to become frustrated or withdrawn due to memory impairments (6). Signs of middle stage Alzheimer's disease include repeating statements or questions, routinely misplacing objects, getting lost in familiar places, losing track of the passing of time (e.g., forgetting which season it is), decreased or poor judgment, withdrawal from work or social activities, or changes in mood and personality (1, 2, 4–6). Personality and behavior changes often include depression, apathy, social withdrawal, mood swings, distrust, irritability, aggression, wandering, or delusions (4). Late stage is the final stage of Alzheimer's disease. The dementia symptoms are severe and individuals lose the ability to function independently or to react appropriately to their environment (6). During this stage, individuals may also lose their ability to control their motor movement and they will likely experience severe personality changes (6). At this stage, it is likely that the individual will need a full-time caregiver (6). Late-stage patients also become vulnerable to infections and other conditions due to memory impairment (e.g., dehydration, malnourishment) (6). Alzheimer's disease is the sixth leading cause of death in the United States, and on average, individuals live for 4 to 8 years after diagnosis (1). It is possible for people with Alzheimer's disease to live as long as 20 years post-diagnosis, but it is less common since it mostly occurs in individuals who are already 65 years or older (1).

RISK FACTORS

The greatest known risk factors for developing Alzheimer's disease is increasing age, as most individuals with Alzheimer's disease are 65 years or older (2). Another risk factor is family history (8). Individuals that have parents or siblings that have developed Alzheimer's disease are more likely to develop the disease (4, 8).

The risk increases as members of the family with a diagnosis increase. Genetics also play a role in developing Alzheimer's themselves (4, 8). Genes for developing Alzheimer's disease have been found to be both deterministic and risk genes, meaning that they both cause the disease and increase the risk of developing the disease (4, 8). The deterministic gene, which almost guarantees the individual will develop the disease, only occurs in less than 1% of the Alzheimer's cases (4). Experiencing a head injury is also a possible risk factor for Alzheimer's disease (8). The most effective ways to prevent head injury are wearing seatbelt in any motor vehicle, wearing helmets, and "fall-proofing" homes for the elderly (8). An example of fall-proofing is placing non-slip mats in strategic areas of the home, especially in the bathroom and in the shower.

Sex, a common risk factor for many diseases (e.g., breast cancer), is not a risk factor for Alzheimer's disease (8). More women than men are diagnosed with Alzheimer's disease mainly due to the fact that women live longer than men – making them more likely to develop Alzheimer's disease (8). Race may also play a role. Latinos and African Americans are more likely than Whites to develop Alzheimer's disease. This is not well understood, but it may be due to their increased rates of vascular disease (4, 8). Heart health and brain health are directly related, as dementia is associated with conditions that damage the heart and blood vessels (e.g., heart disease, stroke, high blood pressure, and high cholesterol) (4, 8). There is also evidence that plaques and tangles (physical evidence of Alzheimer's disease in the brain) are more likely to cause symptoms of dementia if damage to the brain's blood vessels is also apparent (4, 8).

Other lifestyle risk factors include lack of exercise, obesity, smoking, and poorly controlled type 2 diabetes (4, 8). All of these conditions are related to poor heart and brain health and can be modified by living a healthy active lifestyle that focuses on a whole food plant based diet (8). The Physician's Committee for Responsible Medicine recommends exercising regularly, limiting saturated and trans fats, eating plant based foods, eating foods rich in vitamin E, and taking a B12 supplement daily to prevent Alzheimer's disease (9). There is an increased risk for developing Alzheimer's disease in individuals with Down syndrome, which is most likely due to having three copies of chromosome 21, which also includes having three copies of the gene that is associated with Alzheimer's disease (8). Symptoms of Alzheimer's disease usually appear 10 to 20 years earlier in people with Down syndrome compared to individuals without it (8).

EARLY-ONSET ALZHEIMER'S DISEASE

When the condition occurs in individuals younger than 65, it is referred to as early-onset Alzheimer's disease (2). Early-onset Alzheimer's disease is less common, making up less than 5% of the population with Alzheimer's disease (around 200,000 people in the United States), and primarily affects people in their 40s and 50s (3). Since memory complications are less common in those under 65, it can be difficult to diagnosis early-onset Alzheimer's disease. The cause of early-onset is unclear, but there have been a few rare inherited genes that may play a factor in symptoms developing as early as 30 years old, referred to as "familial Alzheimer's disease" (3).

TREATMENT

There is no cure for Alzheimer's disease, but treatments for symptoms are available as research into the condition continues (2). These drug or non-drug treatments may relieve some symptoms or slow the rate of mental decline. Their goal is to increase quality of life for the individuals and their caregivers (10).

Drug treatment

Drug options to help with memory loss include cholinesterase inhibitors and memantine, which treat cognitive symptoms (memory loss, confusion, etc.) (10). These medications may not prevent the degradation of neuronal cells, but they can slow the speed at which they degenerate (10). They are usually well tolerated by patients, and side effects may include nausea, vomiting, and loss of appetite (10). The stage of disease will determine the dosage and type of medication prescribed (10).

Behavioral treatment

Behavioral treatment addresses behavior problems that may arise, such as irritability, anxiety, and depression (7). These symptoms may lead to difficulties caring for an individual with Alzheimer's disease, especially for the caregiver (7). Avoiding drastic changes is an imperative factor of behavioral treatment (7). Change can be stressful and increase the individual's fear and frustration as they are trying to make sense of the situation with their impaired cognitive function (7). Situations that may trigger frustration include moving to a new residence, admission to hospitals, or being asked to alter appearance (7). Sometimes, medications can increase these symptoms of fear and anxiety (7). As a caregiver, behavioral treatment can include avoiding confrontation with the individual, redirecting their attention, creating a calm environment, and allowing the individual adequate rest (7). These behaviors can not only prevent triggering episodes but also make the individual feel more at home. There are also medications that can assist with behavior modification if behavioral treatment is ineffective, including antidepressants for low mood, antipsychotics for delusions and aggression, and anxiolytics for anxiety or restlessness (7). The use of antipsychotics for Alzheimer's disease is a very hazardous option, as it has been associated with an increased risk of stroke and death in older adults with dementia (7).

MEMORY

Our memories define our character and have a completely unique perspective than everyone else's experiences. Creating a memory involves three stages. The first, encoding, occurs when a stimulus results in the formation of a new memory (11–13). This new formation is often referred to as an engram, which is thought to be a physical memory trace in the brain (14). This trace is very susceptible to decay until the next stage occurs, consolidation (14). Consolidation is the process in which a memory becomes stable and is assimilated into previously acquired knowledge (14). The final stage, retrieval, occurs when the memory is recollected (14).

Sometimes when we create a memory, it only lasts for a short period of time, when other memories last a lifetime (15, 16). Memories are temporally defined as long term or short term depending on the length of time the memory lasts. Short-term memories last less than 2 min, whereas long term memories can last 2 min to a lifetime (15, 16). Short-term memories usually are comprised of working memory (15, 16). Working memory typically involves small amounts of information that we only need to retain for a couple seconds, for example, the prices two items when comparing costs while shopping.

Types of memory

There are multiple types of memory and subdivisions within each type. The main two types are explicit (declarative) and implicit (nondeclarative) (15). When a memory is explicit, the individual is consciously aware of the memory, whereas with implicit memory, the individual is not (15). There are two subsets within explicit memory: episodic and semantic. Episodic, or autobiographical memories, include a what, where, and when aspect of the memory (16–18). Semantic memories are facts about the world around us (15). An example of semantic memory may be knowing that baseball is a sport, whereas an episodic memory may be remembering the first time you went to a baseball game. Implicit memories also have two subdivisions: procedural and priming (15). Procedural memories include motor skills and other actions we complete automatically without conscious thought (e.g., walking and writing) (15). Priming occurs when an individual is exposed to a stimulus that influences their response to a later stimulus (15). An example of priming may be seeing a flash of an image on a computer while taking a computer-based memory task.

Forgetting

Why are some memories retained yet others are lost? There are many reasons that we forget information we have learned or events we have experienced (19–21). The act of forgetting can occur actively or passively. Passive forgetting occurs through natural decay, or biological degradation, of neurons within a memory engram (19). Partial decay of an engram can make it challenging to activate the memory during retrieval (19). Active forgetting can occur through several mechanisms: interference, motivated forgetting, or retrieval-induced forgetting (19). Interference, which will be detailed later in this chapter, occurs when competing information makes it difficult to retrieve the correct memory (19). Motivated forgetting often occurs when an individual actively suppresses a memory due to some unpleasant quality (e.g., guilt, shame, and embarrassment) (19). Finally, retrieval-induced forgetting occurs when only parts of a memory are normally recalled, causing the other parts to degrade over time (19).

Memory Interference

As stated in the previous section, memory interference (MI) is a cause of forgetting. There are two types of MI, proactive and retroactive. Proactive interference (PI)

occurs when previously acquired information interrupts the recall of newly learned information (old → new). For example, calling your new boyfriend by your old boyfriend's name. Retroactive interference (RI) occurs in the opposite direction, newly acquired information interrupts the recall of old information (old ← new). Following the previous example, this would be calling your old boyfriend by your new boyfriend's name. Interference can be benign to serious memory disruption depending on the situation. Interference is also linked to similarity of the content. If the competing material is similar, interference is more likely to occur.

In experiments, MI is measured using paired associate learning tasks. These tasks are typically comprised of lists of word pairs or figure pairs (e.g., "bread knife" or ♦♣) that a participant is asked to memorize as a pair. Research participants memorize lists of the word or figure pairs and subsequently recall them (e.g., bread - ___ or ♦ - ___). There are multiple models of paired associate tasks, but ones commonly used include AB–CD, AB–AC, AB–ABr, and AB–DE AC–FG. For the models, the letter pairs (e.g., AB and CD) signify one list each (e.g., AB = List 1, CD = List 2), each letter in the name (e.g., AB–CD) stands for one word (A = bread B = knife), and the combined letters (e.g., AB) represent one word-pair (breadknife). Examples of measuring MI using various models are summarized in tables 1–4.

Alzheimer's disease and MI

Alzheimer's disease causes the decay of neurons which eventually leads to memory impairment (1, 4, 5, 9). Although there are a lot of research fields focusing on how Alzheimer's disease damages memory, there is less research focusing directly on MI effects on patients with Alzheimer's disease.

When investigating patients with Alzheimer's disease and those with mild cognitive impairments without Alzheimer's disease, Dewer et al. found that memory retention is much higher in these patients when there is minimal interference compared to a normal MI paradigm (22). Their findings align with previous literature

TABLE 1 **AB–CD example**

List 1 (AB)	List 2 (CD)
BABY HUNTER	SPIDER CANDLE
SUPPER SHERIFF	ARROW THEATER
WEDDING MOVIE	CHERRY MONEY
APPLE DIAMOND	TIGER HOTEL
MONKEY GARDEN	CANNON HAMMER
FOREST BATTLE	LADY BUTTER

In this model, AB signifies the first list and CD the second list. There are no repeating letters in the title of the model, meaning there are no repeating words within the lists. Each list consists of unique words with no overlap. Participants may be exposed to both lists (learn List 1 then List 2) and then asked to recall only one of them. To measure PI, the participants will learn List 1 (AB), List 2 (CD), then recall List 2. For RI, the participants will learn List 1 (AB), List 2 (CD), then recall List 1.

TABLE 2 AB–AC example

List 1 (AB)	List 2 (AC)
BABY HUNTER	MOVIE WEDDING
SUPPER SHERIFF	APPLE CANNON
MONKEY GARDEN	BABY SALAD
FOREST BATTLE	MONKEY ENGINE
MOVIE CHERRY	FOREST CITY
APPLE TIGER	SUPPER JACKET

Similar to the previous model AB–CD, this model's first list (AB) has no repeating words, but as we can see "AC" has a repeating "A" word. This signifies that the "A" words from List 1 (AB) and List 2 (AC) will repeat, while the "B" and "C" words will not. When testing for PI, the participant will learn List 1, List 2, and then recall List 2. For RI, the participant will learn List 1, List 2, and then recall List 1.

TABLE 3 AB–ABr example

List 1 (AB)	List 2 (ABr)
BABY HUNTER	SHERIFF CHERRY
SUPPER SHERIFF	DIAMOND BABY
WEDDING MOVIE	BATTLE SUPPER
SPIDER CANDLE	FOREST HUNTER
MONKEY GARDEN	MONEY MOVIE
FOREST BATTLE	SPIDER GARDEN
CHERRY MONEY	APPLE WEDDING
APPLE DIAMOND	CANDLE MONKEY

This model repeats all of the words from List 1 (AB) in the second list (ABr) except the words are rearranged into new word pairs. To reiterate, List 1 and List 2 comprised of the same words, but the way in which they are organized is different for List 2. This model can cause severe interference since the words are so similar. Participants learn List 1, List 2, then recall either List 1 or List 2 depending on the interference being measured.

demonstrating that memory dysfunction in patients with Alzheimer's disease is associated with an increased susceptibility to MI (22). The authors hypothesize that this may be due to a decline in the ability to consolidate new memories (22). The interference paradigm utilized in this experiment is strong at predicting which patients with mild cognitive impairments will or will not progress to Alzheimer's disease within 2 years, with 80% sensitivity and 100% specificity (22).

In term of semantic memory, or facts about the world (e.g., baseball is a sport), patients with Alzheimer's disease perform worse on these memory tasks, which may be due to a deficit in working memory and attention (23). As stated previously, working memory is short-term memory that lasts for a very short period of time with a concurrent interfering stimulus. Hartman describes how, in her experiment, there was no evidence that the patients with Alzheimer's disease utilized

TABLE 4 **AB–DE AC–FG example**

List 1 (AB, DE)	Cued recall 1 A__, D__	List 2 (AC, FG)	Cued recall 2 A__, F__	MMFR A__ __ D__ __ F__ __
BABY HUNTER	SPIDER _____	FOREST CITY	ARROW _____	BABY ___ ___
SUPPER SHERIFF	FOREST _____	ARROW THEATER	FOREST _____	CHERRY ___ ___
WEDDING MOVIE	BABY _____	BABY SALAD	TIGER _____	ARROW ___ ___
SPIDER CANDLE	CHERRY _____	TIGER HOTEL	SUPPER _____	SUPPER ___ ___
MONKEY GARDEN	MONKEY _____	MONKEY ENGINE	LADY _____	TIGER ___ ___
FOREST BATTLE	SUPPER _____	LADY BUTTER	CANNON ____	WEDDING ___ ___
CHERRY MONEY	APPLE _____	CANNON HAMMER	BABY _____	MONKEY ___ ___
APPLE DIAMOND	WEDDING ____	SUPPER JACKET	MONKEY _____	LADY ___ ___
				SPIDER ___ ___
				FOREST ___ ___
				APPLE ___ ___
				CANNON ___ ___

This paired associate task is more complex than the other designs because it includes control word pairs within each list, allowing for the measurement of proactive and RI within the same experiment. As before, List 1 (AB–DE) has repeating "A" words as List 2 (AC–FG). In this case, DE and FG are the control word pairs and AB and AC are the interfering word pairs. To use this model for an experiment, participants learn List 1, recall it, learn List 2, recall it, then recall the Modified Modified Free Recall (MMFR) list which is comprised of all of the word pairs from List 1 and List 2 in a pseudorandomized order.

semantic knowledge (relatedness) of word pairs during recall (23). When patients' working memory is impaired, as is typical in Alzheimer's disease, it is more difficult to retain relevant information about the relationships of words in paired associate tasks (23). Despite this experiment's focus on working memory, its results shed light on MI impairments since semantically relating words is a typical strategy utilized when memorizing word pairs in paired associate tasks. When detailing the symptoms of Alzheimer's disease, we noted that difficulty remembering and following instructions is common (23). This may also influence performance on memory tasks. Repeatedly needing external cues or verbal instructions may alter outcome scores, as mentioned elsewhere (23). Another study that focused specifically on proactive and RI compared mildly demented Alzheimer's disease patients, patients with mild cognitive impairment without Alzheimer's disease, and healthy elderly patients on interference tasks (24). When controlling for

overall memory impairment, mild Alzheimer's disease patients demonstrated higher rates of PI, but equal amounts of RI when compared to the cognitively impaired patients (24). As expected, the healthy elderly participants experienced the least amount of interference (24). Vulnerability to semantic interference may reflect early signs of the onset of Alzheimer's disease (24).

EXERCISE AND ALZHEIMER'S DISEASE

As stated previously, physical inactivity is a risk factor for developing Alzheimer's disease and is even considered the highest population attributable risk (25). In one systematic review, the majority of experiments demonstrated that physical activity was inversely associated with risk of developing Alzheimer's disease (26). Exercise has also been demonstrated to improve multiple types of memory, including long-term and short-term memory (27–33). Exercise may even prevent the onset of Alzheimer's disease by decreasing the risk of cardiovascular disease, increasing cerebral blood flow, increasing hippocampal volume, and improving neurogenesis (34). Higher levels of physical activity are associated with a reduced risk of developing the disease (34), with long-term prospective studies demonstrating that walking regularly is associated with a twofold reduced risk in cognitive impairment (25).

During exercise, many chemicals are released in the body, including brain-derived neurotrophic factor, which is directly associated with learning and memory (35). Research also demonstrates that regular physical activity prevents mental decline and improves thinking in populations with vascular cognitive impairment (34). It has been used clinically in the treatment of preclinical and late-stage Alzheimer's disease, as well as a prevention strategy (34). Recent prospective work, that compared sedentary individuals to those who were the most active, demonstrated a 38% reduction in incidence of Alzheimer's disease (36). In animal studies, mice with Alzheimer's disease that completed 16 weeks of treadmill exercise have been shown to elicit changes in therapeutic parameters at the cellular and molecular level, providing biological plausibility to exercise as a therapy (37).

In order to reduce the risk of developing Alzheimer's disease, to lessen the effects for those already suffering from memory loss, individuals should participate in regular physical activity. The American national guidelines suggest at least 150 min per week of moderate to vigorous physical activity (38). Meta-analyses have demonstrated mixed findings on which mode of exercise is best for improving specific types of memory; however, walking, cycling, and jogging are three of the most common exercises implemented, all of which have demonstrated beneficial effects (33, 39). Multicomponent exercise programs have also been effective in improving cognitive function in institutionalized older adults with mild to moderate Alzheimer's disease (40). One particular study found that incorporating a program that included supervised aerobic, muscular resistance, flexibility, and postural exercises for 45–55 min sessions twice per week for 6 months significantly improved patients' cognitive function when compared to a control group (40). These findings suggest that incorporating a variety of physical activities may be an effective non-pharmacological method for improving cognitive function, along with physical function, in those with Alzheimer's disease (40).

CONCLUSION

In summary, individuals with Alzheimer's disease may be more susceptible to MI, due to dysfunction of working memory and semantic memory. This can cause even more confusion, as patients already cope with a myriad of memory problems and other symptoms. The literature on Alzheimer's disease and MI is sparse, and therefore, it is imperative that the field continues to grow and search for methods to attenuate interference in this population – for example, through exercise, which has been demonstrated to increase memory performance. Experiencing MI, or difficulty recalling information because of competing memories, can be very frustrating and debilitating for anyone, but especially for patients with Alzheimer's disease. In experimental settings, paired associate tasks are often utilized to measure interference, but there are also other methods that are suitable. Due to the debilitating nature of Alzheimer's disease, it is important to focus on prevention and delaying the condition by keeping your mind and body active, eating a healthy diet, and wearing safety equipment to avoid head injury.

