

Hypothesis

Apolipoprotein E3 as a Risk Factor for Alzheimer's Disease Under Conditions of Nutritional Imbalance

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Abstract. The presence of one or more copies of the E4 allele of apolipoprotein E (ApoE) is strongly associated with of Alzheimer's disease (AD). The impact of E4 on neurodegeneration is potentiated by dietary oxidative challenge. Our prior studies in transgenic mice demonstrate that, in the face of dietary oxidative challenge, E3 does not provide any further protection than E4 or lack of murine ApoE for aggression, oxidative damage, presenilin-1 expression, and γ -secretase activity, and provides only partial reduction in phospho-tau levels. Extrapolation of these findings to the human condition leads us to hypothesize that the E3 allele may not provide sufficient neuroprotection under conditions of dietary compromise and/or oxidative challenge. Epidemiological evidence is consistent with this possibility. The E3 allele is approximately half as effective compared to E2 at buffering the impact of a single E4 allele. In addition, the risk of AD increases linearly for the genotypes E2/2, E2/3, and E3/3. It has been proposed that that clinical manifestation of AD may in some cases require the convergence of 2 or more risk factors. We hypothesize that the combined impact of dietary oxidative stress and either the ApoE3 or E4 genotype represents one such condition.

Keywords: Alzheimer's disease, apolipoprotein E, diet, nutritional deficiency, oxidative stress

APOE, OXIDATIVE DAMAGE, AND ALZHEIMER'S DISEASE

A major risk factor for Alzheimer's disease (AD) is the presence of the E4 allele of apolipoprotein E (ApoE), which accounts for up to 50% of the cases of AD [1–5]. Considerable effort has been devoted to determining the nature and extent of risk imparted by ApoE4 and what might be done to lessen this risk [6]. Oxidative damage represents an early and perhaps pivotal factor contributing to age-related decline in cogni-

tive performance including that associated with AD [7–12]. Oxidative damage and ApoE4 are inter-related, as the extent of brain oxidative damage in AD is correlated with the presence of E4 [13–16]. ApoE mediates transport and clearance of lipids, including those subjected to oxidative damage. In doing so, ApoE prevents a cascade of neuronal oxidative damage by quenching downstream products of lipid oxidation, preventing secondary glutamate excitotoxicity and sequestering oxidative metals such as iron [4,17,18,20]. However, ApoE4 is thought to be less effective at this latter function [21].

While over 20 other genes are suspected of being related to AD [22], deficiency in ApoE function has a particularly far-reaching impact in that it not only impairs overall brain metabolism and may impair compensato-

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Table 1

Impact of dietary challenge on behavioral and biochemical parameters associated with AD in transgenic mice of various ApoE genotypes. Mice were maintained for 1 month on a complete diet or a deficient diet lacking folate and vitamin E and containing 500 mg iron/kg total diet weight, after which various groups were subjected to Y maze to quantify cognitive performance [79], the intruder-based aggression test [51], following which they were sacrificed and cortical-hippocampal homogenates were assayed for oxidative damage (by TBARs assay), PS-1 expression (by RT-PCR), γ -secretase activity (by fluorescence spectrophotometry), and A β and phospho-tau levels by immunoblot analyses [52,66,86,87]

ApoE ¹	Behavioral parameters ²		Biochemical parameters ³				
	Cognition	Aggression	Oxidative damage	PS-1 expression	γ -secretase activity	A β levels	Phospho-tau levels
E4	-19 \pm 6% ⁴	86 \pm 33% ⁴	1.5 \pm 0.3 ⁴	1.4 \pm 0.1 ⁴	2.2 \pm 0.2 ⁴	4.5 \pm 2.1 ⁴	1.7 \pm 0.1 ⁴
E3	-6 \pm 8%	71 \pm 33% ⁴	1.4 \pm 0.2 ⁴	1.3 \pm 0.1 ⁴	2.1 \pm 0.2 ⁴	1.1 \pm 2.1	1.3 \pm 0.2
E2	-8 \pm 2%	0%	nd	1.3 \pm 0.1 ⁴	1.1 \pm 0.5 ⁴	0.5 \pm 0.5	nd
-/-	-13 \pm 4% ⁴	0%	2.1 \pm 0.1 ⁴	1.5 \pm 0.2 ⁴	2.0 \pm 0.1 ⁴	2.8 \pm 0.6 ⁴	2.6 \pm 0.5 ⁴
+/-	4 \pm 5%	0%	1.6 \pm 0.1 ⁴	nd	nd	nd	nd

¹E2, E3, and E4 refer to mice lacking murine ApoE and homozygously expressing the respective human alleles; +/- and -/- refer to murine ApoE copy number.

