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The role of *APOE* polymorphisms in late-onset dementias

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Abstract. Epidemiologic and laboratory results consistently implicate the *APOE* gene in the pathogenesis of late-onset Alzheimer's disease (AD): the $\varepsilon 4$ allele increases risk in a dose-dependent fashion, while $\varepsilon 2$ confers protection. Individuals are susceptible for AD in varying degrees depending on which combination of *APOE* alleles has been inherited, *APOE* promoter polymorphism and other factors. Deposition of both senile plaques and neurofibrillary tangles, the pathologic hallmarks of AD, are enhanced by $\varepsilon 4$ from the earliest lesions onward – diffuse plaques consisting of Aβ 1–42 and neurofibrillary tangles in the entorhinal cortex. Transgenic *APOE* mice carrying an *APP* mutation and 0, 1 or 2 copies of *APOE* showed dose-related increases in plaque deposition in the hippocampus and cortex, a clear indication that APOEp promotes Aβ deposition. The presence of each additional *APOE* $\varepsilon 4$ allele leads to an earlier onset of the histopathological process of about 1 decade, on average. The association of both types of AD-related changes with the occurrence of $\varepsilon 4$ suggests that the *APOE* polymorphism causally contributes to the pathogenesis of AD.

Key words. Genetic epidemiology; dementia; apolipoprotein E; Alzheimer's disease; polymorphisms; tau protein; amyloid protein; transgenic mice; susceptibility genes.

APOE and risk for Alzheimer's disease

The importance of a genetic risk factor for late-onset Alzheimer's disease (AD) was recognized in 1993 by Allen Roses and his colleagues at Duke University [1, 2]. They identified an increased frequency of the $\varepsilon 4$ allele for apolipoprotein E (gene: *APOE*; protein: APOEp) among patients who were 65 years of age and older. There are three common alleles at the *APOE* locus ($\varepsilon 2$, $\varepsilon 3$, $\varepsilon 4$) encoding normal variants of the APOEp protein (the E2, E3, E4 isoforms). Each person carries two copies of the gene, one from each parent. Thus, six combinations are possible: the $\varepsilon 2/2$, $\varepsilon 2/3$, $\varepsilon 3/3$, $\varepsilon 2/4$, $\varepsilon 3/4$ and $\varepsilon 4/4$ genotypes.

Allelic frequencies vary somewhat from one population to another. For investigated populations $\varepsilon 3$ is more, and $\varepsilon 2$ less, common than $\varepsilon 4$. Allelic frequencies for Caucasian samples are approximately 7 ($\varepsilon 2$), 77 ($\varepsilon 3$) and 16% ($\varepsilon 4$) with corresponding genotypic frequencies of 0.5 ($\varepsilon 2/2$), 11 ($\varepsilon 2/3$), 59 ($\varepsilon 3/3$), 2 ($\varepsilon 2/4$), 25 ($\varepsilon 3/4$) and 3% ($\varepsilon 4/4$). Investigation of relatively rare $\varepsilon 2/2$ is possible only for extremely large samples.

A three-fold excess of the *APOE* $\varepsilon 4$ allele was identified for AD patients aged 65 + with [1], and without [2], close relatives also diagnosed with AD, that is for 'familial' and 'sporadic' disease. The $\varepsilon 4$ allelic frequency was 50% for 30 patients, each from a different

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AD family, compared with 16% for age-matched unrelated controls. For sporadic AD, clinic patients ($n = 72$) had an $\varepsilon 4$ frequency of 42%, 40% for autopsy-confirmed cases ($n = 352$), instead of the expected 10–16% found for unaffected comparison groups. No clear excess was found for early onset cases (19%).