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REFERENCES

1. Aging NIo. Alzheimer's disease & related dementias [Internet]. Available from: https://www.nia.nih.gov/health/alzheimers/basics
2. Association As. What is Alzheimer's? [Internet]. Available from: https://www.alz.org/alzheimers-dementia/what-is-alzheimers#basics
3. Association As. Younger/early onset [Internet]. Available from: https://www.alz.org/alzheimers-dementia/what-is-alzheimers/younger-early-onset
4. Clinic M. Alzheimer's disease [Internet]. Available from: https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447
5. Association As. 10 early signs and symptoms of Alzheimer's [Internet]. Available from: https://www.alz.org/alzheimers-dementia/10_signs
6. Association As. Stages of Alzheimer's [Internet]. Available from: https://www.alz.org/alzheimers-dementia/stages
7. Association As. Treatments for behavior [Internet]. Available from: https://www.alz.org/alzheimers-dementia/treatments/treatments-for-behavior
8. Association As. Causes and risk factors [Internet]. Available from: https://www.alz.org/alzheimers-dementia/what-is-alzheimers/causes-and-risk-factors
9. Medicine PCfR. Alzheimer's disease [Internet]. Available from: https://www.pcrm.org/health-topics/alzheimers
10. Association As. Medications for memory [Internet]. Available from: https://www.alz.org/alzheimers-dementia/treatments/medications-for-memory
11. Puglisi JT, Park DC, Smith AD, Dudley WN. Age differences in encoding specificity. J Gerontol. 1988;43(6):P145–50. http://dx.doi.org/10.1093/geronj/43.6.P145

12. Stickgold R, Walker MP. Sleep-dependent memory consolidation and reconsolidation. Sleep Med. 2007;8(4):331–43. http://dx.doi.org/10.1016/j.sleep.2007.03.011
13. McGaugh JL. Memory – A century of consolidation. Science. 2000;287(5451):248–51. http://dx.doi.org/10.1126/science.287.5451.248
14. Loprinzi PD, Edwards MK, Frith E. Potential avenues for exercise to activate episodic memory-related pathways: A narrative review. Eur J Neurosci. 2017;46(5):2067–77. http://dx.doi.org/10.1111/ejn.13644
15. Institute QB. Types of memory The University of Queensland 2018, July 23 [Internet]. Available from: https://qbi.uq.edu.au/brain-basics/memory/types-memory
16. Moscovitch M, Cabeza R, Winocur G, Nadel L. Episodic memory and beyond: The hippocampus and neocortex in transformation. Annu Rev Psychol. 2016;67:105–34. http://dx.doi.org/10.1146/annurev-psych-113011-143733
17. Scully ID, Napper LE, Hupbach A. Does reactivation trigger episodic memory change? A meta-analysis. Neurobiol Learn Mem. 2017;142(Pt A):99–107. http://dx.doi.org/10.1016/j.nlm.2016.12.012
18. Shields GS, Sazma MA, McCullough AM, Yonelinas AP. The effects of acute stress on episodic memory: A meta-analysis and integrative review. Psychol Bull. 2017;143(6):636–75. http://dx.doi.org/10.1037/bul0000100
19. Davis RL, Zhong Y. The biology of forgetting – A perspective. Neuron. 2017;95(3):490–503. http://dx.doi.org/10.1016/j.neuron.2017.05.039
20. Aguirre C, Gomez-Ariza CJ, Andres P, Mazzoni G, Bajo MT. Exploring mechanisms of selective directed forgetting. Front Psychol. 2017;8:316. http://dx.doi.org/10.3389/fpsyg.2017.00316
21. Anderson MC, Hanslmayr S. Neural mechanisms of motivated forgetting. Trends Cogn Sci. 2014;18(6):279–92. http://dx.doi.org/10.1016/j.tics.2014.03.002
22. Dewar M, Pesallaccia M, Cowan N, Provinciali L, Della Sala S. Insights into spared memory capacity in amnestic MCI and Alzheimer's disease via minimal interference. Brain Cogn. 2012;78(3):189–99. http://dx.doi.org/10.1016/j.bandc.2011.12.005
23. Hartman M. The use of semantic knowledge in Alzheimer's disease: Evidence for impairments of attention. Neuropsychologia. 1991;29(3):213–28. http://dx.doi.org/10.1016/0028-3932(91)90083-K
24. Loewenstein DA, Acevedo A, Luis C, Crum T, Barker WW, Duara R. Semantic interference deficits and the detection of mild Alzheimer's disease and mild cognitive impairment without dementia. J Int Neuropsychol Soc. 2004;10(1):91–100. http://dx.doi.org/10.1017/S1355617704101112
25. Loprinzi PD, Frith E, Ponce P. Memorcise and Alzheimer's disease. Phys Sports Med. 2018;46(2):145–54. http://dx.doi.org/10.1080/00913847.2018.1445932
26. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: An analysis of population-based data. Lancet Neurol. 2014;13(8):788–94. http://dx.doi.org/10.1016/S1474-4422(14)70136-X
27. Angevaren M, Aufdemkampe G, Verhaar HJ, Aleman A, Vanhees L. Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. Cochrane Database Syst Rev. 2008;(3):CD005381. http://dx.doi.org/10.1002/14651858.CD005381.pub3
28. Chang YK, Labban JD, Gapin JI, Etnier JL. The effects of acute exercise on cognitive performance: A meta-analysis. Brain Res. 2012;1453:87–101. http://dx.doi.org/10.1016/j.brainres.2012.02.068
29. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: A meta-analytic study. Psychol Sci. 2003;14(2):125–30. http://dx.doi.org/10.1111/1467-9280.t01-1-01430
30. Cotman CW, Berchtold NC. Exercise: A behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 2002;25(6):295–301. http://dx.doi.org/10.1016/S0166-2236(02)02143-4
31. McMorris T, Hale BJ. Differential effects of differing intensities of acute exercise on speed and accuracy of cognition: A meta-analytical investigation. Brain Cogn. 2012;80(3):338–51. http://dx.doi.org/10.1016/j.bandc.2012.09.001
32. McMorris T, Sproule J, Turner A, Hale BJ. Acute, intermediate intensity exercise, and speed and accuracy in working memory tasks: A meta-analytical comparison of effects. Physiol Behav. 2011;102(3–4):421–8. http://dx.doi.org/10.1016/j.physbeh.2010.12.007
33. Roig M, Nordbrandt S, Geertsen SS, Nielsen JB. The effects of cardiovascular exercise on human memory: A review with meta-analysis. Neurosci Biobehav Rev. 2013;37(8):1645–66. http://dx.doi.org/10.1016/j.neubiorev.2013.06.012

34. Cass SP. Alzheimer's disease and exercise: A literature review. Curr Sports Med Rep. 2017;16(1): 19–22. http://dx.doi.org/10.1249/JSR.0000000000000332
35. Loprinzi PD, Frith E. A brief primer on the mediational role of BDNF in the exercise-memory link. Clin Physiol Funct Imaging. 2019;39(1):9–14. http://dx.doi.org/10.1111/cpf.12522
36. Guure CB, Ibrahim NA, Adam MB, Said SM. Impact of physical activity on cognitive decline, dementia, and its subtypes: Meta-analysis of prospective studies. Biomed Res Int. 2017;2017:9016924. http://dx.doi.org/10.1155/2017/9016924
37. Um HS, Kang EB, Leem YH, Cho IH, Yang CH, Chae KR, et al. Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. Int J Mol Med. 2008;22(4):529–39.
38. Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, et al. The physical activity guidelines for Americans. JAMA. 2018;320(19):2020–8. http://dx.doi.org/10.1001/jama.2018.14854
39. Loprinzi PD, Blough J, Crawford L, Ryu S, Zou L, Li H. The temporal effects of acute exercise on episodic memory function: Systematic review with meta-analysis. Brain Sci. 2019;9(4):pii: E87. http://dx.doi.org/10.3390/brainsci9040087
40. Sampaio A, Marques EA, Mota J, Carvalho J. Effects of a multicomponent exercise program in institutionalized elders with Alzheimer's disease. Dementia (London). 2019;18(2):417–31. http://dx.doi.org/10.1177/1471301216674558

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Sense of Self among Persons with Advanced Dementia

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Abstract: As humans, we have a sense of self, and at best, we are proud of our abilities and feel respected by other persons. Persons with dementia have been regarded losing their self. Quantitative research has shown that this is true, while qualitative research has shown that parts of self are severely affected while other parts remain even among persons with advanced dementia. These persons sometimes keep feeling "still the same" as before getting dementia. Their memory deficits help as does support from other persons. The theory of three aspects of self by the psychologists Rom Harré and Steven Sabat are presented, that is, the feeling that we are, who we are, and who we are together with other persons. Based on empirical research, suggestions will be given about how by promoting experiences of at-homeness, dignity, and being oneself related to others we can help persons with advanced dementia experience themselves as valuable persons.

Keywords: advanced dementia; anosognosia; confirmation; dignity; self

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INTRODUCTION

As human beings, we need to know that we are and who we are. We need to be proud of our abilities and feel respected by other persons. These experiences can often be hard to achieve for persons with dementia, partly due to symptoms of dementia and to a great extent due to other persons' reactions. There are reports that persons with dementia lose their self and also that it is at least partly preserved. A systematic review revealed that studies performed with quantitative methods generally described that among persons with dementia self was affected, while qualitative studies tended to find that self was at least partly preserved (1). These results are in line with Sabat (2) who argued that self should be investigated by sensitive methods.

The Latin root of the term dementia means to be out of one's mind (3), and dementia has been described as leading to affected persons losing their self and becoming non-persons (4). Thus, it seems reasonable that the term dementia has been subject to criticism, and the American Psychiatric Association has replaced it with the phrase "major neurocognitive disorder" in the Diagnostic and Statistical Manual (DSM-5) (5). Sabat (6) argues that we should not reduce persons to brains but see the persons behind the dysfunctional symptoms and remember our shared humanity. Those living with dementia are semiotic persons, that is, their behavior is driven by meaning, as they, for example, have the capacity to show shame and pride and to feel concern for other persons' well-being (7). They are also relational beings and their behavior is an effect of neuropathology, their reaction to these effects, others' ways of treating them and their reaction to that treatment (8). Healthy persons often use negative stereotyping of persons with dementia which may lead to these persons themselves using self-stereotyping (9).

There are several types of dementia diseases, with Alzheimer's disease as the most common type. The disease progresses from a mild stage, via a moderate stage, to an advanced stage in which affected persons are dependent on others in most situations (10). In the literature about care, the term "dementia" is sometimes used and sometimes the type of dementia is identified. Here, I use the term "dementia" when I refer to literature using the terms "Alzheimer's disease" or dementia without further specification.

SYMPTOMS OF DEMENTIA

The cognitive symptoms of dementia can be described as four A's, namely amnesia (impaired memory), apraxia (impaired ability to organize sequences of movements in space), agnosia (impaired perception), and aphasia (impaired language ability) (11). Amnesia affects first the short-term memory and later also the long-term memory. A decreasing autobiographic memory (incident memory about specific personal events including context and personal semantic memory such as names of friends) affects sense of self (12). In interviews, persons with moderate dementia can often provide short accounts of their experiences. They may narrate fragments of their life story: childhood, education, family life and professional life. Several persons describe specific events such as leaving home to go to school (13, 14) and narrate thoughts about their future life, that is, about possible selves (15). Apraxia causes

problems with performing everyday tasks like, dressing, eating, grooming, and walking. Agnosia causes difficulties in recognizing, for example, colors, persons, objects, odors, shapes, and sounds. Aphasia leads to difficulties with both speaking and comprehending speech (16). Persons with dementia use circumlocutions and paraphrases as they forget words, and they need prolonged time to understand the meaning of what has been said, and to formulate answers (17). They also often can compensate by using extralinguistic means such as gestures and tone (18).

SELFHOOD

There is no consensus about the meaning of the concepts of identity, person, personhood, self, and selfhood. The terms are often used interchangeably although there are many different conceptualizations (19–20). Studies on the sense of self in persons with dementia have used various concepts or failed to describe the concepts used. Here, I use the term "self" also when I refer to authors who use the terms "identity" or "personhood". Thus, I use these terms as interchangeable.

The psychologists Sabat and Harré have together (21), separately (17, 22), and together with other researchers (23) published about a social constructionist theory of selfhood in persons with dementia.

The embodied, material human being they label "person," and the linguistic expressions we use to refer to ourselves they label "self." Selfhood is expressed both as speech and behavior in public discourse. Sabat and Harré describe self as tripartite: Self 1, Self 2 and Self 3. Self 1 (the self of personal identity) expresses our embodied experience of being singular continuous persons located in space, time and in a local moral order. We experience this aspect of selfhood in that each of us has one single point of view of the world, that is, our continuous experiences of events that form the narrative of our lives. Through the use of first-person singular pronouns, we take responsibility for our actions, feelings, and experiences as being our own and tell autobiographical stories. We manifest Self 1 when we speak in first-person indexicals ("I," "me," "mine," "my," "our") or indicate Self 1 nonverbally for example by pointing to ourselves (21, 24). Self 1 is a necessary condition to be able to reflect on our personal attributes (Self 2) and exhibit them in appropriate social situations (Self 3).

Self 2 is comprised of how we perceive our physical and mental attributes such as eye pigmentation, height and weight, educational achievements, political and religious convictions, sense of humor, and vocational pursuits. We have beliefs about our attributes, such as that they are adequate or outdated and also emotions related to them such as pride or disdain. Some Self 2 attributes have long histories such as being a daughter, while some may be more recent such as being diagnosed with dementia. Self 2 can be restricted and unrestricted. The restricted Self 2 is about how we perceive ourselves to be in the moment, while the unrestricted Self 2 includes both how we are in the present, how we were in the past and may develop in the future, that is, our relatively constant, temporary or ever-changing attributes, such as traits, skills, and our beliefs regarding these attributes. We manifest multiple Selves 2 (22).

As persons with advanced dementia usually remember past attributes better than recent ones, they may feel proud of already lost attributes (25). For persons

with dementia, new Self 2 attributes include deficits connected to the neuropathology of the disease (24) and results of their attempts to adapt to the disease (26).

Self 3 (social personae) is a complex concept, in that it includes the perspectives of both the displayer and the perceiver. It is the display of Selves 1 and 2 to other persons. How we display Self 3, depends on the situation, on how other persons position us and how we position ourselves to them through actions or inner dialogues (23, 27–28). Persons with dementia may lose their sense of self-worth and feel depersonalized, depressed, and angry when healthy persons behave in manners that can be classified as "malignant positioning" (28–30). Previous research has described both the negative positioning of persons with dementia (27) and the understanding and support that they sometimes receive (13). It is obvious that support from other persons is important for preserving a sense of self (20, 30). If others focus on dysfunctional Self 2 attributes of persons with dementia, their Self 3 (social persona) is restricted to "the patient" (28). If other persons focus on remaining healthy Self 2 attributes, it is possible for the afflicted persons to construct worthy Self 3 that makes them experience pride and satisfaction. (24). We manifest multiple Selves 3 that are constantly reconstructed in the interplay among persons (22).

Studies based on the Harré–Sabat theory of selfhood have shown that among persons with mild to moderate dementia, Self 1 was not affected during the course of dementia, whereas Selves 2 and 3 were (13–15). A few studies using that theory concern persons with advanced dementia (e.g., 24, 31–33). Studies that do not use the Harré–Sabat theory have found that some aspects of sense of self were preserved while other aspects were reduced among persons with advanced dementia (e.g., 34–37). Kontos (38) argued that selfhood is an embodied dimension of human existence persists even with advanced dementia. These persons have several preserved abilities despite losses of cognitive functions, for example being able to assess their own internal state of being such as feeling cold (39) and experiencing pain (40). Here, I use the term "advanced dementia" to describe information in articles about persons with moderate to advanced dementia as well as with advanced dementia.

PERSONS WITH ADVANCED DEMENTIA

Having advanced dementia involves many strains. Neighboring persons, carers, and everyone who meets these persons need to provide them support.

Awareness and lucidity

Although persons with advanced dementia are often described as having lost their self (4), their sensory and perceptual awareness has been found retained (41). These persons show distinct individual reactions to particular kinds of stimuli, and they, for example, differentiate between pleasant and unpleasant experiences, or various pieces of music (42–44). If the self is lost, it is difficult to understand the meaning of *moments of lucidity* (cognitive fluctuations) that have been identified when persons with advanced dementia that seem "not there" suddenly show

that they understand, remember, and care (42, 45). It was evident that lucidity during conversations with a women with advanced dementia occurred when her communication partner supported her by showing that he shared her expressed view, repeating and reformulating her words, using positive words and statements, helping by suggesting words and starting, completing and ending sentences and not emphasizing errors in her speech (46).

Suffering

Living with advanced dementia includes several negative experiences. When writing about *suffering* among persons with advanced dementia, several researchers write about experiences of pain and bodily distress (47). Few writers have explicitly focused on the other various experiences of suffering in persons with advanced dementia although carers often feel that these persons indeed suffer.

Eriksson (48) described three categories of suffering: "suffering of life" (for example grief due to the death of a friend, or feeling abandoned when friends do not pay visits to the nursing home), "suffering of illness" (effects of having dementia such as not feeling at home, having problems communicating), and "suffering of caring" (distress caused by received care). An example of "suffering of illness" is that several persons with advanced dementia have neuropsychiatric symptoms such as apathy, depression, irritability, agitation, sometimes delusions, hallucinations, and sleeping disorders (49). Examples of "suffering of care" are reports that carers have been observed treating persons with advanced dementia as object (50) which reasonably cause them suffering. Living with advanced dementia can also be experienced as a relative well-being (29) mainly through carers' compensating for problems related to the disease (51).

Anosognosia

Many persons with advanced dementia have been reported to suffer from explicit or implicit "mnemonic anosognosia" *anosognosia* that makes them seem unaware of deficits caused by neurodegeneration although they may demonstrate implicit awareness (52–54). Their memory deficits can make their sense of self become a "petrified self," that is frozen in time and sometimes reflects the features that were accurate in early adulthood and perhaps even in childhood. Thus, memory impairments hinder the persons to update information about self. Sometimes, they may register impairment but they cannot integrate the information into a coherent picture of their situation. This may lead to stable but inaccurate evaluation of experiences and actions (54).

Moments of homecoming

Feeling at home is a fundamental aspect of human existence (55), and it is important for our sense of self (56). Thus, losing one's home is losing one's self. To feel at home through the life cycle has been described as feeling related to oneself, significant activities, significant others, significant places, significant things, and to feeling a sense of transcendence (57). Persons with advanced dementia often appear to feel homeless, they may walk around, asking where they are and

searching for their home. They have problems feeling at home both in their former homes and in their new places of residence (58). The understanding of how important the experience of at-homeness is for our sense of self and well-being has led to an endeavor to create care environments where persons with advanced dementia can feel at home. However, although carers try to furnish the rooms as homely as possible it is often hard to document positive effect on the persons' daily life (59). Observation of nursing home residents with advanced dementia showed that they alternated between expressing feeling at home (at-homeness) and not feeling at home (homelessness) both verbally and nonverbally. They showed often short lived moments of homecoming that were characterized by "being released from burdens and demands (e.g., not being required to make choices), being united with actions (e.g., being helped to use previously familiar routines when getting dressed), and being reached by language (e.g., being spoken to with familiar words)" (60). One woman seemed to live and feel at home in two worlds simultaneously, that is, she interpreted some things that happened at present as something that happened earlier in her life (61). In her "remembered-world," she took care of her small children and had coffee with her friends and expressed astonishment about the fact that some nice and friendly persons entered her room, made her bed and invited her to dinner. In her "care home-world," she could tell her carers about her grandchild having taken an exam.

Communication

Persons with advanced dementia may show some retained abilities to communicate, they for example sometimes are using politeness when communicating (62). Their *communication difficulties* are, however, more often acknowledged. They can show lack of interactional synchrony such as integrating verbal and nonverbal communicative cues to a whole and synchronize their actions with their communication partners by adequate turn-taking. First the carer talks, then the person answers, then the carer answers, then the person shifts theme etc. The lack of synchrony makes behavior chaotic and fragmented. The persons' sensitivity to representative meaning and less to affective meaning is reduced due to decreased arousal and attentiveness. They often send vague undifferentiated verbal and nonverbal cues that are difficult to interpret for the communication partner. They also show problems interpreting the communication partners' cues and they need prolonged time for responding to their partner. They may use short sentences but more often single words or even react with primitive reflexes (63). Communication partners sometimes have to impute or attribute meaning to the vague cues, that is, they make guesses that are based on their previous experiences with the person in question and with other similar persons or on empathy or intuition (50, 64). Sometimes, the communication can depend on the communication partners imitating each other (65). When I tried to help a person with advanced dementia to eat and the person did not seem to understand, I demonstrated what I meant by opening my own mouth, chewing and swallowing. I ate an air meal. Suddenly, the person seemed to understand and started eating (The author's experience). Both imputation of meaning to vague cues and imitation can help the persons feel like partners being answered, that is strengthening the Self 3.

