APOLIPOPROTEIN E ALLELES AS RISK FACTORS IN ALZHEIMER’S DISEASE

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KEY WORDS: genetic, linkage, tau, MAP2, aging, etiology, pathogenesis

ABSTRACT
Alzheimer’s disease (AD) is unique in medicine in that millions of people suffer from what appears to be the same form of disease, and unlike most other late-onset diseases, the genetic etiologies have been well identified. Three early onset forms of AD inherited as autosomal dominant traits account for less than 2% of prevalent AD. A major susceptibility locus, apolipoprotein E (APOE, gene; apoE, protein) is associated with risk and age of onset distributions for the common familial and sporadic late-onset AD. The identification of additional genetic susceptibility genes in the etiology of AD and the metabolic mechanisms leading to differences in age of onset and disease pathogenesis are active areas of current research.

INTRODUCTION
Late-onset Alzheimer’s disease (AD) was the first complex, common disorder for which a major, previously unsuspected susceptibility gene affecting the biology of the disease was identified by using linkage and positional cloning technology (1–3). The ability to discover many genes inherited as Mendelian traits by positional cloning (reverse genetics) is one of the remarkable landmark achievements of the past two decades. Thought by many during the 1980s to be impossible, genetic mapping of disease genes is now a commonplace event (4). The central strategy involves the use of highly polymorphic...
DNA markers to delineate a chromosomal region flanked by polymorphisms that are identified through recombination events. This works well for diseases inherited as Mendelian recessive or dominant traits, but it had never been applied successfully to common diseases. Many highly prevalent diseases are now thought to have significant genetic components. AD is the most common form of dementia affecting adults older than 55 years and is also seen aggregated in large families, especially those with large groups of brothers and sisters living into the age range of risk.

Significant improvements in genetic epidemiologic analysis, coupled with the development of relatively dense chromosomal maps, allows identification of chromosomal regions of linkage if informative clinical evaluations of patients’ families are available (4). Table 1 delineates the genes identified for the three major early onset (35–60 years), autosomal dominant forms and for apolipoprotein E (APOE), the susceptibility gene locus for late-onset (55 and older) AD affecting the risk and age of onset distribution in the population (2, 5–9). Each of the early onset genes, amyloid precursor protein (APP), presenilin I, and presenilin II, are integral membrane proteins that probably interact with the apolipoprotein E protein (apoE) within the endosomal-lysosomal pathways of neurons.

NEW LINKAGE STRATEGIES IDENTIFIED APOE

Pericak-Vance et al were the first to apply the Affected Pedigree Member (APM) analysis to a late-onset complex disease, AD (1, 10). This method has the advantage of incorporating family members other than affected siblings, including cousins, uncles, etc. Such family structures are relatively common, but it usually requires special effort to put together a family history, develop contacts within a family, and do the extensive fieldwork needed to examine and collect DNA from family members who live in distant locations. Only

<table>
<thead>
<tr>
<th>Type</th>
<th>Chromosome</th>
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<tr>
<td>Early-onset familial, autosomal dominant mutation, AD1</td>
<td>21</td>
<td>APP</td>
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<td>Late-onset familial and sporadic associated susceptibility gene, AD2</td>
<td>19</td>
<td>APOE genotype</td>
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<td>Early-onset familial, autosomal dominant, AD3</td>
<td>14</td>
<td>presenilin I (S182)</td>
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<td>Early-onset familial, autosomal dominant (Volga-German founder and other), AD4</td>
<td>1</td>
<td>presenilin II (E5.1, STM2)</td>
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<td>Other late-onset susceptibility genes</td>
<td>Several reported, but unconfirmed</td>
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affected individuals are used in the analysis, and the method looks for statistically significant variations of polymorphic alleles from the general population. Because screening is rapid and computationally simple, potential false positives can be turned to an experimental advantage. In regions of actual linkage, nearby probes also may provide a positive APM statistic. Thus, the method allows for the identification of a relatively large physical DNA region of linkage without clear outer boundaries (cross-overs) mapped by standard likelihood [LOD (logarithm of the odds) score] linkage analyses. In practice, the APM method focused AD to a linkage region consisting of approximately 0.2% of the genome on chromosome 19, reducing by 500-fold the amount of genomic DNA to be considered (1, 10). In practice, if pedigrees are collected, it is possible to incorporate several linkage methods simultaneously to try to find an area of linkage for a susceptibility gene.

