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Molecular neuropathogenesis of Alzheimer's disease: an interaction model stressing the central role of oxidative stress

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Abstract

Alzheimer's disease (AD) exhibits a complex etiology that simultaneously manifests as a complex cellular, neurobiological, molecular, anatomic–physiological and clinical entity. Other significant psychiatric conditions, such as depression and schizophrenia, may also present with complex and concurrent clinical and/or molecular phenotypes. These neuropsychiatric pathologies also originate from both environmental and genetic factors. We analyzed the molecular phenotypes of AD and discuss them with respect to the classical theories, which we integrated into mechanisms that share molecular and/or anatomical connections. Based on these mechanisms, we propose an interaction model and discuss the model in light of studies that refute or support it. Given the spectrum of AD phenotypes, we limit the scope of our discussion to a few, which facilitates concrete analysis. In addition, the study of specific, individual pathogenic phenotypes may be critical to defining the complex mechanisms leading to AD, thereby improving strategies for developing novel therapies.

Keywords

Alzheimer's disease; amyloid-β; apolipoprotein E; neurofibrillary tangles; Parkinson's disease; reactive oxygen species

Background

The study of drug interactions, which has evolved enormously in the past two decades, has led to significant progress in our ability to interpret the complex interactions between multiple drugs with respect to their neurobiology [1]. Specifically, many investigations have been performed to define the interaction of neurotransmitters. An example is provided by the studies of Beninger and Gerdjikov [2] and Konradi *et al.* [3], which clarified many

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In memoriam

This review is dedicated to the memory of our colleague and friend Professor Mark A Smith, PhD, FRCPath.

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aspects of the role of dopamine–glutamate in incentive learning. Studies in psychoneuropharmacology have added to our understanding of the extremely complex neuromolecular and cellular interactions that induce the onset and development of Alzheimer's disease (AD) and other neurodegenerative disorders [4].

Cross-sectional studies are necessary to elucidate the molecular and cellular underpinnings of pathological events. Unlike longitudinal studies, which facilitate understanding the developmental dynamics of specific phenomena – that is, neurodegenerative diseases – cross-sectional studies analyze a particular event in a variety of individuals at the same time. To define the etiology of a unique event, it seems reasonable to rely on cross-sectional interaction studies – that is, the investigation of the most important molecular, cellular, clinical and statistical occurrences that may be contributing to or inhibiting an observed occurrence.

A brief example may illustrate this suggestion: ApoE4 is associated with both AD and depression [5]. However, ApoE4 may or may not be the principal cause of the neuromolecular and anatomical occurrences of interest, although ApoE4 is well established as a major risk factor for both early- and late-onset AD. Based on these observations, one can postulate that depression may also be related to early- or late-onset AD. However, chronic depression, anxiety and stress are also correlated with serotonin transporter (5-HTT) genotypes. The short allele of 5-HTT is associated with chronic anxiety and depressive syndromes and predisposes carriers to a lower threshold for adverse and stressful environmental events than long 5-HTT nondepressed individuals [6]. Considering that this is a newly discovered neuromolecular pathway related to depression and that this mood disorder is associated with late-onset AD [7], two new molecular pathways could be investigated: the association of the 5-HTT short allele and ApoE4, and the neurobiological and molecular relationship of late-onset AD and the 5-HTT short allele. Rather than attempting to build up an interaction model of AD neuropathogenesis based on longitudinal studies, our aim is to underline the importance of the interactions amenable to cross-sectional analysis and investigations based on current research in neurobiological and molecular neurosciences. There are additional pathways, beyond the scope of this review, that are either omitted or only briefly mentioned. Once the observed phenotypic interactions encompassed in our model are clarified, it may be possible to improve interrelated therapeutic measures and, using a similar strategy, investigate other pathologically or clinically associated phenomena.

In this review, known participants in AD are discussed: ApoE, Aβ and metals, which can be related to oxidative stress. The complex interactions involving oxidative stress, normal aging, mild cognitive impairment (MCI) and AD are discussed, which are in turn related to ApoE, Aβ and metals. The significant interaction between the ApoE4 isoform, chronic depression and comorbid anxiety and stress is then analyzed. We discuss the complex relationship of ApoE to AD and the arguments for and against the antioxidant function of ApoE4. Next, we integrate oxidative stress with the clinical and pathological entities, where we consider all clinical and neuromolecular findings as representative of stages of the life cycle – that is, aging. Finally, we discuss and analyze a global interaction model (Figure 1).

Functions of ApoE4: brain physiology & pathology

ApoE4 provides a protective function in the brain through its role in lipid transport, although it is most frequently associated with its known neurotoxic potential [8]. The ability of ApoE4 to interact with Aβ and microtubule proteins is the most interesting of its properties in the context of AD and deserves thorough study in the search for new treatments in AD and other neurodegenerative illnesses. A number of therapeutic strategies using ApoE as an

inhibitory target have generated promising results [9]. In addition, numerous reports have posed that this protein has a harmful action in the brain. For example, ApoE and, in particular, the ApoE4 isoform promote damage by interacting with senile plaques and neurofibrillary tangles (NFTs) and contributing to AD progression by creating free radicals [10]. In this process, ApoE binds to Aβ and slows the brain's ability to export Aβ [11]. ApoE is also a factor in aging, depression, oxidative stress [12], AD and MCI, through its role in the transport of cholesterol and other lipids such as low-density, high-density and very low-density lipoproteins. An exciting new finding in transgenic models suggests that increasing ApoE expression may promote plaque clearance and restore cognitive function [13], although the precise mechanism remains to be elucidated. This may not be the result of Aβ/ApoE interaction as indicated by experiments in which the Aβ/ApoE binding was blocked by an interfering peptide, $A\beta_{12–28P}$ [14].

A growing body of evidence reinforces the view that, despite its being a very robust risk factor in AD, ApoE provides a protective function in the brain. Compared with controls, ApoE4 has been reported to induce significant higher antidepressant efficiency of mirtrazapine in depressed, elderly individuals [15]. Additionally, the ApoE4 and ApoE3 isoforms have an important effect on Aβ aggregation in AD. ApoE4 is also important for neurite maintenance, although mice carrying the human *ApoE4* allele also show more neuritic deficits than *ApoE3* carriers [16].

Many apolipoproteins, in addition to ApoE, exhibit a spectrum of protection and/or injury in a variety of neuronal and glial cells in the CNS. For example, apolipoprotein J (clusterin) is a multifunctional glycoprotein that interacts with a variety of molecules that protect the brain from various insults [17]. However, to date no studies have contrasted its role in the brain to ApoE.

ApoE4 is a significant risk factor in late-onset familial and sporadic AD [18,19]. There are studies substantiating age-associated memory impairment in *ApoE4* carrier elderly females who perform worse than males in memory tests, indicating an association between ApoE4 effects and gender [20]. The major protective roles of ApoE are the transport of lipoproteins and distribution of cholesterol and phospholipids, facilitating synaptic plasticity, and as free radical scavengers [21]. Furthermore, as will be shown below, ApoE may control the level of choline acetyltransferase (ChAT), the principal enzyme in the synthesis of acetylcholine. Buttini and colleagues report that ChAT control is dependent on specific ApoE isoform, aging and the overexpression of Aβ peptides, but not on senile plaque formation [22].

ApoE4 accelerates AD onset, worsens age-related neuronal decline and induces damage to cholinergic synapses, which correlate with cognitive decline. This suggests that the critical effects of ApoE isoforms on AD risk precede clinical expression of the disease by many years. ApoE4, in addition to being expressed at a lower level, may fail to act as an effective Aβ chaperone, clearing neurons and glia of excess Aβ through transport. Instead, ApoE4 may actually promote fibrillogenesis and increase the $A\beta_{42/40}$ ratio [23]. ApoE also binds to very low-density lipoprotein, low-density lipoprotein and high-density lipoprotein, acting as a cholesterol transporter [24], and, through its interaction with nitric oxide synthase, may inhibit platelet aggregation [25,26]. All of these functions may protect the endothelium of arteries and contribute to vascular preservation and the survival of neurons. Thus, while the E4 isoform may cause age-related problems, ApoE, in general, plays an important role in maintenance of neuronal health.