The allelic association of *APOE* $\varepsilon 4$ and late-onset AD was initially met with great skepticism. The neuropsychiatric literature is renowned for allelic associations and genetic linkages that cannot be consistently replicated even when comparable diagnostic criteria are employed to identify case subjects. Comparison subjects may represent a different population having different allele frequencies than is found for the population case subjects are derived from, resulting in false associations and failure to replicate real associations. This is especially problematic when alleles at a nearby gene are etiologically relevant for the disease instead of the investigated locus, that is the *APOE* $\varepsilon 4$ association might be due to linkage disequilibrium.

The *APOE* locus was known to be located on chromosome 19, a scant 40,000 bp from the apolipoprotein CII (*APOCII*) locus: an *APOCII* allele had previously been associated with familial AD in 1987 [3], consistent with the suspicion that the APOE $\varepsilon 4$ association with AD might be due to linkage disequilibrium involving *APOCII*. Family studies in the Duke sample in 1991 by Pericak-Vance et al. [4] motivated investigations of *APOE* by demonstrating genetic linkage of the region with late-onset AD. The sample did not replicate associations for *APOCII*. Furthermore, *APOCII* alleles were randomly found with *APOE* alleles. Hence, linkage disequilibrium involving *APOCII*, or more distally located genes on chromosome 19, was an unlikely explanation for the *APOE* $\varepsilon 4$-AD association.

Reinvestigation of the AD families [1] demonstrated a gene dose effect for the *APOE* $\varepsilon 4$ allele [5]: risk was low for subjects without $\varepsilon 4$, that is who carried $\varepsilon 3/3$ or $\varepsilon 2/3$. Higher risk was found for those with one copy of $\varepsilon 4$, i.e. for the $\varepsilon 3/4$ or $\varepsilon 2/4$ genotypes. Subjects homozygous $\varepsilon 4/4$ had the highest risk. When investigated in this way, eight-fold variation in risk was demonstrated. The gene dose effect was found for men and women, and for familial and sporadic AD [6]. The strength of the gene dose effect argued against linkage disequilibrium.

Further investigation demonstrated protection for the $\varepsilon 2$ allele for familial and sporadic AD [6]: $\varepsilon 2/3$ posed lower risk than $\varepsilon 3/3$, and risk was lower for $\varepsilon 2/4$ than $\varepsilon 3/4$. When considered in this way, there was 16-fold variation in risk: $\varepsilon 2/3$ (lowest) $< \varepsilon 3/3$, $\varepsilon 2/4 < \varepsilon 3/4 < \varepsilon 4/4$ (highest). Only a complex pattern of linkage disequilibrium involving neighbouring genes – or the *APOE* alleles themselves – could account for this striking variation.

Epidemiologic evidence favouring the direct involvement of the apoE E4 isoform in the aetiology of AD now derives from several hundred replications for diverse samples, most Caucasian [7, 8]. *APOE* $\varepsilon 4$ may not be a major risk factor for AD in black populations, for example Nigerians or African Americans [9, 10]. However, the replicated association of *APOE* $\varepsilon 4$ with AD among the Japanese [11, 12] rules out linkage disequilibrium: recombination between *APOE* alleles and those found for nearby genes would have occurred since racial divergence in the distant past.

Implications of genotype-specific risks for AD

Inclusion of age information (fig. 1) allows inspection of the proportion of subjects affected at each age from 60 to 90 years for each *APOE* genotype [13; the same subjects as in ref. 6 pooled together]. Each genotype demonstrates a distinct trajectory of onset for late-onset AD. The median age at diagnosis varied by 15 or more years from near age 70 for $\varepsilon 2/3$ compared with age 85+ for $\varepsilon 4/4$. Clearly, the investigated sample in which affected subjects are overrepresented is not even remotely representative of any human population. However, it does indicate the potential for preventive measures altering *APOE*-related risk to delay onset for many years, limiting AD dementia to ages reached by only a fraction of the population.

