

# ApoE genotype accounts for the vast majority of AD risk and AD pathology

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## Abstract

In this review, evidence is provided that apolipoprotein E (apoE) genotype accounts for the majority of Alzheimer's disease (AD) risk and pathology. The three major human isoforms, apoE2, apoE3, and apoE4, are encoded by different alleles ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) and regulate lipid metabolism and redistribution. ApoE isoforms differ in their effects on AD risk and pathology. Clinical and epidemiological data have indicated that the  $\epsilon 4$  allele may account for 50% of AD in the United States. Further, the rarity of AD among carriers of the  $\epsilon 2$  allele suggests that allelic variations in the gene encoding this protein may account for over 95% of AD cases. ApoE4 disrupts memory function in rodents. Further studies have indicated that fragments of apoE may contribute to both plaque and tangle formation. Thus, the epidemiologic and basic science evidence suggest that apoE genotype accounts for the vast majority of AD risk and pathology.

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**Keywords:** ApoE genotype; AD risk; AD pathology

## 1. The risk of AD increases in accordance with apoE genotype such that $E4 > E3 > E2$

In 1993, a group lead by Alan Roses published a series of papers suggesting that the  $\epsilon 4$  allele of the gene encoding apolipoprotein (apo) E has a major association with the risk for Alzheimer's disease (AD) [8,46,47,50,54]. In one paper, Corder et al. [8], showed that the Estimated Onset of Distribution is shifted to considerably younger ages in AD cases with  $\epsilon 4/4$  genotype (50% by age of onset of 66 y/o), than the  $\epsilon 3/4$  (50% by 73 y/o) or the  $\epsilon 3/3$  (50% by 86 y/o), and those without an  $\epsilon 4$  and with an  $\epsilon 2$  allele have an even later age of onset.

## 2. Relative to apoE2, apoE3, and apoE4 may account for 95% of the risk of AD

In a complimentary paper, Saunders et al. [49], presented clinical data regarding the association of the  $\epsilon 4$  allele with AD suggesting that 50% of AD is associated with the  $\epsilon 4$

allele. Further, if the relative rarity of the disease among  $\epsilon 2$  homozygotes is considered, then the apoE genotype may be seen as being responsible for as much as 95% of AD (Table 1). These findings have been widely replicated (for review, see [3]). However, the relationship between apoE genotype and AD hazard (yearly incidence) has been confused by studies which have examined populations over 65 years of age [11] and the diagnosis of autopsy confirmed AD without regard to age of onset [35]. Examination of the relationship between apoE and AD with respect to age shows that apoE genotype indeed accounts for most of the AD risk.

### 2.1. The central role of age in the development of AD

It has long been held that the major factor associated with AD is aging, with family history playing a major secondary role. Clearly, age is associated with AD, with estimations that both the incidence and prevalence increase at an exponential rate, such that the rates of disease occurrence start at a very low level in middle age and double about every 5 years (most clearly between ages 60 and 90, Table 2 [28]). There is some uncertainty as to whether this age-specific rate varies between men and women (see below). The suggestion that the rate may stabilize or decline over 90 years

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Table 1  
ApoE genotype frequency in US population and AD risk

| Genotype | Population <sup>a</sup><br>(%) | AD <sup>b</sup><br>(%) | #Population | #AD    | Risk <sup>c</sup><br>(%) | If all<br>US |
|----------|--------------------------------|------------------------|-------------|--------|--------------------------|--------------|
| ε2/2     | 1                              | 0.1                    | 0.5M        | 0.004M | 0.08                     | 0.4M         |
| ε2/3     | 12                             | 4                      | 5.5M        | 0.18M  | 3.2                      | 1.5M         |
| ε3/3     | 60                             | 35                     | 27.6M       | 1.4M   | 5.1                      | 2.3M         |
| ε3/4     | 21                             | 42                     | 9.6M        | 1.7M   | 18                       | 8.2M         |
| ε4/4     | 2                              | 16                     | 0.9M        | 0.6M   | 67                       | 30.7M        |

Please note that ε2/ε4 subjects are not included in table.

<sup>a</sup> Using estimate of 46 million in US over 60 y/o in 2000.

<sup>b</sup> Assuming 4 million individuals have AD.

<sup>c</sup> Data from [13,46,49].

of age has some support [38], but such “healthy survivor” effects have been discussed at length and apply only to a small number of individuals and may be measurement artifacts, mostly unrelated to AD diagnosis [21–23] or represent the extreme limit of the aging process. Also, family history shows a major association with the risk of developing AD, even beyond the autosomal dominant mutations and apoE genotype. The family risk that is not yet explained by genetic factors could be related to unidentified genes associated with either AD risk or longevity to reach the age of AD risk [52] or other manners of familial association such as cultural factors. However, closer scrutiny of the actual age of onset of dementia in AD patients in relationship to the apoE genotype reveals that it is the *APOE* gene which is the factor determining the vast majority of AD risk. This association is modeled in detail below.