Aβ & ApoE4 as protection against oxidative stress, an early response observed in AD & aging

Recent studies have shown that Aβ, in addition to being the major component of extracellular senile plaques and fibrils in AD [27,28], mediates neuronal toxicity in part through interaction with redox-active metals and oxidative stress [29,30]. Aβ has an opposing function to those traditionally postulated, as suggested in the AD unifying hypothesis [8]. In this hypothesis, the relationship of key processes – that is, calcium excitotoxicity, energy decrease and antioxidant challenge – are linked to the age-related loss of hormonal balance. The energy decrease induced by Aβ formation and tau protein hyperphosphorylation, are neuronal and glial responses to the chronic stress the brain is suffering, particularly with regard to oxidative stress and redox transition metals [29,31]. It should be noted that in transgenic models overexpressing Aβ, mitochondria are damaged, resulting in an increase in oxidative stress or loss of redox homeostasis [32].

Recent studies have explored the effects of Aβ peptides on glucose metabolism in cultured astrocytes and the implications of these effects. These astrocyte-dependent changes are detected before any symptom or tissue marker elements appear in AD and demonstrate two opposing functions of Aβ: neuroprotection via reduced glutathione and neurotoxicity via free radicals [33]. These reports are consistent with the double action of Aβ in neural cells, neuronal viability and/or damage to astrocytes and neurons. Since senile plaques and NFTs are related to the interacting factors mentioned above, it is possible that all of these age-related events result from a disease load that exceeds the genetic threshold of the individual, resulting in progressive pathophysiological events that lead to early or late-onset AD.

Moreover, Heininger emphasizes in his interaction hypothesis that the immune system, as an integral component of the neuroendocrine immunological network, is functionally impaired giving rise to secreted inflammatory mediators – that is, the cytokines TNF-α/IL-1β, and NFκB – which have neurotoxic actions and induce diffuse Aβ to form neuritic plaques [8]. Activation of an immune response results in pathological amyloid cascade processes [34].

Human AβPP is also reported to be a protective factor in preventing neuronal death, which is correlated with Aβ production [35]. In this context, Aβ protects neurons from lipoperoxidation, redox metals and DNA oxidation [36]. Conversely, there are recent studies reporting that Aβ peptide and AβPP modulate metal homeostasis (Cu, Fe and Zn) in the brain [37]. It has not been established which event comes first: metal dyshomeostasis leading to Aβ and AβPP changes; or metal dyshomeostasis as a consequence of genetics (mutant alleles of the critical genes for AD) and metabolic alteration of Aβ (oxidative stress). Nevertheless, there is an interaction between these two phenotypes that feed forward in the amyloid cascade, resulting in aggregation and free radical production [38].

It is also important to note the role of *ApoE* genotypes in this cascade, specifically *ApoE4/E4*. ApoE4 induces fibrillation and aggregation of $A\beta_{1–40}$ and $A\beta_{1–42}$, furthering its deposition and neural toxicity [39,40]. One report highlights the strong interaction of ApoE isoforms with the Aβ peptide [41] and proposes that these ApoE actions are an ‘on/off’, dose-dependent molecular defense mechanism against Aβ toxicity. We suppose that the ‘on phase’, which is dependent on oxidative stress, is transition metal dependent, resulting in robust Aβ loading, increasing sodium dodecyl sulfate-stable concentrations of ApoE3, ApoE2 or ApoE3/Aβ complexes, to a much greater extent than the ApoE4/Aβ complex, with a consequent reduction of ApoE4 levels. With the *ApoE4/E4* genotype, transport or clearance of Aβ to the extracellular compartments is facilitated [42]. On the other hand, the ‘off phase’ downregulates E3 and E2 levels, with resultant increases in normal or higher

levels of ApoE4 and of the Aβ-ApoE4 complex, when the Aβ burden is neither high nor toxic to cells.

It is possible that ApoE4 acts as an antioxidant. Hypothetically the relationship of ApoE4/(ApoE3 + ApoE2) should be constant for homeostasis. Multiple reasons led to the formulation of this putative 'on/off ' system. The most apparent is that while ApoE genotypes are clearly important in the development of AD and associated characteristics, approximately 50% of AD patients have the same ApoE genotype as unaffected individuals.

Indeed, ApoE4 may be an antioxidant [43,44]. ApoE3 and ApoE4 prevent Aβ-induced, glial-mediated neurotoxicity and inflammation, with the involvement of ApoE receptors (ApoE3 > ApoE4). ApoE facilitates deposition of Aβ as amyloid and fibrillar amyloid redistribution (ApoE4 > ApoE3) and the development of cerebral amyloid angiopathy [45]. The ApoE3 and ApoE4 isoforms do not show differences in reducing the Aβ load in cerebral arteries [46] or in increasing amyloid deposition in this AD variety [42]. Transgenic amyloid-producing mice expressing ApoE3 or ApoE4 exhibit less Aβ deposition than ApoE knockout mice, although it is important to note that *ApoE* knockout mice exhibit increased ApoJ. ApoE is involved in neuritic maintenance, since the ApoE knockout mice suffer neuritic and behavioral deficits as a result of aging or treatment with excitotoxins.

Furthermore, these researchers showed that Aβ peptides aggregate in AD rather than existing as monomers. While the oligomeric structure causes the pathology, extracellular senile plaques and fibrillar aggregates, monomeric Aβ serves as an antioxidant [47], particularly against metal-induced oxidative damage (see Figure 2). The fibrillization, aggregation and deposition of neurotoxic, oligomeric Aβ in senile plaques is facilitated by ApoE4. The results obtained in the study indicate that ApoE4 stimulates, in an isoform-specific fashion, the nucleation and aggregation of Aβ deposits and that reversible disaggregation of these deposits and their irreversible conversion to fibrillar deposits are stimulated by ApoE and are affected similarly by the different ApoE isoforms. Similarly, a study testing the affect of $A\beta_{12–28}$ peptide, a strong therapeutic agent that binds to ApoE4 and blocks Aβ binding, significantly reduces plaques and cerebral amyloid deposition in transgenic mice, but does not affect the formation of oligomeric Aβ [48]. The ApoE/Aβ interaction is illustrated in Figure 3.

To induce oxidative stress, including elevated levels of protein oxidation (reflected in protein carbonyls and 3-nitrotyrosine) and lipid peroxidation (reflected in protein-bound 4-hydroxy-2-nonenal), the Aβ structure must have an oxidized sulfur in the methionine residue [49]. Interestingly, mutating the Met35 residue to Leu in the Aβ peptide may completely abolish the presence of oxidative stress markers. In-depth neurochemical investigations to elucidate the neuromolecular mechanisms, including redox metals such as Fe and Cu, Aβ monomers and the shift in redox transition metal homeostasis that are capable of generating free radicals from hydrogen peroxide, are necessary. The alternative peroxynitrite-dependent pathway [31] and the oxidation of Fe(II) to Fe(III; Fenton reaction) and its conversion back to Fe(II) have been investigated previously.

Indeed, there are significant concentrations of redox metals such as Fe, Cu and Zn in senile plaques and NFTs. These transition metals, with the exception of Zn (which can do so indirectly), may disturb redox homeostasis and generate free radicals [29]. They also induce aggregation of either Aβ in senile plaques or tau in paired helical filaments, the precursor of NFTs [31]. Aggregated-oligomeric Aβ, in concert with metals and ApoE, forms deposits in the hippocampus, neocortex and amygdala, and causes neurotoxicity [36].

On the other hand, we suggest that monomeric $A\beta_{1–40}$ inhibits neuronal damage caused by free radical generation by Fe(II), Fe(III) and Cu(II) [50], which produces superoxides and

lipid peroxidation [51]. Monomeric Aβ protects neurons by reducing metal-induced free radical generation, preventing neurotoxicity caused by oligomeric Aβ [52]. Otherwise, oligomeric Aβ promotes robust neuronal toxicity through lipid release from neuronal membranes. This means that neuronal lipid homeostasis is disturbed, leading to a loss of neuronal function. On the other hand, oligomeric Aβ peptide accumulates outside the cell in the AD brain and stimulates lipid release, leading to disruption of cholesterol homeostasis in the CNS, deficiency of intracellular cholesterol storage and hyperphosphorylated tau in some tauopathies [53].

As mentioned above, ApoE4 binds to the Aβ peptide and slows the brain's ability to export Aβ to clear neurons and glia. While soluble monomeric Aβ is easily expelled from the neurons, the oligomeric Aβ structure that binds to ApoE4 and to redox metals such as Fe(II), Fe(III) and Cu(II), easily aggregates as a macromolecular complex, ApoE4–Aβ–redox metals (RMs), which is 'sticky' and insoluble. This complex cannot be cleared easily from the brain and accumulates in the form of senile plaques. The ApoE2 or ApoE3–Aβ–RM complexes are much more efficiently cleared from neurons and glia (two- to three-times faster than ApoE4–Aβ–RM) [11].

