ACS Chemical Neuroscience

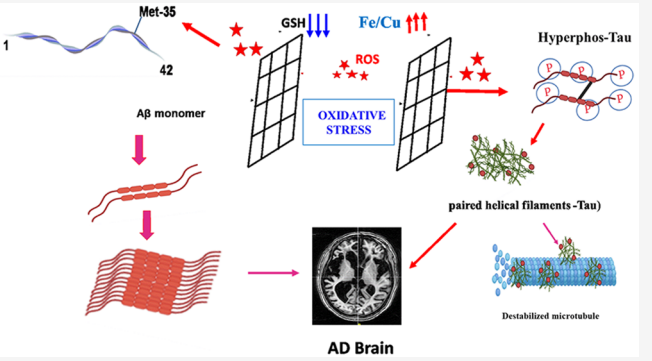
pubs.acs.org/chemneuro
Perspective

Oxidative Stress Occurs Prior to Amyloid Aβ Plaque Formation and Tau Phosphorylation in Alzheimer's Disease: Role of Glutathione and Metal Ions

Rimil Guha Roy, Pravat K Mandal,* and Joseph C. Maroon

Cite This: ACS Chem. Neurosci. 2023, 14, 2944–2954
Read Online

ABSTRACT: Alzheimer's disease (AD) is an insidious and progressive neurodegenerative disorder that affects millions of people worldwide. Although the pathogenesis remains obscure, there are two dominant causal hypotheses. Since last three decades, amyloid beta (Aβ) deposition was the most prominent hypothesis, and the other is the tau hyperphosphorylation hypothesis. The confirmed diagnostic criterion for AD is the presence of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau and the deposition of toxic oligomeric Aβ in the autopsied brain. Consistent with these hypotheses, oxidative stress (OS) is garnering major attention in AD research. OS results from an imbalance of pro-oxidants and antioxidants. There is a considerable debate in the scientific community on which process occurs first, OS or plaque deposition/tau hyperphosphorylation. Based on recent scientific observations of various laboratories including ours along with critical analysis of those information, we believe that OS is the early event that leads to oligomeric Aβ deposition as well as dimerization of tau protein and its subsequent hyperphosphorylation. This OS hypothesis immediately suggests the consideration of novel therapeutic approaches to include antioxidants involving glutathione enrichment in the brain by supplementation with or without an iron chelator.



KEYWORDS: *Alzheimer's disease, Antioxidant, Glutathione, Iron, Copper, Oxidative stress, Clinical trials, Vitamin C, Vitamin E*

INTRODUCTION

Alzheimer's disease (AD) is an irreversible progressive neurological disorder that leads to the gradual deterioration of cognitive functions and activities in daily living. The exact cause of the disease is unknown, and there is no definite cure of AD. Existing medications and recently approved drugs provide only temporary symptomatic relief, and none reverse the disease process.[1] Two causal hypotheses, the amyloid beta (Aβ) cascade[2] and the tau propagation[3] hypothesis, have been considered pivotal in the progression of AD for the past three decades. One of the older hypotheses for AD is that of oxidative stress (OS),[4,5] and it is now garnering increased attention in the research community.

Low levels of OS are required for the proper functioning of the body and are utilized effectively in redox signaling and regulation. This has been termed oxidative eustress. However, very high levels of OS can be disruptive to the same physiological processes and are known as oxidative distress. Oxidative distress causes an imbalance in redox homeostasis, causing more pro-oxidative species in the system in comparison to antioxidants. Though the nomenclature has been revamped to distinguish between oxidative eustress and oxidative distress conditions,[6] we will be referring to OS throughout this Perspective to indicate this shift from redox homeostasis (oxidative distress).

The pathological hallmarks of AD include the presence of intracellular senile plaques (SPs)[7] and the formation of neurofibrillary tangles (NFTs) secondary to hyperphosphorylation of tau protein.[3,8] Ranging from a total 39 to 43 amino acids in length, Aβ is a peptide generated through the cleavage of the amyloid precursor protein (APP). Though widely popular as the main culprit behind the genesis of AD, the Aβ peptide also participates in a wide number of physiological events like memory consolidation, facilitating neuronal growth, gatekeeping against toxins and pathogens,[9] etc. In the CSF of healthy people with normal cognition, the concentration of

Received: July 19, 2023
Accepted: August 4, 2023
Published: August 10, 2023

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944–2954

$A\beta_{40}$ is found to be 2−3 ng/mL, while that of $A\beta_{42}$ is around 0.7−0.8 ng/mL.[10] However, escalated production or decreased clearance of these Aβ peptides or both result in the accumulation of inflammatory SPs that impair cell signaling pathways and result in subsequent synaptic degeneration, neuronal loss, and decline in cognitive functions.[11] Oligomeric Aβ is found to be highly neurotoxic, and primarily the $A\beta_{40}$ and $A\beta_{42}$ isoforms can aggregate and form Aβ plaques in the brain.[12] Aβ plaques, however, are also observed in the autopsied brains of healthy individuals with no history of cognitive impairment before death.[13,14] Thus, the presence of Aβ plaque deposition does not necessarily correlate with abnormal clinical findings.[13,15,16]

Another important protein, tau, implicated in AD is a soluble microtubule-associated protein (MAP). In physiological conditions, tau contributes to the stability of neuronal microtubules and helps to preserve neuronal morphology and physiology.[17] Tau is usually localized in the axons, consists of a family of six isomeric forms (352−441 amino acids in length), and stabilizes microtubules through its phosphorylated isomeric forms.[18,19] The 4R tau isoform promotes microtubule assembly more than the 3R isoform.[20] Hyperphosphorylated tau disturbs microtubule assembly and sequesters MAPs into paired helical filaments (PHFs).[21] These insoluble entities damage cytoplasmic scaffolding and hamper axonal transport, ultimately leading to neurodegeneration.[22] NFTs formed by aggregated tau protein are also a cardinal pathological finding in AD.[23] Autopsy studies from AD-affected neocortical and CA1 regions display a direct correlation between the location of tau pathology with severity and disease progression.[24,25]

Role of Reactive Oxygen Speciesin AD. The brain is a highly energy-demanding organ, and its varied biochemical processes require the continuance of the redox reactions that aid in producing energy for it. This also makes the brain particularly vulnerable to OS. Reactive oxygen species (ROS) in physiological concentrations are important for maintaining the normal working of a cell.[26] There are different types of ROS; however, the major ones are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and nitric oxide (NO).[27] Furthermore, these ROS can pass through the cell membrane and cause lipid, protein, and DNA oxidation and protein glycosylation due to their uncharged polarity.[28] Redox changes like an increase in ROS (e.g., H_2O_2) levels, mitochondrial dysfunction due to lipid peroxidation, and DNA/RNA oxidation are very important events in the development of AD. Dysregulation of redox signaling pathways in the entorhinal cortex due to OS seems to manifest as a very early event in AD.[28] Prior research has also suggested that OS has a profound role in AD progression, rather than being a consequence of it.[29,30] OS also affects membrane fluidity and causes changes in enzymatic and signaling pathways.[31] Nicotinamide adenine dinucleotide phosphate oxidase (NOX) is a neuroinflammatory enzyme expressed during microglial activation and is a primary source of fibrillar-Aβ-induced production of ROS.[32] Transforming growth factor-β (TGF-β) is also implicated in redox signaling, and the NOX4 isoform was observed to be the most responsible for TGF-β-induced ROS generation by TGF-β/Smad/ROS signaling cascade.[33] TGF-β also promotes amyloidogenesis.[34] Phosphorylated MAP kinases like ERK1/2 could also play a detrimental role through the TGF-β/Smad/NOX4/ERK1/2/tau protein cascade,[33,35] NF (nuclear factor)-κB[36] and nuclear factor-erythroid 2 related factor 2 (Nrf2) are also prominent mediators of OS.

The latent period before AD detected in a person is crucial in identifying risk factors and understanding the pathogenesis of AD.[37] The scientific quest about which comes first, OS or amyloid deposition, has been a long-standing quagmire since the OS theory of AD began gaining attention. Many different experimental and clinical studies have pointed to OS as the initiator of AD pathogenesis.[37−40] However, elucidating all the mechanistic targets and signaling pathways impacted by OS in AD is beyond the scope of this manuscript. Some of the common targets have been listed in Table 1. For a more comprehensive account of the molecular and genetic mechanisms of Aβ deposition and tau tangle formation, please refer to refs 18, 41, and 42

Table 1. Some Targets of Action of Amyloid β and Tau Impairment Due to Oxidative Distress[a]

amyloid β peptide	tau	genetics
downregulation of the GluN1 subunit of NMDAR receptors	accumulation of Cdc2/cyclin B1 kinase, leading to cell cycle re-entry	ApoE
endocytosis of EphB2 class of tyrosine kinases	interaction with Aβ leading to activation of IL-1b and neuroninflammation	BACE
upregulation of apoptosin, a mitochondrial protein, leading to mitochondrial dysfunction and neurotoxicity	activation of ERK2, NFkb, p38, etc. leading to hyperphosphorylation of tau	PSEN1
interaction with metals (e.g., Fe, Cu, Zn) leading to Aβ deposition		
downregulation of COX activity on interaction with heme		

[a]Genetic studies have shown AD-related genes (APOE, PSEN1, and BASE) to be related to Aβ deposition.[62,63] Additionally, other targets leading up to Aβ deposition or a consequence of the same involve N-methyl-D-aspartate (NMDAR) receptors,[64] EphB2 tyrosine kinases,[65] metal interaction,[42] etc. Oxidative stress can affect tau by reconfiguring cell cycle checkpoints,[66] neuroinflammatory mechanisms,[67] and other factors that lead to hyperphosphorylation of the protein.