## 2.2. The Gompertz law and modeling biological aging

To examine the risk factors associated with the development of AD, it is necessary to first examine the process of aging and the consequent mortality. Biological aging is the reciprocal of decay and is associated with a progressive increase in mortality rate with duration of life (as opposed to a stable rate of mortality as would be seen in a decay curve). Aging in biological systems may best be seen as a consequence of the massive scale of redundancy achieved by multi-cellular systems to compensate for the natural loss of system elements over time [16], which is described by a Gompertz curve (as opposed to a Weibull curve, as is seen for

Table 2  
Prevalence of AD

|   |
|---|
| Estimated 4 million cases in US (2000)    |
| (2000–46 million individuals over 60 y/o) |
| Estimated 500,000 new cases per year      |
| Increase with age (prevalence)            |
| 1% of 60–65 (10.7 m) = 107,000            |
| 2% of 65–70 (9.4 m) = 188,000             |
| 4% of 70–75 (8.7 m) = 350,000             |
| 8% of 75–80 (7.4 m) = 595,000             |
| 16% of 80–85 (5.0 m) = 800,000            |

aging in mechanical systems, for reviews, see [16,21–23]). The Gompertz law states that mortality starts at a low level in early adulthood, and then doubles every set number of years. There is an exponential increase of death rate beginning at age 30, doubling every 7.5 years for women and every 8.2 years for men, with over 99.5% of the variance in mortality rate explained by an exponential function (a straight line on log-linear plot, Fig. 1c) between 30 and 95 years of age for the US population. Comparing AD incidence per year data (Fig. 1c), it is apparent that the rate of developing AD increases more rapidly (doubling every 5 years) than mortality rate. The rate of developing AD would be expected to exceed that of mortality by 105 years old (though there is some difficulty in assessing such values in small, difficult to find populations). Without considering the controversy about whether there is a different rate of developing AD related to gender (cf. [28]; and for the consideration that females are more vulnerable to AD than males, see [13,64], application of the rate of developing AD to the US Census data yields the result that there is nearly twice as much AD in women solely related to the increased longevity of women (Fig. 1d). The centenary population (individuals in their 100th year) only represents about 1% of the birth cohort (and there are five times as many females at this age as there are males), but, according to these calculations, 80% of centenarians have AD (though the empirical data of Miech et al. [38], support the findings of others that this level is not reached in centenarians because of a plateau in the increasing rate at very old age).

## 2.3. AD attacks brain systems with a high degree of neuroplasticity

It is remarkable that the development of AD is more closely related to aging than mortality is. This pattern of age-accelerated AD development suggests that there is a catastrophic breakdown of a selective brain system with age. To show such a close relationship to aging, that system needs to be highly redundant and composed of parts that must reach a threshold of dysfunction and then deteriorate very quickly. In the case of AD, the redundant system is the network of neurons subserving memory, and when the deterioration of these neurons reaches the point that network capacity is impaired, memory problems will appear, and then the patient will deteriorate relatively rapidly. The highly-redundant brain system that is attacked by AD pathology is thought to be composed of those neuronal systems that have major neuroplastic activity, which underlies memory function [1–3,37,56].

## 2.4. Broad support for the relationship between ApoE genotype and AD

Since the apoE genotype was first shown to have a major impact on the age of development of AD, there have been over 100 studies which have confirmed the relationship