APOE had not been previously considered in pathogenic hypotheses for AD. The gene had been mapped to chromosome 19 in the mid-1980s (11). When linkage of late-onset AD to chromosome 19q was found in 1990, there were several candidate genes to consider, including growth factors, proteases, and other possibilities defined by conventional wisdom. Great etiologic legends could be constructed to implicate many genes in this region. A relevant role for apoE in AD has not been previously considered in any hypothesis.

IDENTIFICATION OF APOE

In an independent series of biochemical experiments, Strittmatter et al identified a protein, apoE, that exhibited variable binding to the amyloid ß-protein fragment (Aß) of the APP gene, one of the peptides characteristic of AD plaque pathology (2, 12). apoE had no known specific relationship to AD, but it was found to be deposited in each of the specific amyloids formed by distinct proteins in several diseases. apoE was just one of many proteins found in neuritic plaques in AD. Because the APOE gene was one of the first genes to be mapped to chromosome 19q and was in the middle of the linkage region delineated by Pericak-Vance et al (1), the next step was to look for an association between the disease and the commonly expressed alleles.

APOE has three common alleles, designated ε2, ε3, and ε4. ε4 has an arginine at positions 112 (of 299) and 158, ε3 has a cysteine at position 112, and ε2 has cysteine residues at positions 112 and 158 (11). Well-established DNA and protein protocols are used to determine APOE genotypes or apoE phenotypes. ε4 is not a rare gene; the allele frequency (percentage of all chromosomes) is approximately 0.15 in the United States, with approximately 30% of individuals carrying at least one ε4 allele. ε2 is the least commonly
inherited allele, but certainly not uncommon: the allele frequency is approximately 0.07 with more than 10% of the population inheriting at least one ε2 allele. The most common allele is ε3 (3, 13).

When an association of the APOE genotypes with AD was examined in familial late-onset AD, the ε4 allele frequency was found to be 0.50, compared to 0.16 for age-matched controls (2). Saunders et al then looked at a very large series of 176 autopsy-confirmed sporadic patients with AD who had the clinical syndrome and pathological confirmation, but no known family history (3, 14–16). The ε4 allele frequency in this sporadic series was 0.40, highly significantly different from controls. Several additional series were examined, including (a) a prospectively ascertained clinical series of 80 consecutive patients whose diagnoses were possible/probable AD, who were attending the Bryan Center Memory Disorders Clinic with control spouses; (b) the first affected twin in a series of independently ascertained twins; and (c) a series of patients randomly selected from early onset AD pedigrees (17). In both additional late-onset series, the ε4 allele frequency was 0.40 and 0.41, respectively, whereas the early onset families did not show an ε4 association. The ε4 association with AD has now been widely confirmed throughout the world by approximately 100 laboratories (18–25). The variability of the worldwide data depends more on the control ε4 allele frequency in each population and the ascertainment biases for collecting AD cases, but the associations with AD are consistent (26, 27). The control ε4 allele frequency in Japan is <0.09 in several series, but the ε4 allele frequency is >0.24 in all series (22, 25, 26, 28, 29). Some early data raised issues of differences in African-Americans, but these data remain unconfirmed (17, 30, 31). Later series have demonstrated that the ε4 allele frequency in African-Americans is also increased similar to the Caucasian populations (32). Epidemiologic studies are just beginning to reach publication. The first involved a population in Eastern Finland where the known ε4 allele frequency was quite high, 0.22 (33). However, in a cross-sectional population-based epidemiologic study, the AD patients had an allele frequency of 0.36; age-matched controls had a frequency of 0.165. It was thought that the known high incidence of fatal myocardial infarction in this population culled out many ε4 carriers before the age of risk for AD.