The opposing functions of Aβ depend on whether it is polymeric, which gives rise to neurodegeneration, apoptosis and death (oligomeric form), or monomeric leading to neuroprotection through its antioxidant function [36,52]. The integrated macromolecular complex (ApoE4–Aβ–Fe[III]), not deposition of Aβ alone, results in neurodegeneration. Based on the following observations we can challenge the notion that the Aβ peptide is the 'most wanted criminal in AD': first, antioxidant actions and neuronal protection are essential functions of the monomeric Aβ peptide; second, oxidative stress is the earliest molecular and metabolic phenotype of AD; and third, oligomeric Aβ peptide, despite being neurotoxic, is increased as a defense against oxidative free radicals [54,55] [Nunomura *et al.*, Unpublished Data]. Based on the observations above, we believe that Aβ is 'not guilty'.

Oxidative stress in the CNS arises from normal and pathological oxidation in the brain, originating from the failure of neuronal antioxidant defenses to prevent damage and maintain cellular homeostasis [21]. Oxygen-derived free radicals are generated during oxidative metabolism and energy production in the body and are involved, on the positive side, in the regulation of signal transduction and gene expression, activation of receptors and nuclear transcription factors, and the antimicrobial and cytotoxic action of immune system cells, neutrophils and macrophages, and, on the negative side, with oxidative damage to cell components, aging and age-related degenerative diseases [56]. Evidence for oxidative stress in the brain and/or cerebrospinal fluid may also support the hypothesis that redox or transition metals and free radical imbalances may induce neurodegenerative illnesses. In various neuropsychiatric illnesses, such as aging [57], MCI and AD, oxidative stress is an inevitable player stemming from the generation of reactive oxygen species (ROS).

There is evidence that the production of ROS results from extraneous physical or chemical perturbations, of which radiation may be the major contributor. One of the important radiation-induced free-radical species is the hydroxyl radical, which indiscriminately attacks neighboring molecules, often at near diffusion-controlled rates. Hydroxyl and other radicals are generated by ionizing radiation either directly, by oxidation of water, or indirectly, by the formation of secondary ROS [58]. This common molecular event is strongly supported as one of the main components in the etiology of neurodegenerative diseases [59].

Consistent with our proposal, the relationship between aging and oxidative stress is one of the most intensely studied fields. However, the relationship between aging and the expression of ApoE in the disease process is not well understood. In the young individual,

compared with middle and old age, ApoE is increased in mouse cerebral cortex [60]. This presents a finding requiring further investigation. Thus, oxidative stress, ApoE (through its three isoforms) and Aβ (or AβPP) may be considered the primary molecular complex in the brain. The actual origin of increased Aβ or hyperphosphorylated tau, the classical markers of AD and MCI, may represent a defensive molecular mechanism against oxidative stress and ApoE4 [12,61].

Fe, Cu, ApoE & Aβ peptide as a macromolecular complex: oxidation & antioxidant activity in AD

The dual action of ApoE isoforms has yet to be completely investigated [62]. ApoE4 is considered to be a major risk factor in AD and contributes to the formation of senile plaques and NFTs through its interaction with Aβ and tau protein, respectively [10]. Furthermore, when ApoE4, particularly in cases of the homozygous polymorph gene, binds to Aβ, it creates a macromolecular complex that impairs low-density receptor protein 1 (an extremely efficient protein that speedily clears CNS cells of Aβ). This complex leads to a slow amyloid build up and results in senile plaque formation. *ApoE4* increases the risk of AD, eight- to ten-times in +/+, compared with the *E3* and *E2* heterozygous/homozygous isoforms [63]. On the other hand, some studies have shown that ApoE isoforms 3 and 4 have a protective role and reduce Aβ peptide neurotoxicity. In AD, ApoE4 patients showed Aβ deposition significantly earlier and in higher levels than non-demented control subjects [16]. Extending this study to *in vitro* tests, the authors demonstrated that all ApoE isoforms inhibit Aβ formation and aggregation and stimulate neurite maintenance causing neurite loss and behavioral deficits with aging in knockout mice.

Additionally, ApoE may strongly attach to Aβ to create a macromolecule that facilitates deposition and neurotoxicity of Aβ. The inhibition of this binding by a synthetic peptide ($A\beta_{12–28P}$) – analogous to, but shorter than $A\beta_{1–42}$ – attenuates neurotoxicity and results in smaller senile plaques than those formed with the macromolecular complex ApoE4–Aβ [48]. This macromolecular complex is very resistant to ApoE4 uncoupling from the Aβ peptide because there are strong hydrogen bonds between the proteins. An example of this type of binding is given in an interesting observation on a virally derived peptide [64]: an argininerich peptide from the Jembrana disease virus (JDV) protein is a structural ‘chameleon’, which binds to other viral proteins with an affinity even higher than the cognate proteins. The authors established the structure of this high affinity complex and found that the C-terminal tyrosine in JVD forms a network of inter- and intra-molecular hydrogen bonds and stacking interactions that simultaneously stabilize the hairpin conformation of the peptide and the viral RNA.

They also reported that histidine helps to stabilize the peptide conformation, facilitating the induced binding. Intermolecular and intramolecular Van der Waal dispersion forces (known as ‘dipole–dipole’) will stabilize the complex JVD-trigger protein in a strong complex, impairing other proteins from separating them. The resultant macromolecule turns out to be a complex protein with very different properties than the original JVD. Other studies have shown that the three histidines in Aβ bind to redox metals and may control the redox activity of Cu and Fe [65].

Fe and Cu, redox transition metals, have a major role in oxidative metabolism. In addition, Fe and Cu are also found bound to senile plaques and NFTs, generating free radicals and inducing oxidative stress. Fe, in particular, is a major source of the extremely potent hydroxyl radical, which originates from the reaction of hydrogen peroxide and Fe(II; Fenton reaction). Additionally, Fe(III) may bind to senile plaques and NFTs and may oxidize Aβ peptides *in situ* – that is, in the binding position [30].

The level of protection in the context of this toxicity was proportional to the neurotrophic actions of the ApoE isoforms, with the E4 isoform being less effective. There was no difference in the protection provided by the E2 and E3 isoforms. Furthermore, in the present study it was shown that the level of defense against toxicity was proportional to the neurotrophic actions of the lipoproteins. It is then appropriate to consider that ApoE acts as a potent growth factor, either in the absence or presence of Aβ, substantiating the potentiality for an important role for ApoE in neurobiology [66]. These assumptions may lead us to suggest an interaction between all (E4, E3 and E2) ApoE isoforms, such as an unconfirmed 'on–off ' mechanism. In addition, we could suggest the improbability that there is a unique, pathological or beneficial role of ApoE4 alone, clinically regarded as the only pathological isoform. We believe that the correlations between ApoE levels and genotype frequencies in neurodegenerative illnesses, such as AD, Parkinson's disease (PD), amyotrophic lateral sclerosis and Huntington's disease, should not be used to establish absolute roles for the ApoE isoforms, but rather as ratios between the 'pathological' and 'protective' ones.

Furthermore, other studies, using ApoE4 knockout mice, have shown that ApoE4 performs important functions in the brain. Interestingly, some of these studies reveal that ApoE4 is indeed necessary for normal cognitive function; the absence of ApoE4 is associated with working memory and attention deficits in PD [67]. In spite of the association of ApoE4 with an increased risk of AD and weaker cognitive function in elderly PD patients, the absence of the *E4/E4* genotype as a risk factor in PD may be correlated with poorer working memory and attention. Based on these results, we suggest that the *E4* allele, at physiological levels, may be necessary for normal cognitive function in some brain areas, while at higher concentrations it becomes neurotoxic in other brain areas.

Interestingly, ApoE4 has a different action on dissimilar anatomical brain areas: ApoE4 carriers with mild AD have been reported to have greater medial temporal lobe pathology (atrophy) and poorer memory retention than noncarriers. Conversely, noncarrier patients with the same grade of AD had decreases in working memory, executive controls and lexical access, with greater fronto-parietal atrophy than ApoE4 carriers [68]. This variation of phenotypes in AD patients suggests an important diversity of actions for different ApoE isoforms. It is probable that an 'on–off ' molecular mechanism may protect or damage cells depending on the biochemical and metabolic background, a condition that remains to be investigated. Furthermore, ApoE4 has an intimate relationship with Aβ. It couples with this peptide alone or in complexes with biometals, modulating homeostasis [38].