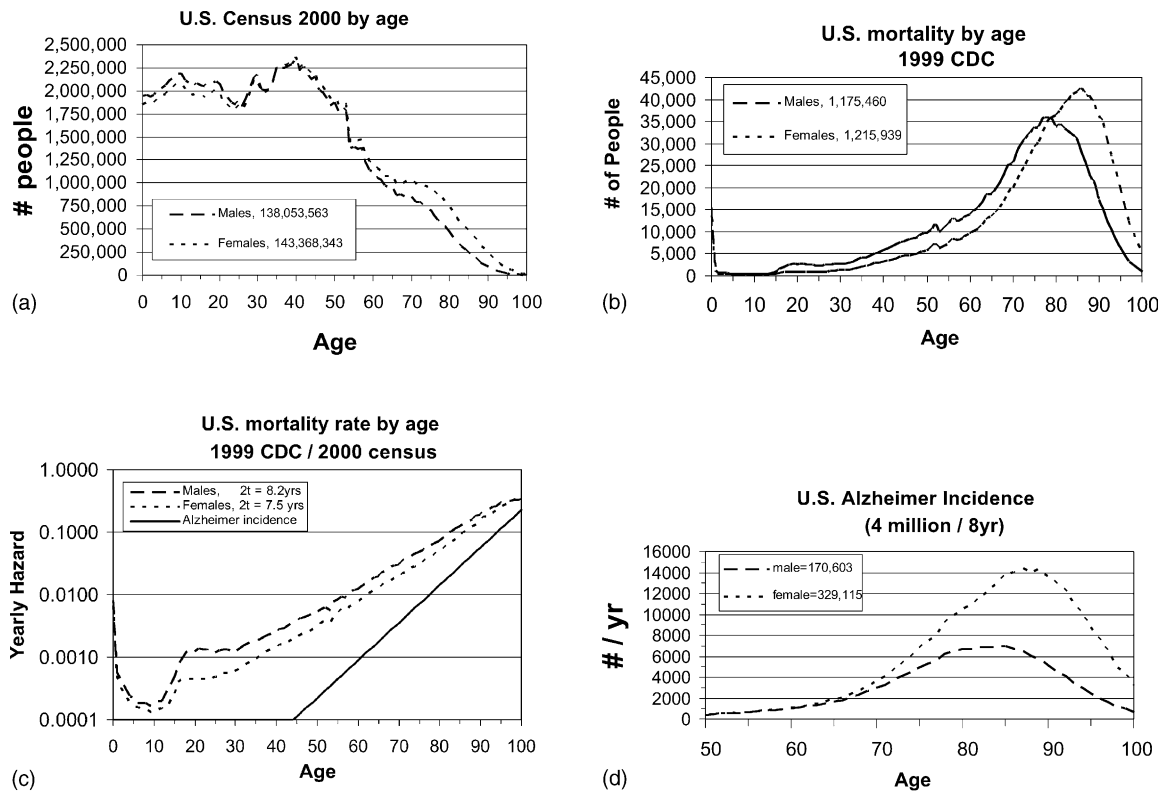


Fig. 1. (a) Census of the US, for 2000. Total: 281,421,906; >60: 45,809,291; >65: 35,003,844; >85: 4,251,678; >100: 62,545. (b) Mortality data from the US, for 1999. (c) Mortality data by year of age using data from the US Center for Disease Control (<http://www.cd.gov>) divided by the US Census data from the year 2000 (<http://www.census.gov>), to yield the yearly hazard rate for dying, according to gender. The doubling times are calculated for the range from 30 to 100 years of age by gender, where an exponential function with these parameters explains over 99.5% of the variance. The solid line shows the estimated yearly hazard for developing AD, assuming a 5 year doubling rate and 4 million affected individuals in the population, with a life expectancy from diagnosis of 8 years. (d) The number of patients estimated to contract Alzheimer's disease each year, by sex (box) and by age (graph) in the US.

between AD and apoE genotype (a PubMed search in November 2003 with “apoE and Alzheimer and epidemiology” revealed 366 publications, most of which support this finding and the few studies that did not find the relationship in selective populations contrasted their findings with other populations that did have the relationship). However, the strength of this relationship varies among epidemiological populations around the world, and it has even been suggested that ε4 might not be a risk factor for AD in some populations [13,20]. Many of the studies can be broken down into two categories, those which examine the prevalence of the disease in the clinical practice setting and those that examine epidemiological samples. The clinical studies are biased by the tendency of younger patients, particularly those less than 75 years of age, to more vigorously seek help for their memory difficulties than very elderly individuals with memory problems. Such studies must contrast their findings with prevalence data from control populations, which may focus on young adults that have not yet lived to the ages with the highest risk (Table 3a and b); therefore, these individuals might still develop AD. Further, apoE allele frequencies vary with populations around the world (Table 3a), which can affect local estimates of the contribution of these alleles to AD prevalence.

In contrast to the case-controlled studies, prospective epidemiological studies need to limit their populations to elderly groups in which AD will occur adequately often to measure, e.g. all individuals over 65 years of age. However, beginning a study with elderly unaffected individuals will screen out the younger individuals with the at risk allele that were highly likely to get AD at a younger age than the study

Table 3  
ApoE genotype (a) in the US (world range)<sup>a</sup> and (b) prevalence in the US (calculated from (a))

|     |  |
|-----|--|
| (a) | e2—7% of the population                                    |
|     | e3—78% of the population (54%–91%)<br>(Pygmies–Sardinians) |
|     | e4—15% of the population (5%–41%)<br>(Mayans–Pygmies)      |
| (b) | e2/2—0.5% of the population                                |
|     | e2/3—11% of the population                                 |
|     | e2/4—2% of the population                                  |
|     | e3/3—61% of the population                                 |
|     | e3/4—23% of the population                                 |
|     | e4/4—2% of the population                                  |

<sup>a</sup> Data from [15].

criterion or the date of enrollment commencement. Also, such epidemiological studies require dementia free status for admission to the study, assuring the relative exclusion of the individuals with high risk alleles, and thus, producing a substantial underestimation of the risk associated with apoE4 (e.g. [11]; see for review [3]). Accordingly, when studying AD, one must focus not just on what the allele frequencies are in the population, but how the gene-frequency changes with age and with respect to the changing incidence of the disease, which is related to the high rates at which the disease depletes the population of unaffected individuals.

2.5. Age-specific estimates of AD risk associated with apoE genotype

For the purpose of modeling the problem of age and changing AD risk with respect to apoE genotype, Gompertz curves were constructed with the acceptance of the estimation that AD incidence doubles every 5 years. It was assumed (without supporting empirical data) that this same

rate would apply across all apoE genotypes. Further, the data from Table 1 were used to approximate the relative base-rates of the Gompertz curves, with the apoE  $\epsilon$ 4/4 genotype estimated to have a risk 7.5 times the mean, the  $\epsilon$ 3/4 to have two times the mean, and the  $\epsilon$ 3/3 genotype to have 0.6 times the mean (these are age-specific hazard factors rather than odds ratios, which would depend on the sample population; also, the risk may vary for gender differently across the apoE genotypes, e.g. [13,38]. AD risk associated with the apoE  $\epsilon$ 2 allele is considered to be much less, but is not shown. The resulting curves show the relative estimate of risk relative to age (Fig. 2a), the effect on the US population of the 2000 Census for probability of AD onset in a year (Fig. 2b), the probability of not having AD (Fig. 2c), and the calculated estimate of the proportion of new AD cases having each genotype according to age of disease development (Fig. 2d). These graphs show that, given the assumptions of the risk and the US population characteristics, the 25% of the population with an  $\epsilon$ 4 allele accounts for 90% of the new AD cases until 80 years of age. While these