²Expressed as percent change versus normal (ApoE+/+) mice on a complete diet.

³Expressed as fold change versus ApoE+/+ mice on a complete diet; nd: not determined.

⁴Indicates value differs statistically ($p < 0.05$) from value obtained for ApoE+/+ mice.

ry regeneration [23,24], but also potentiates several additional risk factors. Deficiency in ApoE function not only impairs clearance of amyloid- β (A β), but it also augments the deleterious consequences of presenilin-1 (PS-1) overexpression and increases γ -secretase activity, both of which increase A β generation [25,27–32]. It is of interest that these three AD risk factors – impaired ApoE function, PS-1 overexpression, and increased A β levels – are inter-related and have in common the promotion of increased neuronal oxidative damage [11, 14–16,33–41].

Transgenic mice in which one or both copies of the single murine ApoE allele has been ablated (ApoE+/- or -/-) have been useful models for studying the impact of ApoE on age-related cognitive decline and AD. More appropriate models include mice in which the murine allele has been replaced with human alleles. Like individuals with AD, ApoE-/- mice and mice expressing human E4 (“E4 mice”) display increased oxidative damage in brain tissue [15].

NUTRITIONAL DEFICIENCY POTENTIATES THE IMPACT OF DEFICIENCY IN APOE FUNCTION IN MOUSE MODELS

Nutritional compromise often accompanies aging and can contribute to neurological deficiencies by multiple mechanisms including increased oxidative stress [8,33,42,47]. Our laboratory has for years studied the impact of nutritional compromise leading to oxidative challenge. Many of our studies have compared the impact of nutritional compromise on normal (i.e., ApoE+/+) mice versus -/- mice, and/or mice expressing human ApoE alleles, since pioneering studies

demonstrated that antioxidant supplementation can alleviate some of the oxidative damage in E4 mice [14, 40]. Studies from our laboratory and others demonstrate that folate deficiency potentiates several AD genetic risk factors, including increasing homocysteine, which potentiates A β neurotoxicity [47–50]. The decline in S-adenosyl methionine (SAM) that accompanies folate deficiency fosters PS-1 overexpression, increases activity of β - and γ -secretases, increases levels of A β [51–54], and compromises glutathione usage, which in turn increases oxidative stress and potentiates the impact of deficiency in ApoE function [3,55].

In these studies, we maintained mice of various ApoE genotype on a complete, vitamin-enriched diet, or instead on a “challenge diet”, which lacked folate and vitamin E, and instead contained a high level of iron to promote oxidative stress, and monitored mice for behavioral and biochemical parameters associated with AD (Table 1). Dietary challenge induced or potentiated the deleterious impact of lack of murine ApoE or expression of E4 on many of these parameters. Notably, however, dietary challenge also exerted a deleterious influence on most of these parameters in mice expressing human E3 (Table 1).

E4 and ApoE-/- mice displayed impaired cognitive performance when subjected to dietary challenge, while other genotypes were apparently unaffected. Notably, a prior study demonstrated subtle impairment in cognitive performance of E3 mice. While most E3 mice displayed superior cognitive performance in most parameters as compared to E4 mice, female E3 mice did not improve during training in spatial learning performance, while E4, ApoE-/-, and ApoE+/+ mice improved [56]. In addition, E3 males made more errors in a Y-maze-based active avoidance task [56]. The par-

ticular tests used may reveal differentially-susceptible aspects of cognition. E3 mice subjected to dietary challenge displayed an increase in aggression at a level similar to that of E4 mice, while *-/-* mice did not display any increase (Table 1). This differential impact is consistent with prior studies indicating that cognitive performance can be improved in rodents independently of aggression [57,58]. Similarly, while both cognitive impairment and aggression accompany AD, they are not necessarily temporally colocalized and may but do not necessarily respond to identical treatments [59–62]. These behavioral manifestations in mice are directly relevant since AD can be accompanied by psychosis and agitation [63], and ApoE4 can also potentiate psychotic symptoms in humans [64]. These findings suggest that aggression, but not cognition, may be one instance in which the presence of the E4 (and E3) genotype, rather than the absence of ApoE function (i.e., ApoE^{-/-} mice) may be the contributing factor [65].