Another impactful phenomenon arising due to OS is mitochondrial dysfunction (MD). MD interferes with glucose and calcium metabolism, influencing neuronal lifespan.[31] Advanced glycation end products (AGEs), a consequence of OS, have been seen to accelerate the deposition of Aβ plaques.[43,44] Clinical studies have found that an imbalance in the amount of the antioxidant GSH and the pro-oxidant iron is observed in patients with mild cognitive impairment (MCI) and AD.[45] Heavy metal ions and pro-oxidants such as iron (Fe), zinc (Zn), and copper (Cu) have been reported to be increased significantly in the hippocampus of the AD-affected brains.[46] In accordance with these clinical findings, a recent study with a 3× transgenic AD mouse model concluded that a shift in redox homeostasis is the upstream event followed by other downstream perturbations like metabolic shifts and SP formation.[47] Summary of the conceptual development of the OS theory, including the major biophysical, neuronal culture, autopsy, human studies, and validation with the transgenic animal model, is presented in Figure 1.

Role of Metal Ions in AD. Metal ions like Cu, Fe, Mn, and Zn are important to maintain the basic functions and the homeostasis of the human body. Fe is the most abundant metal in the brain, followed by Cu and Zn.[48] All these metals are involved in activating signaling pathways, and Zn at low levels

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

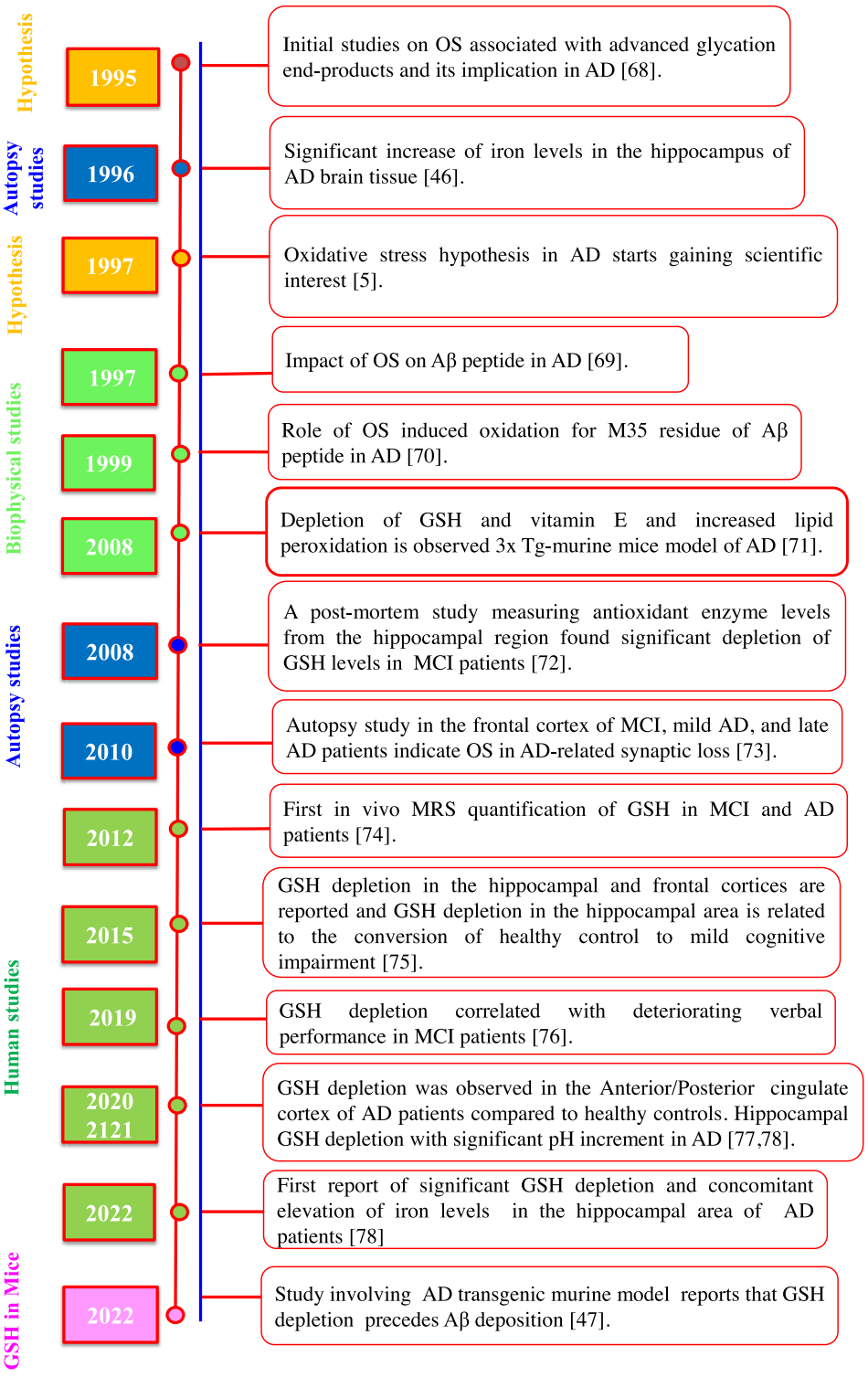


Figure 1. Flowchart illustrating the major chronological development of the OS hypothesis in AD research as mentioned from various research work.[5,46,47,68−79] A review of the medical literature found initial research dating back to the early 1990s with a focus on advanced glycation end-products (AGE) and their role in accelerating the oxidative load on the brain.[68] Subsequently, the OS hypothesis was further advanced.[5,69,70] A study with the AD mouse model reported significant reduction in GSH levels and an increase in lipid peroxidation products in the AD mouse brain.[71] Autopsy studies on human AD brains have also reported significant elevation of iron levels[46] or a reduction in GSH levels. GSH depletion was also observed in other studies, thus establishing it as a major phenomenon in AD pathology.[74−79] Supporting these clinical observations, a study dealing with 3x transgenic AD mice reported OS as the initial factor prior to plaque formation.[47] We could not add other related works to this figure due to space limitations.

has even been found to inhibit Aβ aggregation.[49] However, metal−Aβ complex formation is thought to be an aberrant phenomenon since these complexes have only been detected in AD patients but not under healthy conditions.[50]