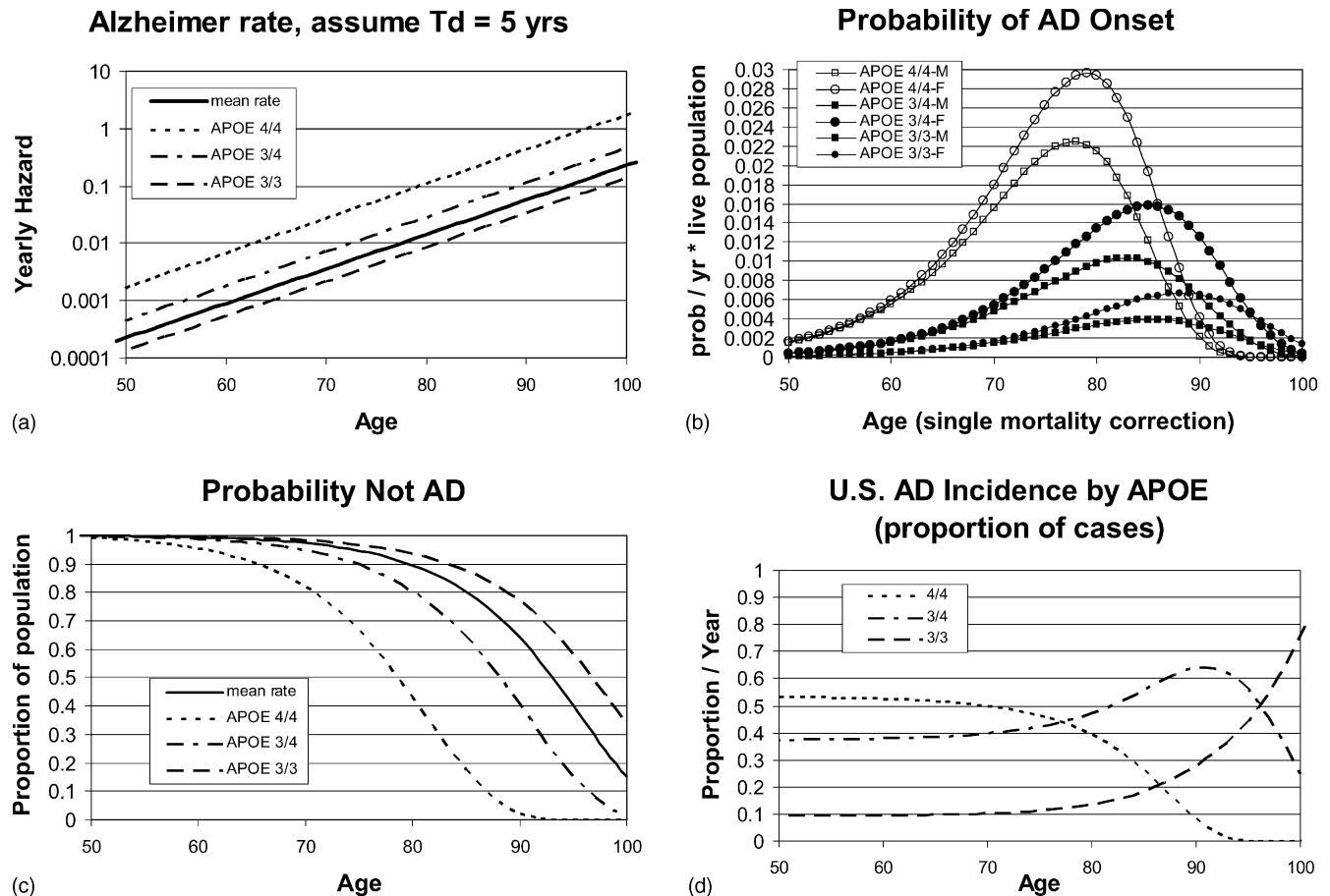


Fig. 2. (a) The rate of development of AD by age for the total population (solid line, from Fig. 1c, mean rate), with estimated rates according to specific ApoE genotypes, using data from Table 1. (b) Squares: males; circles: females; open:  $\epsilon$ 4/4; gray:  $\epsilon$ 3/4; filled:  $\epsilon$ 3/3; age-specific risk  $\times$  proportion of original population at 50 years of age living at the age. (c) Estimate of population not affected by AD, by age, calculated from the rate estimates. (d) Proportion of total non-demented patients estimated to develop AD at each age. Note that the ApoE  $\epsilon$ 4 allele plays a progressively smaller role as the patients with this allele are eliminated from the at-risk population, first the more susceptible homozygous patients, then the heterozygous patients.