Classical biochemical markers were also affected by dietary challenge. When subjected to dietary challenge, E3 mice displayed increases in oxidative damage, PS-1 expression, and γ -secretase activity identical to those of E4 mice (Table 1). However, neither A β nor phospho-tau levels were increased in E3 mice. Genotypic deficiencies were evident in certain of these biochemical parameters prior to dietary challenge. For example, both E3 and E4 but not E2 mice displayed identical increases (1.4 ± 0.1) in PS-1 expression and γ -secretase activity on the complete diet as they did on the deficient diet [52]. Oxidative damage was elevated in E4 and ApoE^{-/-} mice, but not in E3 or ApoE^{+/+} mice, when maintained on the complete diet. The only alteration in these parameters when ApoE^{+/+} were placed on the challenge diet was in PS-1; ApoE^{+/+} mice showed a 1.7 ± 0.1 -fold increase when maintained on the challenge diet [66]. However, the combination of genotype and dietary challenge clearly potentiated these behavioral and biochemical parameters (Table 1).

These comparisons are not intended to suggest that E3 is as detrimental as E4, but are intended to highlight that the protective properties of E3 may be diminished under dietary challenge. Numerous studies of parameters not examined herein clearly demonstrate that E3 provides superior neuroprotection than does E4 [35]. For example, E3 mice display increased dendritic spine density as compared to E4 [67]. The presence of the E3 allele also exerts a dose-dependent reduction in A β deposition [66], and a decrease in γ -secretase-mediated A β generation [25]. These latter findings perhaps explain why E3 mice under dietary challenge did not display increased A β levels despite increases in PS-1 and γ -secretase activity (Table 1) [52].

HYPOTHETICAL RELATIONSHIP OF E3 AND AD

Could the above findings regarding E3 in mouse models subjected to dietary compromise have significance for AD? The vast majority (73%) of the population does not harbor an E4 allele, yet still account for 50% of the cases of AD [5]. Moreover, in tallying cases and relative impact of alleles, investigators typically compare the prevalence of AD in individuals bearing one or more E4 alleles versus those lacking E4 and bearing one or more E3 alleles. This approach is often required to obtain sufficient numbers for statistical analyses, as E3 is the major allele, and only approximately 2% of the population harbors the E4/4 genotype. The unfortunate downside of grouping individuals homozygous and heterozygous for E4 precludes comparison of the E3/4 and E4/4 genotype.

Prior reviews of data from multi-center studies indicate that individuals with E3/4 were approximately 70% less likely to present AD than were those with E4/4; however, individuals with E2/4 were approximately 90% less likely to present AD. While such data are usually presented in the context that E2 and E3 reduce or nullify the impact of a single E4 allele, they also underscore the substantially greater neuroprotective effect of E2 versus E3 [69–71]. The percentage of individuals with AD that are homozygous for E3 slightly exceeded that with E2/4 (5.0% vs. 4.5%) in one of the above studies [71], and rivaled that with E2/4 in the second study (5.2 versus 8.7) [71]. These data indicate that E2 can nullify the impact of a single E4 allele. However, they also suggest that individuals harboring the E3/3 genotype may be equally at risk for AD as are individuals with the E2/4 genotype. Despite that any putative impact of E3 as a risk factor for AD is dwarfed in comparison to that of E4, a virtually linear relationship exists in the percentage of individuals with AD and the number of E3 versus E2 alleles and in the lifetime risk of AD (Fig. 1).

NUTRITIONAL DEFICIENCY MAY POTENTIATE THE IMPACT OF APOE GENOTYPE ON AD

AD has a multifactorial etiology encompassing genetic, nutritional, and environmental risk factors. No one class of risk factors can account for the full incidence of AD [3,36,73]. Clinical manifestation may therefore be dependent upon convergence of two or more risk