At physiological levels, ApoE4 is present at the lowest brain concentration of the three ApoE isoforms, E4, E3 and E2, and ApoE2 and E3 are associated with protective functions. This trend could also be similar for other apolipoproteins (A, B, D, J and so on). We propose that it is possible to find some order in the neuromolecular drive in relation to apolipoprotein roles. Investigating a large series of studies on apolipoproteins' normal and pathological effects, we find very puzzling results: classical studies point to ApoE4 as a high risk to AD, oxidative stress, depression, aging, PD, other dementias, amyotrophic lateral sclerosis and so on; newer studies point to an array of survival and protective effects. If we take these diametrically opposed functions into account, we need to define new parameters to facilitate our investigation: first, are all apolipoproteins, at physiological concentrations, valuable for neuronal and neuroglia survival? Second, why, at either lower or excessive levels, particularly for ApoE4, do apolipoproteins promote neurodegeneration? And third, what are the differences between all apolipoproteins, specifically ApoE4, with respect to their neuromolecular effects? For example, normal ApoE4 functions, although less effectively than ApoE3 and ApoE2, to transport lipids and phospholipids, clear Aβ and cholesterol, facilitate Aβ degradation in the extracellular space by insulin degrading enzyme and control ChAT and nicotinic receptor sites [69].

Apolipoproteins may also be attacked by free radicals and redox metals and participate in the cascade of neuronal damage. Indeed, the work of Perry and colleagues reports that tau, Aβ and ApoE possess metal binding sites, inducing metal-associated cellular redox activity that is dependent on Fe, Cu and other transition redox elements [70]. We have already shown that ApoE4 binds to Aβ peptide, forming macromolecular complexes that aggregate, becoming insoluble, and thus are unable to cross neuronal membranes. In addition, we have presented a few studies that report that only monomeric Aβ peptides may be transported across neurons and neuroglia. Thus, we may suggest that ApoE4 induces aggregation when it binds to oligomeric Aβ peptides, contributing to senile plaque formation, one of the cardinal features of AD.

Some pioneering studies revealed that ApoE binds redox metals such as Fe, Zn and Cu and converts them into oxidizing agents [70]. We suggest that ApoE4 binding affinity to metals is a phenotype yet to be studied. Current studies report ApoE as a free-radical scavenger and as a neuronal protector, binding to Aβ and inducing its clearance. Thus, the antioxidant activity of ApoE may be considered to be a survival function [43]. It is reasonable to suggest that oxidative and nitrosative stress may be the earliest molecular occurrences preceding AD.

This subject is clarified in a number of significant studies [55,71–73]. Indeed, the seminal work of Nunomura *et al.* measuring an oxidized nucleoside RNA product, 8-hydroxyguanosine and nitrotyrosine in AD patients established the association of these molecules with Aβ and NFTs and the development of early-onset AD [55]. It was reported that AD patients with the homozygous *ApoE4* genotype present with significantly more senile plaques and NFTs (~50%) than non-ApoE4 carriers and that oxidative damage – measured by 8-hydroxyguanosine and nitrotyrosine [72] – was significantly elevated early in the development of the disease. Furthermore, oxidative damage was an early event in the course of the disease, and in contrast to the progressively increasing levels of Aβ, oxidative stress decreased over time.

These important results support a new hypothesis that places oxidative stress as an early event in the disease course rather than a consequence of Aβ deposition. The authors suggest a new hypothesis: AβPP and tau act as safeguards that protect against oxidative stress. As oxidative stress is a common molecular event involved in depression, neurodegenerative diseases and many physiological, metabolic [74] and pathogenic phenomena, it is reasonable to propose an interaction or association of mental syndromes, particularly those involving depression, with markers of AD such as ApoE4, Aβ and tau [75].

ApoE4, depression & neuropsychiatric symptoms in AD

A series of clinical and neurobiological investigations into the neuropsychiatric symptoms (NPS) associated with AD have been carried out in the past three decades. The impetus behind this research is related to the stresses and decreased quality of life that these noncognitive syndromes bring to patients and caregivers. NPS may be markers of severe dementia when combined with common cognitive impairment and may indicate the need for hospital care [76].

Despite the fact that substantial progress has been made in the field of neuropsychiatry, there is controversy related to the association of NPS and ApoE4. A study by Levy and coworkers sought to clarify the relationship between ApoE status and noncognitive symptoms in 605 AD patients for whom the *ApoE* genotype and the neuropsychiatric inventory of ten items (including psychosis, mood changes and personality alterations) were assessed prior to treatment [77]. The results showed no significant relationship between homozygous and heterozygous *ApoE4* allele patients and neuropsychiatric and/or noncognitive symptoms. In

another study, Martinez-Barrondo *et al.* did not find any association between ApoE and panic disorder, concluding that the variation of *ApoE* genotypes was not associated with the development of panic disorder [78]. By contrast, in combat related post-traumatic stress disorder subjects, it was found that specific *ApoE* alleles were associated with the NPS of post-traumatic stress disorder. *ApoE2* was associated with significant disruption and impaired memory function and more frequent relapse of symptoms [79]. Chronic anxiety in *ApoE4* homozygotes affects cognitive skills and, compared with heterozygote controls, it correlates significantly with cognitive decline as measured by the Wisconsin Card Sorting Test [80].

Previous studies by Caselli and colleagues showed that age-related memory decline occurs earlier in cognitively healthy *ApoE4* homozygotes than in *ApoE4* heterozygotes and controls [81]. Moreover, an interesting study in caregivers of AD patients showed that increased levels of stress were associated with increased levels of depressive symptoms in caregivers with the *ApoE4* allele. This relationship was not observed in caregivers lacking the *ApoE4* allele. These results suggest that carriers of the *E4* allele may respond differently to psychological stress than individuals without the *E4* allele [82].

In a recent study on the interaction of dementia, depression and ApoE, it was found that *ApoE4* carrier AD patients had a significantly higher rate of depression than non-*ApoE4* carriers. The risk of dementia in nondepressed men with *ApoE4* was not significantly different to nondepressed men lacking *ApoE4*. However, depressed men without *ApoE4* had a 1.6-fold greater risk of dementia, whereas depressed men with *ApoE4* had a 7.1-fold greater risk. The conclusion of this research was that *ApoE4* status modifies the association between depressive symptoms and dementia in elderly men. Thus, older individuals with both depression and *ApoE4* should be assessed for early signs of AD [83,84].

Consistent with this study, other important reports have substantiated the correlation of ApoE and depression and MCI. For example, Geda *et al.* appraised 840 cognitively normal elderly subjects without depression at recruitment and followed them for 42 months [85]. The subjects who developed depression during this period (on the short Geriatric Depression Scale; the cohort group) were compared with those who did not (the control group). All participants were assessed for *ApoE* genotype. Individuals in the depressive cohort were at a significantly increased risk of subsequent MCI, were at the same risk for dementia and demonstrated a synergistic interaction between *ApoE* and depression: *ApoE4> ApoE2> ApoE3*. Thus, depression in patients with MCI increases the risk of AD-type dementia, significantly more than patients without depression [86] and especially in the *ApoE4*-positive genotype [87].

Clinically, it is very difficult to distinguish between depression and dementia in older persons [88], and the similarities of both neurobiological mechanisms suggest the need to differentiate between older patients with depression who start with cognitive symptoms from those patients with cognitive deficits who coincidently present with depression and correlated symptoms [75,89]. Consistent with this conclusion, recent studies on the treatment of AD patients have shown efficacy with antidepressants, particularly the inhibitors of serotonin reuptake [90]. Here we may ask the following question: do chronic depression patients improve if they are treated with acetylcholinesterase inhibitors and/or antagonists of glutamate NMDA receptors, the most common drugs for AD? This question remains unanswered; there are limited reports addressing this issue, necessitating further investigation [91,92].