Table 4  
Ages of maximum probability of AD onset

| Gompertz estimate (AD probability per year) |        | Cache county data [38] (discrete annual hazard of AD) <sup>a</sup> |        |
|---|--------|--|--------|
| Male  |        |  |        |
| 3/3   | 85 y/o | <i>x/x</i>   | 92 y/o |
| 3/4   | 83     | <i>x/4</i>   | 91     |
| 4/4   | 78     | 4/4  | 78     |
| Female                                      |        |  |        |
| 3/3   | 88 y/o | <i>x/x</i>   | 97     |
| 3/4   | 86     | <i>x/4</i>   | 96     |
| 4/4   | 80     | 4/4  | 83     |

<sup>a</sup> *x* = 2 or 3.

calculations are based on several assumptions, the only population study that shows data with this type of presentation is from the Cache County Study, and the data presented by that study show a relatively stronger influence of the  $\epsilon 4$  allele [38] than the relatively conservative assumptions used to formulate the estimates shown here (see Table 4).

### 2.6. Further support for the association between apoE genotype and AD risk

There are still several other points that support the above estimates. For example, to the best of knowledge there is no publication of an autopsy confirmed case of an individual with  $\epsilon 4/4$  genotype over 90 years of age without AD. Also, there is no publication claiming that an individual with  $\epsilon 2/2$  genotype has had typical AD pathology without a separate genetic or clear traumatic antecedent (that we know of). Even though the  $\epsilon 2$  allele is nearly as common as the  $\epsilon 4$  allele in the population, and over-represented among centenarians [14], there are relatively few AD patients studied with this allele. Estimations of the evolution of the apoE genotype from all  $\epsilon 4$  to a majority of  $\epsilon 3$  (which appeared 300,000 years ago [15]) with a recent development of  $\epsilon 2$  (200,000 years ago), which occurred in parallel with a change of diet to include more cholesterol and a progressive increase of longevity, supports the relationship between improved memory in the elderly and increased survival fitness (see [56] for review). Consequently, the epidemiological, clinical, and archeological literature generally supports the point that the major risk factor for AD is apoE  $\epsilon 4$  allele, with the  $\epsilon 2$  allele appearing to be a protective factor. While there might be environmental factors, including diet, cholesterol, and hormones, which may interact with the APOE gene and modulate its expression, they seem to account for a relative small proportion of the variance. Accordingly, the apoE genotype represents the major factor associated with the development of AD.

### 3. ApoE is associated with the pathological hallmarks of AD, i.e. both plaques and tangles

The association of apoE immunoreactivity with amyloid  $\beta$  (A $\beta$ ) peptides in extracellular plaques, intracellular

neurofibrillary tangles (NFT), and cerebral vessel congophilic angiopathy in brains of AD patients [41] suggests a major role for apoE in the amyloid and neuritic pathology in AD. Indeed, the severity of this pathology is influenced by apoE genotype as illustrated below.

#### 3.1. ApoE and A $\beta$

The presence of an  $\epsilon 4$  allele increases the rate and extent of amyloid deposition [10,50,54]. ApoE binds A $\beta$  with high avidity. ApoE isoforms differ in their A $\beta$  binding characteristics [54], and these different A $\beta$  binding characteristics may contribute to the isoform-dependent effects of apoE on amyloid deposition.

#### 3.2. ApoE and neurofibrillary tangles

While there is evidence for isoform-dependent effects of apoE on neurofibrillary pathology, these effects seem less consistent than those on amyloid deposition (or plaque formation) described above [10,17,50,54,61]. The percentage of  $\epsilon 4$  allele is higher in young subjects with initial neurofibrillary pathology (Stage I) than in controls [17]. In addition, this  $\epsilon 4$  percentage correlates with the formation of neurofibrillary pathology in non-demented elderly subjects [61] and with neurofibrillary pathology in AD subjects [36]. ApoE binds tau and the differential ability of apoE isoforms to bind tau [54] may contribute to the isoform-dependent effects of apoE on neurofibrillary pathology. In general, the effects of apoE isoforms on neuritic pathology parallel those on A $\beta$ -related pathology. However, while AD patients with the  $\epsilon 4/\epsilon 2$  genotype showed significantly less amyloid deposition in the neocortex than those with  $\epsilon 4/\epsilon 3$  genotype, the neurofibrillary pathology in these two genotypes was not statistically significant [40]. Similarly, comparing AD patients with the genotypes  $\epsilon 4/\epsilon 2$  and  $\epsilon 3/\epsilon 2$  showed opposite effects on amyloid load and neuritic plaques and neurofibrillary tangles [40]. The involvement of distinct mechanisms involving interactions of apoE with A $\beta$  or tau could contribute to these divergent effects on amyloid deposition and neurofibrillary pathology. As the clinical signs of AD are more related to neurofibrillary pathology than plaques [40],  $\epsilon 2$  would be expected to be more protective in the presence of  $\epsilon 3$  than  $\epsilon 4$ .