At the end state, persons with advanced dementia may become mute (66). Hughes (67) argues that there are other means to understand persons with

dementia than understanding the words they use. It is about sharing a form of life, it is about understanding the context, and feeling what the persons express. This is like using affect attunement, that is, the carer tuning into the persons' affective state to be able to help them to reduce negative affect and increase positive affect (68, 69). In this way, communion between the partners is created. According to Stern (70), the sense of self starts with the parent and infant being in communion, that is, participating in and sharing emotions and vitality effects that, for example, are expressed through intensity and rhythm. Later, the sense of self gradually develops through interaction with others throughout one's history to also include cognitive dimensions. As their cognitive abilities decrease, persons with advanced dementia increasingly need to be in communion with carers to preserve their vulnerable self. Söderlund et al. (71) reported positive results from using the Feil's validation method during one-to-one conversations with persons with advanced dementia. The focus was not the facts about what was expressed but rather the feelings behind what the persons tried to talk about and the aim was to treat them as adults and increase their feelings of self-worth and well-being. Eggers et al. (72) described two somewhat different ways of interpreting the communicative cues of persons with advanced dementia, partly by establishing communion with them through affect attunement and partly by putting various fragments together until a picture of the meaning of utterances and behavior appeared. This was like completing a puzzle.

AGENCY AND COMMUNION

Agency and communion have been described as fundamental modalities in human beings' lives (73). Communion is the urge to be connected and unified with others. The positive themes of communion are love/friendship, dialogue, caring/help, and unity/togetherness, whereas the negative themes are separation, rejection, disillusionment about people, and another's misfortune (74) Agency implies a quest for autonomy, self-realization, and separation from other people. The positive themes of agency are self-mastery, status/victory, achievement/responsibility, and power/impact, and the negative themes are, failure/weakness, losing face, ignorance, and conflict (75). For a positive sense of self, we need agency (individuality) and communion (togetherness). Experiences of agency and communion have been assessed among persons with dementia (76). Although there are no clear-cut borders between the modalities, it seems as communion is more relevant for Self 1 and Self 3 and agency for Self 2.

Strengthening Self 1 communion

Being treated and even feeling as a non-person reasonably means suffering. Therefore, it is important to help persons feel as *persons* with a sense of self. Research has shown that the Self 1 is preserved among persons with advanced dementia. Even when they almost entirely answer "yes" or "no" to questions, they still show that they can experience themselves as "I" (32, 60). Still it seems reasonable to suggest that we can help persons preserve their feeling of being an I by making them feel that they are seen and listened to, that is that they are. This kind

of behavior has often been labeled confirming actions and seems a type of communion.

The concept *confirmation* is based on Martin Buber's philosophy and is described by Cissna and Sieburg (77, p. 254–260) as the process through which we endorse others by showing them recognition, acknowledgement and acceptance of their self-definition. Thereby they get help forming and maintaining human relationship. Cissna and Sieburg emphasize that confirmation includes four key elements: (i) The element of existence (the individual sees self as existing). (ii) The element of relating (the individual sees self as being-in-relation with others). (iii) The element of significance, or worth. (iv) The element of validity of experience. Observations at a small group living for persons with advanced dementia revealed that staff confirmed persons by accepting the ways they spoke and acted by making them feel accepted and allowed to be just as they were. A person who thought the ward is a church was not corrected as long as he appeared to feel good. Another person who found it meaningful to carry things around was allowed to do that as long as no one else was disturbed (78). It seems reasonable that confirmation could be seen as strengthening persons' Self 1.

Strengthening Self 2 agency

Experiences of using motoric and other abilities can be understood as part of agency. Because Self 2 concerns persons' perceptions of their abilities, it seems logical that there is a need that they both get opportunities to use their abilities and also get help to remember how they previously used their abilities. They most easily remember what happened during their childhood (54) and often express they are proud of these memories. In articles about needs of persons with advanced dementia, agency is mentioned (51, 78). To my knowledge, there are few studies presented about agency among persons with advanced dementia. There are, however, articles about topics that could be seen as agency. Together with their respective co-workers Kihlgren (79) and Ekman (80) analyzed video-tapes of morning care sessions with persons with advanced dementia and carers. The carers were taught about the Erikson (81) theory of eight stages of man and encouraged to, in a concrete way, promote the persons' experiences of trust, autonomy, initiative, industry, identity, intimacy, generativity, and integrity during morning care sessions. The comparison of behaviors and speech before and after the intervention showed that the persons with dementia displayed more and more ability (79) and that carers who spoke the persons' mother tongue (Finish) were most successful in helping them use their latent abilities than carers who only spoke Swedish (80).

Persons with advanced dementia may get help to recognize and remember important themes or episodes from their life history. As the ability to recognize is better preserved than the ability to recall, they might remember a phenomenon when reminded although they cannot recall it (17). They can have an unconscious or implicit memory of past experiences (38) and often remember emotions better than facts (81). As persons with dementia best remember their life before the debut of dementia and later their earliest memory, they most easily can express their experiences by referring to memories from their childhood (54). They may for example call their mother when they feel unsecure (82). When they perceive the emotions in a conversation, they can go back in memory to an experience with the same emotions to understand what the conversation is about (83).

Thus, when a person with advanced dementia asks a carer: You are my mother, aren't you, this does not have to mean that she thinks the carer really is her mother. Instead it might mean that the interaction with the carer made the person feel being loved and when she sought an experience with the same emotional tone from the past, she found memories of her mother.

At the last stages of life, persons with advanced dementia often experience eating difficulties, especially swallowing problems (84–85). By participant observations of meals Eggers (86) described fragmentation when the persons with advanced dementia did not seem to recognize what was going on, the persons involved, the things used, or recognize themselves in the meal situation. Carers counteracted fragmentation by showing attentive interest in the interaction, valuing the persons as human beings, considering the symptoms of the dementia, and striving for mutual interpretation of the meal situation.

Strengthening Self 3 communion

Strengthening persons' Self 3 seem to mainly concern themes of communion. Self 3 has been found especially vulnerable when persons with dementia are negatively positioned and do not get support of others (17, 28). This means that actions that promote the wellbeing of these persons are strengthening their Self 3. Few articles about *well-being* concern persons with advanced dementia due to the fact that they cannot take part in studies that require answering complicated questions. They can, however, take part in easy conversations and become observed, thus in qualitative research (87). Kaufmann and Engel (51) included persons with advanced dementia in a study based on a Tom Kitwood's model of needs and described well-being according to the themes *comfort* (small pleasures of life providing relaxation, consolation); *attachment* (company with human beings, animals and objects, support); *inclusion* (being part of a community, feeling recognized); *occupation* (e.g., listening to the radio, exercise, participation in activities); *identity* (role maintenance, recognition, familiar rhythms and habits). Jetten et al. (12) reported that life satisfaction that was lower among persons with mild dementia than among persons with advanced dementia. Reasonably this could be related to anognosia among person with advanced dementia (54). There are several means to improve the sense of well-being among persons with advanced dementia such as using multisensory stimulation for example including music and massage (88), singing (89), dancing (90), animals (91) and dolls (92). Listening to well-known songs or music and to positive stories from their own lives can strengthen the feeling of being important. Music, touch, dance and rocking can mean comfort for persons with advanced dementia (43, 69). The stimulation of the senses can be combined with ordinary nursing actions. A review of 21 intervention studies on persons with advanced dementia, in which aromatherapy, music, simulated presence (for example, listening to a tape recording of their partner), touch and multisensory stimulation, have been used, showed no proven scientific evidence but carers' proven experience that the methods are effective, sometimes they fit, sometimes not (93).

As a positive Self 3 means that the persons with advanced dementia feel proud of themselves, loved and acknowledged other persons' acts toward them are of utmost importance, actions that promote the persons feeling dignified certainly would strengthen self 3. Manthorpe et al. (94) described *dignity* as a phenomenon involving an inherent self-respect and feelings of worthiness, and being respectfully recognized

and confirmed by others. Nordenfelt (95) wrote about four variants of dignity: Menschenwurde (human dignity), which is overall other variants of dignity, that is as it is part of being human it is the same for all people; dignity as merit (e.g., being a leader), dignity as moral stature (e.g., being an altruistic person) and dignity of identity (concerns self-image, self-respect, worth and value ascribed by oneself or others). Persons' dignity of identity can change over time, for example depending on if the persons are afflicted by dementia and how they are treated of others, which can result in physical, psychological, or emotional change or harm. Dignity of identity is connected to sense of self, and it is threatened if the persons have forgotten who they are.

The dignity as moral stature is about our moral actions. We can show that we know about their previous good properties and actions as persons with advanced dementia cannot upgrade their identity (54). Persons with advanced dementia should be cared so they can keep their experiences of human dignity , dignity as merit, dignity as moral stature and dignity of identity. When persons with advanced dementia are seen as having lost their self (4), their human dignity is questioned and there is a risk that they become treated as objects.

SPIRITUAL AND CONSOLING CARE AT THE END OF LIFE

There is so far, no cure for AD. Within approximately 4 to 8 years, dementia usually leads to *dying and death,* although some persons live up to 20 years after being diagnosed (96). Persons may *die with dementia* due to various causes (97) or *due to dementia* as it is a lethal disease (98).

There are few reports about needs during end of life among persons with advanced dementia. A review of 10 articles published 1993–2013 mentioned physical, social, psychological, spiritual, supportive, environmental needs and needs related to individuality. The authors emphasized that as persons with advanced dementia have severe communication difficulties, we need more research about views of stakeholders (99). Analyses of focus-group discussions at four nursing homes showed that dying was silent and silenced, emotions were put into the background and death was talked about after a person's death. The staff did not talk about death neither with each other nor with the residents (100). This seems unfortunate as several residents have revealed that they were aware of the fact that they soon would die. One person emphasized that she was waiting to go to her real heavenly home. Another resident said that she was only living at the ward temporarily until she would meet her deceased spouse again and another one said that she wanted to listen to gospels while dying. Some did not speak about death and dying but reasoned about their funeral (60). At the last stages of life persons with advanced dementia often experience eating difficulties, especially swallowing problems (84–85). Several qualitative studies have reported that persons with advanced dementia at the end of life often exhibit aversive refuse-like eating behavior (101). There have been discussions about whether tube-feeding or comfort feeding should be used (102–103). The American Geriatrics Society (96) has recommended comfort feeding.

Spirituality has been regarded as "an integral, even fundamental, element of what it is to be a human being" (93, p. 765) and if we regard persons with advanced dementia as human beings it follows that they have spiritual needs. Spirituality among persons with advanced dementia has not, however, been

extensively studied. A literature review of scientific articles about spirituality did not report any study about persons with dementia (104) and another review study found expressed spiritual needs in 2 out of 10 articles (99). According to Kverno (93) there is a diversity of definitions of *spirituality* such as being connected to meaning-making. Spirituality should be understood as involving the dimensions of time and/or social space and stresses it's longitudinal, habitual dimension, that is, "the values, meaning and practices most deeply ingrained at the heart of 'who we are' are those which have been repeated and reinforced over and over again from our infancy" (p.773) and held together with other persons. Perkins et al. (105) reported that even persons with advanced dementia were able to engage in *spiritual practices* that provide life meaning in accordance with their values. Observations in a nursing home showed that some persons expressed a need for religious expression or participating in religious rituals (106).

Thinking about or even experiencing that one interacts with deceased dear loved ones can provide satisfaction (60). This finding seems to represent a tendency to gerotranscendence (107) and can be understood as related to the fact that among persons with advanced dementia memory for past events is better preserved than memory of recent events (12). Swinton (108) argued that spiritual practices can be beneficial for Christian persons with advanced dementia. When their memory fails their bodily memory can be reclaimed. He referred to Bergson's writing about a memory synonymous with recollection that represents particular things that have happened in the past and inscribes the past in the present (54). Swinton emphasized that we are embedded in our memories even when we cannot recall them, we are our memories. Persons with other religions or life views of course also need spiritual care adopted to their needs. Nursing home residents with moderate dementia expressed that religion is consolation for them (109). Persons with advanced dementia need consoling care (69, 110). The most important ingredient in consolation for these persons certainly is communion (69, 111) and it may provide them a feeling of being at home (112). However, it is most probable that they can get some moments of homecoming (60).

CONCLUSION

Persons with dementia lose parts of their self which can be noticed when considering symptoms such as amnesia, agnosia, aphasia, and apraxia. Their own sense of self can be preserved during the entire course of dementia partly due to the fact that their amnesia makes it difficult for them to upgrade their life story. Of utmost importance is that other persons understand that persons with advanced dementia still are persons and support them to feel valuable.

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REFERENCES

1. Caddell LS, Clare L. The impact of dementia on self and identity: A systematic review. Clin Psychol Rev. 2010 Feb;30(1):113–26. http://dx.doi.org/10.1016/j.cpr.2009.10.003
2. Sabat SR. Epistemological issues in the study of insight in people with Alzheimer's disease. Dementia. 2002 Nov; 1(3):279–93. http://dx.doi.org/10.1177/147130120200100302
3. Jenkins D, Price B. Dementia and personhood: A focus for care? J Adv Nurs 1996 Jul; 24(1): 84–90. http://dx.doi.org/10.1046/j.1365-2648.1996.16511.x
4. Millett S. Self and embodiment: A bio-phenomenological approach to dementia. Dementia. 2011Nov;10(4):509–22. http://dx.doi.org/10.1177/1471301211409374
5. Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS et al. Classifying neurocognitive disorders: The DSM-5 approach. Nature Rev Neurol. 2014 Nov;10(11):634–42. http://dx.doi.org/10.1038/nrneurol.2014.181
6. Sabat SR. Dementia beyond pathology: What people diagnosed can teach us about our shared humanity. J Bioethical Inq. 2019 Jun;16(2):163–72. http://dx.doi.org/10.1007/s11673-019-09899-0
7. Sabat SR. Capacity for decision-making in Alzheimer's disease: Selfhood, positioning and semiotic people. Aust NZ Psychiat. 2005 Nov;39(11–12):1030–5. http://dx.doi.org/10.1111/j.1440-1614.2005.01722.x
8. Sabat SR. Subjectivity, the brain, life narratives and the ethical treatment of persons with Alzheimer's disease. Am J Bioeth. 2009 Sep;9(9):23–5. http://dx.doi.org/10.1080/15265160903098655
9. Scholl JM, Sabat SR. Stereotypes, stereotype threat and aging: Implications for the understanding and treatment of people with Alzheimer's disease. Aging Soc. 2008 Jan;28(1):103–30. http://dx.doi.org/10.1017/S0144686X07006241
10. Folstein MF, Folstein SE, McHugh PR. Mini-mental state: A practical guide for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975 Nov;12(3):189–98. http://dx.doi.org/10.1016/0022-3956(75)90026-6
11. Kramer JH, Duffy JM. Aphasia, apraxia, and agnosia in the diagnosis of dementia. Dementia. 1996 Jan;7(1):23–6. http://dx.doi.org/10.1159/000106848
12. Jetten J, Haslam C, Pugliese C, Tonks J, Haslam SA. Declining autobiographical memory and the loss of identity: Effects on well-being. J Clin Exp Neuropsychol. 2010 Apr;32(4):408–16. http://dx.doi.org/10.1080/13803390903140603
13. Hedman R, Hansebo G, Ternestedt B-M, Hellström I, Norberg A. How people with Alzheimer's disease express their sense of self: Analysis using Rom Harré's theory of selfhood. Dementia. 2013 Nov;12(6):713–33. http://dx.doi.org/10.1177/1471301212444053
14. Skaalvik MW, Fjelltun AM, Normann HK, Norberg A. Expressions of sense of self among individuals with Alzheimer's disease. Res Theory Nurs Pract. 2016;30(2):161–75. http://dx.doi.org/10.1891/1541-6577.30.2.161
15. Kristensen PJL, Normann HK, Norberg A, Fjelltun A-M, Skaalvik MW. How do people in the early stage of Alzheimer's disease see their future? Dementia. 2017 Feb;16(2):145–57. http://dx.doi.org/10.1177/1471301215584223
16. Helmes E, Østbye T. Beyond memory impairment: Cognitive changes in Alzheimer's disease. Arch Clin Neuropsychol. 2002 Feb;17(2):179–93. http://dx.doi.org/10.1093/arclin/17.2.179
17. Sabat SR. The experience of Alzheimer's disease. Life through a tangled veil. Oxford: Blackwell Publishers Ltd; 2001. p. 34–90.
18. Sabat SR, Cagigas XE. Extralinguistic communication compensates for the loss of verbal fluency: A case study of Alzheimer's disease. Lang Commun. 1997 Oct;17(4):341–51. http://dx.doi.org/10.1016/S0271-5309(97)00019-0
19. Higgs P, Gilleard C. Interrogating personhood and dementia. Aging Ment Health. 2016 Aug; 20(8):773–80. http://dx.doi.org/10.1080/13607863.2015.1118012
20. Zeiler K. A philosophical defense of the idea that we can hold each other in personhood: Intercorporeal personhood in dementia care. Med Health Care Philos. 2014 Feb;17(1):131–41. http://dx.doi.org/10.1007/s11019-013-9515-z