Despite the wide variation in *APOE* genotype-specific relative risks, allelic frequencies dictate that many occurrences of late-onset AD are found for subjects with low-risk $\varepsilon 2/3$ and $\varepsilon 3/3$ genotypes [13]. The largest prevalence of cases is found for the $\varepsilon 3/4$ genotype, not $\varepsilon 4/4$. Hence, routine screening for *APOE* genotype will not reliably distinguish persons at risk from those destined to remain unaffected. The differential diagnosis of AD may usefully employ *APOE* genotype information as demented patients carrying $\varepsilon 4$ are subsequently found to have definite AD with high probability [14].

Similar strong genotypic patterns of risk were found for subjects with and without a family history for late-onset AD (not shown) [6], hinting that the distinction between 'familial' and 'sporadic' disease is blurred. The existence of other factors that modify risk is indicated by the survival of some highest-risk $\varepsilon 4/4$ subjects, and many with $\varepsilon 3/4$, to late ages without dementia [15, 16]. Most pedigrees identified as late-onset AD families have high frequencies of the $\varepsilon 4$ allele for affected, and unaffected, members, and few $\varepsilon 2$ alleles. The occurrence of multiple cases of AD in close relatives thus likely involves the sharing of high-risk *APOE* alleles and other risk factors.

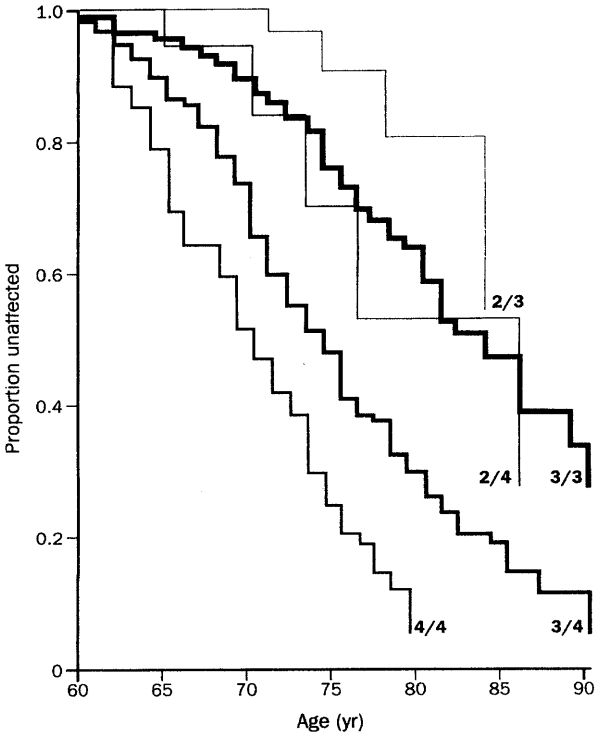

Figure 1. Risk of remaining unaffected by AD in relation to *APOE* genotype. Age of onset for subjects with each indicated *APOE* genotype. Onset curves were estimated by Kaplan-Meier product limit distributions. Data are limited to late-onset AD and should not be generalized for all populations at risk. Epidemiologically based distributions will be needed before risks can be estimated in the general population.

A shift away from the Mendelian perspective, useful for early-onset familial AD, is implied by the genotype-specific trajectories of risk similar for 'familial' and 'sporadic' disease: everyone inherits susceptibility for late-onset AD to varying degrees. This is in contrast to early-onset familial AD, where rare, highly penetrant disease-causing mutations are transmitted in an autosomal dominant fashion. The alleles are not causal in a deterministic sense. However, variation in susceptibility related to APOEp in conjunction with other factors, for example head trauma [17, 18], is strong enough to account for a large fraction of late-onset AD.

Of particular interest is the recently identified −491 A/T polymorphism in the transcriptional regulatory region for the *APOE* gene [19]. The AA genotype for the −491 A/T promoter polymorphism was more frequently found among late-onset AD cases carrying 0, 1 and 2 copies of the ε4 allele. Hence, the higher constitutive levels of apoE for the homozygous promoter polymorphism may increase risk for AD regardless of which combination of APOEp isoforms is expressed. That both the amount and type of APOEp influence susceptibility implicates APOEp in AD pathogenesis.