On the other hand, many reports show that selective serotonin reuptake inhibitors have a therapeutic effect in AD, an observation that supports the relationship or association

between the neurobiology of depression/anxiety/stress and AD. The work of Rocchi *et al.*, which underlines the disagreement and controversy behind many neurobiological, clinical and etiologic data in AD [93], is consistent with our proposal that investigating the interaction between these fields would facilitate the search for new treatments in AD that involve a unique clinical AD phenotype. An integrated therapy based on an understanding of various etiologies would potentially be more effective. However, despite the uncertainty over the relationship of NPS and *ApoE* genotype in AD, we may find a robust effect of these apolipoproteins [94]. Given the possible link between depression, cognitive decline and AD symptoms [95–97], and the ApoE4 influence on AD and other neurodegenerative disorders, it is possible that depression and ApoE4 are associated. An alternative possibility is that chronic depression and stress may induce hippocampal atrophy.

Aging, age-associated cognitive impairment, depressive disorder, MCI & AD: independent of or linked to oxidative stress, Aβ, ApoE & tau

Oxidation reactions provide the basis of life, but it may result in oxidative stress early or late in life, leading to AD and other neuropathologies [98]. Redox active transition metals are critical for brain metabolism, but may result in severe oxidative stress and accelerated neurodegeneration [99]. ApoE2, ApoE3 and ApoE4 are examples of twofold function lipoproteins. We have suggested an ‘on–off ’ model to be investigated. Aβ and tau proteins are normal defenses against oxidative stress in the normal state and are considered to be pivotal in aging and AD. The interaction model is presented in Figures 1–3, based on Nakamura *et al.* [65] and Rodrigues *et al.* [4].

For more than a decade, neurologists and researchers have known that *ApoE4* carriers develop AD at earlier ages and are susceptible to an increased risk of cognitive decline after traumatic brain injury or stroke. Moreover, some studies show that patients carrying AD risk factor genes, particularly the *ApoE4* allele, develop NPS after disease onset at much earlier ages than healthy subjects [100]. These same researchers identified NPS in 120 probable AD patients: 28% had major depression, 17% minor depression, 30% delusions and 14% hallucinations. However, they did not find significant differences between the three *ApoE* genotypes, so they concluded that *ApoE* genotypes do not modify the risk of developing AD-associated NPS. We suggest that NPS indeed occur earlier, since *ApoE4* is associated with earlier sporadic AD [59]. Consequently, neurodegenerative symptoms, either cognitive or emotional, appear earlier than in non-*ApoE4* AD patients.

Prolonged stress, leading to elevated levels of cortisol, impairs memory in humans [101–103]. This effect is significantly greater in elderly AD individuals that are *ApoE4* carriers than in non-*ApoE4* carriers. In these studies, it was not reported whether prolonged stress and *ApoE4* interact to increase the risk of developing AD [104]. Nevertheless, its importance to our hypothesis is founded on the interactions between the four elements: cortisol, *ApoE4*, prolonged stress and memory [95].

New reports detailing the relationship of oxidative stress and depression have provided additional and significant information regarding these simultaneous events. One example is the depression-induced oxidative stress found in rats. This study demonstrated that chronic, mildly stressed, depression-induced rats exhibit a significant decrease in glutathione peroxidase activity, a decrease in glutathione and a decrease in vitamin C in brain cortex. By contrast, lipid peroxidation was significantly elevated compared with controls. The administration of mood stabilizers, antipsychotics and antidepressant drugs such as lamotrigine, escitalopram and aripiprazol, respectively, normalized the results. This study gives strong evidence for the interaction between oxidative stress and depression [105].

The relationship between stress, depression and *ApoE* genotype has also been addressed. Some studies reported lower than normal glucocorticoid levels in ApoE-deficient and stress-induced mice [106]. Another group of investigators observed the opposite: higher corticosteroid levels in ApoE-deficient elderly mice than wild-type control mice in response to repeated stress. Fiocco and colleagues reported in a long-term study of humans that *ApoE4* carriers were not found to secrete higher serum cortisol than non-*E4* carriers after 8 years of observation, although declarative memory performance over these years showed significant differences based on *ApoE* genotype [107]. Felician and Sandson presented the results of a longitudinal study of the interaction of five factors: increased Aβ and tau, decreased acetylcholine in the synaptic cleft, oxidative stress, inflammatory responses with cytokine production, and estrogen deficiency [108]. The authors related these multiple pathogenic events to coherent forms of AD treatment: estrogens, acetylcholine supplements, NSAIDs, antioxidants and acetylcholinesterase inhibitors. However, none of these therapies have a curative effect, but may just delay the appearance of clinical AD symptoms.

On the other hand, glucocorticoids enhance oxidative stress, inducing neuronal apoptosis and death in the hippocampal CA3 region [109]. Remarkably, in some recent investigations, the Aβ peptides, specifically $A\beta_{1–42}$ and $A\beta_{1–16}$, were shown to decrease the generation of ascorbate-dependent hydroxyl radicals, particularly via free Fe(III) and Cu(II), and accelerated by superoxide dismutase 1 [65]. These authors also suggest that histidine residues from Aβ peptides control the activity of transition metals, thus meeting the criteria of antioxidant agents that may be bound to Cu(II) and Fe(III) and attenuate neuronal damage from the Fenton/Haber–Weiss cycle [50].

Research linking depression and chronic stress/anxiety is common, and their relationship to AD is currently being investigated [95,96]. Linking multiple factors suggests that positive results would be more likely with a combinatorial therapy than using a single treatment or drug. Indeed, combinatorial drug therapies are effective with chronic depression. On the other hand, brain aging has been considered not only a risk with respect to AD, but it also acts in concert with oxidative stress [110], producing age-associated memory impairment, MCI and AD [4].

The ‘free radical theory of aging’ is one of the most accepted theories to date. Throughout life, free radicals – ROS and reactive nitrogen species – occur inevitably due to the organism’s aerobic respiration [111] and exposure to ultraviolet rays [4,112]. Many studies have demonstrated age-related increases in DNA oxidation and damage through detection of increased 8-oxo-2-deoxyguanosine, which appears to arise from age-related decreases in the brain’s ability to deal with oxidative stress [113]. In addition, increases in free radical production in the brain and the consequent damage may be used as a sign of the rate of aging [114].

Disruption of Fe homeostasis and the effect on Fe-regulatory proteins may be another of the signs of aging deserving study. Thus, the production of ROS through the Fenton reaction and the increase in Fe uptake via Fe-regulatory proteins may be a consequence of Fe depletion. If not compensated for by antioxidants, such as glutathione peroxidase, superoxide dismutase 1, and catalase, this impaired homeostasis may lead to apoptosis and/or necrosis of neurons and glia [115].

Aging is correlated with a reduction in redox-metal ions (accumulation of Fe, Cu and Zn) and the expression of the aforementioned protein enzyme antioxidant defenses may be impaired by long-term DNA damage stemming from genetic susceptibility, oxidative stress and stochastic mutations accrued throughout life [4]. Furthermore, Aβ accumulates owing to its increased production in an attempt to combat ‘aging oxidative stress’ [31,55,70,74], the

result of elevated synthesis of the AβPP and/or inefficient clearance of Aβ from the brain. The negative cascade arises because the Aβ peptide, in the presence of redox active transition metals, generates abundant ROS and reactive nitrogen species, reinforcing 'aging oxidative stress' [57,72]. This 'aging oxidative stress cascade' provides a molecular mechanism that may explain whether there is a slow or fast progression into age-associated cognitive impairment, MCI and/or AD [4,116,117]. Taking these studies into consideration, we may ask whether AD is an 'age-related' or 'aging-related' type of 'demented senility' [118]. It is also relevant to include chronic depression, anxiety and psychological stress as AD interaction factors.

Various depression and stress syndromes can lead to hippocampal atrophy, resulting from activation of the glucocorticoid cascade, whereby increased glutamate and subsequent calcium stimulation can damage and kill neurons and glia [119]. In AD, hippocampal, amygdala and frontal cortex volume reductions are common neuropathological findings [120]. The similar patterns of brain damage in depression and in AD may suggest that the underlying cellular and molecular mechanisms may be the same, opening a new pathway for investigation into this common neuropathological occurrence. In the field of psychoneuroimmunology, various interactions between depression and AD have been shown, particularly regarding brain tissue inflammation and oxidative stress. Both of these conditions are promoted by these occurrences. Furthermore, chronic depression and stress increase brain cortisol levels, elevating adrenocorticotrophic hormone concentrations and inducing lesser immunologic defenses.