21. Sabat SR, Harré R. The construction and deconstruction of self in Alzheimer's disease Aging Soc. 1992;12(4):443–61. http://dx.doi.org/10.1017/S0144686X00005262
22. Harré R. The singular self: An introduction of the psychology of personhood. London: Sage; 1998.
23. Harré R, Moghaddam FM, Cairnie TP, Rothbart D, Sabat SR. Recent advances in positioning theory. Theory Psychol. 2009 Feb;19(1):5–31. http://dx.doi.org/10.1177/0959354308101417
24. Sabat SR. Surviving manifestations of selfhood in Alzheimer's disease: A case study. Dementia. 2002 Feb;1(1):25–36. http://dx.doi.org/10.1177/147130120200100101
25. Sabat SR, Fath H, Moghaddam FM, Harre' R. The maintenance of self esteem: Lessons from the culture of Alzheimer's sufferers. Cult Psychol. 1999 Mar;5(1):5–31. http://dx.doi.org/10.1177/1354067X9951001
26. de Boer ME, Hertogh CM, Dröes RM, Riphagen II, Jonker C, Eefsting JA. Suffering from dementia – The patient's perspective: A review of the literature. Int Psychogeriatr. 2007 Dec;19(6):1021–39. http://dx.doi.org/10.1017/S1041610207005765
27. Sabat SR, Napolitano L, Fath H. Barriers to the construction of a valued social identity: A case study of Alzheimer's disease. Am J Alzheimers Dis. 2004 May–Jun;19(3):177–85. http://dx.doi.org/10.1177/153331750401900311
28. Sabat SR. Mind, meaning and personhood in dementia: The effects of positioning. In: Hughes JC, Louw S, Sabat SR, editors. Dementia: Mind, meaning and the person. Oxford: Oxford University Press; 2006. p. 287–302.
29. Kitwood T, Bredin K. Towards a theory of dementia care: Personhood and well-being. Ageing Soc. 1992 Jul;12(3):269–87. http://dx.doi.org/10.1017/S0144686X0000502X
30. Hampson C, Morris K. Dementia: Sustaining self in the face of cognitive decline. Geriatrics. 2016 Oct;1(4):1–6. http://dx.doi.org/10.3390/geriatrics1040025
31. Sabat SR, Harré R. The Alzheimer's disease sufferer as a semiotic subject. Philos Psychiatr Psychol. 1994 Sep;1(3):145–60.
32. Batra S, Sullivan J, Williams BR, Geldmacher DS. Qualitative assessment of self-identity in people with advanced dementia. Dementia. 2016 Sep;15(5):1260–78. http://dx.doi.org/10.1177/1471301215601619
33. Viaña JNM, Gilbert F. Deep brain stimulation for people with Alzheimer's disease: Anticipating potential effects on the tripartite self. Dementia. 2019 Oct–Nov;18(7–8):2836–2855. http://dx.doi.org/10.1177/1471301218761147
34. Tappen RM, Williams C, Fishman S, Touhy T. Persistence of self in advanced Alzheimer's disease. Image J Nurs Scholarship. 1999 Feb;31(2):121–5. http://dx.doi.org/10.1111/j.1547-5069.1999.tb00445.x
35. Cohen-Mansfield J, Golander H, Arnheim G. Self-identity in older persons suffering from dementia: Preliminary results. Soc Sci Med. 2000 Aug;51(3):381–94. http://dx.doi.org/10.1016/S0277-9536(99)00471-2
36. Clare L, Rowlands J, Bruce E, Surr C, Downs M. "I don't do like I used to do": A grounded theory approach to conceptualising awareness in people with moderate to severe dementia living in long-term care. Soc Sci Med. 2008 Jun;66(11):2366–77. http://dx.doi.org/10.1016/j.socscimed.2008.01.045
37. Eustache ML, Laisney M, Juskenaite A, Letortu O, Platel H, Eustache F, Desgranges B. Sense of identity in advanced Alzheimer's dementia: A cognitive dissociation between sameness and selfhood? Conscious Cogn. 2013 Dec;22(4):1456–67. http://dx.doi.org/10.1016/j.concog.2013.09.009
38. Kontos P. Ethnographic reflections on selfhood, embodiment and Alzheimer's disease. Aging Soc. 2004 Nov;24(6):829–49. http://dx.doi.org/10.1017/S0144686X04002375
39. Sabat SR, Collins M. Intact social, cognitive ability, and selfhood: A case study of Alzheimer's disease. Am J Alzheimer's Dis Other Demen. 1999 Jan;14(1):11–19. http://dx.doi.org/10.1177/153331759901400108
40. Benedetti F, Arduino C, Vighetti S, Asteggiano G, Tarenzi L, Rainero I. Pain reactivity in Alzheimer patients with different degrees of cognitive impairment and brain electrical activity deterioration. Pain. 2004 Sep;111(1–2):22–9. http://dx.doi.org/10.1177/153331759901400108
41. Clare L. Awareness in people with severe dementia: Review and integration. Aging Ment Health. 2010 Jan;14(1): 20–32. http://dx.doi.org/10.1080/13607860903421029
42. Norberg A, Melin E, Asplund K. Reactions to music, touch and object presentation in the final stage of dementia: An exploratory study. Int J Nurs Stud. 1986 Jul;40(5):473–9. http://dx.doi.org/10.1016/S0020-7489(03)00062-2

43. Asplund K, Norberg A, Adolfsson R, Waxman H. Facial expressions in severely demented patients. – A stimulus-response study of four patients with dementia of the Alzheimer type. Int J Ger Psychiatr. 1991 Aug; 6(8):599–06. http://dx.doi.org/10.1002/gps.930060809
44. Magai C, Cohen C, Gomberg D, Malatesta C, Culver C. Emotional expression during mid- to late-stage dementia. Int Psychogeriatr. 1996 Fall;8(3):383–95. http://dx.doi.org/10.1017/S104161029600275X
45. Normann HK, Asplund K, Karlsson S, Sandman PO, Norberg A. People with severe dementia who exhibit episodes of lucidity: A population-based study. J Clin Nurs. 2006 Nov;15(11):1413–17. http://dx.doi.org/10.1111/j.1365-2702.2005.01505.x
46. Normann HK, Norberg A, Asplund K. Confirmation and lucidity during conversations with a woman with severe dementia. J Adv Nurs. 2002 Aug;39(4):370–6. http://dx.doi.org/10.1046/j.1365-2648.2002.02298.x
47. Midtbust MH, Alnes RE, Gjengedal E, Lykkeslet E. A painful experience of limited understanding: Healthcare professionals' experiences with palliative care of people with severe dementia in Norwegian nursing homes. BMC Palliat Care. 2018 Feb 13;17(1):25. http://dx.doi.org/10.1186/s12904-018-0282-8
48. Eriksson K. Theories of caring as health. In: Gaut DA, Boykin A, editors. Caring as healing: Renewal through hope. New York: National League for Nursing Press; 1994. p. 3–20.
49. Rozum WJ, Cooley B, Vernon E, Matyi J, Tschanz JAT. Neuropsychiatric symptoms in severe dementia: Associations with specific cognitive domains the Cache County Dementia Progression Study. Int J Ger Psychiatry. 2019 Jul;34(7):1087–94. http://dx.doi.org/10.1002/gps.5112
50. Athlin E, Norberg A. Caregivers' attitudes to and interpretations of the behaviour of severely demented patients during feeding in a patient assignment care system. Int J Nurs Stud. 1987 Feb;24(2):145–53. http://dx.doi.org/10.1016/0020-7489(87)90056-3
51. Kaufmann EG, Engel SA. Dementia and well-being: A conceptual framework based on Tom Kitwood's model of needs. Dementia. 2016 Jul;15(4):774–88. http://dx.doi.org/10.1177/1471301214539690
52. Agnew SK, Morris RG. The heterogeneity of anosognosia for memory impairment in Alzheimer's disease: A review of the literature and a proposed model. Aging Ment Health. 1998 Feb;2(1):7–19. http://dx.doi.org/10.1080/13607869856876
53. Starkstein SE, Jorge R, Mizrahi R, Robinson RG. A diagnostic formulation for anosognosia in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2006 Jun;77(6):719–25. http://dx.doi.org/10.1136/jnnp.2005.085373
54. Mograbi DC, Brown RG, Morris RG. Anosognosia in Alzheimer's disease – The petrified self. Conscious Cogn. 2009 Dec;18(4):989–1003. http://dx.doi.org/10.1016/j.concog.2009.07.005
55. Dekkers W. Dwelling, house and home: Towards a home-led perspective on dementia care. Med Health Care Philos. 2011 Aug;14(3):291–300. http://dx.doi.org/10.1007/s11019-011-9307-2
56. de Jonge DM, Jones A, Phillips R, Chung M. Understanding the essence of home: Older people's experience of home in Australia. Occup Ther Int. 2011 Mar;18(1):39–47. http://dx.doi.org/10.1007/s11019-011-9307-2
57. Zingmark K, Norberg A, Sandman P O. The experience of being at home throughout the life span. Investigation of persons aged from 2 to 102. Int J Aging Hum Dev. 1995 Feb; 41(1): 47–62. http://dx.doi.org/10.2190/N08L-42J5-31D2-JUQA
58. Zingmark K, Norberg A, Sandman P-O,. Experience of at-homeness and homesickness in patients with Alzheimer's disease. Am J Alzheimer's Care Related Disorder Res. 1993 May;8(3):10–16. http://dx.doi.org/10.1177/153331759300800302
59. Kok JS, Berg IJ, Blankevoort GCG, Scherder EJA. Rest-activity rhythms in small scale homelike care and traditional care for residents with dementia. BMC Geriatr. 2017 Jul;17(1):137. http://dx.doi.org/10.1186/s12877-017-0525-1
60. Norberg A, Ternestedt BM, Lundman B. Moments of homecoming among people with advanced Dementia disease in a residential care facility. Dementia. 2017 Jul;16 (5):629–41. http://dx.doi.org/10.1186/s12877-017-0525-1
61. Cipriani G, Danti S, Vedovello M, Nuti A, Lucetti C. Understanding delusion in dementia: A review. Geriatr Gerontol Int. 2014 Jan;14(1):32–9. http://dx.doi.org/10.1111/ggi.12105
62. Temple V, Sabat S, Kroger R. Intact use of politeness in the discourse of Alzheimer's sufferers. Lang Communic. 1999 Apr;19(2):163–80. http://dx.doi.org/10.1016/S0271-5309(98)00020-2

63. Athlin E, Norberg A. Interaction between the severely demented patient and his caregiver during feeding. A theoretical model. Scand J Caring Sci. 1987 Sep–Dec;1(3–4):117–23. http://dx.doi.org/10.1111/j.1471-6712.1987.tb00612.x
64. Berg A, Hallberg IR, Norberg A. Nurses' reflections about dementia care, the patients, the care and themselves in their daily caregiving. Int J Nurs Stud.1998 Oct;35(5): 271–82. http://dx.doi.org/10.1016/S0020-7489(98)00040-6
65. Astell AJ, Ellis MP. The social function of imitation in severe dementia. Infant Child Dev. 2006 May/Jun; 15(3): 311–19. http://dx.doi.org/10.1002/icd.455
66. Walmsley BD, McCormack L. The dance of communication: Retaining family membership despite severe non-speech dementia. Dementia. 2014 Sep;13(5):626–41. http://dx.doi.org/10.1177/1471301213480359
67. Hughes JC. 'Y' feel me?' How do we understand the person with dementia? Dementia. 2013 May;12(3):348–58. http://dx.doi.org/10.1177/1471301213479597
68. Häggström, TM, Jansson, L, Norberg A. Skilled carers' ways of understanding people with Alzheimer's disease. Sch Inq Nurs Pract. 1998 Fall;12(3):239–66.
69. Norberg A. Consoling care for people with Alzheimer's disease or another dementia in the advanced stage. Alzheimer's Care Quarterly. 2001Spring;2(2):46–52.
70. Stern DN. The international world of the infant. New York: Basic Books;1985.
71. Soderlund M, Cronqvist A, Norberg A, Ternestedt, Hansebo G. Conversations between persons with dementia disease living in nursing homes and nurses – Gualitative evaluation of an intervention with the validation method. Scand J Caring Sci. 2016 Mar;30(1):37–47. http://dx.doi.org/10.1111/scs.12219
72. Eggers T, Ekman S-L, Norberg A. Nursing staff's understanding expressions of people with advanced dementia disease. Res Theory Nurs Pract. 2013 Feb;27(1):19–34. http://dx.doi.org/10.1891/1541-6577.27.1.19
73. Bakan D. The duality of human existence: An essay on psychology and religion. Chicago, IL: IMcNally, 1966.
74. McAdams DP. Power intimacy, and the life story: Personological inquiries into identity. New York: Guilford Press. 1988.
75. McAdams DP, Hoffman B J, Mansfield ED, Day R. Themes of agency and communion in significant autobiographical scenes. J Personal. 1996 Jan;64(2):339–77. http://dx.doi.org/10.1111/j.1467-6494.1996.tb00514.x
76. Hedman R, Norberg A, Hellström I. Agency and communion in people with Alzheimer's disease, as described by themselves and their spousal carers. Dementia (London). 2019 May;18(4):1354–72. http://dx.doi.org/10.1177/1471301217706268
77. Cissna KNL, Sieburg E. Patterns of interactional confirmation and disconfirmation. In: Wilder-Mott C, Weakland JH editors. Rigor and imagination essays from the legacy of gregory Bateson. New York: Praeger Publishers; 1981. p. 253–281.
78. Zingmark K, Sandman PO, Norberg A. Promoting a good life among people with Alzheimer's disease. J Adv Nurs. 2002 Apr; 38(1):50–8. http://dx.doi.org/10.1046/j.1365-2648.2002.02145.x
79. Kihlgren M, Kuremyr D, Norberg A, Bråne G, Engström B, Karlsson I, Melin E. Nurse-patient interaction after training in integrity promoting care at a long-term ward. Analysis of video-recorded morning care sessions. Int J Nurs Stud. 1993 Feb;30(1):1–13. http://dx.doi.org/10.1016/0020-7489(93)90088-C
80. Ekman SL, Robins Wahlin TB, Norberg A, Winblad B. Relationship between bilingual demented immigrants and bilingual/monolingual caregivers. Analysis of video-recorded morning care sessions in institutions by means of the Erikson theory of 'eight stages of man'. Int J Aging Human Dev.1993 Feb; 37(1): 37–54.
81. Erikson EH, Erikson JM. The life cycle completed (Extended version). NewYork; WW Norton & Company; 1998.
82. Miesen BML. Alzheimer's disease, the phenomenon of parent fixation and Bowlby's attachment theory. Int J Ger Psychiatry. 1993 Feb;8(2):147–53. http://dx.doi.org/10.1002/gps.930080207
83. Piefke M, Weiss PH, Zilles K, Markowitsch HJ, Fink GR. Differential remoteness and emotional tone modulate the neural correlates of autobiographical memory. Brain. 2003 Mar;126(3): 650–68. http://dx.doi.org/10.1093/brain/awg064
84. Shinagawa S, Honda K, Kashibayashi T, Shigenobu K, Nakayama K, Ikeda M. Classifying eating-related problems among institutionalized people with dementia. Psychiatry Clin Neurosci. 2016 Apr;70(4):175–81. http://dx.doi.org/10.1111/pcn.12375

85. Kai K, Hashimoto M, Amano K, Tanaka H, Fukuhara R, Ikeda M. Relationship between eating disturbance and dementia severity in patients with Alzheimer's disease. PLoS One. 2015 Aug 12; 10(8):e0133666. http://dx.doi.10.1371/journal.pone.0133666. eCollection
86. Eggers T, Norberg A, Ekman, SL. Counteracting fragmentation in the care of people with moderate and severe dementia. Clin Nurs Res. 2005 Nov;14 (4): 343–69. http://dx.doi.org/10.1177/1054773805277957
87. Wolverson EL, Clarkea C, Moniz-Cook ED. Living positively with dementia: A systematic review and synthesis of the qualitative literature. Aging Ment Health. 2016 Jul;20(7):676–99. http://dx.doi.org/10.1080/13607863.2015.1052777
88. Bunn F, Lynch J, Goodman C, Sharpe R, Walshe C, Preston N, et al. Improving living and dying for people with advanced dementia living in care homes: A realist review of Namaste Care and other multisensory interventions. BMC Geriatr. 2018 Dec; 18(1): 303. http://dx.doi.org/10.1186/s12877-018-0995-9
89. Götell E, Brown S, Ekman SL. The influence of caregiver singing and background music on vocally expressed emotions and moods in dementia care: A qualitative analysis. Int J Nurs Stud. 2009 Apr;46(4):422-30. http://dx.doi.org/10.1186/s12877-018-0995-9
90. Palo-Bengtsson L, Ekman SL. Social dancing in the care of persons with dementia in a nursing home setting: A phenomenological study. Sch Inq Nurs Pract. 1997 Spring;11(2):101-18; discussion 119-23.
91. Kårefjärd A, Nordgren L. Effects of dog-assisted intervention on quality of life in nursing home residents with dementia. Scand J Occup Ther. 2019 Oct;26(6):433-40. http://dx.doi.org/10.1080/11038128.2018.1467486
92. Cantarella A, Borella E, Faggian S, Navuzzi A, De Beni R. Using dolls for therapeutic purposes: A study on nursing home residents with severe dementia. Int J Geriatr Psychiatry. 2018 Jul;33(7): 915–5. http://dx.doi.org/10.1002/gps.4872
93. Kverno KS, Black BS, Nolan MT, Rabins PV. Research on treating neuropsychiatric symptoms of advanced dementia with non-pharmacological strategies, 1998–2008: A systematic literature review. Int Psychogeriatr. 2009 Oct;21(5):825–43. http://dx.doi.org/10.1017/S1041610209990196
94. Manthorpe J, Iliffe S, Samsi K, Cole L, Goodman C, Drennan V, et al. Dementia, dignity and quality of life: Nursing practice and its dilemmas. Int J Older People Nurs. 2010 Sep;5(3):235–44. http://dx.doi.org/10.1111/j.1748-3743.2010.00231.x
95. Nordenfelt L. The varieties of dignity. Health Care Anal 2004 Jun;12(2):69–81. http://dx.doi.org/10.1023/B:HCAN.0000041183.78435.4b
96. American Geriatrics Society feeding tubes in advanced dementia position statement. American Geriatrics Society Ethics Committee and Clinical Practice and Models of Care Committee. J Am Geriatr Soc. 2014 Aug;62(8):1590–3. http://dx.doi.org/10.1111/jgs.12924
97. Koopmans RT, van der Sterren KJ, van der Steen JT. The "natural" endpoint of dementia: Death from cachexia or dehydration following palliative care? Int J Geriatr Psychiatry. 2007 Apr; 22(4):350–5. http://dx.doi.org/10.1002/gps.1680
98. Sampson EL, Candy B, Davis S, Gola AB, Harrington J, King M, et al. Living and dying with advanced dementia: A prospective cohort study of symptoms, service use and care at the end of life. Palliat Med. 2018 Mar;32(3):668–81. http://dx.doi.org/10.1177/0269216317726443
99. Perrar KM, Schmidt H, Eisenmann Y, Cremer B, Voltz R. Needs of people with severe dementia at the end-of-life: A systematic review. J Alzheimers Dis. 2015;43(2):397–13. http://dx.doi.org/10.3233/JAD-140435
100. Osterlind J, Hansebo G, Andersson J, Ternestedt B-M, Hellström I. A discourse of silence: Professional carers reasoning about death and dying in nursing homes. Ageing Soc. 2011 May; 31 (4): 529–44. http://dx.doi.org/10.1017/S0144686X10000905
101. Pasman HR, Onwuteaka-Philipsen BD, Kriegsman DM, Ooms ME, Ribbe MW, van der Wal G. Discomfort in nursing home patients with severe dementia in whom artificial nutrition and hydration is forgone. AMA Arch Intern Med. 2005 Aug;165(15):1729–35. http://dx.doi.org/10.1001/archinte.165.15.1729

102. Palecek EJ, Teno JM, Casarett DJ, Hanson LC, Rhodes RL, Mitchell SL. Comfort feeding only: A proposal to bring clarity to decision-making regarding difficulty with eating for persons with advanced dementia. J Am Geriatr Soc. 2010 Mar;58(3):580–4. http://dx.doi.org/10.1111/j.1532-5415.2010.02740.x
103. Douglas JW, Lawrence JC, Turner LW. Social ecological perspectives of tube-feeding older adults with advanced dementia: A systematic literature review. J Nutr Gerontol Geriatr. 2017 Jan–Mar;36(1): 1–17. http://dx.doi.org/10.1080/21551197.2016.1277174
104. Kalish N. Evidence-based spiritual care: A literature review. Curr Opin Support Palliat Care. 2012 Jun;6(2):242–6. http://dx.doi.org/10.1097/SPC.0b013e328353811c
105. Perkins C, Egan R, Llewellyn RL, Peterken B. Still living, loving, and laughing: Spiritual life in the dementia unit. J Relig Spiritual Aging. 2015 Jul; 27(4): 270–87. http://dx.doi.org/10.1080/15528030.2015.1037532
106. Schmidt H, Eisenmann Y, Golla HI, Voltz R, Perrar KM. Needs of people with advanced dementia in their final phase of life: A multi-perspective qualitative study in nursing homes. Palliat Med. 2018 Mar;32(3):657–67. http://dx.doi.org/10.1177/0269216317746571
107. Tornstam L. Gerotranscendence and the functions of reminiscence. J Aging Ident. 1999 Sep;4(3): 155–66. http://dx.doi.org/10.1080/15528030.2013.855966
108. Swinton J. What the body remembers: Theological reflections on dementia. J Relig Spiritual Aging. 2014 Apr;26(2–3):160–72. http://dx.doi.org/10.1080/15528030.2013.855966
109. Higgins P. "It's a consolation": The role of Christian religion for people with dementia who are living in care homes. J Relig Spiritual Aging. 2014 Oct;6(4):320–39. http://dx.doi.org/10.1080/15528030.2014.880773
110. Tornøe KA, Danbolt LJ, Kvigne K, Sørlie V. The power of consoling presence – Hospice nurses' lived experience with spiritual and existential care for the dying. BMC Nurs. 2014 Sep 3;13:25. http://dx.doi.org/10.1186/1472-6955-13-25. eCollection 2014.
111. Norberg A, Bergsten M, Lundman B. A model of consolation. Nurs Ethics. 2001 Nov;8(6):544–53. http://dx.doi.org/10.1191/096973301682553832
112. Rasmussen BH, Jansson L, Norberg A. Striving for becoming at-home in the midst of dying. Am J Hosp Palliat Care. 2000 Jan–Feb;17(1):31–43. http://dx.doi.org/10.1177/104990910001700109

14

Depression in Alzheimer's Disease: The Roles of Cholinergic and Serotonergic Systems

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Abstract: Although depression and Alzheimer's disease fundamentally result from distinct pathophysiological events, their coincidence is far from a rare occurrence. In addition to the difficulty in the diagnosis of depression in the patients with a cognitive impairment, care givers and even physicians are mostly unaware that depression and Alzheimer's disease can coexist. While depression has already a devastating impact on quality of life by itself, coinciding depression and Alzheimer's disease may advance to a cataclysmic magnitude. This chapter underlines obstacles in the recognition of depression in the Alzheimer's patients following a brief introduction to the concept of depression. Depression and Alzheimer's disease appear to intersect in the cholinergic and serotonergic systems which may engender an exquisite strategy in the treatment of both disorders. Therefore, potential cholinergic and serotonergic targets are also emphasized.

