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Apolipoprotein E and Oxidative Stress in Brain with Relevance to Alzheimer Disease

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Abstract

Inheritance of apolipoprotein E4 (*APOE4*) is a major risk factor for development of Alzheimer disease (AD). This lipoprotein, in contrast to apoE2, has arginine residues at positions 112 and 158 in place of cysteines in the latter isoform. In apoE3, the Cys at residue 158 is replaced by an arginine residue. This differential amino acid composition of the three genotypes of *APOE* have profound influence on the structure, binding properties, and multiple functions of this lipoprotein. Moreover, AD brain is under a high degree of oxidative stress, including that associated with amyloid β -peptide (A β) oligomers. Lipid peroxidation produces the highly reactive and neurotoxic molecule, 4-hydroxynonenal (HNE) that forms covalent bonds with cysteine residues (Cys) [as well as with Lys and His residues]. Covalently modified Cys significantly alter structure and function of modified proteins. HNE bound to Cys residue(s) on apoE2 and apoE3 lessens the chance of HNE damage other proteins. apoE4, lacking Cys residues, is unable to scavenge HNE, permitting this latter neurotoxic molecule to lead to oxidative modification of neuronal proteins and eventual cell death. We posit that this lack of HNE scavenging activity in apoE4 significantly contributes to the association of *APOE4* inheritance and increased risk of developing AD. ApoE knock-out mice provide insights into the role of this lipoprotein in oxidative stress. Targeted replacement mice in which the mouse gene of *ApoE* is separately replaced by the human *APOE2*, *APOE3*, or *APOE4* genes, while keeping the mouse promoter assures the correct location and amount of the human protein isoform. Human *APOE* targeted replacement mice have been used to investigate the notion that oxidative damage to and death of neurons in AD and its earlier stages is related to *APOE* genotype. This current paper reviews the intersection of human *APOE* genotype, oxidative stress, and diminished function of this lipoprotein as a major contributing risk factor for development of AD. Discussion of potential therapeutic strategies to mitigate against the elevated risk of developing AD with inheritance of the *APOE4* allele also is presented.

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Keywords

Apolipoprotein E; oxidative stress; Alzheimer disease; key cysteine residues; targeted replacement mice

1. Introduction

Apolipoprotein E (apoE) has several functions in the brain, among which are cholesterol transport and efflux of key moieties from brain to blood, for example efflux of amyloid β -peptide [A β] in cooperation with the low density lipoprotein receptor-like protein 1 [LRP-1] (Munoz et al., 2019; Mahley, 1988; Herz and Beffert, 2000). apoE exists in three isoforms, encoded by three different gene alleles, *APOE2*, *APOE3*, and *APOE4*. In humans, the levels of isoforms of apoE occur in the order, apoE3 > apoE4 > apoE2. In brain, apoE is localized in astrocytes, but can be transported to neurons.

There are key conformational and structural differences among the three isoforms of apoE (Figure 1): apoE2 has two cysteine residues (residues 112 and 158), while apoE3 has residue 158 replaced by a positively charged arginine residue, and apoE4 has both cysteine residues replaced by positively charged arginine residues (Munoz et al., 2019; Yamazaki et al., 2019). This differential amino acid sequence among the three isoforms of apoE leads to differential conformations (Figure 2), which lead to differential interactions among these isoforms with amyloid β -peptide and hyperphosphorylated tau protein resulting in neurotoxicity (Strittmatter et al., 1993; Winkler et al., 1999; Liu et al., 2017; Richey et al., 1995; Shi et al., 2017). These differences in conformation are easily understood in terms of electrostatics. Namely, either one or two positively charged arginines will be forced away from or toward nearby positively charged Lys or negatively charged glutamate or aspartate residues, respectively, thereby changing the 3-dimensional structure of the apoE3 or apoE4 relative to apoE2.

These same principles affect how apoE3 or apoE4 interact with other proteins or peptides. Indeed, interaction of apoE isoforms with A β is critical to the increased risk of developing Alzheimer disease (AD), with persons inheriting the *APOE4* allele having a significantly different interaction between apoE4 and A β and also a greater risk of age-dependent development of this dementing disorder (Strittmatter et al., 1993; Liu et al., 2017; Richey et al., 1995; Winkler et al., 1999). In persons with AD, the presence of apoE4 appears to strengthen the pathology of this disorder, including A β fibril deposition and A β oligomer production, neurofibrillary tangle formation, neuronal death, decreased synaptic plasticity associated with learning and memory, loss of lipid bilayer compositional asymmetry and lipid homeostasis, and oxidative stress (Markesbery, 2010; Bader-Lange et al., 2008; Cedazo-Minguez and Cowburn, 2001; Tudorache et al., 2017; Mahley et al., 2006; Cedazo-Minguez, 2007). Oxidative damage can induce or is associated with each of these pathological alterations in AD brain (Butterfield et al., 2001; Butterfield and Halliwell, 2019).

As discussed further below, another factor that can influence the role of apoE isoforms on development of AD may be related to oxidative stress that varies with apoE isoforms. Brains

from persons with AD and its earlier stage, amnesic mild cognitive impairment (MCI), are under significant oxidative and nitrosative stress (Hensley et al., 1995; Keller et al., 2005; Butterfield et al., 2001; 2006a; 2012; Butterfield and Halliwell, 2019; Butterfield and Boyd-Kimball, 2019).

The current paper reviews the relationships of among oxidative stress, *APOE* allele status, and AD.