Progression for affected subjects

APOE polymorphism determines susceptibility for late-onset AD without strongly influencing the clinical course of the disease in affected persons [20–24]. Survival from the onset of the symptoms of AD was similar for the affected subjects carrying ε3/3, ε3/4 and ε4/4 in the sample used to define the gene dose effect on AD risk [5, 6, 20]. Prognosis depended on age at onset and gender: women and those affected at relatively younger ages had better survival. The majority of studies considering *APOE* ε4 and progression, in terms of survival or cognitive decline, have reached similar conclusions.

There are two major implications to be drawn: First, survival biases do not account for the overrepresentation of ε4 in AD patients. Second, preclinical ε4-related processes do not simply continue in clinical disease causing more rapid decline among ε4 carriers. There may be a transition near the time of clinical onset from *APOE*-dependent neurodegeneration occurring over decades across a threshold to a phase where rapid autoprogressive degenerative processes predominate. Tremendous benefit might be derived from preventive strategies that delay crossing this threshold.

APOE and other disorders

Investigation of *APOE* polymorphism and the risk for dementia was facilitated by a decade or more of prior studies involving cardiovascular disease. APOEp is a 34-kDa protein involved in lipid and cholesterol transport. The ε4 allele for *APOE* had previously been associated with alterations in serum lipid profiles [25], atherosclerosis [25–28] and coronary heart disease in late middle age [28–31]. Hence, the association of this same allele with AD has often been attributed to vascular dementia.

While *APOE* ε4 is now an established risk factor for AD, it seems unlikely that ε4 increases susceptibility for ischaemic stroke and vascular dementia at ages 75+ when AD is common [32–34]. Instead, expression of dementia may be found earlier in the evolution of AD pathology for patients with a history of stroke. Snowdon et al. [35] investigated cognitive function information on 61 nuns who subsequently died at ages 76 to 100 years and met the neuro-

pathologic criteria for AD. Those with brain infarcts had poorer cognitive function, and a higher prevalence of dementia, than those without infarcts. For the 41 participants who did not meet the neuropathologic criteria for AD, brain infarcts were only weakly associated with poor cognitive function and dementia. Hence, vascular pathology contributed to the occurrence of dementia.

Nagy et al. [36] systematically investigated cortical AD and vascular pathology for 88 subjects for whom there was recent cognition information. There were greater cognitive deficits for a given plaque density for patients with mixed dementia and other central nervous system (CNS) disorders. This gives further support to the notion that the expression of dementia, and its severity, depend on the cumulative deficits imposed by AD changes plus other lesions. Information is limited on the extent to which *APOE* $\varepsilon 4$ contributes to this process by, for example, enlarging the region of damage around infarcts via reduced free-radical quenching.

Risk of death

APOE polymorphism generates differing risks for death at late ages. The $\varepsilon 2$ allele is more frequent among French and Finnish centenarians, and $\varepsilon 4$ is less common, compared with younger persons in those populations [37, 38]. Alterations in allelic frequencies may occur at ages 85+: There was threefold variation in mortality depending on *APOE* genotype at ages 85+ in a Swedish population sample followed for 7 years (the Kungsholmen Project) [39]. Mortality was associated with $\varepsilon 4$ and the absence of $\varepsilon 2$ (i.e. $\varepsilon 2/3 < \varepsilon 3/3 <$ the $\varepsilon 3/4$ genotype). There was no variation in mortality at ages 75 to 84.

Closer investigation of the cohort involved genotype-specific incidence of cardio- and cerebrovascular disease and prognosis, i.e. mortality, in affected subjects [33]. For ages 85+, incidence was unrelated to genotype. There were genotypic differences in prognosis ($P = 0.02$): 3-month mortality was 8% for subjects who carried $\varepsilon 2/3$, 21% for $\varepsilon 3/3$, and 40% for $\varepsilon 3/4$ – a fivefold variation. Diagnoses of atrial fibrillation and congestive heart failure predominated.