Keywords: Alzheimer's disease; cholinergic system; coincidence; depression; serotonergic system

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INTRODUCTION

Depression is an affective disorder as old as mankind. Hippocrates has defined depressive psychological state as "melancholia," which means "black bile" in Greek, by the words of "despair, apathy, unwillingness, insomnia, anxiety, incompetence, gloominess, sadness and fear" in around 400 BC (1). The brief description of depression is being possessed by unhappiness, moodiness and unwillingness (2). However, its description as a medical condition is continually evolving with the efforts of classification to make differential diagnosis clearer. Depression is an additional mental burden in Alzheimer's patients who already struggle with cognitive impairments. Both depression and Alzheimer's disease lower quality of life and harden daily activities of the patients who are mostly elders. Alzheimer's disease often does not have a good prognosis whereas depression is a reversible, but recurrent disorder. Almost one-third of the Alzheimer's patients suffer depression (3, 4). This chapter focuses on the interrelation between depression and Alzheimer's disease, and discusses common properties of these neuropsychiatric disorders.

HOW DO WE RECOGNIZE DEPRESSION?

Diagnostic and Statistical Manual of Mental Disorders (DSM) by the American Psychiatric Association is the main guideline to diagnose depressive disorders whereas *International Classification of Diseases* (ICD) by the World Health Organization is the global system for reporting health conditions. According to the fifth edition of DSM, depressive disorders are categorized as major depressive disorder, dysthymic disorder, disruptive mood disorder, premenstrual dysphoric disorder, substance or drug related depressive disorder, depressive disorder due to a medical condition, and otherwise undifferentiated depressive disorder (5). With the system of ICD, the most commonly diagnosed depressive disorders are single depressive episode, recurrent depressive disorders, and persistent mood disorders (6). Depression is the most prevalent psychiatric disorder among general population (7). The patients with depression often have low self-esteem, suffer overwhelming unwillingness, and are afflicted by attention and concentration deficits which result in cognitive impairments (6, 8). Depression may exceed being an affective problem and lead to physical abnormalities. It can coincide with and aggravate existing physical pathologies (2, 6, 8). On the other side, chronic diseases can also generate depressive disorders (2, 9, 10). Together with its complications including the aggravation of physical health conditions, depression is a serious public health concern which also creates an important economic burden (10–12). Concerningly, depression has become the leading cause of disability (13). This insidious pandemic urges a better understanding of its pathophysiological mechanism and so, development of more advanced treatment options.

COGNITIVE DYSFUNCTION MAY LOOK ALIKE DEPRESSION

Although depression occurs frequently, it should not be mistaken as its diagnosis is unchallenging, particularly for the patients with cognitive impairment. Depressive symptoms are summarized above; however, the cognitive impairment can easily be confused with depression and *vice versa*. Lowered self-esteem, self-blame, forgetfulness and indecisiveness are shared behavioral symptoms of depression and cognitive impairment. Furthermore, hypomimia, apathy, psychomotor slowness, fatigue, and reluctance to communicate are the signs a physician may notice in a depressive patient which again resemble cognitive dysfunction (14, 15). Therefore, in the patients with cognitive impairment, diagnosis of depression often requires scrutinization of medical history and discrimination of the affective disorder with overlapping signs of cognitive inability. Depression can emerge at any age, but its prevalence is higher in adults, especially between 55 to 74 years old (16). Also, women are reported to experience depression about two times more than men (11, 16), indicating that sex is a risk factor for depression (17). Besides, there are numerous other factors that create a tendency toward depression such as divorce, separation, loneliness, and low socioeconomic status. (18).

THE COINCIDENCE OF DEPRESSION AND ALZHEIMER'S DISEASE

Epidemiological and longitudinal studies indicate that there is a relation between Alzheimer's and depression. However, it is debatable whether depression is a symptom arisen from the neurodegeneration or a reaction against cognitive inabilities. Some authors are defending that depression is a preceding pathology and a risk factor for Alzheimer's disease whereas some others suggest that depression co-occurs in Alzheimer's disease, and it becomes apparent as a component of Alzheimer's (4, 19).

A yearly increase in elderly population is predicted in almost all countries (20). Because age is an individual risk factor for Alzheimer's disease (3), an aging population means more patients with Alzheimer's disease. About 5% of the people over 65 years have dementia and an additional 5% increases every 5 years. Thus, the prevalence of dementia is as high as 40% after 95 years of age (21). Today, it is estimated that there are 35 million demented people all around the world and this number is projected to be 115 million in 2050 (22).

Alzheimer's disease is the most common form of dementia (23). One-ninth people over 65 years of age and one-third over 85 years struggle with Alzheimer's disease (3). Alzheimer's disease affects more than 5 million people only in the United States and it is reported to be the fourth cause of death (24). Furthermore, coinciding pathologies can significantly increase the incidence of the disease. For example, the risk for Alzheimer's disease is 3 to 5 times more in Down's syndrome (25).

Depression is an affective disorder that can afflict who takes a medical treatment and also, it is more prevalent in inpatients and elders under nursing care (3). Depression is a notable problem for overall elderly health. It has higher recurrence rates in elders than middle-aged people (26). Depression soars the death ratio independently of any medical interventions in patients under the nursing care (27). Suicide is the gravest consequence of depression and depressive elders have the highest suicide rates in all ages (28). Startlingly, suicidal ideation has been reported in 45% of the patients with concurring depression and Alzheimer's disease (29).

Depression is considered to be a "syndrome" rather than a "disease" which presently lacks a definitive biomarker and hence, is diagnosed by subjective questionnaire inventories. These inventories mainly aim to inspect neuropsychiatric symptoms such as negative emotional state, changes in personality and psychotic signs. The emotional and psychotic symptoms are relatively common in the Alzheimer's patients (30). Dysphoria, anxiety, aggressiveness, psychomotor agitation, loss of interest, and sleep disorders are the most frequent depressive symptoms. These symptoms encumber the care of Alzheimer's patients which is already difficult without them (Figure 1).

Clinical studies suggest that depression coincides with Alzheimer's disease in more than a half of the patients (29). Besides, depression in Alzheimer's disease often resembles severe depression, but with a variety of ambiguous symptoms.

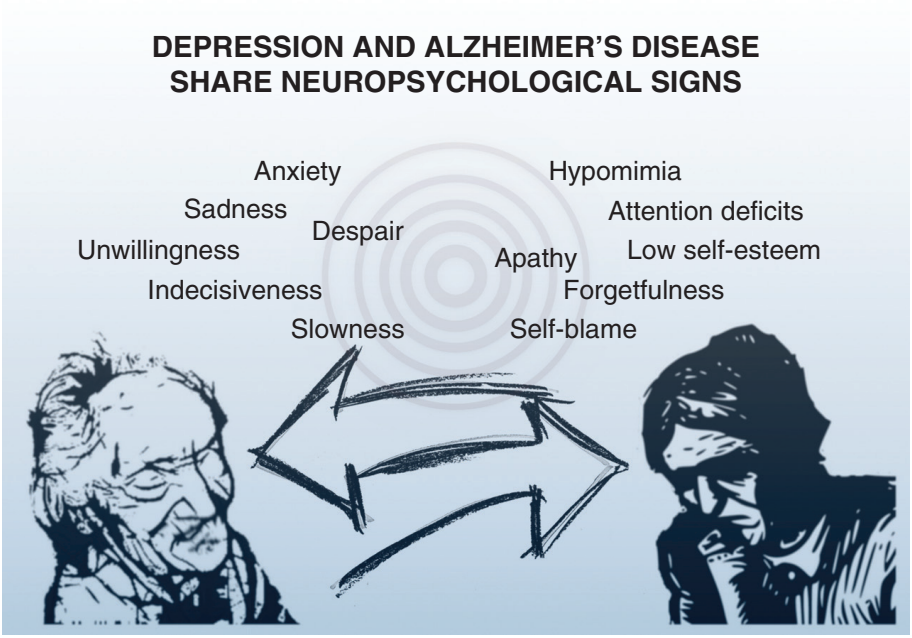

Figure 1 **Depression and Alzheimer's disease are often manifested with similar neuropsychological symptoms and signs.**

For example, depression in Alzheimer's disease can be represented with social isolation, self-abnegation or aggressiveness (3, 4). However, apathy and loss of interest remains to be the most common symptoms in coinciding depression and Alzheimer's disease (4, 31).

Diagnosis of depression in Alzheimer's disease is a strenuous task due to the lack of an objective and repeatable laboratory test to identify depression. Because of similarities with the patients with Alzheimer's disease, depression inventories can be misguiding. Accordingly, it is important to emphasize that the incidence of depression gradually increases from mild to moderate cognitive impairment whereas decreases sharply in severe dementia (32). This decrease in incidence is a clear indicator of the obstacle in the diagnosis of coexisting depression in Alzheimer's disease.

WHAT DOES LIE BEHIND COINCIDING ALZHEIMER'S DISEASE AND DEPRESSION?

As mentioned above, the coincidence of Alzheimer's and depression is not a rare occurrence, and it creates a serious challenge to the diagnosis and quality of life. Although the two pathologies converge on behavioral and cognitive disturbances, they apparently do not originate in a common pathophysiological basis. However, they have a number of overlapping features that may explain the high comorbidity of Alzheimer's disease and depression.

The first report hinting the relation between Alzheimer's and depression dates back to late 1920s. Herz and Fünfgeld (33) have described depression as a preceding disorder that is immediately followed by deteriorations in memory in Alzheimer's patients. Numerous subsequent researches confirmed this link up to now, but post-mortem studies are peculiarly important since there is not a definitive ante-mortem diagnostic tool for Alzheimer's disease (34). Because longitudinal studies are invaluable means to reveal if there is a relation between seemingly distinct conditions, we will briefly discuss the prominent longitudinal studies in which post-mortem diagnosis was established.

In 2004, Milwain and Nagy (35) examined 89 histopathologically confirmed Alzheimer's patients with depression and found that the patients in the intermediate stage of the disease scored lower in CAMCOG, a neuropsychological battery to assess cognition (36), than the patients without depression. The worsened cognition in depressive Alzheimer's patients was implying a deterioration in the neuropathology, although this was not evidenced in that report. Rapp et al. (37) have investigated the post-mortem brains of 95 patients with clinically diagnosed Alzheimer's disease, of which 44 had a life-time history of major depressive disorder and 51 without depression. They noted that the Alzheimer's patients who suffered a life-time depression had about two times more amyloid plaques and neurofibrillary tangles in their hippocampi. In another longitudinal study that presents patient data from almost 40 years, Brunnström et al. (38) emphasized that the onset of dementia is lower in the depression sufferer Alzheimer's patients compared to those without depression.

Alzheimer's disease and depression appear to have a reciprocal relationship. Depression is an individual risk factor for Alzheimer's disease (39) even when the latency between the two pathologies is as late as more than 25 years (40). Preceding depression is a specifically notable predictor of Alzheimer's disease for the patients who do not carry apolipoprotein E (ApoE) ϵ4 allele which is an Alzheimer's-associated polymorphism (41). Thereby, non-ApoE4 allele carriers with depression have higher risk for Alzheimer's disease when compared with whom without depression. Furthermore, depression can have an outrageously high frequency in Alzheimer's disease. Usman et al. (42) have reported that depression was observed in three-fourths of the Alzheimer's patients without considering sex as a variable, and the prevalence was as high as 90% in females. This is particularly important because depression does not only aggravate amyloid pathology, but also worsens the clinical progress in Alzheimer's disease (43).

The mentioned interrelation points out some shared molecular features in Alzheimer's and depression even though they apparently originate from diverse pathological processes. Disturbances in the neurotransmitter systems and hypothalamic-pituitary-adrenal axis are prevailing peculiarities shared in the two pathologies. Indeed, the functions of the brain cannot be accredited to any individual neurotransmitter or neuromodulator because all systems should be operational in a stupendous harmony to achieve an efficient function. However, aberrations in the cholinergic, monoaminergic and serotonergic transmission are evident in both Alzheimer's disease and depression which compose a pathophysiological intersection.

Cholinergic system in the central nervous system consists of two sub-systems as nicotinic and muscarinic. Although cholinergic projections are clustered in distinct regions, both nicotinic and muscarinic receptors are widely distributed throughout the brain and hence, cholinergic transmission involves in numerous brain functions that are carried out by diverse brain areas (44). The nicotinic system works out through the neuronal nicotinic acetylcholine receptors which are simply cation channels (45) whereas the muscarinic system employs any of the five muscarinic acetylcholine receptors (M1-5) which all are G-protein coupled receptors (46). It is long known that cholinergic dysfunction is a problem in Alzheimer's disease (47). The cholinergic hypothesis of Alzheimer's disease proposes that the deterioration in the cholinergic signaling is responsible for learning and memory deficits, a condition which also can be experimentally mimicked by the administration of anti-cholinergic drugs (48). This hypothesis is supported by symptom relieving effects of acetylcholine esterase inhibitors whereas disapproved by the presence of cholinesterase inhibitor-resistant patients (48). Nevertheless, the cholinergic system is evidently disturbed in a remarkable portion of the patients and it shows a correlation with cognitive inabilities (49). This is probably because the cholinergic neurons are particularly affected by the amyloid accumulation (50). With regard to the cholinergic system, the relation between Alzheimer's and depression seems to be paradoxical, considering that reduced cholinergic signaling is linked to cognitive decline. The involvement of the cholinergic system in depression is known for almost 50 years (51) and preliminary studies have underlined the hyperactivity in the cholinergic signaling in depression (52). Consecutive researches have noted that enhanced cholinergic transmission leads to depression (53, 54) and

antagonizing nicotinic signaling can exert an anti-depressant-like effect (55). Contrarily, the activation of a sub-type of nicotinic receptors, alpha7 receptor, has been shown to alleviate depression in mice through restoring the hippocampal function (56). Therefore, instead of a widespread contribution of the cholinergic system to depression, its influence on hippocampus should be taken into account. As illustrated in Figure 2, decreased cholinergic innervation diminishes the hippocampal neurogenesis and function, and improving the cholinergic transmission by means of cholinesterase inhibitors reverses this consequence (53). In regard to alterations in the cholinergic system, the hippocampus is the crossroad where cognitive deficits meet with depressive behaviors. This probably explains the finding of that cholinesterase inhibitors improve neuropsychiatric symptoms in some Alzheimer's patients (57, 58). On the other side, it should be kept in mind that cholinergic hyperactivity created by cholinesterase inhibitors can result in depression (59) and hence, fine dose adjustment and a strict follow-up are particularly important issues in the Alzheimer's patients with a history of depression.

The other neurotransmitter system that bridges between Alzheimer's disease and depression is the serotonergic system that is named after its neurotransmitter, serotonin (5-hydroxytryptamine; 5-HT). Serotonin has 7 families of receptors (5-HT_{1-7}) which are all G-protein coupled except for the ionotropic 5-HT_3

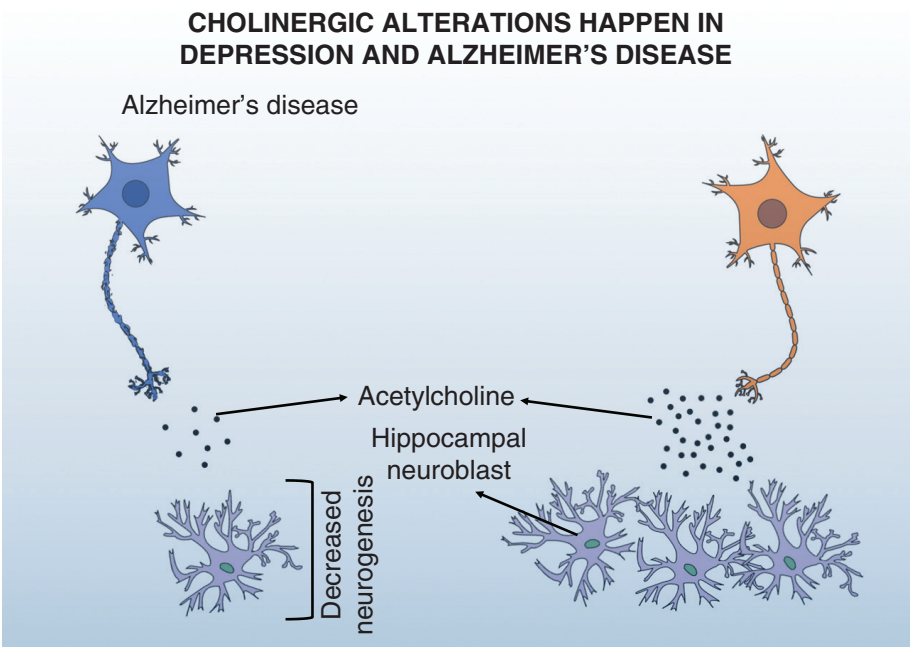

Figure 2 **The cholinergic depletion reduced hippocampal neurogenesis that contributes to cognitive impairments.**

receptor (60). The most commonly prescribed anti-depressants, selective serotonin reuptake inhibitors (SSRIs), aim the restoration of serotonin in the central nervous system. The alleviation of depression with SSRIs constitutes the foundation for the serotonin hypothesis of depression in which depressed mood and its complications are accredited to reduced serotonergic neurotransmission and neuromodulation (61). Besides the serotonin transporter (SERT), three sub-types of serotonin receptors, 5-HT_{1A}, 5-HT_{1B} and HT_{2A}, which are mainly localized in the limbic system, appear to bear higher importance in depression (61). Recently, 5-HT_4 and 5-HT_6 receptors have been suggested to have a role in depression. The stimulation of 5-HT_4 receptors has been shown to lead to an anti-depressant-like effect that is similar to fluoxetine (62, 63). Moreover, 5-HT_4 stimulation has been found to restore cognitive abilities that are altered in depression (63). An opposite link has been revealed for 5-HT_6 receptors which are abundant in the hippocampus (64). The inhibition of these receptors has been suggested to exert an anti-depressant-like effect (65). Indeed, the strongest relation between Alzheimer's disease and depression may lie behind the serotonergic system. The patients with Alzheimer's disease display depleted serotonin and 5-hydroxyindoleacetic acid, the main metabolite of serotonin, in their frontal and temporal cortices (49). Amyloidogenic activity increases in the post-menopausal period and this may be originated in decreased serotonergic signaling due to decreased estrogen (66) which is in accordance with the epidemiological data of higher prevalence of Alzheimer's disease in women (67). The Alzheimer's patients have a decreased 5-HT_{1A} receptor expression particularly in their hippocampi and raphe nuclei, and the hippocampal receptor decrement is correlated with worsened clinical symptoms (68, 69). Similarly, 5-HT_2 receptors decrease up to 69% in Alzheimer's disease as documented by decreased setoperone binding, a 5-HT_2 ligand that has particular affinity to 5-HT_{2A} receptors (70), and by decreased altanserin binding, a 5-HT_{2A} ligand (71). More recently discovered 5-HT_4 and 5-HT_6 receptors, which are novel anti-depressant treatment targets, also involve in the pathophysiology of Alzheimer's disease. Similar to that for depression, 5-HT_4 agonism alleviates Alzheimer's amyloidogenic pathology whereas 5-HT_6 antagonism augments memory and learning in the Alzheimer's patients (72). Moreover, a decrease in SERT accompanies the decrease in the receptors of interest which results in an extensive disruption in the serotonergic signaling in Alzheimer's disease (73). Overall, the serotonergic system, as summarized in Figure 3, plays a crucial role in both Alzheimer's disease and depression and constitutes a highly promising treatment target which may ease depressive mood while soothing cognitive deficits in the Alzheimer's patients with depression.