2. Oxidative and Nitrosative Stress in Brain

Due to the high levels of polyunsaturated fatty acid content, the high exposure to inspired oxygen, and the presence of redox-active transition metal ions, i.e., Fe^{2+} , Cu^+ , oxidative damage in brain is a prominent risk. The principal types of oxidative damage are protein oxidation [indexed by protein carbonyls; 3-nitrotyrosine], lipid peroxidation [indexed by 4-hydroxy-2-*trans*-nonenal (HNE), among other indices] and DNA oxidation [indexed by 8-hydroxy-2-deoxyguanosine] (Halliwell and Gutteridge, 2015).

Protein carbonyls arise due to: a) free radical-mediated scission of the primary amino acid sequence of proteins, with subsequent covalent binding to both cleaved ends by paramagnetic oxygen, forming aldehyde or ketone moieties; b) Oxidation of side chains of many amino acids, i.e., 4-oxohistidine; c) Covalent binding by Michael addition to cysteine, histidine, and lysine residues of target proteins by HNE, thereby bringing one or more aldehyde moieties to the protein; and 4) formation of advance glycation end products, resulting from lysine residues reacting with reducing sugars and subsequent Amadori chemistry to form aldehyde or ketone moieties (Butterfield and Stadtman, 1997).

Nitrosative stress is most often indexed by elevated levels of 3-nitrotyrosine [3-NT] (Estevez et al., 1998; Ferrer-Sueta et al., 2018; Butterfield, 2014). In the presence of nitric oxide [NO], produced from arginine in a reaction catalyzed by nitric oxide synthase [NOS]. Cytosolic-resident NOS II, also known as inducible NOS [i-NOS], is released from microglia upon activation of this brain cell. Mitochondria and certain enzymatic reactions lead to elevated superoxide free radicals. Reaction of NO with O_2^- in an extremely fast radical-radical recombination reaction forms the non-radical peroxynitrite [ONOO⁻], which through a series of reactions leads to formation of nitrogen dioxide [NO₂], also a free radical. In the presence of a free radical within 0.4 nanometers of a tyrosine residue, the H-atom of the OH-group in the 4-position of the aromatic group of tyrosine is abstracted, leaving a free radical on the O-atom in this position, which translocates to the 3-position due to chemical principles. Then, the NO₂ free radical rapidly reacts with the free radical on the 3-position to form 3-nitrotyrosine. One important implication of 3-NT is that the steric interference of the NO₂ group makes tyrosine kinase-catalyzed reactions more difficult if not impossible to accomplish, since the active site of the enzyme cannot easily access the OH group on the 4-position of tyrosine. Inhibition of soluble- or receptor- tyrosine kinases severely hinders required cellular signaling processes (Butterfield and Stadtman, 1997; Butterfield, 2014).

Lipid peroxidation occurs by the following mechanism (Figure 3) (Esterbauer et al., 1991; Butterfield and Halliwell, 2019: a) A free radical ($R\cdot$) attack within a Van der Waals distance of labile H-atoms on acyl chains of phospholipids leads to H-atom abstraction to form RH and a carbon-centered radical on the acyl chain of the lipid; b) Binding of paramagnetic molecular oxygen [zero dipole moment, therefore highly soluble in the hydrophobic environment of the lipid bilayer] to the fatty acid-resident, C-centered free radical by radical-radical recombination forms a lipid peroxy free radical; c) Abstraction of another allylic H-atom from the acyl chains of phospholipids by the lipid peroxy free radical leads to formation of the lipid hydroperoxide and another acyl chain-resident C-centered free radical. That is, this is a chain reaction that can continue as long as oxygen and nearby allylic H-atoms near lipid peroxy free radicals are present. The lipid hydroperoxide, through a series of organic reactions, leads to formation of highly reactive HNE. This is why it only takes a small concentration of initiating free radicals to greatly amplify both the free radical levels in the lipid bilayer and subsequent formation of highly reactive HNE.

3. Oxidative Damage in Brains from Persons with Alzheimer Disease and Mild Cognitive Impairment and Down Syndrome and Down Syndrome with Alzheimer-like Neuropathology and Dementia

Many researchers reported long ago and more recently that A β oligomers led to oxidative damage to neurons in culture, in animal models of AD, and in human brains (Butterfield et al., 2001; Pedersen et al., 2000; Nunomura et al., 2001; Smith et al., 1996; Bowling and Beal, 1995; Montine et al., 2005; Reddy, 2006; Cardoso et al., 2016; Verdile et al., 2015; Martins et al., 2018; Di Domenico et al., 2017b; Sultana et al., 2013). Basically, small hydrophobic A β oligomers insert into lipid bilayers of neurons where lipid oxidation ensues, producing HNE that modifies key proteins by covalent Michael addition as noted above (Sultana et al., 2013; Di Domenico et al., 2017b; Mattson, 2009). As reviewed recently, AD and MCI brains demonstrate elevated indices of protein carbonyls, protein-bound HNE, and 3-NT (Butterfield and Halliwell, 2019; Butterfield and Boyd-Kimball, 2019; Keller et al., 2005). Others showed oxidative stress is elevated and synaptic protein levels are lowered in elderly cognitively intact individuals who display Alzheimer disease pathology (Scheff et al., 2016), demonstrating that a very early preclinical stage of AD, oxidative damage is already present (Aluise et al., 2010). Redox proteomics, developed in the Butterfield laboratory (Butterfield et al., 2012), leads to identification of oxidatively modified and almost always dysfunctional proteins. Table 1 summarizes the results of many redox proteomics studies that identified cellular functions damaged in brains from persons with MCI, early-stage AD, late-stage AD, Down syndrome (DS), and Down syndrome with AD-like neuropathology and dementia following oxidative dysfunction of proteins that perform these functions (Butterfield and Halliwell, 2019; Butterfield and Boyd-Kimball, 2019). In persons with DS, often in the decade between 40-50 years of age, transition to AD-like neuropathology and dementia occurs (Di Domenico et al., 2018; Di Domenico et al., 2019; Cenini et al., 2012; Head et al., 2018).