Oxidative stress may be another interaction factor related to AD, depression and inflammation [121]. Eren *et al.* studied depression-induced oxidative stress and the protective effects of lamotrigine, aripiprazole and escitalopram, considering oxidative stress as an indirect depression-derived occurrence [105]. Additionally, there is more than a decade's worth of studies on depression, stress and anxiety that have established their robust effect on the hippocampus, particularly in chronic syndromes. Stress hormones induce structural plasticity in hippocampal formation, suppressing neurogenesis from the dentate gyrus granule cells. While acute hormonal damage may be reversible, chronic psychosocial stress leads to hippocampal atrophy of dendrites in the pyramidal neurons of the CA3 region. The glucocorticoid and mineralocorticoid receptors are triggered by glucocorticoid hormones, which act in concert with excitatory amino acids or NMDA receptors and, with the increase of calcium efflux to intracellular media, cause neuron death [122].

Based on the above we can conclude that aging, oxidative stress, depression (anxiety and stress), MCI, AD and other neurodegenerative diseases are a part of a continuum of brain dyshomeostasis and metabolic responses, with the main factor being redox active transition metals, Aβ, ApoE and tau, in addition to AβPP, presenilin 1 and 2, and others that constitute the genetic origin of those neuropathologies. ApoE4 is also strongly associated with anxiety, depression and stress [82] and indirectly with NPS. Oxidative stress is currently considered to be one of the universal factors in the etiology of AD and other neurodegenerative illnesses, in addition to its involvement in aging and acute neurological processes [4]. Oxidative stress occurs when the antioxidant defenses against free radicals can no longer maintain the metabolic homeostasis in the brain, a situation that may result in the activation of signaling cascades [74] ending in cellular apoptosis and/or necrosis. The strong relationship between AD and chronic depression (also stress/anxiety) has been reported, and AD markers such as senile plaques and NFTs are increased in the hippocampus in patients with a lifetime history of major depression [123]. On the other hand, ApoE isoforms may alter regional gray matter volumes in remitted depression in human brains [124], demonstrating an association between brain tissue volumes and *ApoE* genotypes. Depression-related reduction in brain volume reduction is induced, at least in part by

glucocorticoids, providing a further basis for the association of ApoE isoforms and major chronic depression/anxiety. Given that oxidative stress is also associated with glucocorticoids, all of these factors may interact and may act through similar neurobiological mechanisms.

Indeed, all of these phenotypes are intimately related. For example, in normal aging the hippocampus is affected in specific areas of synaptic transmission [125]. This regional specificity constitutes a selective pattern of both degenerative change and functional sparing in different physiological parameters in the same hippocampal cells. Thus, it is possible to see aging and neurodegeneration as similar processes. Squire reports studies relating the hippocampus with a memory system that links distributed sites in the neocortex and other areas, such as the amygdala (emotional memory) [126]. Finally, AD may be a result of synergy of these mentioned elements, a reality that challenges the current model.

Discussion: the interaction model

The figures can be viewed as if they are independent from one another. Nevertheless, when we observe that one event is significant in two or more molecular, cellular, statistical, pharmacological or clinical processes, we will try to 'integrate' these conceptually, as indeed they should be. To do otherwise, we would be opposing the 'natural order', which is to conserve energy, to protect the organisms from dispersion and uneven responses and to utilize the fewest molecular, cellular and/or biochemical agents or actions to obtain the most robust effects/responses for protection and survival purposes.

Some studies in neurological sciences have already gravitated toward this rather phenomenological analysis, which is a deeper investigative process than the Cartesian method [4,7,8,127]. The end result is the interaction hypotheses with which our work is consistent. The important work of Behl *et al.* has clearly shown that glucocorticoids in rats and *in vitro* induce vulnerability to neuronal death from oxidative stress, due to enhanced glutamate toxicity and Aβ deposition in senile plaques [109]. We have already reported that oxidative stress is the earliest event in the development of AD [55,73] [Nunomura *et al.*, Unpublished Data]. Additionally, we have discussed studies of elevated glucocorticoid levels and their association with chronic stress/anxiety/depression syndromes [101,102,122] and hippocampal atrophy, with aging being a progressive risk [101,103,128]. Finally, we briefly mention, as an additional illustration, a recently described molecular event linking inflammation in AD and age-related changes in TNF receptor (TNF-R) expression [129]. The Aβ peptide, a pivotal molecule in AD, was reported to stimulate the TNF-α cascade through TNF-R1 and TNF-R2 expression. As the extrinsic pathway of AD, apoptosis is related to the TNF superfamily of membrane receptors. Age affects TNF-R expression, making neurons in the aging brain more susceptible to Aβ toxicity [130].

Conclusion

AD shares molecular mechanisms with neuropsychiatric phenotypes (Figures 1–3). Treatments focused on similar mechanisms may lead to the development of novel AD therapies. As there is a more or less temporal continuum in human life affecting their expression and functions, we suggest that these pathological mechanisms may be influenced by the life cycle, and hence the age-related nature of the diseases – that is, depression early, AD late [4]. Thus, incorporating the concepts in the interaction model provides a novel framework for developing new treatments for AD and other neurodegenerative/ neuropsychiatric disorders.

The association between depression and AD has been viewed as the former flowing from the latter. However, it is also possible that this comorbidity is actually a separate, parallel process both related to ApoE. Figures 1–3 show the dynamic interactions linking these entities. In this vein, the lack of understanding of the association between ApoE and the 5-HTT allele points to the need for cross-sectional studies in our attempt to elucidate the sources of pathology and to develop effective therapies.

In addition, understanding that AD is the result of a process rather than simply the production of a 'toxic' molecule is crucial. Rather than acting as a 'poison', Aβ seems to be a response and signaling cascade, as recently demonstrated through interaction with the cellular prion protein and redox suppression of Cu. In addition, another key player in the development of AD is ApoE, which also acts through multiple cellular pathways.

Another important aspect of these conditions is the temporal occurrence. The most significant risk factor for the development of AD is age. Even in the early-onset cases, AD does not develop until the fourth decade of life, despite the presence of a fully penetrant pathogenic mutation. This implies that one or more cellular systems needs to be compromised before disease onset. Thus, similar to cancer, it appears that two, or more, hits must occur to promote the disease state.

The model presented herein attempts to show the complex interacting systems that involve Aβ and ApoE in neurodegenerative and neuropsychiatric conditions. In addition, contemplating the different forms that the Aβ molecule adopts – that is, monomeric versus oligomeric versus fibrillar – and the different functions they perform, from anti- to pro-oxidant, may help to explain the age-related development of disease.

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Future perspective

Recent years have seen an increasing appreciation of the role of oxidative stress in neurodegenerative disease, which is extending to other neuropsychiatric conditions. In addition, our view of ApoE involvement has progressed, largely through the use of transgenic model systems. The outstanding issues relate to the discrepancies between the models available and the actual diseases and conditions they represent. Determining how the differences in human versus mouse proteins, in particular ApoE, have influenced experimental outcomes will be crucial for interpreting translational studies on therapeutics. In addition, characterizing the whole spectrum of AD will be required to develop rational treatments that account for underlying differences in the pathogenesis. Finally, ferreting out the various mechanisms underlying aging and the relative importance of each will facilitate our understanding of the aging process on an individual basis with respect to genetics and environment.

Executive summary

Background

- Cross-sectional studies are required for elucidating the cellular/molecular basis of pathology in neurodegenerative disease.

Functions of ApoE4: brain physiology & pathology

- ApoE plays critical roles in neuroprotection and pathogenesis, presumably related to its role as a cholesterol transporter. The ApoE4 isoform is the least effective in this role.

Aβ & ApoE4 as protection against oxidative stress, an early response observed in Alzheimer's disease & aging

- Although Aβ is associated with the pathology found in Alzheimer's disease (AD), senile plaques and fibrils, Aβ also plays a protective role through the maintenance of redox homeostasis. Note that oxidative stress is the earliest pathological event in AD.
- The Aβ/ApoE interaction modulates the action of Aβ and varies by ApoE isoform, with ApoE4 yielding the least protection.

Iron, copper, ApoE & Aβ peptide as a macromolecular complex: oxidation & antioxidant activity in AD

- Aβ/ApoE action is related to metal homeostasis, particularly iron and copper, which factor into oxidative damage.
- ApoE, in addition to its association with AD, is relevant to neuropsychiatric conditions, with ApoE4 being associated with depression.