In addition to current treatment targets of cholinergic and serotonergic systems in Alzheimer's disease and depression, they share some other pathophysiological features such as disturbances in the hypothalamic-pituitary-adrenal axis, inflammation and oxidative stress (74). However, it is not clear whether these disorders are reasons or consequences. Nonetheless, it is evident that the treatments targeting either cholinergic or serotonergic systems can reduce coinciding immunohumoral and oxidative disruptions to some degree in both diseases (75, 76).

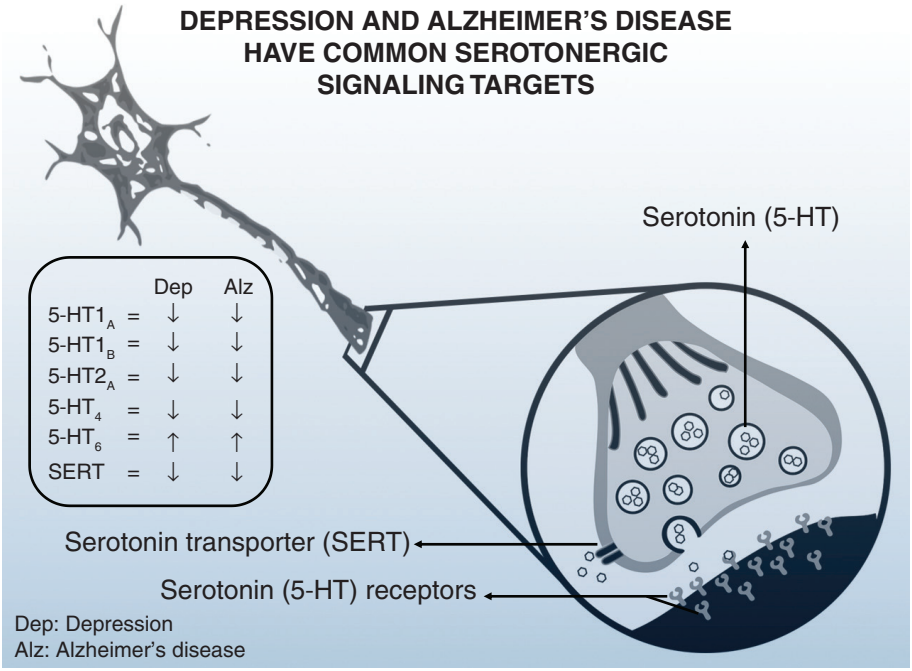

Figure 3 **The serotonergic system is similarly altered in both depression and Alzheimer's disease.**

CONCLUSION

Alzheimer's disease and depression are debilitating disorders which need further scrutinization to understand their pathophysiological properties and to develop novel treatment options. Alzheimer's disease is an incurable neurodegenerative disorder, at least for now, and undiagnosed/untreated depression in Alzheimer's patients creates a serious problem since it worsens neurodegeneration while causing further cognitive deficits and lowering quality of life. Considering abovementioned shared molecular features of both disorders, awareness among clinicians of the possibility of depression in Alzheimer's patients would let them prescribe not only against cognitive symptoms, but also affective disturbances which can benefit to both Alzheimer's disease and depression.

Acknowledgments: Images used in the figures have been labeled for "reuse with modification" according to Google Search by Google LLC.

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REFERENCES

1. Toohey P. Some ancient histories of literary melancholia. Ill Classic Stud. 1990;15(1):143–61.
2. Liu Y, Tang X. Depressive syndromes in autoimmune disorders of the Nervous System: Prevalence, etiology, and influence. Front Psychiatry. 2018 Sep;9:451. http://dx.doi.org/10.3389/fpsyt.2018.00451
3. Alzheimer's Association. 2016 Alzheimer's disease facts and figures. Alzheimers Dement. 2016 Apr;12(4):459–509. http://dx.doi.org/10.1016/j.jalz.2016.03.001
4. Galts CPC, Bettio LEB, Jewett DC, Yang CC, Brocardo PS, Rodrigues ALS, et al. Depression in neurodegenerative diseases: Common mechanisms and current treatment options. Neurosci Biobehav Rev. 2019 Jul;102:56–84. http://dx.doi.org/10.1016/j.neubiorev.2019.04.002
5. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5. Washington, DC: American Psychiatric Association Publishing; 2013.
6. Bernaras E, Jaureguizar J, Garaigordobil M. Child and adolescent depression: A review of theories, evaluation instruments, prevention programs, and treatments. Front Psychol. 2019 Mar;10:543. http://dx.doi.org/10.3389/fpsyg.2019.00543
7. Lim GY, Tam WW, Lu Y, Ho CS, Zhang MW, Ho RC. Prevalence of depression in the community from 30 countries between 1994 and 2014. Sci Rep. 2018 Dec;8(1):2861. http://dx.doi.org/10.1038/s41598-018-21243-x
8. Varinthra P, Liu IY. Molecular basis for the association between depression and circadian rhythm. Tzu-chi Med J. 2019;31(2):67–72. http://dx.doi.org/10.4103/tcmj.tcmj_181_18
9. Demir EA, Oz M, Alp MI, Gergerlioglu HS, Nurullahoglu KE, Yerlikaya FH. Co-administration of cisplatin and curcumin does not alter mood-associated behaviors. Bratisl Lek Listy. 2016;117(2):106–11. http://dx.doi.org/10.4149/BLL_2016_021
10. Wang J, Wu X, Lai W, Long E, Zhang X, Li W, et al. Prevalence of depression and depressive symptoms among outpatients: A systematic review and meta-analysis. BMJ Open. 2017 Aug;7(8):e017173. http://dx.doi.org/10.1136/bmjopen-2017-017173
11. Lu Y, Mak K-K, van Bever HPS, Ng TP, Mak A, Ho RC-M. Prevalence of anxiety and depressive symptoms in adolescents with asthma: A meta-analysis and meta-regression. Pediatr Allergy Immunol. 2012 Dec;23(8):707–15. http://dx.doi.org/10.1111/pai.12000
12. Caliskan H, Sentunali B, Ozden FM, Cihan KH, Uzunkulakoglu M, Cakan O, et al. Marble burying test analysis in terms of biological and non-biological factors. J Appl Biol Sci. 2017;11(1):54–7.
13. Demir EA, Gergerlioglu HS, Oz M. Antidepressant-like effects of quercetin in diabetic rats are independent of hypothalamic–pituitary–adrenal axis. Acta Neuropsychiatr. 2016 Feb;28(1):23–30. http://dx.doi.org/10.1017/neu.2015.45
14. Rampello L, Nicoletti G, Raffaele R. Dopaminergic hypothesis for retarded depression: A symptom profile for predicting therapeutical responses. Acta Psychiatr Scand. 1991 Dec;84(6):552–4. http://dx.doi.org/10.1111/j.1600-0447.1991.tb03193.x
15. Hausmann A, Fleischhacker WW. Differential diagnosis of depressed mood in patients with schizophrenia: A diagnostic algorithm based on a review. Acta Psychiatr Scand. 2002 Aug;106(2):83–96. http://dx.doi.org/10.1034/j.1600-0447.2002.02120.x
16. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018 Nov;392(10159):1789–858.
17. Kessler RC. Epidemiology of women and depression. J Affect Disord. 2003 Mar;74(1):5–13. http://dx.doi.org/10.1016/S0165-0327(02)00426-3

18. Mojtabai R, Stuart EA, Hwang I, Eaton WW, Sampson N, Kessler RC. Long-term effects of mental disorders on marital outcomes in the National Comorbidity Survey ten-year follow-up. Soc Psychiatry Psychiatr Epidemiol. 2017 Oct;52(10):1217–26. http://dx.doi.org/10.1016/S0165-0327(02)00426-3
19. Diniz BS, Butters MA, Albert SM, Dew MA, Reynolds CF, 3rd. Late-life depression and risk of vascular dementia and Alzheimer's disease: Systematic review and meta-analysis of community-based cohort studies. Br J Psychiatry. 2013 May;202(5):329–35. http://dx.doi.org/10.1192/bjp.bp.112.118307
20. United Nations, Department of Economic and Social Affairs PD. World population ageing [Internet]. 2017 [cited 2019 Jun 10]. Available from: https://www.un.org/en/development/desa/population/
21. Qiu C, De Ronchi D, Fratiglioni L. The epidemiology of the dementias: An update. Curr Opin Psychiatry. 2007 Jul;20(4):380–5. http://dx.doi.org/10.1097/YCO.0b013e32816ebc7b
22. Dartigues JF. Alzheimer's disease: A global challenge for the 21st century. Lancet Neurol. 2009 Dec;8(12):1082–3. http://dx.doi.org/10.1016/S1474-4422(09)70298-4
23. Bilgic Y, Demir EA, Bilgic N, Dogan H, Tutuk O, Tumer C. Detrimental effects of chia (Salvia hispanica L.) seeds on learning and memory in aluminum chloride-induced experimental Alzheimer's disease. Acta Neurobiol Exp (Wars). 2018;78(4):322–31. http://dx.doi.org/10.21307/ane-2018-031
24. Crous-Bou M, Minguillón C, Gramunt N, Molinuevo JL. Alzheimer's disease prevention: From risk factors to early intervention. Alzheimers Res Ther. 2017 Dec;9(1):71. http://dx.doi.org/10.1186/s13195-017-0297-z
25. Wiseman FK, Al-Janabi T, Hardy J, Karmiloff-Smith A, Nizetic D, Tybulewicz VLJ, et al. A genetic cause of Alzheimer disease: Mechanistic insights from Down syndrome. Nat Rev Neurosci. 2015 Sep;16(9):564–74. http://dx.doi.org/10.1038/nrn3983
26. Mitchell AJ, Subramaniam H. Prognosis of depression in old age compared to middle age: A systematic review of comparative studies. Am J Psychiatry. 2005 Sep;162(9):1588–601. http://dx.doi.org/10.1176/appi.ajp.162.9.1588
27. Rovner BW. Depression and increased risk of mortality in the nursing home patient. Am J Med. 1993 May;94(5A):19–22.
28. Reynolds CF, Kupfer DJ. Depression and aging: A look to the future. Psychiatr Serv. 1999 Sep;50(9):1167–72. http://dx.doi.org/10.1176/ps.50.9.1167
29. Olin JT, Katz IR, Meyers BS, Schneider LS, Lebowitz BD. Provisional diagnostic criteria for depression of Alzheimer disease: Rationale and background. Am J Geriatr Psychiatry. 2002;10(2):129–41. http://dx.doi.org/10.1097/00019442-200203000-00004
30. Lyketsos CG. Neuropsychiatric symptoms (behavioral and psychological symptoms of dementia) and the development of dementia treatments. Int Psychogeriatrics. 2007 Jun;19(3):409–20. http://dx.doi.org/10.1097/00019442-200203000-00004
31. Chi S, Yu J-T, Tan M-S, Tan L. Depression in Alzheimer's disease: Epidemiology, mechanisms, and management. J Alzheimers Dis. 2014 Sep;42(3):739–55. http://dx.doi.org/10.3233/JAD-140324
32. Forsell Y, Jorm AF, Winblad B. Variation in psychiatric and behavioural symptoms at different stages of dementia: Data from physicians' examinations and informants' reports. Dementia. 1993;4(5):282–6. http://dx.doi.org/10.3233/JAD-140324
33. Herz E, Fünfgeld E. Zur Klinik und Pathologie der Alzheimerschen Krankheit. Arch Psychiatr Nervenkr. 1928 Dec;84(1):633–64. http://dx.doi.org/10.1007/BF01814526
34. Pietrzak K, Czarnecka K, Mikiciuk-Olasik E, Szymanski P. New perspectives of Alzheimer disease diagnosis – The most popular and future methods. Med Chem (Los Angeles). 2018 Jan;14(1):34–43.
35. Milwain EJ, Nagy Z. Depressive symptoms increase the likelihood of cognitive impairment in elderly people with subclinical Alzheimer pathology. Dement Geriatr Cogn Disord. 2005;19(1):46–50. http://dx.doi.org/10.2174/1573406413666171002120847
36. Huppert FA, Brayne C, Gill C, Paykel ES, Beardsall L. CAMCOG – A concise neuropsychological test to assist dementia diagnosis: Socio-demographic determinants in an elderly population sample. Br J Clin Psychol. 1995 Nov;34(Pt 4):529–41. http://dx.doi.org/10.1111/j.2044-8260.1995.tb01487.x
37. Rapp MA, Schnaider-Beeri M, Grossman HT, Sano M, Perl DP, Purohit DP, et al. Increased hippocampal plaques and tangles in patients with Alzheimer disease with a lifetime history of major depression. Arch Gen Psychiatry. 2006 Feb;63(2):161. http://dx.doi.org/10.1001/archpsyc.63.2.161

38. Brunnström H, Passant U, Englund E, Gustafson L. History of depression prior to Alzheimer's disease and vascular dementia verified post-mortem. Arch Gerontol Geriatr. 2013 Jan;56(1):80–4. http://dx.doi.org/10.1016/j.archger.2012.10.008
39. Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: Systematic review, meta-analysis, and metaregression analysis. Arch Gen Psychiatry. 2006 May;63(5):530–8. http://dx.doi.org/10.1001/archpsyc.63.5.530
40. Green RC, Cupples LA, Kurz A, Auerbach S, Go R, Sadovnick D, et al. Depression as a risk factor for Alzheimer disease. Arch Neurol. 2003 May;60(5):753. http://dx.doi.org/10.1001/archneur.60.5.753
41. Sun X, Chiu CC, Liebson E, Crivello NA, Wang L, Claunch J, et al. Depression and plasma amyloid β peptides in the elderly with and without the apolipoprotein E4 allele. Alzheimer Dis Assoc Disord. 2009 Jul;23(3):238–44. http://dx.doi.org/10.1097/WAD.0b013e31819cb3ac
42. Usman S, Chaudhary HR, Asif A, Yahya MI. Severity and risk factors of depression in Alzheimer's disease. J Coll Physicians Surg Pak. 2010 May;20(5):327–30.
43. Zahodne LB, Ornstein K, Cosentino S, Devanand DP, Stern Y. Longitudinal relationships between Alzheimer disease progression and psychosis, depressed mood, and agitation/aggression. Am J Geriatr Psychiatry. 2015 Feb;23(2):130–40. http://dx.doi.org/10.1016/j.jagp.2013.03.014
44. Thiele A. Muscarinic signaling in the brain. Annu Rev Neurosci. 2013 Jul;36(1):271–94. http://dx.doi.org/10.1146/annurev-neuro-062012-170433
45. Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: Native subtypes and their relevance. Trends Pharmacol Sci. 2006 Sep;27(9):482–91. http://dx.doi.org/10.1016/j.tips.2006.07.004
46. Carruthers SP, Gurvich CT, Rossell SL. The muscarinic system, cognition and schizophrenia. Neurosci Biobehav Rev. 2015 Aug;55:393–402. http://dx.doi.org/10.1016/j.neubiorev.2015.05.011
47. Davies P, Maloney AJ. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet (London, England). 1976 Dec;2(8000):1403. http://dx.doi.org/10.1016/S0140-6736(76)91936-X
48. Craig LA, Hong NS, McDonald RJ. Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. Neurosci Biobehav Rev. 2011 May;35(6):1397–409. http://dx.doi.org/10.1016/j.neubiorev.2011.03.001
49. Garcia-Alloza M, Gil-Bea FJ, Diez-Ariza M, Chen CPL-H, Francis PT, Lasheras B, et al. Cholinergic–serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease. Neuropsychologia. 2005 Jan;43(3):442–9. http://dx.doi.org/10.1016/j.neuropsychologia.2004.06.007
50. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: Targeting the cholinergic system. Curr Neuropharmacol. 2016 Jan;14(1):101–15. http://dx.doi.org/10.2174/1570159X13666150716165726
51. Janowsky D, Davis J, El-Yousef MK, Sekerke HJ. A cholinergic-adrenergic hypothesis of mania and depression. Lancet. 1972 Sep;300(7778):632–5. http://dx.doi.org/10.1016/S0140-6736(72)93021-8
52. Dilsaver SC, Coffman JA. Cholinergic hypothesis of depression: A reappraisal. J Clin Psychopharmacol. 1989 Jun;9(3):173–9. http://dx.doi.org/10.1097/00004714-198906000-00003
53. Dagytė G, Den Boer JA, Trentani A. The cholinergic system and depression. Behav Brain Res. 2011 Aug;221(2):574–82. http://dx.doi.org/10.1016/j.bbr.2010.02.023
54. Bassi S, Seney ML, Argibay P, Sibille E. Elevated hippocampal cholinergic neurostimulating peptide precursor protein (HCNP-pp) mRNA in the amygdala in major depression. J Psychiatr Res. 2015 Apr;63:105–16. http://dx.doi.org/10.1016/j.bbr.2010.02.023
55. Aboul-Fotouh S. Behavioral effects of nicotinic antagonist mecamylamine in a rat model of depression: Prefrontal cortex level of BDNF protein and monoaminergic neurotransmitters. Psychopharmacology (Berl). 2015 Mar;232(6):1095–105. http://dx.doi.org/10.1007/s00213-014-3745-5
56. Zhao D, Xu X, Pan L, Zhu W, Fu X, Guo L, et al. Pharmacologic activation of cholinergic alpha7 nicotinic receptors mitigates depressive-like behavior in a mouse model of chronic stress. J Neuroinflammation. 2017 Dec;14(1):234. http://dx.doi.org/10.1007/s00213-014-3745-5
57. Holmes C, Wilkinson D, Dean C, Vethanayagam S, Olivieri S, Langley A, et al. The efficacy of donepezil in the treatment of neuropsychiatric symptoms in Alzheimer disease. Neurology. 2004 Jul;63(2):214–19. http://dx.doi.org/10.1212/01.WNL.0000129990.32253.7B

58. Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. Neurology. 2000 Jun;54(12):2269–76. http://dx.doi.org/10.1212/WNL.54.12.2269
59. Reynolds CF, Butters MA, Lopez O, Pollock BG, Dew MA, Mulsant BH, et al. Maintenance treatment of depression in old age. Arch Gen Psychiatry. 2011 Jan;68(1):51. http://dx.doi.org/10.1001/archgenpsychiatry.2010.184
60. Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. Serotonin: A review. J Vet Pharmacol Ther. 2008 Jun;31(3):187–99. http://dx.doi.org/10.1111/j.1365-2885.2008.00944.x
61. Fakhoury M. Revisiting the serotonin hypothesis: Implications for major depressive disorders. Mol Neurobiol. 2016 Jul;53(5):2778–86. http://dx.doi.org/10.1007/s12035-015-9152-z
62. Mendez-David I, David DJ, Darcet F, Wu M V, Kerdine-Römer S, Gardier AM, et al. Rapid anxiolytic effects of a 5-HT_4 receptor agonist are mediated by a neurogenesis-independent mechanism. Neuropsychopharmacology. 2014 May;39(6):1366–78. http://dx.doi.org/10.1038/npp.2013.332
63. Darcet F, Gardier AM, David DJ, Guilloux J-P. Chronic 5-HT4 receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression. Neurosci Lett. 2016 Mar;616:197–203. http://dx.doi.org/10.1016/j.neulet.2016.01.055
64. Ly S, Pishdari B, Lok LL, Hajos M, Kocsis B. Activation of 5-HT6 receptors modulates sleep-wake activity and hippocampal theta oscillation. ACS Chem Neurosci. 2013 Jan;4(1):191–9.
65. Wesołowska A. Potential role of the 5-HT6 receptor in depression and anxiety: An overview of preclinical data. Pharmacol Rep. 2010;62(4):564–77. http://dx.doi.org/10.1021/cn300184t
66. Bethea CL, Reddy AP, Christian FL. How studies of the derotonin system in macaque models of menopause relate to Alzheimer's disease. J Alzheimers Dis. 2017 Apr;57(4):1001–15. http://dx.doi.org/10.3233/JAD-160601
67. Beam CR, Kaneshiro C, Jang JY, Reynolds CA, Pedersen NL, Gatz M. Differences between women and men in incidence rates of dementia and Alzheimer's disease. J Alzheimers Dis. 2018 Jul;64(4):1077–83. http://dx.doi.org/10.3233/JAD-180141
68. Kepe V, Barrio JR, Huang S-C, Ercoli L, Siddarth P, Shoghi-Jadid K, et al. Serotonin 1A receptors in the living brain of Alzheimer's disease patients. Proc Natl Acad Sci. 2006 Jan;103(3):702–7. http://dx.doi.org/10.1073/pnas.0510237103
69. Lanctôt KL, Hussey DF, Herrmann N, Black SE, Rusjan PM, Wilson AA, et al. A positron emission tomography study of 5-hydroxytryptamine-1A receptors in Alzheimer disease. Am J Geriatr Psychiatry. 2007 Oct;15(10):888–98. http://dx.doi.org/10.1097/JGP.0b013e3180488325
70. Blin J, Baron JC, Dubois B, Crouzel C, Fiorelli M, Attar-Lévy D, et al. Loss of brain 5-HT_2 receptors in Alzheimer's disease. Brain. 1993 Jun;116(3):497–510. http://dx.doi.org/10.1093/brain/116.3.497
71. Hasselbalch SG, Madsen K, Svarer C, Pinborg LH, Holm S, Paulson OB, et al. Reduced 5-HT2A receptor binding in patients with mild cognitive impairment. Neurobiol Aging. 2008 Dec;29(12):1830–8. http://dx.doi.org/10.1016/j.neurobiolaging.2007.04.011
72. Geldenhuys WJ, Van der Schyf CJ. Role of serotonin in Alzheimer's disease. CNS Drugs. 2011 Sep;25(9):765–81. http://dx.doi.org/10.2165/11590190-000000000-00000
73. Smith GS, Barrett FS, Joo JH, Nassery N, Savonenko A, Sodums DJ, et al. Molecular imaging of serotonin degeneration in mild cognitive impairment. Neurobiol Dis. 2017 Sep;105:33–41. http://dx.doi.org/10.1016/j.nbd.2017.05.007
74. Wenzlera S, Knöchela C, Balabana C, Krafta D, Kopfa J, Alves G, et al. Integrated biomarkers for depression in Alzheimer's disease: A critical review. Curr Alzheimer Res. 2016 Jun;13(999):1. http://dx.doi.org/10.2174/1567205013666160603011256
75. McKay MS, Zakzanis KK. The impact of treatment on HPA axis activity in unipolar major depression. J Psychiatr Res. 2010 Feb;44(3):183–92. http://dx.doi.org/10.1016/j.jpsychires.2009.07.012
76. Gałecki P, Mossakowska-Wójcik J, Talarowska M. The anti-inflammatory mechanism of antidepressants – SSRIs, SNRIs. Prog Neuro Psychopharmacol Biol Psychiatry. 2018 Jan;80(Pt C):291–4. http://dx.doi.org/10.1016/j.pnpbp.2017.03.016

15

A Collaborative Approach to Treatment of Alzheimer's Disease from a Psychological Perspective

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Abstract: After the age of 60, earlier in many cases, patients who experience perseverations, forgetfulness, and difficulties with daily living are often referred by their physicians for a neuropsychological evaluation. A neuropsychological evaluation consists of a variety of tests that illustrate a patient's cognitive functioning that include attention, concentration, verbal memory, visual memory, problem-solving, and cognitive flexibility. It further clarifies a range of diagnostic criteria that distinguish Alzheimer's disease (AD) from other mental health-related disorders. Depression presents in a very similar pattern to early stages of AD. Therefore, the neuropsychological evaluation will rule in or out diagnostic criteria and pinpoint which medication should be recommended. A collaborative approach between psychologists, physicians, and caretakers is crucial in obtaining an accurate diagnosis to develop an appropriate treatment plan. Results from the neuropsychological assessment provide physicians with information to develop a medication regimen that helps treat a patient's cognitive and behavioral symptoms. Additionally, this information provides caretakers with psychoeducation to help understand the current functioning of their loved ones.

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The neuropsychological test findings coupled with a medical intervention is imperative to help patients and their families to develop adaptive methods that may help minimize the difficulties of daily living.

Keywords: Alzheimer's; attention; cognitive rehabilitation; dementia; emotional functioning; memory; neuropsychology

INTRODUCTION

Evaluating dementia, particularly Alzheimer's disease (AD), varies across settings. In this chapter, we will introduce what is neuropsychology, neuropsychological testing for possible or probable AD, as well as the importance of collaboration between the doctors involved in treatment of the patient with AD. In particular, we will describe what a medical doctor can expect from neuropsychological testing. Among countless batteries that are performed by neuropsychologists, we will emphasize the following phenomena: depression, substance use, decision-making, co-occurring mental health disorders, as well as the role that technology plays in neuropsychological testing. The importance of neuropsychological testing is priceless to patients. Results from neuropsychology testing can aid in confirming and/or ruling out often otherwise misdiagnosed syndromes that may require a different treatment plan and hence may impede and/or lead to success for appropriate treatment. For instance, neuropsychological testing may confirm AD and rule out depression and vice versa. Our hope is that readers will be more familiar with neuropsychological testing and its role in treatment for patients as well as their loved ones.

WHAT IS NEUROPSYCHOLOGY?

Neuropsychology is a scientific term that encompasses studying the central nervous system, including the brain and spinal cord, and how it influences an individual's cognitions and behaviors. This field within psychology has experienced a growth for over the last 40 years, with interest for about 120 years within the modern scientific psychology (1). Neuropsychology utilizes specialized knowledge of foundational neuroanatomy, principles of neuroscience, brain development, neurological disorders and etiologies, neurodiagnostic techniques, normal and abnormal brain functioning, and neuropsychological and behavioral manifestations of neurological disorders. In addition to this diverse amount of knowledge, neuropsychologists gather relevant historical information, conduct a neuropsychological examination, analyze and integrate data and findings, and provide feedback to the referral source. Neuropsychologists attempt to answer the question of "What mechanisms are responsible for human thinking, learning, and emotion, how do these mechanisms operate, and what are the effects of changes in brain states upon human behavior?" (1). This specialty applies principles of assessment and evidence-based interventions to the study of human behavior as it relates to normal and abnormal functioning of the central nervous system. The combination of extensive

training, knowledge, and education with the process of obtaining and analyzing information allows for neuropsychologists to solve questions about the mind that otherwise cannot be solved.

As the main focus for neuropsychology is to determine the brain and behavior relationship with each patient exhibiting their own unique presentation, this has pushed to develop the field toward becoming an essential discipline for diagnosing and treating cognitive mental health disorders. Neuropsychology utilizes mechanisms to elicit activation in the multiple areas of the brain to determine their individual levels of functioning. In particular, these areas are referred to as cognitive domains. These cognitive domains studied by neuropsychologists include "perception, attention, learning, memory, processing of spoken and written language, thinking, reasoning, or belief formation, with the aim of learning more about the normal functional architectures of the cognitive processing systems used to carry out these activities" (2). As a result of testing the functioning in all the above-mentioned domains, there are a wide range of disorders that may be diagnosed including dementia, vascular disorders, Parkinson's disease, and other neurodegenerative disorders, traumatic brain injury, seizure disorders, learning disabilities (LD), neuropsychiatric disorders, infectious disease affecting the central nervous system (CNS), neurodevelopmental disorders, metabolic disease and neurological effects of medical disorders or treatment. Achieving a diagnosis allows individuals, their loved ones, their caretakers, and their physicians to gain awareness of the presenting problem and understand effective treatment recommendations to manage the diagnosis.

It is important to understand the direct correlation between cognitions and behaviors for neuropsychologists. This relationship that is observed is an individual's natural response to their environment, which may experience fluctuations. Neuropsychologist's main role is to attempt to distinguish where there are abnormalities and how it may cause dysfunction that may impact an individual's ability to properly function in their daily lives. Dysfunctions that a patient may experience may present in one or more of the domains of cognitive functioning. Each domain plays a crucial role in how individuals function in their personal and professional lives. These dysfunctions may present at different severities. In general, the minimal symptoms are not noticed by the patients; however, the more pronounced are usually noticed by their significant others. Many times, when a cognitive domain begins to decline, the patient may have minimal or no presenting symptoms, while others may have a severe presentation from the beginning. Such presentation depends on the domain that is impacted and possible other co-occurring medical complications. While there may be an overlap between the areas that present with similar problems, each has their own focus on how they contribute to the brain and cognition relationship.

One of the main impacts to the cognition and behavior relationship is a type of brain damage or trauma. The damage or trauma can range from a direct impact to the head to an organic occurrence in the brain. Regardless of the type of brain trauma, when it occurs, it can be localized or generalized throughout the brain. The area of where the impact occurs will influence the patient's presentation. It may be difficult to pinpoint where the impact occurred, but the neuropsychological evaluation will pinpoint impairments within each cognitive domain. The neuropsychological evaluation is pertinent as it will discern clusters of symptoms that may belong to psychiatric symptoms, medical conditions, as well as emotional presentation.

The primary reason for a neuropsychological evaluation is to create a diagnosis and eliminate other disorders that may seem relevant but may not apply to the individual's presentation. Neuropsychological assessments specifically target parts of the brain through measuring the different cognitive domains of functioning. Cognitive functions are processes that help individuals carry out tasks requiring attention, memory, language, judgment, and problem solving. While it is well known that certain domains of cognitive functioning have their main centers, they also may be spread across several parts of the brain. Neuropsychological assessments elicit activity in different parts of the brain when activated with test tasks. A thorough analysis of the integrated data from the neuropsychological evaluation, will not only pinpoint given cognitive strengths but also weaknesses. Such analysis is most likely driven by patterns within the testing battery which as a result renders a diagnosis. This is why a neuropsychological evaluation contains a set of tests that measure similar cognitive functioning, rather than utilizing screening assessments. On that note, the neuropsychological evaluation is a lengthy process which oftentimes requires several hours of testing. While the several hours may seem long and daunting, it presents accurate data, rather than screening alone. Each neuropsychological battery is designed for an individual's ability to withstand long testing hours. In those situations a focused neuropsychological battery is conducted and a single cognitive domain may be tested (i.e., attention). Hence it is not uncommon that testing may vary in length from one to several hours, depending on the setting. For instance, testing may be shorter within an inpatient bedside setting and longer for a patient seeking an evaluation for a high-profile employment position, in an outpatient setting. In spite of the long hours of testing, it is our experience that patients and their family members appreciate the time that they engage in the testing, as this allows for a thorough evaluation. Patient's family members especially their children, appreciate the knowledge as they may carry similar genetic load and hence can utilize measures for prevention.

Individual results of the neuropsychological evaluation are compared to norms that will then indicate the level of functioning of this individual. The neuropsychological evaluation will render normative data that will indicate whether a patient is in the above average, average, or impaired range of functioning. The neuropsychological evaluation provides the clinician with twofold data. First, it delineates the pre-morbid functioning level that allows the neuropsychologist to understand whether the impairment is significant, as this may vary from one individual to another. For instance, if the tested individual has never experienced difficulties in school, it is expected that their premorbid functioning would demonstrate intact cognitions that are impaired during the time of testing. It is crucial to highlight here, the importance of having accurate norms to which the results are compared. The neuropsychological testing norms on several tests include norms not only for AD but also for patients with stroke, head trauma, LD, PTSD, and schizophrenia as compared to a control group. To further delineate neuropsychologists' work it is also important to note that each neuropsychological assessment presents not only an understanding of the cognitive domains in the human brain but also its characteristics that then determine diagnostic criteria and the appropriate treatment plan.

For the purpose of diagnosing probable AD, clinical neuropsychology is the main discipline to determine its presentation and severity. As aforementioned, AD impacts several areas of the brain and may present as several different diagnoses. It is imperative then to utilize the neuropsychological assessment results to determine an appropriate diagnosis and rule out other possibilities, as those treatment recommendations may be drastically different.

NEUROPSYCHOLOGICAL TESTING FOR POSSIBLE OR PROBABLE AD

AD is a neurodegenerative disorder. It is the most common type of dementia, with an estimate of 5 million individuals in the United States (65 or older) who were diagnosed with AD and related dementias in 2014 and an estimated 13.9 million projected to be diagnosed with the disease by the year 2060 (3). According to the World Health Organization, worldwide there are an estimated 47 million individuals affected by the disease currently (4). According to Braak and Braak (5), AD is "characterized by neuronal atrophy, synapse loss, and the abnormal accumulation of amyloidogenic plaques and neurofibrillary tangles in medial temporal lobe limbic structures (e.g., entorhinal cortex and hippocampus) and the association cortices of the frontal, temporal, and parietal lobes" (6). Several brain regions are affected by AD including the anterior cingulate cortex, the frontal lobes, as well as the medial temporal lobes (7).

According to Groth-Marnat (8), AD is referred to as a "cortical" dementia due to it affecting the cortical regions in the brain, as opposed to other types of dementia that affect the subcortical regions in the brain and cause impairments in attention and visuoconstruction. To further explain, the cortical dementias are characterized by more difficulties with memory and learning and sub-cortical effect more problems within attention and visuoconstruction.

The first cognitive symptom of AD is an impairment in episodic memory functioning, and later on as the disease progresses it impacts the frontal cortex, which impairs executive functioning (9). Based on a study conducted by Reed and colleagues (9), it was found that "Pathologically defined cases of Alzheimer's disease had verbal and non-verbal memory scores that averaged about 1 SD lower than their executive function scores." According to Smith and Bondi (10), the types of cognitive deficits observed in AD include impairments in areas such as memory and episodic learning, language and semantic knowledge, visuospatial, and executive functioning such as difficulties with problem-solving and abstract thinking as well as set-shifting. According to the Diagnostic and Statistical Manual of Mental Disorders (5th Edition), procedural memory and social cognition may be preserved for a longer period of time. Smith and Bondi further indicate that individuals with AD can experience anosognosia, in which they lack awareness of the cognitive problems they may experience (10). This lack of awareness may result in delayed diagnosis and treatment. Most patients are tested due to family concerns who notice the most salient characteristics such as perseveration and lack of new learning.

Physicians may refer patients who experience forgetfulness, changes in their behavior and functioning, and/or a decline in the ability to complete activities of daily living independently, for a neuropsychological evaluation. When the referral question is for memory decline, it is important to conduct a thorough and detailed clinical interview. In such cases where the referred individual may have difficulties reporting relevant information and is deemed to be an unreliable historian, it is important when possible that the clinician attempts to obtain collateral information. This information may be obtained from the referred individual's caregiver or family member regarding the onset/frequency/severity of the individual's symptoms and functioning. Information obtained from the clinical interview guides the clinician to choose which test or series of tests need to be administered to the referred patient to address the referral question.

A standard neuropsychological testing battery to assess for possible or probable AD should include measures assessing several domains of functioning including: Attention, Processing Speed, Memory, Language, Executive Functioning and Dementia Severity (11). Simple attention continues to remain intact as patients with intact verbal skills, who present with severe impairments, are able to repeat 5 digits forward correctly (12). With regards to language abilities, individuals with AD often demonstrate impairments in verbal fluency, semantic fluency, and naming objects. Tests are administered to assess areas of visual and verbal memory, as well as the individual's performance in areas of immediate and delayed memory, and ability to learn new information. With regards to memory, patients with AD have difficulty encoding new information, the learning curve is flat across trials, and new information is not consolidated (13). Additionally, recognition is impaired and delayed recall of information is also poor, even after a short period of time (13). According to Delis et al. 1991, "…AD patients rapidly forget information over time and are equally impaired (relative to age-matched controls) on recognition and free recall components of the tasks. This pattern of performance is consistent with impaired consolidation rather than ineffective retrieval of new information" (14).

In addition to other criteria that must be met for a diagnosis of Major Neurocognitive Disorder Due to AD, the Diagnostic and Statistical Manual of Mental Disorders (5th Edition) indicates that there must be "clear evidence of decline in memory and learning and at least one other cognitive domain (based on detailed history or serial neuropsychological testing)" (15).

DEPRESSION AND AD

Neuropsychological tests help with determining if the patients have symptoms of dementia, depression, or both. As discussed above, depression and dementia share similar characteristics, but neuropsychologists know the differences. Neuropsychologists have the clinical capacity to rule out and state either diagnosis. Such luxury helps medical doctors and/or prescribing psychologists to design the most efficient treatment plan available for a patient.

There are many alternate diagnoses that may interfere with an accurate assessment of probable or possible AD. Depression is among one of the most common misdiagnoses due to the number of overlapping symptoms it shares with AD.

The most common overlapping symptoms include loss of interest in enjoyable activities and hobbies, social withdrawal, problems with memory, lack of sleep or over-sleeping, and impaired concentration (16). The difficulty in differentiating AD from depression depends on several factors, which may include inaccurate information provided by the patient and/or family members, normal effects of aging, dementia and depression comorbid conditions (17). The causal relationship between dementia and depression is supported by findings that people with dementia appear to have a higher prevalence of depression (18). However, in many cases patients may not have AD or dementia and may just have depression. In such cases, particular attention is placed on depressive symptoms which are less common in dementia alone. These symptoms may include feelings of hopelessness, expressions of guilt, feelings of worthlessness, and thoughts of self-harm (19).

There have been correlations found between mild, moderate, and major depressive disorder and insomnia with regard to AD risk (20). Insomnia and depression share a complex relationship. Depression may cause sleep problems and sleep problems may cause or contribute to depressive disorders (21). However, in terms of diagnosing AD when lack of sleep and depression occurs, the challenge becomes greater, as the symptoms overlap. Some of the symptoms such as memory loss, poor judgment and taking longer to complete normal daily tasks, all may contribute to lack of sleep, depression, and/or AD itself. Again this is why a full neuropsychological evaluation is necessary as it encompasses evaluating sleep patterns, symptoms of depression, as well as highlights hallmarks of AD. It is also crucial to state here that a neuropsychologist needs to work very closely with the patient's primary care physician, psychiatrist, and/or prescribing psychologist, as the medications that the patient is taking may also have a crucial impact on his/her functioning. "A survey in the United States of a representative sampling of 2206 community dwelling adults (age 62–85 years) was conducted by in-home interviews and use of medication logs between 2010 and 2011" (22). "At least 1 prescription medication was used by 87%, 5 or more prescription medications were used by 36%" (22). In summary, it is well known that an adult population that is most susceptible to AD takes about 1–5 different medications a day. Those medications include not only medication related to systemic failures and/or inadequacies including steroids, diabetes medication, vascular problems, to list a few and they may cause side effects stimulating not only metabolic system problems, arrythmias, but also sleep problems.