A prominent dysfunction in brain from persons with MCI, AD and of DS and DS with AD is glucose dysmetabolism. This diminution of glucose metabolism leads to decreased levels of

ATP, which would cause loss of cell potential and the opening of voltage-gated ion channels, including Ca^{2+} channels and decreased function of ion-motive ATPases that would otherwise normally pump Ca^{2+} back out of neurons and maintain cell potentials (Mark et al. 1995). Once large amounts of Ca^{2+} accumulate inside neurons, numerous biochemical pathways are disrupted, as are mitochondria, leading to neuronal death by both apoptosis and necrosis (Butterfield and Halliwell, 2019). When enough neurons die, major brain areas involved in cognition, such as hippocampus and frontal cortex, would decrease in thickness and loss of cognition would become apparent.

This above scenario, proposed decades ago by both authors of this current paper, was recently validated by studies involving persons carrying the mutated genes that lead to autosomal dominantly inherited, familial AD, i.e., persons in the dominantly inherited Alzheimer network, DIAN and other large population studies of AD (Gordon et al., 2018; Martins et al., 2018). Researchers involved in the DIAN network, located across several continents, used various PET and MRI imaging modalities to monitor three imaging-detected pathological brain alterations of thousands of such persons regularly for more than two decades before onset of symptoms to determine the order of events that occurred: a) $\text{A}\beta$ deposition outside neurons [and since fibrillar $\text{A}\beta$ arises from and is in equilibrium with $\text{A}\beta$ oligomers, fibrillar $\text{A}\beta$ provides a surrogate marker for $\text{A}\beta$ oligomers, some of the latter cansolubilize in neuronal lipid bilayers,]; glucose metabolism; and thickness of frontal cortical and hippocampal regions of the brains. These authors reported that the first imaging-detected pathological alteration observed was $\text{A}\beta$ fibrillar deposition [and as noted above $\text{A}\beta$ fibrils are formed in stages originating from $\text{A}\beta$ oligomers, which would lead to $\text{A}\beta$ oligomer-associated oxidative damage to neurons], followed at a later time by the second PET-detected pathological alteration, glucose dysmetabolism [which would lead to diminution of ATP and the subsequent Ca^{2+} -related events leading to neuronal death as noted above], and the last imaging-detected pathological alteration, MRI revelation of thinning of frontal cortical and hippocampal regions of the brain, occurred when enough neurons had died.

In contrast to what is observed in familial AD as described above, persons carrying the *APOE4* allele demonstrate glucose dysmetabolism as young adults well before deposition of $\text{A}\beta$ (Reiman et al., 2004). Even though it is widely asserted that the most robust correlation of apoE4 in brain is with $\text{A}\beta$ oligomers (Yamazaka et al., 2019), glucose dysmetabolism in young adults carrying the *APOE4* allele suggests at least two tentative hypotheses: a) There may be multiple mechanisms that lead to glucose dysmetabolism, i.e., mechanisms other than through $\text{A}\beta$ -mediated HNE formation; and b) apoE4, noted below to be associated with oxidative damage (Ramaswamy et al., 1999;2000), may, in ways independent of $\text{A}\beta$, lead to HNE formation and subsequent HNE formation. More experimentation will be required to determine if these hypotheses have merit.

Other cellular functions that are dysfunctional as a result of oxidative modification noted in Table 1 also contribute to neuronal death in AD and MCI brains (Butterfield et al., 2006; Sultana et al., 2013; Di Domenico et al., 2017b; Butterfield and Halliwell, 2019; Butterfield and Boyd-Kimball, 2019;).

4. Oxidative Stress and ApoE in Alzheimer Disease-Relevant Settings

4.1 Human Studies.

As discussed below, the degree of oxidative damage in AD brain reportedly is directly associated with *APOE* allele in the order *APOE2* < *APOE3* < *APOE4* (Dose et al., 2016). Ramassamy and Poirier and colleagues used the thiobarbituric acid reactive substances [TBARS] method to assess elevated lipid peroxidation in hippocampus of AD subjects and noted the greatest elevation of TBARS signal in AD brain with ApoE4 (Ramassamy et al., 2000). These researchers also reported lower activities of catalase and glutathione peroxidase [GPx] and lower levels of glutathione [GSH] in brains of *APOE4* subjects compared to those parameters in brain of subjects homozygous for *APOE3*. In an earlier study, these researchers used frontal cortex of AD subjects to investigate similar endpoints (Ramaswamy et al., 1999). However, while increased TBARS signals were observed as previously, in contrast to their later study, the authors reported elevated activities of catalase GPx). Two potential concerns of these studies are: a) TBARS signals are not good measures of lipid peroxidation, since several other moieties beyond dialdehyde units yield TBARS signals as well; however, partially mollifying this concern and as discussed below, lipid peroxidation-specific signals also evince elevated lipid peroxidation in brain of *APOE4* AD subjects and targeted-replacement mice. b) The length of time between patient death and acquisition of brain [the post-mortem interval, PMI] was excessive. Generally, higher confidence in measures of oxidative stress or other pathological indices arise with short [fewer than 4 h] PMIs (Butterfield and Halliwell, 2019).