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

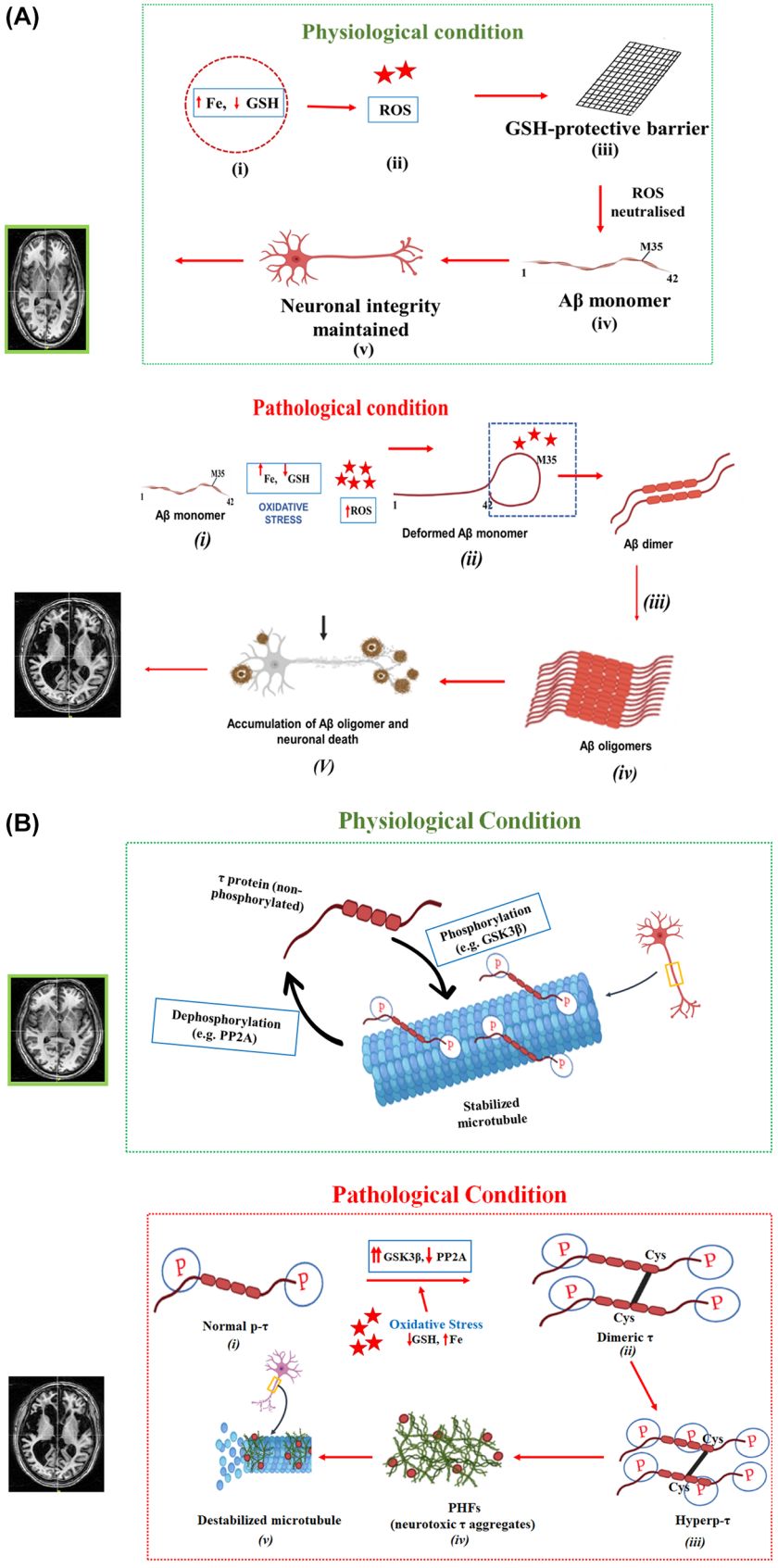


Figure 2. Model highlighting the impact of OS on amyloid plaque formation and tau hyperphosphorylation and the role that GSH plays in the process. Though other antioxidants also act rigorously to combat OS, we are solely focused on GSH and its contribution in ameliorating OS. Representative magnetic resonance imaging (MRI) images of normal and AD brain are taken from NINS laboratory. (A) Amyloid related processes. Physiological conditions: Under normal physiological conditions and basal levels of OS (i), (ii), GSH forms a protective barrier against ROS and effectively filters radicals, thus neutralizing ROS and reducing oxidative burden in the system (iii). The Aβ monomer is not constrained

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

Figure 2. continued

and retains its structural integrity and functionality (iv). Absence of plaque formation maintains neuronal integrity (v); healthy brain architecture and normal functionality is thus preserved. Pathological conditions: Depleted GSH levels and an increasing iron load lead to an imbalance in redox homeostasis, ultimately leading to a concurrent accumulation of free radicals (i). Depleted GSH levels, in this case, cannot form an effective filtration barrier against ROS (ii). Some of the radicals gain the propensity to interact with the M-35 residue of Aβ peptide and oxidation of the M-35 residue of Aβ (v) gives rise to structural modification.[45,100] Oxidation of M-35 subsequently leads to the formation of Aβ dimers (iii) and the formation of higher order oligomeric Aβ structures (iv). This extracellular toxic oligomeric Aβ accumulation on neurons leads to neuronal death (v) and eventually neurodegeneration in the AD brain.[7] (B) Tau related processes. Physiological conditions: In normal conditions, when redox homeostasis is maintained, the rate of phosphorylation and dephosphorylation of tau is also under homeostasis. Tau is usually phosphorylated in its proline-rich region by kinases.[92] However, phosphorylation is negatively related to tau function, and hence to maintain proper function of tau, it needs to be dephosphorylated. This cyclic homeostatic process enables tau protein to remain bound to the axonal microtubules and carry out its normal physiological function by promoting microtubule assembly and stabilizing microtubules. Pathological conditions: Under OS conditions, depleted levels of the GSH and an increase in the pro-oxidant iron can stimulate the structural conversion of normal phosphorylated tau (i). The cysteine residues of tau can form intermolecular disulfide bridges, leading to a dimeric state. This can lead to accumulation and formation of NFTs[96−99] (ii). OS also affects phosphorylation−dephosphorylation signaling pathways, dysregulating the rate of enzymatic reactions. GSK3β, the major phosphorylating enzyme, is highly upregulated, while PP2A, the major dephosphorylating enzyme, is downregulated. This disturbance in balance leads to hyperphosphorylated tau (iii). Hyperphosphorylated tau dissociates from microtubules and can form insoluble aggregates, PHFs (iv), which can then destabilize microtubules, ultimately leading to neurodegeneration (v).

Even though low levels of Zn might end up tackling AD, at high levels Zn might promote fibrillar Aβ formation.[49] Additionally, the amyloid precursor protein (APP) has a Cu-binding site and binding of Cu to this site leads to oxidative modification of the APP.[51,52] The binding of Cu^{2+} to APP reduces it to Cu^{+}, giving rise to ROS such as $O_2^{\bullet-}$ or $^{\bullet}OH$.[53] Interaction of Cu with Aβ in senile plaques is also a reason for the formation and stabilization of Aβ aggregates.[52,54] Additionally, freely available Cu and Fe can effectively catalyze the generation of ROS through the Fenton reaction.[42] Both Fe^{2+} and Cu^{+} can react with H_2O_2 and generate OH^{-} and $^{\bullet}OH$, both highly reactive radicals. A post-mortem study quantifying iron from AD hippocampal tissue found increased accumulation of Fe^{3+} to be associated with senile plaque and NFT formation.[55] Increased iron load and toxicity has also been implicated in ferroptosis, a regulated cell death mechanism caused by the accumulation of lipid peroxidation products.[56,57] Introduced as a concept in 2012, ferroptosis is mainly caused by a deficiency in the antioxidant defense systems (particularly that of the GSH system), resulting in oxidative death due to increased Fe and polyunsaturated fatty acids (PUFAs).[58] Both Aβ and tau are associated with Fe and increasing Fe levels are primarily implicated in AD pathogenesis and progression.[59] Increased Fe levels also seem to be able to catalyze H_2O_2-dependent oxidation. Cu−Aβ complexes can also catalyze the formation of H_2O_2 yielding intermediate states of $O_2^{\bullet-}$.[60] Cytoplasmic H_2O_2 can interact with Fe^{2+} and lead to more ROS production.[61] These factors eventually cause neuronal death and enhance AD development.

OXIDATIVE STRESS IMPACT

Aβ Cascade. The relationship between OS and the Aβ hypothesis in AD is not a new observation. Aβ is produced by both neurons and astrocytes in the brain and is deposited in the extracellular space and cleared by the cerebrospinal fluid glymphatic and the vascular systems.[10] Aβ adopts a random coil structure in the extracellular aqueous environment and a helical form with a kink in the membrane associated environment.[80] In vitro nuclear magnetic resonance (NMR) studies have indicated that the membrane microenvironment has a profound role in Aβ conformation. Any abrupt changes in the membrane composition lead to oligomeric Aβ formation.[80] Upon interaction with metals (Fe, Cu, and Zn), Aβ can form covalently cross-linked oligomeric structures which become precursors for Aβ fibrillization.[81,82] An increase in brain Fe and Cu leads to increased formation via Fenton's reaction. Autopsy studies have also indicated the presence of depleted GSH levels in the hippocampal area of MCI patients.[72] A schematic diagram for the oligomerization of Aβ due to OS induced methionine-35 (M-35) oxidation and subsequent toxic plaque formation of Aβ is presented in Figure 2A.

Tau Hyperphosphorylation. Tau is a microtubule associated protein (MAP) and plays an important role in maintaining neuronal morphology and integrity. Its main function is to bind to tubulin monomers and stabilize their assembly into the neuronal microtubule network.[83] Neuronal tau protein has six isoforms in the brain.[84,22] Tau protein with 441 amino acids is the longest isoform and constitutes 4 distinct regions: the N-terminal region (NTR), the proline-rich region (PRR), the microtubule-binding region (MBR), and the carboxy-terminal region (CTR).[85] Splicing out exon 10 results in an isoform with three MBR repeats (3R) while inclusion results in isoforms with four repeats (4R).[86] In the adult brain, the ratio of 3R/4R tau isoforms is maintained at 1:1. Disturbance of this ratio has been observed in AD and other neurodegenerative diseases.[87] The NTR and PRR regions contain amino acid sequences that contribute to the intrinsically disordered nature of tau and are involved in signaling and regulation of cellular processes.[88] Under normal conditions, tau gets phosphorylated at its serine (Ser)/threonine (Thr) residues.[89,22] About 20% of the tau-protein can undergo phosphorylation as it consists of a total of 80 Ser or Thr residues and five Tyr residues which are also potential sites of phosphorylation.[90,22] Additionally, the open structure of tau also makes it accessible to many kinases.[91] Phosphorylation of tau is facilitated by kinases that transfer a phosphate group from adenosine triphosphate. There are three major types of kinases that are involved in the phosphorylation of tau: (a) proline-directed kinases (e.g., glycogen synthase kinase 3β (GSK 3β); cyclin dependent kinase 5 (cdk5)), (b) non-proline-directed kinases (e.g., tau-tubulin kinase), and (c) tyrosine kinases (e.g., Fyn kinase, Abl kinase).[92] On the other hand, protein phosphatase 2A (PP2A) is the major dephosphorylating enzyme, along with other phosphatases (e.g., PP1, PP5, and PP2B).[92] The activity of tau is maintained by preserving the balance between the rates of phosphorylation and dephosphorylation, and phosphorylated tau can bind to

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

microtubules, thus stabilizing neuronal morphology and integrity.