These findings indicate that *APOE* genotype is a prognostic factor for cardio- and cerebrovascular disease at ages 85+ accounting for most *APOE* mortality variation. This is intriguing, given the good survival for cohort members carrying $\varepsilon 2/3$ following the occurrence of cardio- and cerebrovascular disease at ages 85+ (more frequent for men than women). It suggests a subgroup at low risk for AD with the $\varepsilon 2/3$ genotype who are at high risk for dementia. This subgroup may explain part of the difficulty in replicating the protective effect for $\varepsilon 2$ and the apparent attenuation of risk for $\varepsilon 4$ at advanced ages.

APOE and the amyloid cascade hypothesis of AD

Extracellular senile plaques consisting primarily of amyloid protein and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein are the neuropathological hallmarks of AD. *APOE* polymorphism is associated with both plaque load and neurofibrillary tangle formation.

The identification of the β-amyloid precursor protein gene (APP) mutations which cause AD has strongly implicated mismetabolism of APP as a central event in the aetiology and pathogenesis of the disease [40, 41]. One theory of later years presumes that Alzheimer pathogenesis is triggered by changes in the metabolism of APPp and derived Aβ (amyloid β-protein) as primary events – the amyloid cascade hypothesis of AD – and that tangles develop later in the pathological cascade [42]. This hypothesis, which posits a central role for Aβ in the aetiology and pathogenesis of the disease, has been proposed as a number of observations support a pivotal role for the Aβ peptide in AD.

The Swedish *APP* 670/671 double mutation has proved to be an important tool in validating the amyloid hypothesis [43]. In studies where *APP* with the Swedish mutation was transfected into cultured cells, a greatly enhanced production of total Aβ was demonstrated [44, 45]. The majority of Aβ released from cells was Aβ 1–40, but 10% was slightly longer and consists of Aβ extending to amino acid 42 [46]. Both Aβ 1–40 and Aβ 1–42 were increased in plasma from symptomatic and presymptomatic mutation carriers from the Swedish family. Also cultured fibroblasts bearing different presenilin mutations release increased levels of Aβ 1–42 into the cell medium [47]. Aβ 1–42 has been shown to be the Aβ variant most prone to aggregate, and Aβ 1–42 can also seed aggregation of the more soluble Aβ 1–40 into fibrils [48]. Thus, these studies strongly suggest that altered balance of APPp processing favouring Aβ 1–42 production provides the link between *APP* and presenilin mutations and the clinical Alzheimer phenotype in these families. *APP* and presenilin mutations have one common denominator: they all increase Aβ 1–42 production in culture media or body fluid such as blood [49].

APOEp is the principal apolipoprotein in the brain and is involved in the transport of lipids and cholesterol in the brain, where they are needed for neuronal repair, as well as at the periphery. Synthesis by astrocytes is upregulated in response to inflammation and damage in adjacent neurons. Neuropathologic examination of brains from sporadic late-onset AD patients who were

homozygous for the $\varepsilon 4$ allele has demonstrated a higher amyloid burden than is seen in $\varepsilon 3$ homogygotes for a similar disease duration [50, 51]. The *APOE* $\varepsilon 4$ allele has been associated with death and deposition of Aβ following severe head trauma in young persons [52].

APOE $\varepsilon 4$ gene dosage correlates with an increase in Aβ 1–40 but not Aβ 1–42/43-immunoreactive plaques and with cortical Aβ 1–40 levels, while levels of Aβ 1–42 showed no significant association with genotype [53–56] (the major component of diffuse plaques).