Others used 3-nitrotyrosine and 8-OHdG to demonstrate elevated oxidative stress early in the progression of AD, but less oxidative stress late in the disorder when A β deposition was greater, and that these effects were greater in subjects with *APOE4* (Numomura et al., 2001). These authors contributed to AD understanding by noting that oxidative stress is important early in the disease, an observation that was reinforced by demonstration that oxidative stress occurs in brain of subjects with amnesic MCI, i.e., no dementia (Keller et al., 2005; Butterfield et al., 2006a,b; Butterfield et al., 2007a,b).

Increased risk for development of AD may be dependent on several, potentially interacting variables, including *APOE4* genotype, gender, body mass index, inflammation, insulin resistance, and in some cases exposure to particulates in air pollution (Calderon-Garciduenas and de la Monte, 2017). Each of these risk factors also are associated with elevated oxidative damage. Others showed that *APOE4* was associated with elevated risk of developing AD in persons with mutations in *glutathione-S-transferase* [GST] compared to the risk of *APOE4* alone, and since GST is dependent on GSH, this study suggests oxidative damage also is associated with these mutations in this transferase (Bernardini et al., 2005; Pinhel et al., 2008). Similarly, mutations in the gene for *uncoupling protein-4* (UCP4) interact with *APOE4* to affect risk of development of AD (Montesanto et al., 2016), and a C47T mutation in manganese superoxide dismutase [MnSOD] interact with *APOE4* to increase risk of developing AD (Gamarra et al., 2015). Since UCP proteins are related to mitochondrial metabolism, MnSOD is resident in mitochondria, and mitochondrial routinely leak

superoxide free radicals especially from Complex I, then these studies are consistent with the notion that elevated oxidative stress is a key factor in AD pathogenesis.

As noted elsewhere in this paper, the apoE4 isoform is linked to both senile plaque and neurofibrillary tangle elevation as well as to elevated oxidative damage in brain. apoE of molecular mass 35 kDa undergoes proteolytic fragmentation in brain in patterns that are apoE-isoform dependent (Munoz et al., 2019). The 25 kDa, N-terminal fragment of apoE is more prevalent in *APOE3* individuals and is neuroprotective, while full-length ApoE4 and the N-terminal residues 1-271 and 1-272 are neurotoxic but by different mechanisms (Huang et al., 2001; Chang et al., 2005), including Ca^{2+} accumulation and mitochondrial dysfunction that can lead to oxidative damage (Butterfield and Halliwell, 2019).

Integration of transcriptomic and genomic analyses suggested several potential mechanisms for apoE4-mediated damage in AD, prominent among which were mechanisms related to oxidative stress (Caberllo et al., 2016). The researchers reported that homozygous *APOE4/4* carriers with AD demonstrated an intersection point of pathways at Notch signaling, and the latter is related to presenilin-mediated contribution to gamma-secretase cleavage of APP to form A β 42. As noted above, this peptide is associated with oxidative damage in AD and MCI brains. Consistent with this assertion, examination of CSF of individuals as a function of age and *APOE* genotype showed that levels of phosphorylated tau and isoprostanes, the latter a marker of lipid peroxidation, were statistically correlated in ApoE4 (Glodzik-Sobanska et al., 2009). Cerebrospinal fluid in AD and MCI demonstrates a specific oxidative signature (Di Domenico et al., 2016).

4.2. Apoe Knockout Mice Studies.

A few studies of oxidative stress in *Apoe* knockout mice have been performed general under the principles that apoE4 is associated with elevated oxidative stress in AD brain as noted above, and the extent of oxidative damage in AD brain correlated to the presence of apoE4, consistent with the notion that of an association of between *APOE4* allele and free radical damage in AD brain. Consequently, in one study, *Apoe4* null mice were subjected to oxidative stress by folate deprivation and/or elevated iron in the diet (Shea et al. 2002). Interestingly, glutathione and other antioxidant levels in brain were elevated, suggested by the authors to be responses to the elevated oxidative stress. A combination of folate deprivation and excess dietary iron led to oxidative damage not rescued by the elevated glutathione, supporting the idea that Apoe deficiency is associated with oxidative damage in brain. A second study by the same group (Tchantchou et al., 2005) showed protection to brain from *Apoe* knockout mice that had been pretreated with N-acetylcysteine, a glutathione precursor. A similar approach was taken by others, who also found elevated oxidative damage in brain of *Apoe*-deficient mice, but in contrast to the two studies noted above, these authors found a decrease in small antioxidants, i.e., vitamin E in such mice (Ramasamy et al. 2001).

We showed that synaptosomal membrane preparations isolated from Apoe knockout mice subsequently treated with amyloid beta-peptide (1-40) had elevated indices of protein oxidation and lipid peroxidation (Lauderback et al., 2001) and displayed mitochondrial dysfunction and caspase activation (Keller et al., 2000) compared to similarly treated

synaptosomes from wild-type mice. These results are consistent with a role for apoE in maintaining homeostasis by modulation of synaptosomal oxidative stress, caspase inactivation, and mitochondrial dysfunction. As noted below, the *APOE4* allele is unable to provide this modulation relative to the other *APOE* alleles.

4.3. Human APOE Targeted-replacement Mice Studies.

ApoE knock-out [null] mice or targeted replacement mice, in which the mouse gene for *ApoE* is knocked out and replaced by the human gene for *APOE2*, *APOE3*, or *APOE4*, while keeping the mouse promoter [thereby ensuring that the human apoE isoform is directed to the correct locations and in the usual amounts], are excellent resources with which insights into the role of human apoE in brain can be investigated *in vivo*. Indeed, as described further below, such mice have been used to validate most human studies.