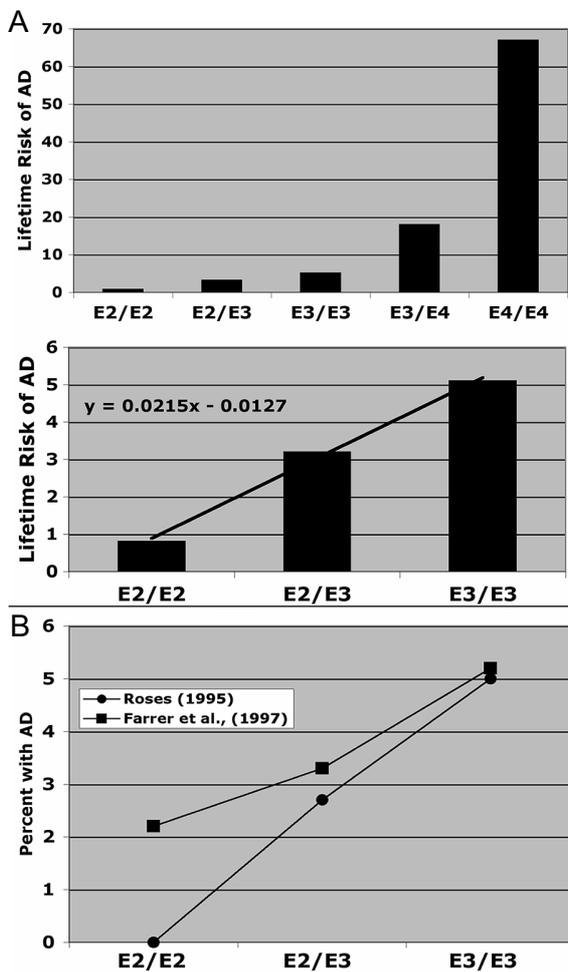


Fig. 1. Differential impact of E2 and E3 on prevalence of AD. Panel A presents the lifetime risk of AD for various ApoE genotypes. The upper graph includes genotypes with E4, while the lower graph excludes E4. Note that, while the potential contribution of other alleles to a lifetime risk of AD is minimal compared to that of E4, the genotypes of E2/E2, E2/E3, and E3/E3 present a linear increase in the lifetime risk of AD. Data from [5]. Panel B presents data from two meta-studies as indicated which demonstrate that a virtually linear relationship exists in the percentage of individuals with AD and the number of E3 versus E2 alleles.

factors, and such has been suggested for the impact of ApoE on AD [74]. In this regard, even the E4/E4 genotype is not a predictor in isolation; the fact that E4 exhibits an incomplete penetrance (60% [2];) suggests the need for one or more additional contributing factors (else would not E4/E4 exhibit 100% penetrance?). The findings reviewed herein demonstrate that key nutritional compromise, including that of folate, may be one of these additional risk factors [75]. Genetic deficiencies in folate usage may also represent a contributing factor even in the presence of otherwise sufficient

dietary intake. For example, the C677T polymorphism of 5',10' methylene tetrahydrofolate reductase (which utilizes folate to regenerate methionine from homocysteine) is an ApoE4-dependent AD risk factor [76–79]. Transgenic mice expressing this polymorphism were more severely compromised in the above behavioral and biochemical parameters than were wild-type mice when each were maintained on the challenge diet [78]. Detailed studies in humans are wanting, although vitamin E-induced neuroprotection was compromised in AD patients with E4 [80]. Genetic predispositions placing an individual at risk for age-related disorders including AD may remain latent pending age-related decline in nutrition and/or homeostasis [45–49].

Rather than simply considering E4 to be detrimental and E2 and E3 to be beneficial, the differential impact of ApoE alleles represents a continuum, with E4 the most detrimental and E2 the most beneficial [22, 70]. Retrospective epidemiological analyses as presented above indicate that E3 is capable of contributing to AD, especially in concert with other deleterious influences such as nutritional compromise. The transgenic mouse studies reviewed herein demonstrate not only that dietary oxidative challenge can potentiate the risk associated with E4, but also suggest that it can in some cases exert a similar deleterious interaction with E3. While murine model data can at best only be extrapolated to the human condition, they are nevertheless consistent with the early contribution of oxidative stress to AD [11], the ability for E4 to account for only 50% of the cases of AD, and the linear relationship of E3 versus E2 for risk of AD [71,73]. Extrapolation of these findings in mice to the human condition, coupled with the experimental data presented herein, lead us to hypothesize that the E3 allele may not provide sufficient neuroprotection under conditions of dietary compromise and/or oxidative challenge. Notably, this article has not addressed additional deleterious effects associated with ApoE metabolism, which may also indirectly impact cognition [81]. Lifestyle approaches suggested to counter the recognized “at-risk” E4 genotype [13, 75,82–85] should therefore perhaps not be ignored by individuals bearing the other alleles.

DISCLOSURE STATEMENTS

Authors' disclosures available online (<http://www.jalz.com/disclosures/view.php?id=346>).

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