#### 3.3. ApoE, progressive supranuclear palsy (PSP), and AD pathology

ApoE genotype also has an impact on AD pathology when it occurs in patients that have other conditions, including PSP and Down Syndrome. For example, most patients with PSP have minimal or no AD pathology (Braak stage III or less), but some patients do show AD pathology (plaques and neurofibrillary pathology) [59]. While the  $\epsilon 4$  allele frequency is similar in PSP patients with minimal or no

AD pathology (Braak stage III or less) (11%) and controls, it is significantly higher in PSP patients with concomitant AD pathology (64%) or with pathologic aging (many cortical senile plaques, mostly diffuse amyloid deposits, and few or no neurofibrillary tangles) (38%) [59]. Comparison between  $\epsilon$ 4-positive and  $\epsilon$ 4-negative PSP patients shows that  $\epsilon$ 4-positive PSP patients have significantly more senile plaques and neurofibrillary tangles in association cortices and more senile plaques in primary cortices.

#### 4. In transgenic mice, expression of apoE4 causes neuropathology and behavioral deficits

##### 4.1. Higher plaque loads in hAPP/E4 than in hAPP/E3 mice

Consistent with the human data described above, apoE has isoform-dependent effects on plaque formation in transgenic mice lacking murine apoE and expressing human amyloid precursor protein with familial AD mutations (hAPP) and human apoE isoforms in astrocytes [24] or neurons [6]. Importantly, these mice express apoE3 and apoE4 at similar levels and were shown to have comparable levels of A $\beta$ 1-x (approximation of total A $\beta$ ) and A $\beta$ 1–42 in the hippocampus [44] at 6 months of age and neocortex at 12–15 months of age [6].

##### 4.2. ApoE4 and hAPP/apoE4 mice show loss of synaptophysin-immunoreactive presynaptic terminals

Mice lacking murine apoE with and without hAPP show a progressive age-dependent loss of synaptophysin-immunoreactive presynaptic terminals. In human apoE singly transgenic mice, apoE3 protects against this loss but apoE4 does not. This protection is even seen at 19–24 months of age [6]. In hAPP/apoE bigenic mice, apoE3 is able to delay the age-dependent decline in synaptophysin-immunoreactive presynaptic terminals. At 6–7 months of age, hAPP/apoE3 mice have significantly higher levels of synaptophysin-immunoreactive presynaptic terminals than hAPP/apoE4 mice in the neocortex and at 12–15 months of age in both hippocampus and cortex [6]. At 19–24 months of age, apoE3 is not protecting anymore against the loss and the levels of synaptophysin-immunoreactive presynaptic terminals at this age in hAPP, hAPP/apoE3, and hAPP/apoE4 mice are comparable [6]. The isoform-dependent effects of apoE on plaque loads do not correlate with isoform-dependent effects of apoE on synaptophysin-immunoreactive presynaptic terminals [6] or cognitive function [44], supporting the possibility that apoE has also important plaque-independent effects in AD. Consistent with the human data, apoE2 is also neuroprotective; apoE2 prevented dendritic spine loss in the hippocampus of young PDAPP and Tg2576 mice [31].

##### 4.3. Neuronal expression of apoE4 increases hyperphosphorylation of tau

Isoform-dependent effects of apoE with the microtubule-associated proteins tau [54] and MAP2c have been reported, suggesting a role for apoE in neuronal cytoskeletal function. ApoE4 could contribute to AD pathology by hyperphosphorylation of cytoskeletal proteins and inducing neurofibrillary pathology. Overexpression of apoE4 in neurons using relatively strong promoters increased hyperphosphorylation of tau, which was not seen when apoE4 was expressed in non-neuronal cells, such as astrocytes [55]. The extent of tau hyperphosphorylation correlated with apoE4 expression levels and increased with age. In addition, in transgenic mice expressing apoE4 in neurons but not in wild-type mice ubiquitin-containing inclusions were observed in the hippocampus, and prominent gliosis was observed in the neocortex, hippocampus, and amygdala [55]. A recent study suggests that increased tau phosphorylation in transgenic mice expressing apoE4 in neurons might be caused by the generation of carboxyl-terminal-truncated fragments of apoE [19].

##### 4.4. ApoE4 and cognitive function

ApoE has isoform-dependent effects on cognitive function in transgenic mice. As they age, female apoE4, but not *ApoE*<sup>-/-</sup>, mice develop progressive impairments in spatial learning and memory in the water maze [42,43]. These cognitive impairments are independent of the cellular source of apoE as they are observed in mice expressing apoE4 in neurons or astrocytes. Spatial learning and memory are severely impaired in AD and can be assessed in mice using the water maze test. At 6 months of age, male and female *ApoE*<sup>-/-</sup>, apoE3, and apoE4 mice learned to locate both the visible and the hidden platform, and there were no significant differences in the learning curves. At 18 months of age, only *ApoE*<sup>-/-</sup> ( $P < 0.01$ ) and apoE3 ( $P < 0.05$ ) females learned to locate the hidden platform, whereas apoE4 females did not. In the probe trial, 6- and 18-month-old female *ApoE*<sup>-/-</sup> and apoE3 mice showed memory retention. They showed an increased preference to search the quadrant where the hidden platform was located previously (target quadrant). In contrast, apoE4 females did not. ApoE4-induced cognitive deficits were detected even earlier using a modified version of the water maze test, in which the platform location is hidden from the start and changed daily [42]. In this version of the water maze, apoE4 females showed striking learning impairments by 6 months of age. Importantly, no spatial learning deficits were detected in age- and gender-matched *ApoE*<sup>-/-</sup> mice in this more sensitive test. ApoE4 also exerts detrimental effects on non-spatial learning and memory; 6-month-old male and female apoE4, apoE3, wildtype, and *ApoE*<sup>-/-</sup> mice were assessed in a novel-object recognition test. The percentage of time the mice spent exploring the novel versus the familiar object relative to the total