In pathological conditions like AD, tau gets hyperphosphorylated and aggregates into PHFs, which finally leads to the formation of NFTs.[93,94] NFT deposits destabilize microtubules, causing them to disintegrate and leading to neuronal death.[95] Dimerization of tau due to OS conditions has also been observed,[96−99] which also leads to NFT formation. However, it is ambiguous whether hyperphosphorylation and dimer formation of tau protein are linked or happen independently of each other. More research is warranted in this areas. A schematic representation of physiological and pathological processes of tau is presented in Figure 2B.

Mitochondrial Dysfunction. Oxidative stress and mitochondrial (mt) dysfunction form a vicious cycle in the pathogenesis of AD. While OS can lead to structurally and functionally defective mitochondria, damaged mitochondria are susceptible to producing large amounts of ROS, thus increasing the overall oxidative burden on the brain.[101] In this section, we briefly describe how OS can impair mt function.

Mitrocondia are popularly called the "powerhouse of the cell", and are required to produce adenosine triphosphate (ATP), which is essential to carry out cellular processes. However, the mitochondrial respiratory chain system that leads to the production of the energy currency also produces ROS (e.g., superoxide) by itself.[102] Superoxide radicals are highly reactive and can cause lesions in mt-DNA.[103] Once damaged, mt-DNA fails to express the crucial proteins required for the proper functioning of the electron transport chain. This eventually leads to the production of more ROS and ultimately mitophagy and apoptosis.[103]

Studies have reported that mitochondria are dysfunctional in early stage AD.[104] The Aβ peptide has been observed to proactively gain access to the mt-matrix in both AD brain and transgenic APP mice when compared to healthy controls.[105] The mt-enzyme Aβ-binding alcohol dehydrogenase is inhibited upon interaction with Aβ, leading to the release of ROS and affecting major metabolic pathways.[105] ATP synthase, creatine kinase, and aconitase, all very important metabolic enzymes, have also been observed to be nitrated, carbonylated, or HNE-modified (HNE = 4-hydroxy 2-nonenal, a lipid peroxidation byproduct).[106] These findings further corroborate the role of increased OS in MD as well as impaired neuroenergetics in AD.[107] Furthermore, increased ROS also affect mitochondrial permeability and structure.[108] OS can also cause mt-Ca^{2+} overloading, leading to a transient increase in ROS production.[109] Increased mt-Ca^{2+} also impairs normal mitochondrial processes and causes loss of synaptic function and ultimately neuronal death.[110,102]

Dysregulated Fe metabolism also plays a role in mt dysfunction. Iron dysregulation hampers the electron transport chain, affecting mt bioenergetics.[111] In a mouse model study, increase in exogenous Fe^{3+} has been seen to promote mitochondrial fragmentation in hippocampal neurons.[112] This is in direct conjunction with the Fe-induced overload of Ca^{2+} in the mitochondria. Thus, iron overload is also associated with mt dysfunction and aggravation of AD pathogenesis.

THERAPEUTIC POTENTIAL IN AD WITH ANTIOXIDANTS OR COMBINATION DRUGS

Major efforts and resources have been spent on clinical trials primarily based on the Aβ deposition and tau phosphorylation hypotheses. Many clinical trials with different mechanistic targets have been undertaken, but most of them have met with potentially severe adverse effects, including intracerebral hemorrhage and cerebral edema.

A human monoclonal antibody targeted against aggregated soluble and insoluble forms of Aβ was used in two (EMERGE and ENGAGE) randomized, double-blind, placebo-controlled, global phase 3 studies. Both trials were halted based on futility analysis of data.[113] Another antiamyloid drug was found to reduce amyloid burden in early AD patients but was only partially effective at slowing global decline in cognition.[11] There were, however, adverse effects, as evidenced by amyloid-related imaging abnormalities (ARIA) due to vasogenic edema (ARIA-E) and intracranial hemorrhage (ARIA-H). Another clinical trial involving an antiamyloid drug also reported significant adverse events.[114] A recent report from a meta-analysis on anti-Aβ drugs revealed drug-induced decreased brain volume. β-Secretase inhibitors like verubecestat accelerated hippocampal atrophy (mean difference: −37.1 μL [−19.6% relative to change in placebo]) and whole brain atrophy (−3.3 mL [−21.8% relative to change in placebo]).[115]

We have presented clinical trials based on antioxidant therapies in AD in Table 2. Though some studies with antioxidants (vitamins C and E) show positive results, other trials show negative results; hence, the results are mixed. Vitamin C is an antioxidant and prevents the oxidation of xenobiotic compounds by donating two electrons. In turn, vitamin C gets oxidized to dehydroascorbic acid.[116] GSH is directly involved in maintaining vitamins E and C in their reduced forms,[117] which is required for the normal functioning of vitamins E and C as antioxidants. A study with selenium and probiotics, which also have antioxidative effects, was observed to increase cognition in AD patients. Some clinical trials with curcumin have also shown improvement in memory deficits. However, the results of these studies were mostly inconclusive. We posit that clinical trials with long-term supplementation to the MCI or early AD patients with the master antioxidant GSH or γ-glutamyl cysteine are necessary to fully elucidate the optimal antioxidative therapies in AD research.

Iron homeostasis goes awry in AD and there is also depletion of GSH, which makes the brain much more susceptible to OS. Recent clinical studies involving healthy control (HC), MCI, and AD patients showed significant depletion of antioxidant GSH as well as an increase of iron levels in the hippocampus using state-of-the art MR spectroscopic and susceptibility imaging measurements when HC is converted to MCI.[79] Accordingly, GSH enhancement in the brain was proposed to alleviate AD symptoms.[118] Clinical trials with desferrioxamine (DFO), an iron chelator, has found success in slowing down the progression of AD.[119] DFO also has an affinity for Cu and Zn. Iron chelators delivered using nanoparticles have been observed to cross the blood−brain barrier (BBB) to deliver the drug to the brain while also effectively preventing metal-associated oxidative damage.[120] Thus, future combination therapy involving combined GSH and iron chelator may be proposed for upcoming clinical trials for AD therapeutic development.[121]

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

Table 2. List of Antioxidant Based Clinical Trials Pertaining to AD

compounds	subjects	intervention/dosage	outcomes	ref
vitamin E, selegiline, vitamin E + selegiline	patients with moderate AD (N = 341)	vitamin E (2000 IU/day), selegiline (10 mg/day); vitamin E + selegiline administered for 2 years	patients with moderately severe cognitive impairment reported a reduction in the functional progression of the disease	124
vitamin E, memantine, vitamin E + memantine	patients with mild to moderate AD (N = 613)	vitamin E (1000 IU twice daily), memantine (10 mg twice daily over 4 weeks) for 5 years	in patients with mild to moderate AD, vitamin E resulted in slower functional decline; no significant differences were observed in the groups receiving memantine alone or vitamin E + memantine	125
vitamin E, vitamin C	patients with mild to moderate AD (N = 23)	vitamin E (400 IU/day), duration not known; vitamin C (1000 mg/day) for 1 year	daily intake of vitamin C combined vitamin E did not significantly affect the course of AD over a year	126
vitamin E, vitamin C, α-lipoic acid	patients with mild to moderate AD (N = 78)	placebo, vitamin E (800 IU/day), vitamin C (500 mg/day), α-lipoic acid (900 mg/day), or CoQ10 (400 mg, 3 times/day) for 4 months	no significant differences observed in CSF antioxidative biomarker levels	127
Gingko biloba	subjects with normal cognition (N = 2587) or MCI (N = 482)	placebo, *Gingko biloba* extract (120 mg, twice daily) with a median follow-up time of 6.1 years	*Gingko biloba* extract was ineffective in reducing the incidence rate of dementia or AD (or possibly MCI) in the subjects; is it MCI or AD as title says MCI?	128
desferriox-amine (DFO)	patients with probable AD (N = 48)	no treatment, placebo, DFO (125 mg intramuscularly twice daily, 5 days per week, for 24 months)	DFO treatment significantly reduced the rate of decline in daily skills	129
selenium, probiotics, selenium + probiotics	patients with AD (N = 79)	Se (200 mg/day) plus probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* (2×10^9 CFU/day each)	selenium and probiotic cosupplementation was reported to improve cognitive function and metabolic profiles in AD patients	130
curcumin	nondemented individuals (N = 40)	curcumin (90 mg twice daily), for 18 months; what type of trial?	daily oral supplementation likely lead to improved memory and attention	131

CONCLUSION

AD is a highly debilitating and lethal disease affecting millions of people worldwide, with increasing prevalence. Although many clinical trials based on the Aβ hypothesis have been conducted, none have resulted in major advances in treatment. Through literature review and our own laboratory and clinical observations, we suggest that OS is the dominant factor for the pathogenesis of AD. We believe clinical trials should be considered evaluating combinations of drugs directed at OS and brain GSH enrichment with or without iron chelators[45,122] on an urgent basis and data should be presented to researchers.[123] We sincerely hope this work will invite constructive discussion and collaboration to support urgently needed clinical trial for the dreaded disease afftecting millions of people worldwide.