Aging, age-associated cognitive impairment, depressive disorder, mild cognitive impairment & AD: independent of or linked to oxidative stress, Aβ, ApoE & tau as phases in a life cycle?

- Oxidative stress may provide the link between aging, cognitive impairment, depression and AD.

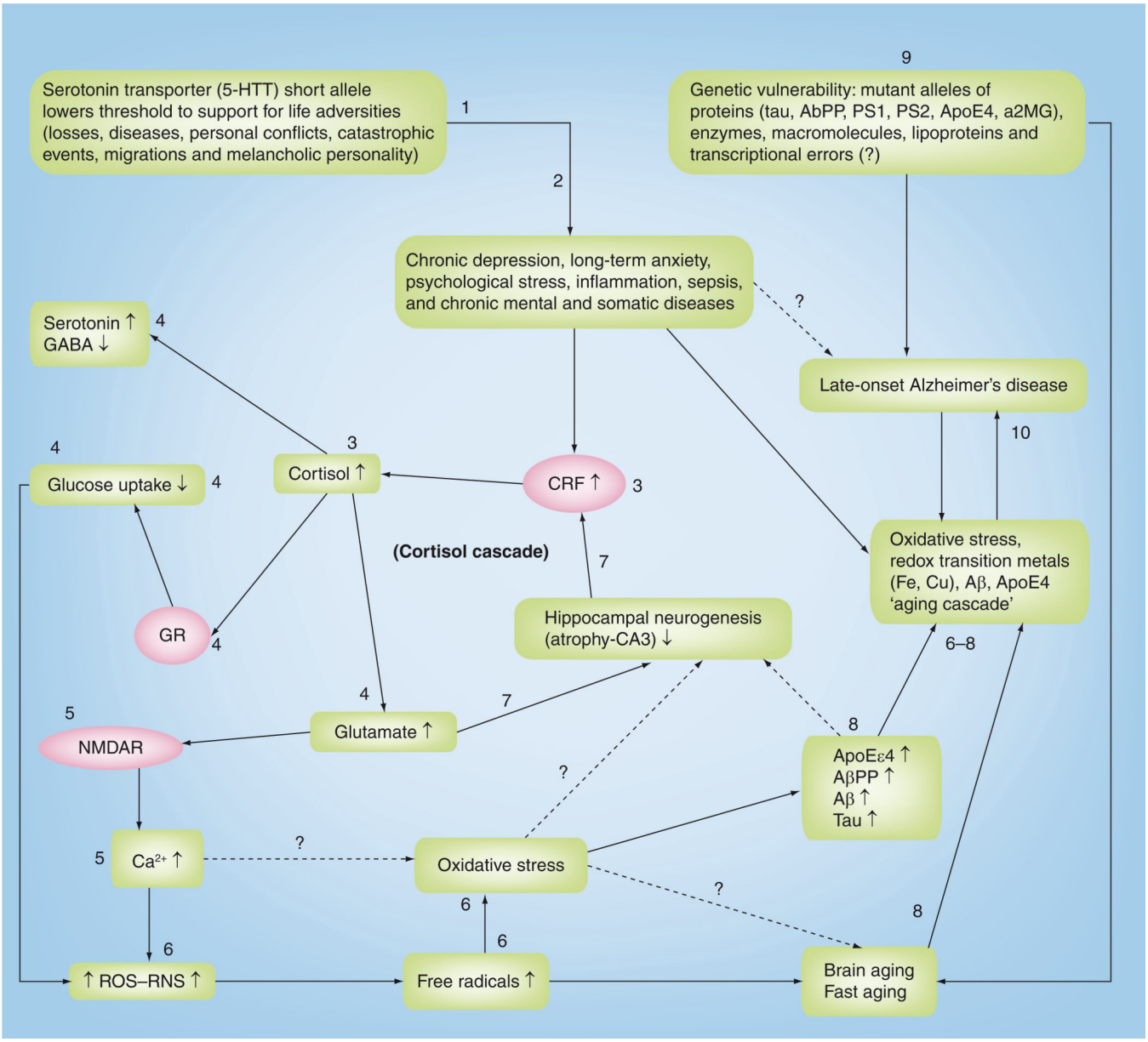

Figure 1. Alzheimer's disease interaction model: pathogenesis

(1) Genetic vulnerability (short alleles of serotonin transporters) may lead to weak resistance. Support for life adversities, such as severe personal losses, catastrophic events and diseases. **(2)** In vulnerable individuals, it may result in chronic major depression, stress and anxiety; and/or inflammatory or somatic diseases. **(3)** Long-term suffering from these depressive and consequent events raises CRF levels, which induces the formation of the cortisol cascade with consequent elevation of cortisol in the brain. **(4)** The corticoid cascade is set with elevation of cortisol in the brain leading to increased glutamate and serotonin and to a reduction of GABA levels. GR and mineralocorticoid receptor concentrations are also stimulated. Reduced glucose uptake also stimulates ROS, RNS and free radical generation. **(5)** Glutamate excites NMDA receptors that leads to increased intracellular [Ca^{2+}]. **(6)** Calcium elevation may elevate ROS and RNS, in turn these reactive species produce free radicals, which may result in oxidative stress. **(7)** Continuing the cascade: it is possible that

these events result in hippocampal damage and atrophy, diminishing neurogenesis and synaptogenesis. Oxidative stress may lead by itself to hippocampal atrophy in CA3 and hippocampal atrophy results in CRF elevation. This closes the cycle feeding back the cortisol cascade. **(8)** Oxidative stress leads to the elevation of AβPP, Aβ and tau proteins for protection against oxidation. However, these peptides cause damage to the hippocampus, contributing to its atrophy and feeding-back to CRF increase, leading to the corticoid cascade. Furthermore, chronic production of free radicals may induce brain aging and accelerated aging. **(9)** Increases in ApoE4, AβPP, Aβ, tau and accelerated brain aging may also induce oxidative stress. Redox transition metals (Fe and Cu) are increased leading to the aging cascade, closing the circle. **(10)** Genetic vulnerability due to specific proteins (i.e., tau, ApoE4, Aβ, PS1 and PS2), other enzymes, macromolecules and lipoproteins may cause inadequate antioxidant defenses, resulting in protein misfolding and consequent deposition. With aging and genome damage many ‘errors’ accumulate, and eventually initiate the AD cascade.

?: Potential consequences that are not proven; CA3: Cornus-amonis 3; CRF: Corticotrophin release factors; GR: Glucocorticoid receptor; NMDAR: NMDA receptor; RNS: Reactive nitrogen species; ROS: Reactive oxygen species.