As noted previously, apoE is a multifunctional protein with potential functions in microtubule assembly and stability, intracellular signaling, glucose metabolism, A β deposition and oxidative stress, among other functions (Saunders, 2000). With respect to oxidative stress and apoE, Gandy and co-workers (Yao et al., 2004) reported that factors such as aging and gender couple with apoE isoform to modulate metabolism of A β and lipid peroxidation [assessed by F2-isoprostanes that derive mostly from arachidonic acid]. Specifically, *APOE3* and *APOE4* human targeted replacement mice or the corresponding *ApoE* knockout mice with subsequently added transgenes for *APOE3* or *APOE4* revealed that the transgenic *APOE4* mice led to elevated F2-isoprostanes in brain not observed in *APOE3* mice. Moreover, the changes in *APOE4* mice correlated with levels of A β 42 in likely an oligomeric form.

The antioxidant nature of apoE depends on its allele, with apoE4 being the least functional antioxidant. Nitrosative stress was observed in greater level in brains of *APOE4* mice than those of *APOE3* mice (Colton et al., 2002; Brown et al. 2002). Mattson and co-workers demonstrated that apoE4 bound less of the lipid peroxidation product HNE than did apoE3 (Pedersen et al., 2000). These researchers suggested that the Cys residue at 158 bound HNE, thereby keeping this neurotoxic agent from damaging neuronal proteins. In contrast, since apoE4 is devoid of Cys, no HNE was bound, permitting HNE to bind to neuronal proteins. The researchers opine that this may be a prominent mechanism by which *APOE4* is a major risk factor for AD. Presaging this work by Mattson and co-workers, Montine and colleagues demonstrated that apoE3 was more crosslinked by HNE than was apoE4 in CSF from adult subjects with or without dementia (Montine et al., 1996).

Consistent with these above studies, and consonant with the notion that inheritance of the *APOE4* gene is a major risk factor for AD, the Butterfield laboratory studied the role of different human *APOE* alleles in modulating A β 42-induced protein oxidation [protein carbonyls; 3-nitrotyrosine] and lipid peroxidation [protein-bound HNE] in synaptosomes isolated from brains of human *APOE2*, *APOE3*, and *APOE4* targeted-replacement mice (Lauderback et al., 2002). After addition of A β 42 to synaptosomes from these mice, increased reactive oxygen species formation and elevated levels of protein carbonyls and 3-nitrotyrosine and protein bound HNE were observed in synaptic membranes in the order apoE4 > apoE3 > apoE2. These results reflect the function of apoE as a modulator of

oxidative stress, and the order by which this occurs is related to the number of Cys residues in the protein, i.e., apoE2 > apoE3 > apoE4, and we opine these results have mechanistic implications for the intersection of the oxidative stress associated with A β 42 oligomers (Butterfield et al., 2001; Butterfield and Halliwell, 2019) and apoE4 in the elevated risk for neurodegeneration and development of AD (Lauderback et al., 2002; Pedersen et al., 2000).

apoE is involved in efflux of A β peptide from brain employing LRP-1 as described above. This transport protein is oxidatively modified, and therefore likely dysfunctional, in brains of AD subjects, which could contribute to the accumulation of A β in the brain parenchyma (Owen et al., 2010). Consistent with this notion, others reported that in *LRP-1* knockout mice, there is increased susceptibility to A β -induced neurotoxicity (de Oliveira et al., 2014) and that following intracerebroventricular injection of aggregated A β 42, apoE protein levels lost their temporal regulation in brain that was accompanied by loss of GSH and elevation of lipid peroxidation (Navigatore-Fonzo et al., 2017).

Thioredoxin-1, an endogenous antioxidant protein, is involved in regulation of apoptosis through inhibition of apoptosis signal-regulating kinase (Ask-1). Compared to *APOE3* targeted replacement mice, *APOE4* mice have decreased levels of thioredoxin-1, which was associated with disrupted lysosomal integrity evinced by relocalization of the key lysosomal cathepsin D from this organelle to cytosol, making degradation of autophagic mediation of lysosomal degradation of proteins less efficient (Persson et al., 2017). These researchers opine that down-regulation of thioredoxin-1 plays a role in the neurotoxicity associated with the presence of the *APOE4* allele.

Ozone exposure has been suggested to be a potential risk factor for late-onset AD, but the mechanisms for this potential risk factor remain unknown. To address this gap, Jiang and co-workers (Jiang et al., 2019) reported ozone exposure to old *APOE3* mice led to impaired memory, which was not observed in old *APOE4* or young *APOE3* or *APOE4* mice. Further, *APOE4* mice had elevated expression of antioxidant enzymes and diminished oxidative stress [also reported by Ramasamy et al., as noted above]. No explanation of these results were provided, but it is conceivable, as suggested elsewhere in this current review, that the decreased cysteine content of apoE4 provides fewer opportunities for HNE modification of this protein [i.e., antioxidant scavenging] than is the case for apoE3 [Figure 1] (Pedersen et al., 2000; Lauderback et al., 2002).

Alzate's group has investigated the role of mortalin in AD (Londono et al., 2012). This protein is a chaperone that is transported from the cytosol to the matrix of mitochondria by the TOM and TIM transporters, and in the matrix, mortalin reportedly serves as an antioxidant protein, protecting mitochondria. This group used proteomics to identify that mortalin is regulated by apoE in hippocampi of *APOE* targeted replacement mice, with *APOE4* mice showing increased levels compared to *APOE3* mice, and mortalin in *APOE4* mice appeared to be highly phosphorylated or oxidized (Osorio et al., 2007). In AD hippocampi decreased expression in subjects with an *APOE4* genotype was observed. The authors propose that the differential regulation of the normally protective mortalin in AD by the *APOE* genotype is a cellular defense against oxidative stress, and consistent with this

finding, *APOE* knockout mice brains were reported to have oxidatively modified mortalin (Choi et al., 2004).