To investigate the role of *APOE* on amyloid deposition, *APOE* knockout mice were generated. These experiments recently provided in vivo evidence that APOEp promotes Aβ deposition in the brain [57]. Mice were created that lacked one or both alleles of the *APOE* gene. These mice, and normal mice with two *APOE* alleles, were crossed with transgenic mice overexpressing a human mutant *APP* gene. These transgenic *APP* mice normally show increased deposition of Aβ in plaquelike formations in the hippocampus and cortex. In the offspring it was shown that lack of APOEp dramatically reduced Aβ deposition. Furthermore, this reduction was seen in a dose-dependent manner in mice with 0, 1 or 2 *APOE* alleles. Levels of APPp and Aβ were unchanged. This suggests that the APOE $\varepsilon 4$p isoform promotes Aβ aggregation, not increases in levels of Aβ. Thus APOEp acts as a strong glue that makes the Aβ fibrils form plaque, and $\varepsilon 4$ is most likely a better glue then E3 or E2.

APOE and tau

Tau is a microtubule-binding protein, and APOE $\varepsilon 3$p (and $\varepsilon 2$p) may play a role in supporting and stabilizing microtubule formation and preventing hyperphosphorylation of tau [58]. The APOEp $\varepsilon 4$ isoform (without cysteine residues at positions 112 and 158) does not bind tau. Hence, tau-tubulin interactions are weaker, potentially compromising neurons. This hypothesis is supported by the following two studies: The tau concentration measured by enzyme-linked immunosorbent assay (ELISA) is elevated in cerebrospinal fluid (CSF) from AD patients [59], suggesting that tau in CSF may be a useful biochemical diagnostic marker for AD. Of 18 AD cases studied in a follow-up study with a mean interval of 14 months [60], 12 demonstrated increased values of tau in CSF obtained at two occasions, and 6 showed decreased values. All AD patients with increased tau were $\varepsilon 4(+)$ carriers. Of the AD cases with decreased tau levels, only three individuals were $\varepsilon 4(+)$. This was a statistically significant difference ($P < 0.05$).

Furthermore, disease duration and cognitive functioning were investigated in relation to CSF tau levels and *APOE* genotypes in members of the Swedish mutation family and in two *PS1* mutation families, M146V and H163Y. Normal and abnormal cognitive functionings were quantified using eight neuropsychological tests. There was a highly significant correlation between long duration of disease and high tau levels in $\varepsilon 4(+)$ mutation carriers ($r = 0.980$; $P < 0.01$), but not in $\varepsilon 4(-)$ mutation carriers. Furthermore, highly significant correlations were found between impaired cognitive functions and high tau levels in $\varepsilon 4(+)$ mutation carriers, but not in $\varepsilon 4(-)$ mutation carriers. The highest correlation was found for block design ($r = 0.985$) [61]. These findings suggest that there may be APOEp isoform-specific differences in tau regulation in AD, as previously hypothesized.

Regional brain AD pathology

Considering the evolution of AD pathology, *APOE* polymorphism influences the occurrence of late-onset dementia by determining the rate at which the regular regional spreading of AD pathology proceeds [62–65]. The earliest stage I–II lesions are characterized by transentorhinal and entorhinal neurofibrillary changes. Stages III and IV are marked by severe involvement of both the entorhinal and transentorhinal regions. Isocortical destruction occurs during stages V and VI. This progression in cortical pathology correlates with the gradual worsening of clinical symptoms.

Subjects already expressing stage I–II lesions at ages 20–40 usually are $\varepsilon 4(+)$ and those without these lesions at advanced ages are usually $\varepsilon 4(-)$ (H. Braak, personal communication). Ohm et al. [64, 65] observed a significant positive correlation between both neurofibrillary changes and β-amyloid deposits and the $\varepsilon 4$ gene dose, estimating that each occurrence of an *APOE* $\varepsilon 4$ allele leads to an earlier onset of the histopathological process of about 1 decade – a result in good agreement with earlier clinical studies [13]. These findings implicate *APOE* alleles in the timing of the earliest expression of AD pathology and its subsequent evolution. They do not indicate that the sequenced spreading of neurofibrillary changes is altered.

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