AUTHOR INFORMATION

Corresponding Author

Pravat K Mandal − *Neuroimaging and Neurospectroscopy (NINS) Laboratory, National Brain Research Centre, Gurgaon 122052, India; Florey Institute of Neuroscience and Mental Health, Melbourne 3052 VIC, Australia;* orcid.org/0000-0003-4999-2808; Email: pravat.mandal@gmail.com, pravat@nbrc.ac.in, pravat.mandal@florey.edu.au

Authors

Rimil Guha Roy − *Neuroimaging and Neurospectroscopy (NINS) Laboratory, National Brain Research Centre, Gurgaon 122052, India*

Joseph C. Maroon − *Department of Neurosurgery, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania 15213, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acschemneuro.3c00486

Author Contributions

P.K.M. (Principal Investigator) conceived the idea for this perspective, contributed to the literature search and analysis, wrote the first draft of manuscript, contributed to discussion figure preparations and overall quality check and execution. R.G.R. contributed to literature search and analysis, figure preparation, and writing the manuscript. J.C.M. was involved in analysis of literature and discussion and participated in writing.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

P.K.M. (Principal Investigator) thanks Indo-Australia strategic funding (BT/Indo-Aus/10/31/2016/PKM) for funding support. Dr. Mandal thanks to all AD, MCI, PD patients, and healthy control subjects who participated in various ongoing AD/PD related research study in the NINS lab for last 15 years at NBRC. This intense research program has helped to develop Indian Brain Template (BRAHMA), BHARAT BIG DATA analytics, SWADESH DATA BASE development, and KALPANA data processing methodology development (granted US patent). Dr. Mandal thanks Avantika Samkaria (Researcher, NINS Lab) for her immense help related to data management and table of content figure design. Special thanks to the learned reviewers for constructive comments. Ms Gayatri Kumaran, (Researcher, NINS lab) for helping to

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

remodel figures and providing valuable comments. Ms Aditi Kaushik (Researcher at the NINS lab) is appreciated for proof reading.

REFERENCES

(1) Casey, D. A.; Antimisiaris, D.; O'Brien, J. Drugs for Alzheimer's disease: are they effective? *P T* **2010**, *35* (4), 208−211.
(2) Hardy, J. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci* **1997**, *20* (4), 154−159.
(3) Kosik, K. S.; Joachim, C. L.; Selkoe, D. J. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83* (11), 4044−4048.
(4) Nunomura, A.; Perry, G.; Aliev, G.; Hirai, K.; Takeda, A.; Balraj, E. K.; Jones, P. K.; Ghanbari, H.; Wataya, T.; Shimohama, S.; et al. Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol Exp Neurol* **2001**, *60* (8), 759−767.
(5) Markesbery, W. R. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol. Med.* **1997**, *23* (1), 134−147.
(6) Sies, H.; Jones, D. P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21* (7), 363−383.
(7) Murphy, M. P.; LeVine, H., 3rd. Alzheimer's disease and the amyloid-beta peptide. *J. Alzheimers Dis* **2010**, *19* (1), 311−323.
(8) Simic, G.; Babic Leko, M.; Wray, S.; Harrington, C.; Delalle, I.; Jovanov-Milosevic, N.; Bazadona, D.; Buee, L.; de Silva, R.; Di Giovanni, G.; et al. Tau Protein Hyperphosphorylation and Aggregation in Alzheimer's Disease and Other Tauopathies, and Possible Neuroprotective Strategies. *Biomolecules* **2016**, *6* (1), 6.
(9) Bishop, G. M.; Robinson, S. R. Physiological roles of amyloid-beta and implications for its removal in Alzheimer's disease. *Drugs Aging* **2004**, *21* (10), 621−630.
(10) Brothers, H. M.; Gosztyla, M. L.; Robinson, S. R. The Physiological Roles of Amyloid-beta Peptide Hint at New Ways to Treat Alzheimer's Disease. *Front Aging Neurosci* **2018**, *10*, 118.
(11) Ahn, H. J.; Zamolodchikov, D.; Cortes-Canteli, M.; Norris, E. H.; Glickman, J. F.; Strickland, S. Alzheimer's disease peptide beta-amyloid interacts with fibrinogen and induces its oligomerization. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (50), 21812−21817.
(12) Yang, H.; Li, J.; Li, X.; Ma, L.; Hou, M.; Zhou, H.; Zhou, R. Based on molecular structures: Amyloid-beta generation, clearance, toxicity and therapeutic strategies. *Front Mol. Neurosci* **2022**, *15*, 927530.
(13) Goldman, W. P.; Price, J. L.; Storandt, M.; Grant, E. A.; McKeel, D. W., Jr.; Rubin, E. H.; Morris, J. C. Absence of cognitive impairment or decline in preclinical Alzheimer's disease. *Neurology* **2001**, *56* (3), 361−367.
(14) Haroutunian, V.; Perl, D. P.; Purohit, D. P.; Marin, D.; Khan, K.; Lantz, M.; Davis, K. L.; Mohs, R. C. Regional distribution of neuritic plaques in the nondemented elderly and subjects with very mild Alzheimer disease. *Arch Neurol* **1998**, *55* (9), 1185−1191.
(15) Swerdlow, R. H. Is aging part of Alzheimer's disease, or is Alzheimer's disease part of aging? *Neurobiol Aging* **2007**, *28* (10), 1465−1480.
(16) Swerdlow, R. H. Pathogenesis of Alzheimer's disease. *Clin Interv Aging* **2007**, *2* (3), 347−359.
(17) Guo, T.; Noble, W.; Hanger, D. P. Roles of tau protein in health and disease. *Acta Neuropathol* **2017**, *133* (5), 665−704.
(18) Guo, T.; Zhang, D.; Zeng, Y.; Huang, T. Y.; Xu, H.; Zhao, Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. *Mol. Neurodegener* **2020**, *15* (1), 40.
(19) Mohandas, E.; Rajmohan, V.; Raghunath, B. Neurobiology of Alzheimer's disease. *Indian J. Psychiatry* **2009**, *51* (1), 55−61.
(20) Goedert, M.; Jakes, R. Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerization. *EMBO J.* **1990**, *9* (13), 4225−4230.
(21) Gong, C. X.; Iqbal, K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Curr. Med. Chem.* **2008**, *15* (23), 2321−2328.
(22) Johnson, G. V.; Stoothoff, W. H. Tau phosphorylation in neuronal cell function and dysfunction. *Journal of cell science* **2004**, *117* (24), 5721−5729.
(23) Perl, D. P. Neuropathology of Alzheimer's disease. *Mt Sinai J. Med.* **2010**, *77* (1), 32−42.
(24) Bierer, L. M.; Hof, P. R.; Purohit, D. P.; Carlin, L.; Schmeidler, J.; Davis, K. L.; Perl, D. P. Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol* **1995**, *52* (1), 81−88.
(25) Arriagada, P. V.; Growdon, J. H.; Hedley-Whyte, E. T.; Hyman, B. T. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **1992**, *42* (3), 631.
(26) Manoharan, S.; Guillemin, G. J.; Abiramasundari, R. S.; Essa, M. M.; Akbar, M.; Akbar, M. D. The Role of Reactive Oxygen Species in the Pathogenesis of Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease: A Mini Review. *Oxid Med. Cell Longev* **2016**, *2016*, 8590578.
(27) Ferreira, M. E.; de Vasconcelos, A. S.; da Costa Vilhena, T.; da Silva, T. L.; da Silva Barbosa, A.; Gomes, A. R.; Dolabela, M. F.; Percario, S. Oxidative Stress in Alzheimer's Disease: Should We Keep Trying Antioxidant Therapies? *Cell Mol. Neurobiol* **2015**, *35* (5), 595−614.
(28) Juan, C. A.; Perez de la Lastra, J. M.; Plou, F. J.; Perez-Lebena, E. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *Int. J. Mol. Sci.* **2021**, *22* (9), 4642.
(29) Butterfield, D. A.; Boyd-Kimball, D. Oxidative Stress, Amyloid-beta Peptide, and Altered Key Molecular Pathways in the Pathogenesis and Progression of Alzheimer's Disease. *J. Alzheimers Dis* **2018**, *62* (3), 1345−1367.
(30) Butterfield, D. A. Perspectives on Oxidative Stress in Alzheimer's Disease and Predictions of Future Research Emphases. *J. Alzheimers Dis* **2018**, *64* (s1), S469−S479.
(31) Padurariu, M.; Ciobica, A.; Lefter, R.; Serban, I. L.; Stefanescu, C.; Chirita, R. The oxidative stress hypothesis in Alzheimer's disease. *Psychiatr Danub* **2013**, *25* (4), 401−409.
(32) Wilkinson, B. L.; Landreth, G. E. The microglial NADPH oxidase complex as a source of oxidative stress in Alzheimer's disease. *J. Neuroinflammation* **2006**, *3*, 30.
(33) Chen, Y. Y.; Wang, M. C.; Wang, Y. N.; Hu, H. H.; Liu, Q. Q.; Liu, H. J.; Zhao, Y. Y. Redox signaling and Alzheimer's disease: from pathomechanism insights to biomarker discovery and therapy strategy. *Biomark Res.* **2020**, *8*, 42.
(34) Wyss-Coray, T.; Masliah, E.; Mallory, M.; McConlogue, L.; Johnson-Wood, K.; Lin, C.; Mucke, L. Amyloidogenic role of cytokine TGF-beta1 in transgenic mice and in Alzheimer's disease. *Nature* **1997**, *389* (6651), 603−606.
(35) Ferrer, I.; Blanco, R.; Carmona, M.; Ribera, R.; Goutan, E.; Puig, B.; Rey, M. J.; Cardozo, A.; Vinals, F.; Ribalta, T. Phosphorylated map kinase (ERK1, ERK2) expression is associated with early tau deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Brain Pathol* **2001**, *11* (2), 144−158.
(36) Kaur, U.; Banerjee, P.; Bir, A.; Sinha, M.; Biswas, A.; Chakrabarti, S. Reactive oxygen species, redox signaling and neuroinflammation in Alzheimer's disease: the NF-kappaB connection. *Curr. Top Med. Chem.* **2015**, *15* (5), 446−457.
(37) Liu, Z.; Li, T.; Li, P.; Wei, N.; Zhao, Z.; Liang, H.; Ji, X.; Chen, W.; Xue, M.; Wei, J. The Ambiguous Relationship of Oxidative Stress, Tau Hyperphosphorylation, and Autophagy Dysfunction in Alzheimer's Disease. *Oxid Med. Cell Longev* **2015**, *2015*, 352723.
(38) Martinez, E.; Navarro, A.; Ordonez, C.; Del Valle, E.; Tolivia, J. Oxidative stress induces apolipoprotein D overexpression in hippocampus during aging and Alzheimer's disease. *J. Alzheimers Dis* **2013**, *36* (1), 129−144.