amount of time they explored either object in the retention session was used to evaluate object recognition memory. In the training session, all groups of mice spent a comparable amount of time exploring each object. Female *ApoE*<sup>-/-</sup> mice and all the other genotypes had intact object recognition memory and spent significantly more time exploring the novel object. However, female apoE4 mice showed significant deficits in the retention session and spent a significantly smaller proportion of time exploring the novel object. These results demonstrate that apoE4 induces deficits not only in spatial but also in non-spatial learning and memory. The greater detrimental effects of apoE4 in female than male mice are consistent with the epidemiological interaction of apoE4 and female gender on increased risk to develop AD. However, male mice are only relatively protected against the detrimental effects of apoE4. When tested for spatial learning and memory in the water maze, male hAPP/apoE3 mice showed good memory retention but male hAPP and hAPP/apoE4 mice failed to spend more time in the target quadrant than in the other quadrants. Similar results were obtained in female mice except that singly transgenic apoE4 female mice also failed to spend more time in the target quadrant.

The beneficial effects of environmental enrichment on cognition are also dependent on apoE genotype; environmental enrichment improves spatial learning and memory of apoE3, but not apoE4, transgenic mice [32].

The effects of apoE4 on neuropathology and behavior in mice are consistent with their effects on neuropathology and behavior in humans. The neuropathological data in humans supporting a crucial role for apoE4 in the neuropathological hallmarks of AD are described above (see Section 3). With regard to the behavioral data in humans, apoE4 increases the risk of cognitive impairments and of developing AD [8,9,13,45,64]. In addition, in cognitively non-demented individuals, those with  $\epsilon$ 4 show reduced brain metabolism and increased brain activation during recall [53]. Using fMRI, women at risk for AD showed an increased parietal activation during a fluency task and upon retesting a further decrease in ventral temporal lobe activation on a picture naming task, as compared to a control group [60]. Finally, apoE4 interacts with female gender, further increasing AD risk in women [13,64]. This interaction might involve androgen receptors. Using mice deficient in mouse apoE (*ApoE*<sup>-/-</sup>) and expressing human apoE4 or apoE3 in the brain at comparable levels, apoE4 expression in female and male mice reduced cytosolic AR levels in the neocortex [44]. Male apoE4 mice might be less susceptible to effects of apoE4 on DHT binding to ARs because of their higher circulating levels of endogenous androgens. Importantly, androgens and AR-dependent pathways protected against detrimental effects of apoE4 on learning and memory in female mice [44]. Improved memory in androgen-treated female apoE4 mice was associated with increased binding of DHT to cytosolic ARs, suggesting that apoE4 contributes to cognitive decline by reducing DHT binding to ARs in

the brain and that stimulating AR-dependent pathways can reverse apoE4-induced cognitive deficits.

## 5. Plaque formation appears to require the presence of apoE

### 5.1. Differential interaction of apoE isoforms with A $\beta$ peptides in vitro

Overproduction and deposition of A $\beta$  have been suggested to play a central role in AD pathogenesis [18,51]. In vitro, lipid-free apoE3 and apoE4 can form a sodium dodecyl sulfate (SDS)—and guanidine hydrochloride—stable complex with A $\beta$  peptides, with apoE4 complexes forming more rapidly and effectively [7,54]. Prolonged incubation (several days) of lipid-free apoE with A $\beta$  peptide results in insoluble, high-molecular-weight complexes that precipitate as fibers. Again, apoE4 forms a denser, more extensive matrix of amyloid monofibrils and does so more rapidly and effectively than apoE3 [33,48,62]. In addition, lipid-free apoE4 enhances zinc- and copper-induced A $\beta$  aggregation [39]. Thus, lipid-free apoE appears to display isoform-specific differences in binding to the A $\beta$  peptide, with apoE4 binding more rapidly and effectively under certain conditions. Increased amyloid fibril formation associated with apoE4 might trigger or exacerbate neurodegeneration and the development of AD.

However, when incubated with A $\beta$  peptide, lipidated apoE3 or apoE4 isolated from stably transfected apoE-expressing cells yielded different results [29,30]. ApoE3 bound with a 20-fold greater affinity than apoE4 to the A $\beta$  peptide, suggesting that the lipidation status of apoE modifies its ability to interact with A $\beta$  peptides [29,30]. The avid binding of lipidated apoE3 to the A $\beta$  peptide may enhance clearance of the complex, preventing the conversion of A $\beta$  into a neurotoxic species [30].