In summary, a crucial part of the neuropsychological evaluation is evaluating sleep habits and to differentiate sleep difficulties related to emotional problems, side effects of medications, as well as poor sleep hygiene as they all require different interventions. Furthermore, Alhola and Polo-Kantolastate that attention, working memory, long term memory and decision making abilities can be impaired due to sleep deprivation (23). Moreover, metabolic functioning may also be impaired due to lack of sleep. While the body sleeps, a plumbing method called the glymphatic system opens up channels in the brain and allows fluid to flow rapidly throughout the brain, in order to flush out toxins or interstitial protein deposits (24) that may be accumulating in the brain (25). During sleep deprivation this accumulation of proteins or toxins may cause cognitive impairments, as our brain is unable to function at an optimal level. Although AD may not be the actual cause of these cognitive impairments for patients who lack

sleep, the diagnosis of dementia becomes a viable conclusion. Most likely, this is due to the fact that when older individuals do not have quality sleep as well as struggle with other medical complications, they may simply develop functional impairment that may constitute dementia.

Anxiety can interfere with a person's everyday life and it is also the most common symptom throughout the different stages of dementia (26). Anxiety and agitation become more apparent in the early stages of AD as people begin to recognize their losses and the seriousness of the disease. They may become anxious about being left alone or abandoned, while any changes in the daily routine can also trigger anxiety and agitation (27). The prevalence of depression in AD is fairly high, with rates reported as high as 87% and averaging 30%. Therefore, it is conceivable that comorbid anxiety and depression may affect a large percentage of patients with AD (28). The correlation is unknown between AD and anxiety; however, a recent study has shown that anxiety predicts risk of Alzheimer's disease (n = 26,193 out of seven studies, hazard ratio 1.53, 95% CI 1.16–2.01, $P < 0.01$) and vascular dementia (n = 4916 out of two studies, odds ratio1.88, 95% CI 1.05–3.36, $P < 0.01$) (29). Although anxiety does not directly associate with the likelihood of having dementia or AD, it may reflect neurodegeneration in patients who experience symptoms of AD or dementia.

WHAT DO NEUROPSYCHOLOGISTS HAVE TO OFFER MEDICAL DOCTORS AND HOW IMPORTANT IS THEIR COLLABORATION

Neuropsychologists are able to collaborate with primary care physicians and other specialty driven doctors in a variety of ways, including answering referral questions and establishing and clarifying diagnostic criteria related to dementia. Neuropsychologists use countless tests that are established to be a crucial part of dementia assessments in various situations. A few are described below.

The most common question with which patients, their family members, and their doctors come to neuropsychologists on an outpatient basis is regarding the gravity of dementia. In most cases, medical doctors complete the Mini Mental Status Exam (MMSE) to screen a patient's memory and attention. Typically, patients with dementia fail such questions. In other words, when patients are provided three words to learn and later recall, they do not remember the three words or they remember only one of the three words provided. In most situations, the patient that failed the memory and/or orientation tests are sent to a neurologist so an MRI and/or CT scan can be ordered to indicate the presence of any notable changes in the patient's brain scan. Sometimes, impaired patients may have normal MRIs or an MRI may show significant changes even while the patient's cognitive functioning seems intact. This is most likely explained by neuroplasticity. In such situations when the MRI and/or CT results do not correlate with the patient's self-report, neuropsychological testing is taken into consideration.

Another well-known issue establishing the need for completion of neuropsychological testing is the fact that patients remember previously learned tasks from the Mini-Mental Status Exam administered by their doctors. Repetition of similar

tasks, for instance remembering three words, results in inconclusive data as the screening does not evaluate new learning but rather recall of previously learned information. Similar phenomenon is observed when adult children do not recognize symptoms of dementia in their parents. This happens due to repetition of similar conversations concerning daily tasks and/or activities that do not change.

Patients also tend to minimize their impairments when explaining them to their doctors, and their family members are unable to provide correct information about the patients' functioning because their loved ones have their routines. Usual rituals for family members often include calling the patient and asking them the same questions over and over. The questions they ask are most likely about the patient's eating habits and medication-taking rituals. Since going for a walk with the dog is not a novel task, the patients appear healthy and free from any cognitive problems. Neuropsychologists spend a lot of time with a patient's family, explaining to them that such routine is a very common phenomenon that promotes lack of accurate judgment about a family member's cognitive functioning. In fact, if the medical doctor and/or neuropsychologist hear any complaints relating to a family member's decreased cognitive functioning, such as perseverations and forgetfulness, the patient is most likely already in the advanced stages of dementia. Unfortunately, many patients reside alone and their beginning signs of dementia are not detected or are explained away as part of the normal aging process.

THE ROLE OF ALCOHOL CONSUMPTION IN DEMENTIA

Not all medical doctors choose to speak to their patients about their alcohol consumption. Most doctors that treat AD are younger than their patients. Both of these factors may often result in ambiguity and discomfort that may impede the doctor–patient dialog about alcohol use. However, most doctors of clinical psychology are well-equipped and clinically trained to discuss the topic of alcohol use with their patients.

Despite the toughness of this issue, it is well known that patients with AD are not immune to drinking alcohol. In fact, many dementia patients drink. Drinking causes falls, broken bones, and brain injuries. It also causes cognitive problems such as poor planning and organizational skills, poor decision-making and judgment, problems with impulsivity, difficulty controlling emotions, problems with attention and slower reasoning, lack of sensitivity to others' feelings, and behavior that is socially inappropriate. On the other hand, a systematic review by Peters and colleagues indicates that "in older people, small to moderate amounts of alcohol consumption are associated with reduced incidence of dementia and Alzheimer's disease (AD)" (30).

While there are more problems related to alcohol consumption, dementia and AD patients are getting contraindicated recommendations about drinking alcohol from their primary care physicians. In contrast, clinical psychologists with neuropsychology specialties are not only trained to treat alcohol use but are also able to perform neuropsychological tests that can characterize the type and extent of a patient's cognitive deficit as well as denote which symptoms belong to AD and which ones to alcohol use. In this way, rehabilitation efforts are maximized.

The importance of neuropsychological testing in matters of AD and alcohol consumption was delineated by Robert Heirene, Bev John, and Gereth Roderique-Davies. The authors reviewed the available data and agreed that cognitive screening is limited and is trumped by far by comprehensive neuropsychological testing following screening to ensure a correct diagnosis (31). The authors stated that neuropsychological testing establishes crucial characteristics of alcohol related cognitive decline that mostly includes a decline in executive functioning and episodic, autobiographical, procedural, and working memory. They also discussed the issue of confabulation that may be related to both dementia itself and an alcohol related impairment (30).

AD AND DECISION-MAKING CAPACITY

Another type of neuropsychological referral encompasses denoting the gravity of AD symptoms, as well as the patient's decision-making capacity. Indeed, the vast majority of outpatient referrals relate to the individual who resides at home, and his/her family members worrying about their cognitive functioning. Patients with detected and undetected AD spend time alone at home. During those moments, they are vulnerable to burglary, deceit, and even death because their judgment is impaired. For instance, there have been individuals who opened the door to a stranger, soliciting them to withdraw money from the bank; as well as patients selling health insurance, buying homes, and even moving to another state without having a previous discussion or agreement with the rest of their family members about their plans. This demonstrates the lack of judgment, rational thinking, and insight which may be characteristic of individuals with dementias.

Another very important aspect for the need to complete neuropsychological testing regarding decisional capacity is the fact that many AD patients are in the hospital and are unable to make decisions regarding their health. Unfortunately, without completion of neuropsychological tests, many AD patients seem to cognitively function well to not only their family members but also to their doctors. Those are the patients that refuse certain treatments that may be very vital to their life. Those are the patients that lie in their hospital beds, seem unconcerned about their health, and lack an understanding about the gravity of their illnesses. Those are the patients that have shallow humor and tend to answer their doctors' questions with short answers that indicate their disinterest. For example, they tend to state that they do not care for politics hence they do not know who is the current president, and they tend to fail orientation tests even when the correct date was written by a nurse in front of them.

Families of AD patients have the tremendous task of accepting that their parents, uncles, aunts, and other family members who used to be sharp, have lost the ability to make decisions. The family members also vicariously deal with their own stress responses, facing the possibility that they may have similar genetic loads and that they may also develop AD. All of the above mentioned constraints lead to missing the symptoms, which then leads to the most dangerous problem: not doing anything.

After completion of the neuropsychological testing, the report and recommendations are delineated. One of the possible recommendations may be cognitive rehabilitation. Another recommendation may encompass an evaluation for a

cognitive enhancer. Recommendations may also include possible counseling/psychotherapy and or a support group for the family members/caregivers to process their fears, help them develop skills to deal with their family member that has AD, and start the process of establishing a power of attorney.

AD AND OTHER MENTAL HEALTH PROBLEMS

Doctors of psychiatry often refer their patients for neuropsychological evaluations to rule out AD and possibly diagnose depression. They also seek neuropsychological evaluations to determine possible attention problems and undiagnosed LD that can all resemble early dementia.

It is our experience that more than a few referrals from psychiatrists include ruling out stress in patients as young as 50 years old that presented with dementia like symptoms. Those patients seem to have dementia, but neuropsychological evaluations are able to detect severe stress that results in their poor job performance. In such situations a conclusive diagnosis is crucial, as the treatment for stress as well as burnout differs from treatment for dementia.

Neuropsychological evaluations can also detect LD that have not previously been diagnosed. Nowadays, with more and more jobs requiring computer skills, this phenomenon occurs, even with those jobs that are considered computer-free. For instance, a 55-year-old janitor is now required to login to the computer to check memos, request days off, or perform required training. In this situation, a LD may mirror early dementia and hence lead to inappropriate treatment. Again, a neuropsychologist will not only test this individual's cognitive functioning but also recommend training, teach compensation skills, etc.

AD AND TECHNOLOGY

Typically, the older population does not do well with computerized testing, even though these kinds of tests are well-prepared for inexperienced computer users. Since computers were first used at schools in the eighties in the USA, patients that have never used a computer to study or work are included in this population. Hence, most patients that are at least 50 years old who lack the required computer skills, may be considered in this group. At our clinic, any time a computerized test was administered to an older population member, it has been observed that the cognitive abilities tested on the computer were lower than on paper and pencil tests. Hence, doctors using computer screening tests for cognitions may obtain inaccurate results. When in doubt about a patient's results, it is recommended that the patient be referred for a full neuropsychological testing battery.

AD AND MARIJUANA USE

Much of the older population in the United States recreationally consumes and/or smokes marijuana, as it is now prescribed as well as highly available. When asked what their purpose for smoking is, most people state that it is healthy and

that it helps them with sleeping and managing their pain. Research on smoking marijuana and its effects on cognitions vary, but in our practice it has been observed on several occasions, that severe delirium followed ingestion of cannabis. Cannabis use can result with hallucinations, as it is a hallucinogenic agent. It may also quicken cognitive decline, especially memory problems. While this has little to do with neuropsychological testing and AD, it is crucial to state here that all healthcare providers should consider referring any, but especially older patients, to neuropsychologists because the underlying causes of marijuana ingestion can be detected, processed, and potentially diminished; all of which would better their patient's quality of life. In other words, it is our belief that if an individual decides to smoke marijuana, he or she has reasons to do so. Those reasons may be depression, anxiety, withdrawal, loneliness, or many other problems. No matter the reason, in the therapeutic room of a skilled psychologist, those problems can be addressed, diminished, and/or possibly resolved.

AD AND THE N-648

The N-648 is a form that is used by medical doctors and psychologists to help immigrants obtain United States citizenship without passing the English proficiency and United States history exams. We believe that this form cannot be completed without normative data concerning the patient's memory. It is the neuropsychological testing that can actually determine whether an individual with probable or possible AD is capable of learning English and United States history, which are required to become a United States citizen.

CONCLUSION

Neuropsychological testing is underutilized and it is our hope that its use will be prevalent and treated as a necessity especially in patients with AD. Time spent on neuropsychological testing can change the patient's life tremendously. For instance, a depressed patient that actually has dementia after a neuropsychological assessment will not be prescribed anti-depressants. Conversely, a patient with dementia will be prescribed cognitive enhancers rather than anti-depressants. As mentioned above, testing may last from an hour to several hours, but the knowledge after it is completed is priceless and appreciated by the families we have served thus far. The neuropsychological testing not only helps the patient but also their families. We have spent countless hours working with our patients' families to not only help them with appropriate care, but also with the fact that they may be facing similar future presenting concerns. Those family members that worked with us had a tremendous advantage over their parents, as their anticipation and the possibility to carry similar genetic load allowed them to plan for the future and to initiate anything they can to avoid and/or delay AD. The neuropsychological reports written in our clinic are not only for the patients and their doctors, but also caregivers who can better understand the level of

impairment, as well as learn skills to be a better support system. In other words, a few hours of neuropsychological testing is worthwhile for everyone that is involved with the AD patient.

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REFERENCES

1. Graham Beaumont J. Introduction to neuropsychology. 2nd ed. New York: The Guilford Press; 2008. 382 p.
2. Coltheart M. Cognitive neuropsychology. Scholarpedia. 2008 Feb;3(2):3644. http://dx.doi.org/10.4249/scholarpedia.3644
3. Matthews KA, Xu W, Gaglioti AH, Holt JK, Croft JK, Mack D, et al. Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015–2060) in adults aged > 65 years. Alzheimers Dement. 2019 Jan;15(1):17–24. http://dx.doi.org/10.1016/j.jalz.2018.06.3063
4. World Health Organization. 10 facts on dementia [Internet]. Geneva: World Health Organization; 2017 Apr [cited 2019 Jul 1]. Available from: https://www.who.int/features/factfiles/dementia/en/
5. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 1991 Sept; 82(4):239–59. http://dx.doi.org/10.1007/BF00308809
6. Salmon DP, Bondi MW. Neuropsychological assessment of dementia. Annu Rev Psychol. 2009 Jan;60:257–82. In: Albert MS, Moss MB, Tanzi R, Jones K. Preclinical prediction of AD using neuropsychological tests. J Int Neuropsychol Soc. 2001 Jul;7(5):631–9. http://dx.doi.org/10.1017/S1355617701755105
7. Albert MS, Moss MB, Tanzi R, Jones K. Preclinical prediction of AD using neuropsychological tests. J Int Neuropsychol Soc. 2001 Jul;7(5):631–9. http://dx.doi.org/10.1017/S1355617701755105
8. Groth-Marnat G. Handbook of psychological assessment. 5th ed. Hoboken NJ: Wiley; 2009. 517 p.
9. Reed BR, Mungas DM, Kramer JH, Ellis W, Vinters HV, Zarow C, et al. Profiles of neuropsychological impairment in autopsy-defined Alzheimer's disease and cerebrovascular disease. Brain. 2007 Mar;130(Pt 3):731–9. http://dx.doi.org/10.1093/brain/awl385
10. Smith GE, Bondi MW. In: Morgan JE, Ricker JH, editors. Chapter 39: Normal Aging, mild cognitive impairment, and Alzheimer; Disease: Textbook of clinical neuropsychology. 1st ed. New York: Taylor & Francis; c2008. p.762–780.
11. Weintraub S, Salmon D, Mercaldo N, Ferris S, Graff-Radford N, Chui H, et al. The Alzheimer's Disease Centers' Uniform Data Set (UDS): The neuropsychologic test battery. Alzheimer Dis Assoc Disord. 2009 Apr;23(2):91–101. http://dx.doi.org/10.1097/WAD.0b013e318191c7dd
12. Deutsch Lezak M, Howieson DB, Bigler ED, Tranel D. Neuropsychological assessment. 5th ed. New York: Oxford University Press; 2012. 1200 p.
13. Zec RF. Neuropsychological functioning in Alzheimer's disease. In: Park RW, Zec RF, Wilson RS, editors. Neuropsychology of Alzheimer's disease and other dementias. 1st ed. New York: Oxford University Press; 1993. p. 3–80.
14. Delis DC, Massman PJ, Butters N, Salmon DP, Cermak LS, Kramer JH. Profiles of demented and amnesic patients on the California Verbal Learning Test: Implications for the assessment of memory disorders. Psychol Assess. 1991;3(1):19–26. http://dx.doi.org/10.1037/1040-3590.3.1.19
15. American Psychiatric Association. Dilip V. Joeste and Dan G. Blazer. Diagnostic and statistical manual of mental disorders. Arlington, VA: American Psychiatric Association; 2017. 611 p.

16. Mayo Clinic. Alzheimer's or depression: Could it be both? [Internet]. Rochester: Mayo Clinic; [cited Jul 1]. Available from: https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/in-depth/alzheimers/art-20048362
17. Rotomskis A, Margevičiūtė R, Germanavičius A, Kaubrys G, Budrys V, Bagdonas A. Differential diagnosis of depression and Alzheimer's disease with the Addenbrooke's Cognitive Examination- Revised (ACE-R). BMC Neurol. 2014 Apr;15:57. http://dx.doi.org/10.1186/s12883-015-0315-3
18. Lyketsos CG, Steele C, Baker L, Galik E, Kopunek S, Steinberg M, et al. Major and minor depression in Alzheimer's disease: Prevalence and impact. J Neuropsychiatry Clin Neursci. 1997 Fall;9(4):556–61. http://dx.doi.org/10.1176/jnp.9.4.556
19. Thorpe L. Depression vs. dementia: How do we assess? Can Rev Alzheimers Other Dementias. 2009 Sept;12(3):17–21.
20. Pistacchi M, Gioulis M, Contin F, Sanson F, Marsala SZ. Sleep disturbance and cognitive disorder: Epidemiological analysis in a cohort of 263 patients. Neurol Sci. 2014 Dec;35(12):1955–62. http://dx.doi.org/10.1007/s10072-014-1870-x
21. National Sleep Foundation. Depression and sleep [Internet]. Washington, DC: National Sleep Foundation; [cited Jul 1]. Available from: https://www.sleepfoundation.org/articles/depression-and-sleep
22. Rochon PA. Drug prescribing for older adults. Toronto: UpToDate; 2019.
23. Alhola P, Polo-Kantola P. Sleep deprivation: Impact on cognitive performance. Neuropsychiatr Dis Treat. 2007 Oct;3(5):553–67.
24. Burke SL, Maramaldi P, Cadet T, Kukull W. Associations between depression, sleep disturbance and apolipoprotein E in the development of Alzheimer's disease: Dementia. Int Psychogeriatr. 2016 Sept;28(9):1409–24. http://dx.doi.org/10.1017/S1041610216000405
25. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, et al. Sleep initiated fluid flux drives metabolite clearance from the adult brain. Science. 2013 Oct;342(6156):373–7. http://dx.doi.org/10.1126/science.1241224
26. Alzheimer's Society. Anxiety and dementia [Internet]. London: Alzheimer's Society; [cited Jul 1]. Available from https://www.alzheimers.org.uk/about-dementia/symptoms-and-diagnosis/anxiety
27. Johns Hopkins Medicine. Beyond memory loss: How to handle the other symptoms of Alzheimer's [Internet]. Baltimore: Johns Hopkins Medicine; [cited Jul 1]. Available from: https://www.hopkinsmedicine.org/health/conditions-and-diseases/alzheimers-disease/beyond-memory-loss-how-to-handle-the-other-symptoms-of-alzheimers
28. Teri L, Ferretti LE, Gibbons LE, Logsdon RG, McCurry SM, Kukull WA, et al. Anxiety in Alzheimer's disease: Prevalence and comorbidity. J Geron Med Sci. 1999 Aug;54(7):M348–52. http://dx.doi.org/10.1093/gerona/54.7.M348
29. Becker E, Lorena Orellana Rios C, Lahmann C, Rucker G, Bauer J, Boeker M. Anxiety as a risk factor of Alzheimer's disease and vascular dementia. Brit J Phys. 2018 Nov;213(5):654–60. http://dx.doi.org/10.1192/bjp.2018.173
30. Peters R, Peters J, Warner J, Beckett N, Bulpitt C. Alcohol, dementia and cognitive decline in the elderly: A systematic review. Age Ageing. 2008 May;37:505–12. http://dx.doi.org/10.1093/ageing/afn095
31. Heirene R, John B, Roderique-Davies G. Identification and evaluation of neuropsychological tools used in the assessment of alcohol-related cognitive impairment: A systematic review. Front Psychol. 2018 Dec;9(2618):1–42. http://dx.doi.org/10.3389/fpsyg.2018.02618

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