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

(39) Schuessel, K.; Schafer, S.; Bayer, T. A.; Czech, C.; Pradier, L.; Muller-Spahn, F.; Muller, W. E.; Eckert, A. Impaired Cu/Zn-SOD activity contributes to increased oxidative damage in APP transgenic mice. *Neurobiol Dis* **2005**, *18* (1), 89−99.
(40) Dias-Santagata, D.; Fulga, T. A.; Duttaroy, A.; Feany, M. B. Oxidative stress mediates tau-induced neurodegeneration in Drosophila. *J. Clin Invest* **2007**, *117* (1), 236−245.
(41) Cioffi, F.; Adam, R. H. I.; Broersen, K. Molecular Mechanisms and Genetics of Oxidative Stress in Alzheimer's Disease. *J. Alzheimers Dis* **2019**, *72* (4), 981−1017.
(42) Bai, R.; Guo, J.; Ye, X. Y.; Xie, Y.; Xie, T. Oxidative stress: The core pathogenesis and mechanism of Alzheimer's disease. *Ageing Res. Rev.* **2022**, *77*, 101619.
(43) Gella, A.; Durany, N. Oxidative stress in Alzheimer disease. *Cell adhesion migration* **2009**, *3* (1), 88−93.
(44) Vitek, M. P.; Bhattacharya, K.; Glendening, J. M.; Stopa, E.; Vlassara, H.; Bucala, R.; Manogue, K.; Cerami, A. Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91* (11), 4766−4770.
(45) Mandal, P. K.; Roy, R. G.; Samkaria, A. Oxidative Stress: Glutathione and Its Potential to Protect Methionine-35 of Aβ Peptide from Oxidation. *ACS omega* **2022**, *7* (31), 27052−27061.
(46) Deibel, M. A.; Ehmann, W. D.; Markesbery, W. R. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. *J. Neurol Sci.* **1996**, *143* (1−2), 137−142.
(47) Pontrello, C. G.; McWhirt, J. M.; Glabe, C. G.; Brewer, G. J. Age-Related Oxidative Redox and Metabolic Changes Precede Intraneuronal Amyloid-beta Accumulation and Plaque Deposition in a Transgenic Alzheimer's Disease Mouse Model. *J. Alzheimers Dis* **2022**, *90* (4), 1501−1521.
(48) Liu, Y.; Nguyen, M.; Robert, A.; Meunier, B. Metal Ions in Alzheimer's Disease: A Key Role or Not? *Acc. Chem. Res.* **2019**, *52* (7), 2026−2035.
(49) Mezzaroba, L.; Alfieri, D. F.; Colado Simao, A. N.; Vissoci Reiche, E. M. The role of zinc, copper, manganese and iron in neurodegenerative diseases. *Neurotoxicology* **2019**, *74*, 230−241.
(50) Faller, P. Copper and zinc binding to amyloid-beta: coordination, dynamics, aggregation, reactivity and metal-ion transfer. *Chembiochem* **2009**, *10* (18), 2837−2845.
(51) Multhaup, G.; Schlicksupp, A.; Hesse, L.; Beher, D.; Ruppert, T.; Masters, C. L.; Beyreuther, K. The amyloid precursor protein of Alzheimer's disease in the reduction of copper(II) to copper(I). *Science* **1996**, *271* (5254), 1406−1409.
(52) Holubiec, M. I.; Gellert, M.; Hanschmann, E. M. Redox signaling and metabolism in Alzheimer's disease. *Front Aging Neurosci* **2022**, *14*, 1003721.
(53) Ruiz, F. H.; Gonzalez, M.; Bodini, M.; Opazo, C.; Inestrosa, N. C. Cysteine 144 is a key residue in the copper reduction by the beta-amyloid precursor protein. *J. Neurochem* **1999**, *73* (3), 1288−1292.
(54) Ho, T.; Ahmadi, S.; Kerman, K. Do glutathione and copper interact to modify Alzheimer's disease pathogenesis. *Free Radic Biol. Med.* **2022**, *181*, 180−196.
(55) Smith, M. A.; Harris, P. L.; Sayre, L. M.; 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), 9866−9868.
(56) Chen, X.; Kang, R.; Kroemer, G.; Tang, D. Ferroptosis in infection, inflammation, and immunity. *J. Exp Med.* **2021**, *218* (6), e20210518.
(57) Li, J.; Cao, F.; Yin, H. L.; Huang, Z. J.; Lin, Z. T.; Mao, N.; Sun, B.; Wang, G. Ferroptosis: past, present and future. *Cell Death Dis* **2020**, *11* (2), 88.
(58) Dixon, S. J.; Lemberg, K. M.; Lamprecht, M. R.; Skouta, R.; Zaitsev, E. M.; Gleason, C. E.; Patel, D. N.; Bauer, A. J.; Cantley, A. M.; Yang, W. S.; et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* **2012**, *149* (5), 1060−1072.
(59) Zhang, G.; Zhang, Y.; Shen, Y.; Wang, Y.; Zhao, M.; Sun, L. The Potential Role of Ferroptosis in Alzheimer's Disease. *J. Alzheimers Dis* **2021**, *80* (3), 907−925.
(60) Cheignon, C.; Tomas, M.; Bonnefont-Rousselot, D.; Faller, P.; Hureau, C.; Collin, F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biol.* **2018**, *14*, 450−464.
(61) Zhu, X.; Raina, A. K.; Lee, H. G.; Casadesus, G.; Smith, M. A.; Perry, G. Oxidative stress signalling in Alzheimer's disease. *Brain Res.* **2004**, *1000* (1−2), 32−39.
(62) Serrano-Pozo, A.; Das, S.; Hyman, B. T. APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *Lancet Neurol* **2021**, *20* (1), 68−80.
(63) Sherrington, R.; Rogaev, E. I.; Liang, Y.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Chi, H.; Lin, C.; Li, G.; Holman, K.; et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **1995**, *375* (6534), 754−760.
(64) Mishizen-Eberz, A. J.; Rissman, R. A.; Carter, T. L.; Ikonomovic, M. D.; Wolfe, B. B.; Armstrong, D. M. Biochemical and molecular studies of NMDA receptor subunits NR1/2A/2B in hippocampal subregions throughout progression of Alzheimer's disease pathology. *Neurobiol Dis* **2004**, *15* (1), 80−92.
(65) Lacor, P. N.; Buniel, M. C.; Furlow, P. W.; Sanz Clemente, A.; Velasco, P. T.; Wood, M.; Viola, K. L.; Klein, W. L. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J. Neurosci.* **2007**, *27* (4), 796−807.
(66) Vincent, I.; Jicha, G.; Rosado, M.; Dickson, D. W. Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. *J. Neurosci.* **1997**, *17* (10), 3588−3598.
(67) Terrill-Usery, S. E.; Mohan, M. J.; Nichols, M. R. Amyloid-beta(1−42) protofibrils stimulate a quantum of secreted IL-1beta despite significant intracellular IL-1beta accumulation in microglia. *Biochim. Biophys. Acta* **2014**, *1842* (11), 2276−2285.
(68) Smith, M. A.; Sayre, L. M.; Monnier, V. M.; Perry, G. Radical AGEing in Alzheimer's disease. *Trends Neurosci* **1995**, *18* (4), 172−176.
(69) Butterfield, D. A. beta-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. *Chem. Res. Toxicol.* **1997**, *10* (5), 495−506.
(70) Varadarajan, S.; Yatin, S.; Kanski, J.; Jahanshahi, F.; Butterfield, D. A. Methionine residue 35 is important in amyloid beta-peptide-associated free radical oxidative stress. *Brain Res. Bull.* **1999**, *50* (2), 133−141.
(71) Resende, R.; Moreira, P. I.; Proenca, T.; Deshpande, A.; Busciglio, J.; Pereira, C.; Oliveira, C. R. Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease. *Free Radic Biol. Med.* **2008**, *44* (12), 2051−2057.
(72) Sultana, R.; Piroddi, M.; Galli, F.; Butterfield, D. A. Protein levels and activity of some antioxidant enzymes in hippocampus of subjects with amnestic mild cognitive impairment. *Neurochem. Res.* **2008**, *33* (12), 2540−2546.
(73) Ansari, M. A.; Scheff, S. W. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *J. Neuropathol Exp Neurol* **2010**, *69* (2), 155−167.
(74) Mandal, P. K.; Tripathi, M.; Sugunan, S. Brain oxidative stress: detection and mapping of anti-oxidant marker 'Glutathione' in different brain regions of healthy male/female, MCI and Alzheimer patients using non-invasive magnetic resonance spectroscopy. *Biochem. Biophys. Res. Commun.* **2012**, *417* (1), 43−48.
(75) Mandal, P. K.; Saharan, S.; Tripathi, M.; Murari, G. Brain glutathione levels–a novel biomarker for mild cognitive impairment and Alzheimer's disease. *Biol. Psychiatry* **2015**, *78* (10), 702−710.
(76) Oeltzschner, G.; Wijtenburg, S. A.; Mikkelsen, M.; Edden, R. A. E.; Barker, P. B.; Joo, J. H.; Leoutsakos, J. S.; Rowland, L. M.; Workman, C. I.; Smith, G. S. Neurometabolites and associations with cognitive deficits in mild cognitive impairment: a magnetic resonance spectroscopy study at 7 T. *Neurobiol Aging* **2019**, *73*, 211−218.
(77) Shukla, D.; Mandal, P. K.; Tripathi, M.; Vishwakarma, G.; Mishra, R.; Sandal, K. Quantitation of in vivo brain glutathione conformers in cingulate cortex among age-matched control, MCI, and AD patients using MEGA-PRESS. *Hum Brain Mapp* **2020**, *41* (1), 194−217.