Studies of human targeted replacement mice taken together demonstrate that the presence of *APOE4* genotype increases susceptibility to A β -associated oxidative damage, likely due to its lack of Cys residues that are known to bind to the neurotoxic lipid peroxidation product, HNE. Such consideration provides one highly plausible explanation for the increased risk of developing AD with persons carrying the *APOE4* allele. Consistent with this notion, examination of medical records showed carriers of *APOE2* genotype, with two key Cys residues (Figure 1), is associated with less cognitive decline with aging than persons with *APOE3* (1 key Cys residue) or *APOE4* (no key Cys residues) genotypes (Shinohara et al., 2016).

5. Conclusions and Future Directions

Brains from persons with Alzheimer disease and its earlier stage, amnesic mild cognitive impairment, are characterized by elevated indices of oxidative damage to proteins, lipids, and DNA (Butterfield et al., 2001). Cognitive dysfunction in AD is correlated with the levels of oligomeric A β 42 (Cleary et al., 2005), the latter associated with elevated markers of oxidative damage (Butterfield and Halliwell, 2019). Similarly, brains from persons with Down syndrome, in which there is elevated levels of A β oligomers, often transition to AD-like neuropathology and dementia between 40-50 years of age (Di Domenico et al., 2018; Di Domenico et al., 2019; Cenini et al., 2012; Head et al., 2018).

Inheritance of *APOE4* leads to elevated risk of developing AD. This current review assesses the hypothesis that a significant factor that leads to this elevated risk of developing AD by inheritance of *APOE4* is the inability of this isoform to scavenge the neurotoxic lipid peroxidation product, HNE, due to the absence of the two critical Cys residues in the protein (Pedersen et al., 2000; Lauderback et al., 2002). Consistent with this hypothesis, and as shown in Table II, elevated oxidative stress in brain occurs in persons who inherit the ApoE4 isoform. Moreover, in human targeted replacement mice, lipid peroxidation and tyrosine nitration, emanating from oxidative stress and inflammatory processes, brains from *APOE4* mice demonstrate elevated indices of these oxidative and nitrosative damages compared to brains from *APOE3* mice. As stated above, the presence of the two key Cys residues, or residue, in apoE 2 and apoE3, respectively, permit scavenging the highly reactive and neurotoxic lipid peroxidation product, HNE that is produced in membranes by oligomeric A β 42. These scavenging processes not available to *APOE4*, thereby, we opine, resulting in lipid peroxidation and nitration of tyrosine residues, oxidative damage that leads to neuronal death and AD. We hypothesize that these considerations make significant contributions to the known risk of developing AD by those persons who inherit the *APOE4* genotype.

Diagnosing AD before symptoms appear arguably is critical to slowing or stopping the progression of this dementing disorder. A very recent study (Schindler et al., 2019) demonstrated that using sophisticated analyses of plasma-resident A β 42 and A β 40 in more than 150 asymptomatic people allowed a model to be developed that predicted reasonably accurately who had deposition of A β in their brains using PET scanning. Relevant to this

review article, addition of age and *APOE* genetic status to their model greatly improved the prediction accuracy of who would eventually deposit A β in their brains.

So, even if this or other models that might arise can predict pathology of AD and who may be of great risk of developing this dementing disorder, what can be done to mitigate against an elevated risk of developing AD with inheritance of the ApoE4 genotype? The response to this question is confounded by the differential interactions of many therapeutic approaches in AD by *APOE* genotype and by gender (Hanson et al., 2015). It is unethical to change one's genetic makeup, except in rare circumstances where the gene involved directly causes the devastating disorder, i.e., Huntington disease; Duchenne muscular dystrophy). Moreover, apoE is multifunctional; therefore, targeting knockout of the *APOE4* gene likely would lead to unintended serious consequences.

Recent studies suggest that apoE4 leads to microglial dysfunction, resulting in accumulation of phosphotau and neuronal death (Shi et al., 2014; Shi et al., 2019). This exciting result may lead to pharmacological approaches to protect microglia in *APOE4* individuals.

Alternatively, and in agreement with preclinical studies that suggest that a high antioxidant diet, continuous lifelong stimulation of synapse formation by learning new tasks, and significant exercise regimens increase cognition and lower brain indices of AD (Opii et al., 2008), persons who inherit one or more copies of the *APOE4* allele would be strongly suggested to follow these programs to lower the risk of developing AD. Clearly, no promise that development of AD can be prevented is implied here; rather, that the risk for AD development likely can be materially lowered.

In the meantime, *APOE4* targeted-replacement mice offer preclinical models with which to test promising therapeutic strategies of the future.