### 5.2. Plaque formation appears to require the presence of ApoE in transgenic mice

Studies of transgenic mice expressing human apoE3 or apoE4 have provided insight into the role of apoE in A $\beta$  metabolism in vivo. When transgenic mice expressing human APP with a familial AD mutation (V717F) were crossed onto an apoE-null background, A $\beta$  deposition in the brain decreased dramatically, suggesting that apoE is required for A $\beta$  deposition at least in transgenic mice [4,5]. A similar conclusion had been reached when transgenic mice (Tg2576) expressing human APP with double mutations (K670N and M671L) were crossed with apoE-null mice [27]. However, expression of human apoE3 or apoE4 in the absence of mouse apoE further decreased A $\beta$  deposition, suggesting that human apoE stimulates A $\beta$  clearance [12,24]. Interestingly, apoE3 might clear more A $\beta$  than apoE4 [12,24].

## 6. Proteolytic fragments of apoE may contribute both to plaque and tangle formation

### 6.1. The carboxyl-terminal fragment of apoE may contribute to plaque formation

It was first reported that a carboxyl-terminal fragment (residues 216–299) of apoE (the full-length apoE has 299 amino acids) was co-purified with A $\beta$  from senile plaques [62,63]. In vitro, this fragment from recombinant apoE could form amyloid-like fibrils, which were Congo-red positive [63]. These results suggest that senile plaques may contain amyloid fibrils formed from both A $\beta$  and the carboxyl-terminal apoE fragment [62,63]. Thus, the carboxyl-terminal fragment of apoE may contribute to plaque formation, although the protease that cleaves apoE has not been identified.

### 6.2. The amino-terminal 22-kDa thrombin-cleavage fragment of apoE is neurotoxic in vitro

It was found that the amino-terminal 22-kDa thrombin-cleavage fragment (amino acids 1–191) of apoE4 is more neurotoxic than the corresponding fragment of apoE3 in vitro [57,58]. This neurotoxicity may be mediated by lipoprotein receptors on the cell surface and by increasing intracellular calcium levels through NMDA glutamate receptor [57,58]. The involvement of the lipoprotein receptors in the 22-kDa thrombin-cleavage fragment of apoE-induced neurotoxicity is supported by the observation that the synthetic peptides of the LDL receptor binding domain of apoE is also neurotoxic in vitro [57,58]. However, subsequent study suggests that the apoE fragments generated in AD brains are different from the 22-kDa thrombin-cleavage products [25]. In fact, the lipid-binding domain (amino acids 244–272) is present in all the fragments generated in AD brains [25] and is essential for the development of neurotoxicity in transgenic mice [19].

### 6.3. The carboxyl-terminal-truncated fragments of apoE accumulate in AD brains and can induce NFT-like inclusions in neuronal cells in vitro

ApoE4 is more susceptible to proteolytic cleavage than apoE3 in vitro [19,25]. Notably, apoE fragments were also present at much higher levels in brains of AD patients than in brains of age- and sex-matched non-demented controls with the corresponding apoE genotypes [19,25]. Carboxyl-terminal-truncated fragments of apoE also appeared to accumulate in neurofibrillary tangles in AD brains [19]. In vitro, the carboxyl-terminal-truncated fragments of apoE are toxic when expressed in neuronal cells or added to neuronal cultures, leading to cell death and to the formation of cytoplasmic NFT-like inclusions in some of the cells [19]. Thus, the carboxyl-terminal-truncated apoE may contribute to NFT formation [26,34].

### 6.4. The carboxyl-terminal-truncated apoE4 causes AD-like neuronal and behavioral deficits in transgenic mice

Transgenic mice expressing high levels of the carboxyl-terminal-truncated apoE4 [apoE4( $\Delta$ 272–299)] died at 2–4 months of age [19]. The cortex and hippocampus of these mice displayed AD-like neurodegenerative alterations, including abnormally phosphorylated tau (p-tau) and Gallyas silver-positive neurons containing cytosolic straight filaments with diameters of 15–20 nm, resembling pre-neurofibrillary tangles. Transgenic mice expressing lower levels of the truncated apoE4 survived longer but showed impaired learning and memory at 6–7 months [19]. Thus, carboxyl-terminal-truncated fragments of apoE4, which occur in AD brains, are sufficient to elicit AD-like neurodegeneration and behavioral deficits in vivo [19].

## 7. Summary

This review has concentrated on the evidence that supports the relationship between apoE genotype and AD risk. The apoE molecule has been shown to be associated with several factors that lead to AD pathology. In the context of the debate about the association between apoE genotype and AD, it has been shown that this genotype can independently account for as much as 95% of the AD cases in the US. Another consideration is that apoE- $\epsilon$ 2 homozygotes may not even develop AD in the absence of other specific, rare genetic or environmental factors. Therefore, the apoE genotype may be considered to be responsible for the vast majority of AD.

The dominant role of apoE needs to be considered in a broader context. For example, those individuals with apoE  $\epsilon$ 3 and/or  $\epsilon$ 4 alleles have a risk for AD that is age- and gender-related, and there are other factors, including head-trauma and diet, that can affect the age of onset of AD in these individuals. Further, certain interventions, possibly including NSAID or statin use, may substantially change age of onset, and some of these interventions may differ in their impact, depending on apoE genotype.

The overwhelming role of apoE genotype in AD argues that this genetic factor should be studied in greater depth for how it can be used chemically to improve early detection and diagnosis of AD, as well as how information about this genotype can improve prevention and early intervention strategies. However, the importance of apoE does not lessen the importance of other known and unknown factors that could also play important roles in preventing AD or improving the treatment of patients with this important condition.

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