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

(78) Shukla, D.; Mandal, P. K.; Mishra, R.; Punjabi, K.; Dwivedi, D.; Tripathi, M.; Badhautia, V. Hippocampal Glutathione Depletion and pH Increment in Alzheimer's Disease: An in vivo MRS Study. *J. Alzheimers Dis* **2021**, *84* (3), 1139−1152.
(79) Mandal, P. K.; Goel, A.; Bush, A. I.; Punjabi, K.; Joon, S.; Mishra, R.; Tripathi, M.; Garg, A.; Kumar, N. K.; Sharma, P.; et al. Hippocampal glutathione depletion with enhanced iron level in patients with mild cognitive impairment and Alzheimer's disease compared with healthy elderly participants. *Brain Commun.* **2022**, *4* (5), fcac215.
(80) Mandal, P. K.; Pettegrew, J. W. Alzheimer's disease: soluble oligomeric Abeta(1−40) peptide in membrane mimic environment from solution NMR and circular dichroism studies. *Neurochem. Res.* **2004**, *29* (12), 2267−2272.
(81) Peters, D. G.; Connor, J. R.; Meadowcroft, M. D. The relationship between iron dyshomeostasis and amyloidogenesis in Alzheimer's disease: Two sides of the same coin. *Neurobiol Dis* **2015**, *81*, 49−65.
(82) Atwood, C. S.; Scarpa, R. C.; Huang, X.; Moir, R. D.; Jones, W. D.; Fairlie, D. P.; Tanzi, R. E.; Bush, A. I. Characterization of copper interactions with alzheimer amyloid beta peptides: identification of an attomolar-affinity copper binding site on amyloid beta1−42. *J. Neurochem* **2000**, *75* (3), 1219−1233.
(83) Buée, L.; Bussière, T.; Buée-Scherrer, V.; Delacourte, A.; Hof, P. R. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Research Reviews* **2000**, *33* (1), 95−130.
(84) Brandt, R.; Trushina, N. I.; Bakota, L. Much more than a cytoskeletal protein: physiological and pathological functions of the non-microtubule binding region of tau. *Frontiers in Neurology* **2020**, *11*, 590059.
(85) Trushina, N. I.; Bakota, L.; Mulkidjanian, A. Y.; Brandt, R. The Evolution of Tau Phosphorylation and Interactions. *Front Aging Neurosci* **2019**, *11*, 256.
(86) Cherry, J. D.; Esnault, C. D.; Baucom, Z. H.; Tripodis, Y.; Huber, B. R.; Alvarez, V. E.; Stein, T. D.; Dickson, D. W.; McKee, A. C. Tau isoforms are differentially expressed across the hippocampus in chronic traumatic encephalopathy and Alzheimer's disease. *Acta Neuropathologica Communications* **2021**, *9* (1), 86.
(87) Liu, Z.; Li, T.; Li, P.; Wei, N.; Zhao, Z.; Liang, H.; Ji, X.; Chen, W.; Xue, M.; Wei, J. The ambiguous relationship of oxidative stress, tau hyperphosphorylation, and autophagy dysfunction in Alzheimer's disease. *Oxidative medicine and cellular longevity* **2015**, *2015*, 352723.
(88) Dyson, H. J.; Wright, P. E. Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell Biol.* **2005**, *6* (3), 197−208.
(89) Alonso, A. D.; Cohen, L. S.; Corbo, C.; Morozova, V.; ElIdrissi, A.; Phillips, G.; Kleiman, F. E. Hyperphosphorylation of tau associates with changes in its function beyond microtubule stability. *Frontiers in cellular neuroscience* **2018**, *12*, 338.
(90) Avila, J.; Jiménez, J. S.; Sayas, C. L.; Bolós, M.; Zabala, J. C.; Rivas, G.; Hernández, F. Tau structures. *Frontiers in aging neuroscience* **2016**, *8*, 262.
(91) Mandelkow, E.; Von Bergen, M.; Biernat, J.; Mandelkow, E. M. Structural principles of tau and the paired helical filaments of Alzheimer's disease. *Brain pathology* **2007**, *17* (1), 83−90.
(92) Xia, Y.; Prokop, S.; Giasson, B. I. Don't Phos Over Tau": recent developments in clinical biomarkers and therapies targeting tau phosphorylation in Alzheimer's disease and other tauopathies. *Molecular Neurodegeneration* **2021**, *16* (1), 37.
(93) Daebel, V.; Chinnathambi, S.; Biernat, J.; Schwalbe, M.; Habenstein, B.; Loquet, A.; Akoury, E.; Tepper, K.; Müller, H.; Baldus, M.; et al. β-Sheet core of tau paired helical filaments revealed by solid-state NMR. *J. Am. Chem. Soc.* **2012**, *134* (34), 13982−13989.
(94) Von Bergen, M.; Barghorn, S.; Biernat, J.; Mandelkow, E.-M.; Mandelkow, E. Tau aggregation is driven by a transition from random coil to beta sheet structure. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **2005**, *1739* (2−3), 158−166.
(95) DeTure, M. A.; Dickson, D. W. The neuropathological diagnosis of Alzheimer's disease. *Mol. Neurodegener* **2019**, *14* (1), 32.
(96) Saito, T.; Chiku, T.; Oka, M.; Wada-Kakuda, S.; Nobuhara, M.; Oba, T.; Shinno, K.; Abe, S.; Asada, A.; Sumioka, A.; et al. Disulfide bond formation in microtubule-associated tau protein promotes tau accumulation and toxicity in vivo. *Hum. Mol. Genet.* **2021**, *30* (21), 1955−1967.
(97) Prifti, E.; Tsakiri, E. N.; Vourkou, E.; Stamatakis, G.; Samiotaki, M.; Papanikolopoulou, K. The Two Cysteines of Tau Protein Are Functionally Distinct and Contribute Differentially to Its Pathogenicity in Vivo. *J. Neurosci.* **2021**, *41* (4), 797−810.
(98) Bhattacharya, K.; Rank, K. B.; Evans, D. B.; Sharma, S. K. Role of cysteine-291 and cysteine-322 in the polymerization of human tau into Alzheimer-like filaments. *Biochem. Biophys. Res. Commun.* **2001**, *285* (1), 20−26.
(99) Schweers, O.; Mandelkow, E. M.; Biernat, J.; Mandelkow, E. Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein tau controls the in vitro assembly of paired helical filaments. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92* (18), 8463−8467.
(100) Butterfield, D. A.; Sultana, R. Methionine-35 of abeta(1−42): importance for oxidative stress in Alzheimer disease. *J. Amino Acids* **2011**, *2011*, 198430.
(101) Wang, X.; Wang, W.; Li, L.; Perry, G.; Lee, H. G.; Zhu, X. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochim. Biophys. Acta* **2014**, *1842* (8), 1240−1247.
(102) Guo, C.; Sun, L.; Chen, X.; Zhang, D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen Res.* **2013**, *8* (21), 2003−2014.
(103) Van Houten, B.; Woshner, V.; Santos, J. H. Role of mitochondrial DNA in toxic responses to oxidative stress. *DNA Repair (Amst)* **2006**, *5* (2), 145−152.
(104) Cadonic, C.; Sabbir, M. G.; Albensi, B. C. Mechanisms of Mitochondrial Dysfunction in Alzheimer's Disease. *Mol. Neurobiol* **2016**, *53* (9), 6078−6090.
(105) Chen, X.; Yan, S. D. Mitochondrial Abeta: a potential cause of metabolic dysfunction in Alzheimer's disease. *IUBMB Life* **2006**, *58* (12), 686−694.
(106) Swomley, A. M.; Butterfield, D. A. Oxidative stress in Alzheimer disease and mild cognitive impairment: evidence from human data provided by redox proteomics. *Arch. Toxicol.* **2015**, *89* (10), 1669−1680.
(107) Wang, W.; Zhao, F.; Ma, X.; Perry, G.; Zhu, X. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. *Mol. Neurodegener* **2020**, *15* (1), 30.
(108) Wei, Y. H.; Lu, C. Y.; Wei, C. Y.; Ma, Y. S.; Lee, H. C. Oxidative stress in human aging and mitochondrial disease-consequences of defective mitochondrial respiration and impaired antioxidant enzyme system. *Chin J. Physiol* **2001**, *44* (1), 1−11.
(109) Gorlach, A.; Bertram, K.; Hudecova, S.; Krizanova, O. Calcium and ROS: A mutual interplay. *Redox Biol.* **2015**, *6*, 260−271.
(110) Matuz-Mares, D.; Gonzalez-Andrade, M.; Araiza-Villanueva, M. G.; Vilchis-Landeros, M. M.; Vazquez-Meza, H. Mitochondrial Calcium: Effects of Its Imbalance in Disease. *Antioxidants (Basel)* **2022**, *11* (5), 801.
(111) Onukwufor, J. O.; Dirksen, R. T.; Wojtovich, A. P. Iron Dysregulation in Mitochondrial Dysfunction and Alzheimer's Disease. *Antioxidants (Basel)* **2022**, *11* (4), 692.
(112) Park, J.; Lee, D. G.; Kim, B.; Park, S. J.; Kim, J. H.; Lee, S. R.; Chang, K. T.; Lee, H. S.; Lee, D. S. Iron overload triggers mitochondrial fragmentation via calcineurin-sensitive signals in HT-22 hippocampal neuron cells. *Toxicology* **2015**, *337*, 39−46.
(113) Budd Haeberlein, S.; Aisen, P. S.; Barkhof, F.; Chalkias, S.; Chen, T.; Cohen, S.; Dent, G.; Hansson, O.; Harrison, K.; von Hehn, C.; et al. Two Randomized Phase 3 Studies of Aducanumab in Early Alzheimer's Disease. *J. Prev Alzheimers Dis* **2022**, *9* (2), 197−210.
(114) Mintun, M. A.; Lo, A. C.; Duggan Evans, C.; Wessels, A. M.; Ardayfio, P. A.; Andersen, S. W.; Shcherbinin, S.; Sparks, J.; Sims, J. R.; Brys, M.; et al. Donanemab in Early Alzheimer's Disease. *N Engl J. Med.* **2021**, *384* (18), 1691−1704.