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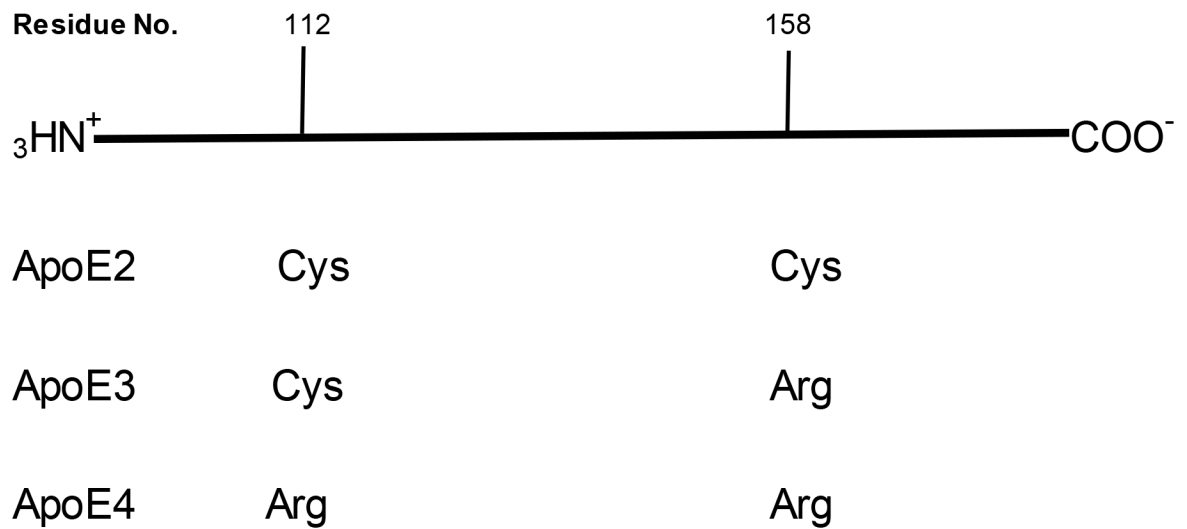


Figure 1. Schematic representation of the amino acid sequence of apoE2, apoE3, and apoE4, showing the differential cysteine content among the three isoforms at residues 112 and 158. Cys residues can covalently bind HNE to prevent this highly reactive and neurotoxic molecule from binding to cellular proteins, thereby protecting these proteins, while Arg cannot perform this function. See text.

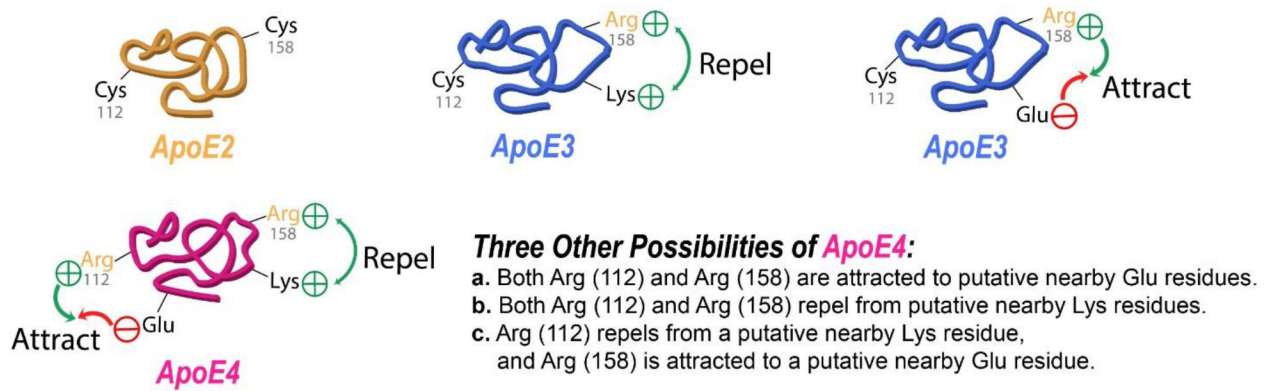


Figure 2.

Schematic representation of idealized conformations in apoE2, apoE3, and apoE4 to illustrate different putative conformations possible by replacement of Cys residues by positively charged Arg and the resultant repulsion or attraction between these Arg residues and positively charged or negatively charged amino acids, respectively. In the case of apoE4 there exist four possibilities.

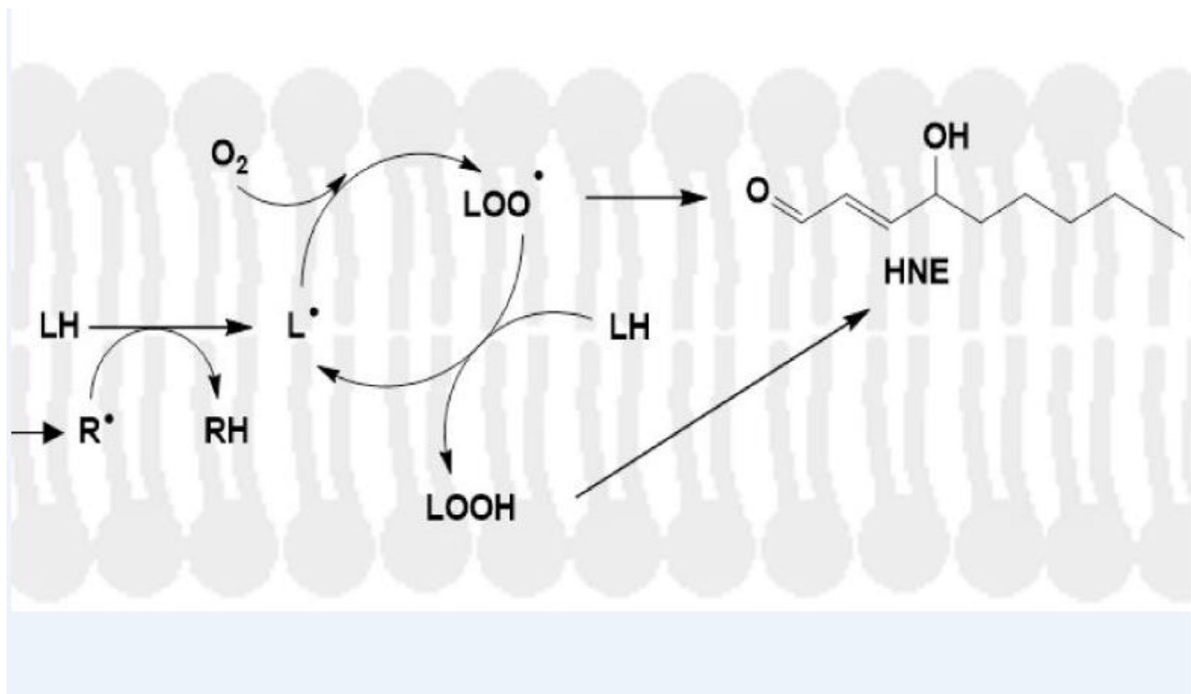


Figure 3.