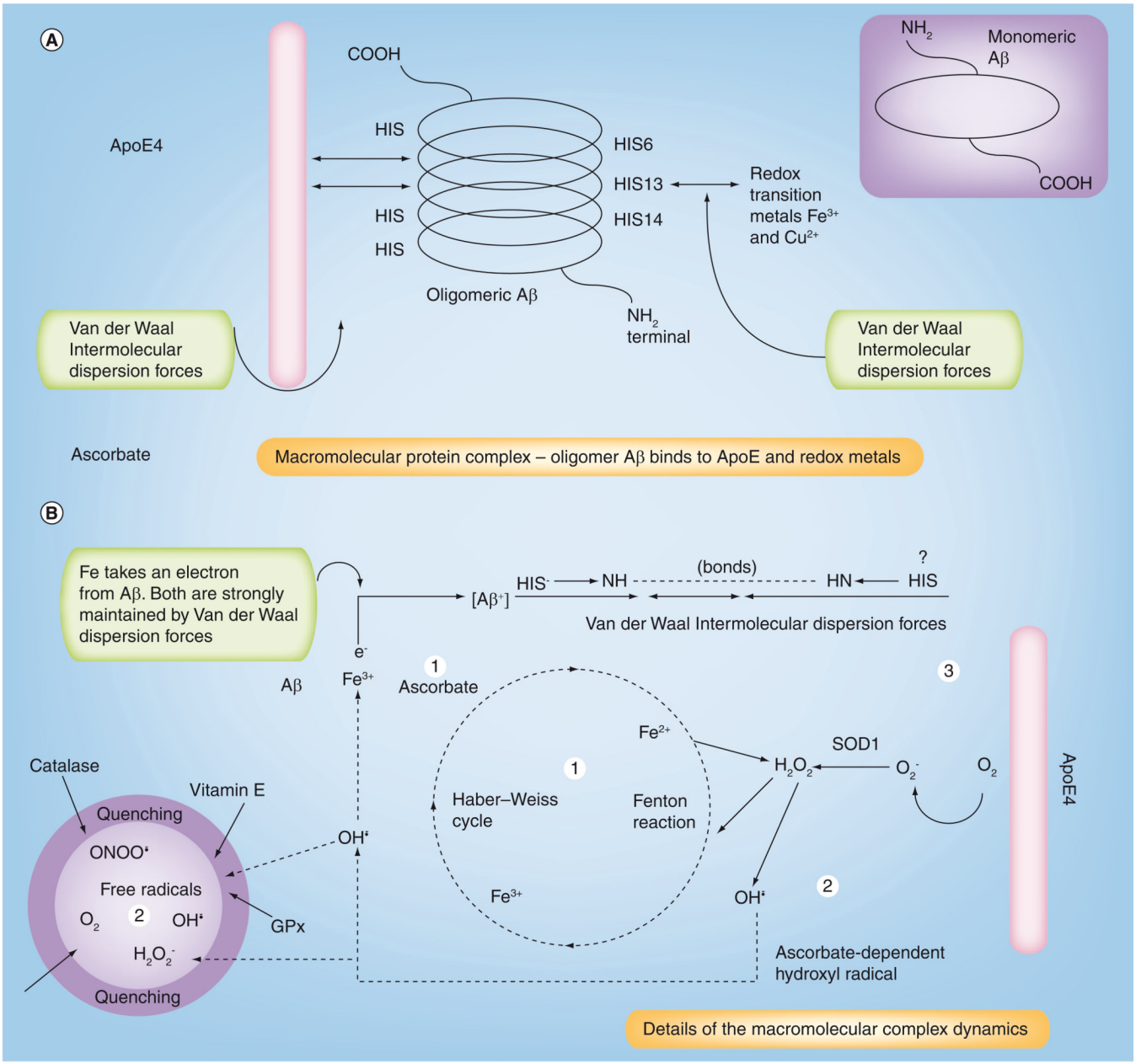

Figure 2. Gross and fine models of Aβ/ApoE dynamics

(A) Macromolecular model: interaction between Aβ, ApoE and redox transition metals. A hypothesis of how oligomeric Aβ peptide is insoluble and how it may be bound to ApoE4 and redox transition metals (Fe^{3+} and Cu^{2+}). This interaction may result in macromolecular complexes and deposition of the fibrillar forms in the brain. The connection of monomeric Aβ peptides could be carried out by histidine sites, which also mantain the bonds with transition metals and ApoE4. **(B)** Details of macromolecular complex dynamics. **(1)** Fe^{3+} may link to oligomeric Aβ by suppression of one electron; and is reduced to Fe^{2+} (Haber–Weiss cycle). Being a transition metal, Fe^{2+} turns, by the Fenton reaction, to Fe^{3+}. This mantains metals bound to Aβ by Van der Waal dispersion forces. **(2)** These reactions generate free radicals as hydroxyl radicals and others that may damage cells. Many antioxidant molecules may hold homeostasis by quenching the free radicals. **(3)** Histidine

residues in both Aβ and ApoE4, through the Van de Waal (hydrogen) forces, may maintain the adhesion of both molecules. The macromolecular dynamics may contribute to the linkage of both proteins and redox transition metals.

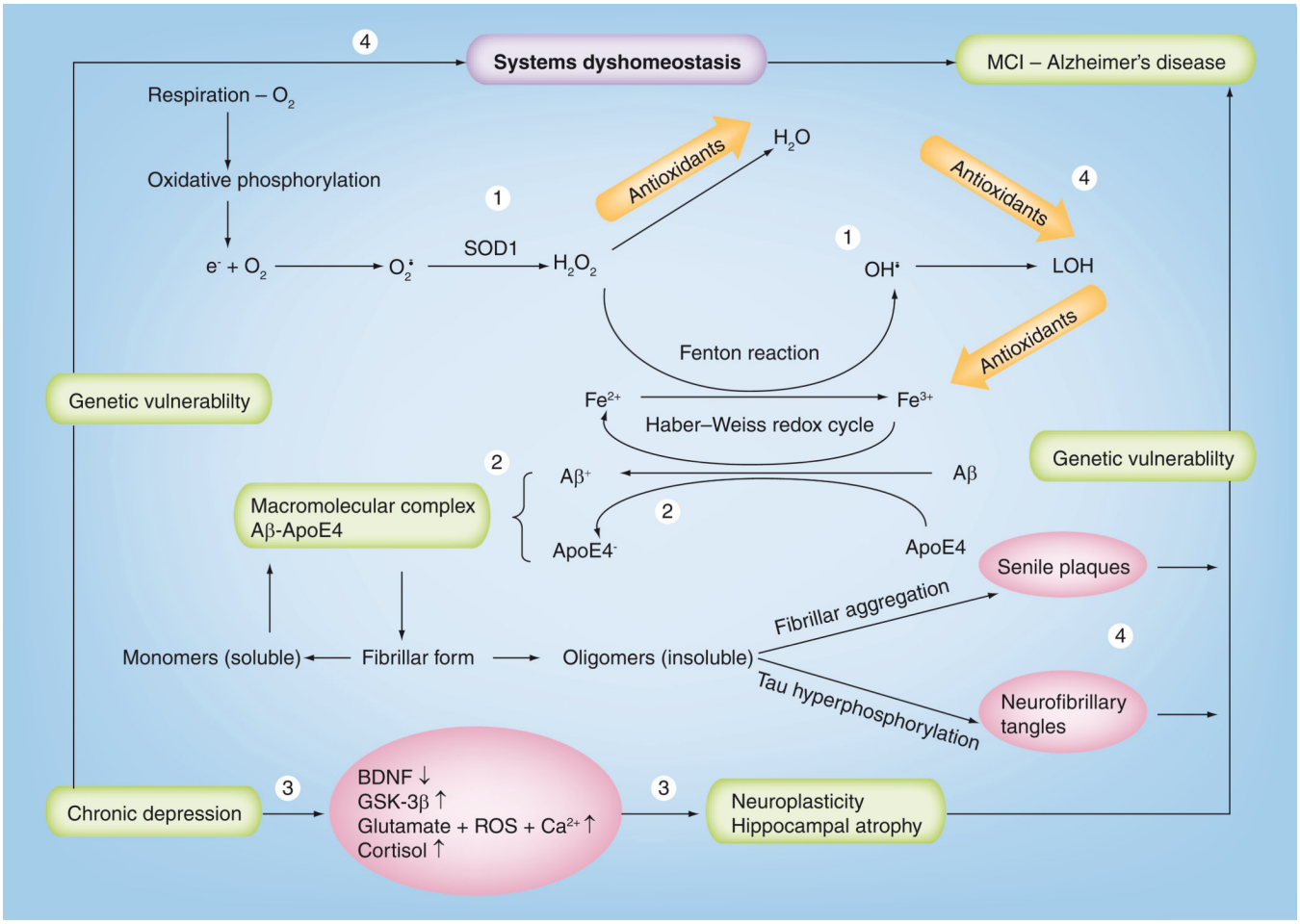

Figure 3. Interaction of transition metals, Aβ, ApoE4, major depressive disorder and Alzheimer's disease

The major molecular components associated with AD, Aβ and ApoE, are intimately connected to metal homeostasis and result in both AD and depression. **(1)** Respiration leads to O_2 and reduced oxygen to H_2O_2. Protection from oxidation is made through antioxidants to water. Alternatively, the Fenton reaction that generates hydroxyl radicals and induces Fe^{2+} to change to Fe^{3+}. Antioxidants avoid severe oxidation from hydroxyl radicals reducing the oxidized iron through the Haber–Weis reaction. **(2)** There is a supposition that the Haber–Weiss reaction may oxidize Aβ to $A\beta^+$; and ApoE4 is simultaneously reduced to $ApoE4^-$. This may induce the buildup of macromolecular complexes and formation of fibrilar and oligomeric Aβ peptides that deposit as senile plaques. Furthermore, fibrillar forms may dissociate to monomers that are soluble. **(3)** Chronic depression may lead to a loss of neuroplasticity and hippocampal atrophy (Figure 1). In genetically vulnerable individuals, the system is seriously damaged. **(4)** Hippocampal atrophy and dyshomeostasis may also result in AD in genetically predisposed individuals.

AD: Alzheimer's disease; MCI: Mild cognitive impairment; ROS: Reactive oxygen species.