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954

(115) Alves, F.; Kalinowski, P.; Ayton, S. Accelerated Brain Volume Loss Caused by Anti−β-Amyloid Drugs: A Systematic Review and Meta-analysis. *Neurology* **2023**, *100*, e2114.
(116) Nermin, M. Y. Vitamin C. In *Vitamin C*, Jean Guy, L., Ed.; IntechOpen, 2018; Chapter 2.
(117) Aliev, G.; Obrenovich, M. E.; Reddy, V. P.; Shenk, J. C.; Moreira, P. I.; Nunomura, A.; Zhu, X.; Smith, M. A.; Perry, G. Antioxidant therapy in Alzheimer's disease: theory and practice. *Mini Rev. Med. Chem.* **2008**, *8* (13), 1395−1406.
(118) Mandal, P. K.; Shukla, D.; Tripathi, M.; Ersland, L. Cognitive Improvement with Glutathione Supplement in Alzheimer's Disease: A Way Forward. *J. Alzheimers Dis* **2019**, *68* (2), 531−535.
(119) Farr, A. C.; Xiong, M. P. Challenges and Opportunities of Deferoxamine Delivery for Treatment of Alzheimer's Disease, Parkinson's Disease, and Intracerebral Hemorrhage. *Mol. Pharmaceutics* **2021**, *18* (2), 593−609.
(120) Liu, G.; Men, P.; Perry, G.; Smith, M. A. Nanoparticle and iron chelators as a potential novel Alzheimer therapy. *Methods Mol. Biol.* **2010**, *610*, 123−144.
(121) Haddad, M.; Herve, V.; Ben Khedher, M. R.; Rabanel, J. M.; Ramassamy, C. Glutathione: An Old and Small Molecule with Great Functions and New Applications in the Brain and in Alzheimer's Disease. *Antioxid Redox Signal* **2021**, *35* (4), 270−292.
(122) Mandal, P. K.; Dwivedi, D.; Joon, S.; Goel, A.; Ahasan, Z.; Maroon, J. C.; Singh, P.; Saxena, R.; Roy, R. G. Quantitation of Brain and Blood Glutathione and Iron in Healthy Age Groups Using Biophysical and In Vivo MR Spectroscopy: Potential Clinical Application. *ACS Chem. Neurosci.* **2023**, *14* (12), 2375−2384.
(123) Mandal, P. K.; Jindal, K.; Maroon, J. C.; Chhikara, R.; Samkaria, A.; Joshi, M.; Roy, S.; Arora, Y. Brain Imaging Databases. *ACS Chem. Neurosci.* **2023**, *14* (11), 1930−1934.
(124) Sano, M.; Ernesto, C.; Thomas, R. G.; Klauber, M. R.; Schafer, K.; Grundman, M.; Woodbury, P.; Growdon, J.; Cotman, C. W.; Pfeiffer, E.; et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J. Med.* **1997**, *336* (17), 1216−1222.
(125) Dysken, M. W.; Sano, M.; Asthana, S.; Vertrees, J. E.; Pallaki, M.; Llorente, M.; Love, S.; Schellenberg, G. D.; McCarten, J. R.; Malphurs, J.; et al. Effect of vitamin E and memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative randomized trial. *JAMA* **2014**, *311* (1), 33−44.
(126) Arlt, S.; Muller-Thomsen, T.; Beisiegel, U.; Kontush, A. Effect of one-year vitamin C- and E-supplementation on cerebrospinal fluid oxidation parameters and clinical course in Alzheimer's disease. *Neurochem. Res.* **2012**, *37* (12), 2706−2714.
(127) Galasko, D. R.; Peskind, E.; Clark, C. M.; Quinn, J. F.; Ringman, J. M.; Jicha, G. A.; Cotman, C.; Cottrell, B.; Montine, T. J.; Thomas, R. G.; et al. Antioxidants for Alzheimer disease: a randomized clinical trial with cerebrospinal fluid biomarker measures. *Arch Neurol* **2012**, *69* (7), 836−841.
(128) DeKosky, S. T.; Williamson, J. D.; Fitzpatrick, A. L.; Kronmal, R. A.; Ives, D. G.; Saxton, J. A.; Lopez, O. L.; Burke, G.; Carlson, M. C.; Fried, L. P.; et al. Ginkgo biloba for prevention of dementia: a randomized controlled trial. *JAMA* **2008**, *300* (19), 2253−2262.
(129) McLachlan, D. R. C.; Dalton, A. J.; Kruck, T. P.; Bell, M. Y.; Smith, W. L.; Kalow, W.; Andrews, D. F. Intramuscular desferrioxamine in patients with Alzheimer's disease. *Lancet* **1991**, *337* (8753), 1304−1308.
(130) Tamtaji, O. R.; Heidari-Soureshjani, R.; Mirhosseini, N.; Kouchaki, E.; Bahmani, F.; Aghadavod, E.; Tajabadi-Ebrahimi, M.; Asemi, Z. Probiotic and selenium co-supplementation, and the effects on clinical, metabolic and genetic status in Alzheimer's disease: A randomized, double-blind, controlled trial. *Clin Nutr* **2019**, *38* (6), 2569−2575.
(131) Small, G. W.; Siddarth, P.; Li, Z.; Miller, K. J.; Ercoli, L.; Emerson, N. D.; Martinez, J.; Wong, K. P.; Liu, J.; Merrill, D. A.; et al. Memory and Brain Amyloid and Tau Effects of a Bioavailable Form of Curcumin in Non-Demented Adults: A Double-Blind, Placebo-Controlled 18-Month Trial. *Am. J. Geriatr Psychiatry* **2018**, *26* (3), 266−277.

https://doi.org/10.1021/acschemneuro.3c00486
ACS Chem. Neurosci. 2023, 14, 2944−2954