Mechanism of lipid peroxidation. A free radical, for example associated with $A\beta_{42}$ oligomers inserted into a lipid bilayer (Butterfield and Halliwell, 2019), attacks a lipid acyl chain-resident, labile allylic hydrogen atom to form the RH compound and a carbon-centered lipid free radical, L^\bullet . Oxygen, which is paramagnetic with two unpaired electrons and has zero dipole moment, meaning it is highly soluble in the lipid bilayer, binds to the carbon-centered radical on the acyl chain to form the lipid peroxy free radical, LOO^\bullet . This free radical, in turn, abstracts another allylic hydrogen on a fatty acid chain of the lipid to form $LOOH$, the lipid hydroperoxide, and another lipid-resident, carbon-centered free radical, i.e., propagating the chain reaction. $LOOH$, through a series of chemical reactions, spontaneously will form HNE that can covalently bind to and change the conformation and function of membrane-resident proteins (Subramaniam et al., 1997; Sultana et al., 2013). In addition, HNE can diffuse from the lipid bilayer to covalently modify cytosolic proteins (Butterfield and Stadtman, 1997). Modified from Butterfield and Boyd-Kimball, 2019.

Table 1.

Altered Biochemical Pathways Assessed from Redox Proteomics-Identified, Oxidatively Modified^a Brain Proteins in Amnesic Mild Cognitive Impairment, Early-Stage AD, Late-Stage AD, Down Syndrome, and Down Syndrome with AD Neuropathology and Dementia^b

Glucose Metabolism
Synaptic Plasticity, Vesicle-mediated Transport and Cell Structure
Cellular Redox Homeostasis and Detoxification
Excitotoxicity
Protein Synthesis
Protein Folding and Degradation
Cell Signaling
Neuroinflammation

^aProteins modified by protein carbonyls, bound HNE, or resident 3-nitrotyrosine

^bRefs.: Butterfield and Halliwell, 2019; Butterfield and Boyd-Kimball, 2019

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TABLE II.

apoE and Oxidative Stress^a in AD and MCI and in *APOE* KO and TR^b Mice

Human CNS Studies		<i>APOE</i> KO Mice Brain		<i>APOE</i> TR Mice Brain	
Observation	Comment and/or Ref.	Observation	Comment and/or Ref.	Observation	Comment and/or Ref.
Oxidative stress varies apoE2<apoE3<apoE4	Dose et al., 2016.	Elevated oxidative stress compared to WT mice.	Rescued by NAC ^d . Tchanchou et al. 2005	Elevated F2-isoprostanes, greater in brains of <i>APOE4</i> mice than <i>APOE3</i> mice, and results correlated with oligomeric A β	Yao et al., 2004
Increased LPO; decreased GSH;	Ramasamy et al., 2000	Decreased in small antioxidants such as vitamin E.	Ramasamy et al. 2001.	Elevated nitrosative stress in <i>APOE4</i> mice compared to ApoE3 mice	Colton et al., 2002; Brown et al., 2002
Elevated risk of AD with mutations in <i>GST</i> , <i>UCP4</i> , or <i>MnSOD</i>	All related to decreased function with consequent elevated oxidative stress. Bernadini et al., 2005; Pinhel et al., 2008; Montesanto et al., 2016; Gamarra et al., 2015	Synaptosomal membranes showed elevated oxidative stress and elevated dysfunction of mito. compared to WT following A β addition.	Lauderback et al. 2001; Keller et al., 2000.	apoE4 had less HNE bound than did apoE3, consistent with idea that apoE2 and apoE3 have antioxidant properties, and increased risk of AD with <i>APOE4</i> could be related to this characteristic	Pedersen et al., 2000; Montine et al., 1996
ApoE undergoes proteolysis, with protective NTF fragment of 25kD more prevalent in ApoE3; full-length ApoE is neurotoxic	Munoz et al., 2019; Huang et al., 2001; Chang et al., 2005			A β added to synaptic membranes showed elevated oxidative stress in order apoE4>apoE3>apoE2, i.e., inverse of number of key Cys residues in apoE isoforms	Lauderback et al., 2002
Brain-resident presenilin component of γ -secretase-mediated APP cleavage to form A β 42 highly present in ApoE4/4 individuals	Predicted to lead to more oxidative stress and neurotoxicity. Caberlotto et al., 2016			ICV injection of A β led to loss of temporal regulation of apoE levels along with loss of GSH and increased lipid peroxidation	Navigatore-Fonzo et al., 2017
CSF-resident p-Tau and F2-isoprostanes elevated in ApoE4 individuals	Glodzik-Sobanska et al., 2009			<i>APOE4</i> brain has decreased levels of Thx-1, affecting lysosomal integrity	Persson et al., 2017

^aOxidative stress indices cited: LPO = lipid peroxidation indexed by HNE = 4-hydroxynonenal or F2-isoprostanes; 3-NT = 3-nitrotyrosine; PC = protein carbonyls. 8-OHdG = 8-Hydroxy-2-deoxyguanosine; GSH = glutathione

^bKO = Mouse *ApoE* gene deleted

^cTR = Human Targeted Replacement *ApoE* gene

^dNAC = N-acetylcysteine