APOE AS A GENETIC SUSCEPTIBILITY LOCUS FOR AD

In 42 late-onset families in which all individuals 60 years of age or older were analyzed, Corder et al demonstrated that individuals with two ε4 alleles had an increased risk and earlier age of onset than individuals who inherited one ε4 allele (34). Similarly, with one ε4 allele, the onset of disease was earlier than
with no ε4 alleles. Thus, the effect of ε4 was dose related, not an autosomal dominant trait as had frequently been assumed in virtually all prior LOD score analyses (4).

In an expanded series containing more than 700 familial and sporadic AD patients, as well as age- and sex-matched nonaffected controls, Corder et al then demonstrated that ε2 delays the onset and decreases the risk of AD (35). Figure 1 illustrates a series of curves estimating the proportion of individuals remaining unaffected at each age as a function of APOE genotype (35–37). Subsequent studies have examined patients under 60 years of age and demonstrated that inheritance of ε4 also accounts for a significant proportion of AD in this earlier onset group (38, 39). For purposes of illustration, the autosomal dominant AD1 and AD3 curves are also included (5, 6).

The analyses of the age of onset as a function of APOE genotype provides some insight into the biology of APOE and AD (Figure 1). The mean age of onset of AD varies from less than 70 years in ε4 homozygotes to more than 90 years in ε2 homozygotes.

![Figure 1](image_url)  
*Figure 1* Age of onset distribution as a function of inherited autosomal dominant forms of AD and APOE genotypes, the representation of proportion of each genotype remaining unaffected as a function of age. The data for APOE are derived from Corder et al (35). The median age of onset for APOE4/4 is less than 70 years of age, whereas APOE2/3 is older than 90 years of age. Each genotype represents a different proportion of the population. APP mutations and presenilin 1 mutations are rare and uncommon, respectively (5, 6). APOE4/4 represents approximately 2% of the general population; 3/4, 21%; 3/3, 60%; 2/3, 11%; and 2/4, 5%. APOE2/2 is not shown because it represents less than 0.5% and there was only a single control with that genotype.
years in ε2/ε3 individuals (35, 37). These data provide a genetic basis for age as a risk factor for AD. The APOE distributions predict the demographics of AD when applied to populations with varying ε4 allele frequencies. In the United States, the ε4 allele frequency is approximately 15%, so by Hardy-Weinberg equilibrium analyses, approximately 2% of the population is homozygous for ε4 (0.15^2 = 0.02). In Japan, where the allele frequency of ε4 is about 7%, only 0.5% of the population would be ε4 homozygotes (22, 39, 40). Thus, there would be less ε4/ε4 AD predicted for the Japanese population. The allele frequency of ε2 is also less in Japan, so a greater proportion of ε4 carriers would be ε4/ε3. These demographics would also predict that the average age of AD would be 7–10 years older than in the United States. This is exactly what has been found by population studies, an average age of 78 years in Japan versus 70 years in the United States (40).

There also appears to be an interaction of the APOE genotype with the age of onset in families with early onset AD due to mutations of APP. Although there are fewer than 20 known families worldwide with this rare form of AD, an interaction of APOE genotypes on the age of onset has already been reported for three of the largest pedigrees (41–43). Asymptomatic individuals who are at least one standard deviation older than the mean age of onset in the pedigree carry the ε2/ε3 genotype (42, 43). A similar interaction may also be present with the presenilin II gene (44).

IS AD A DISEASE, OR JUST THE INEVITABLE CONSEQUENCE OF AGING?

Figure 1 illustrates the etiologies of AD and the effect of each genotype on the distribution of age of onset. It might be inferred from such data that AD is an inevitable consequence of age. Combined with recent clinical data, however, the etiology and pathogenesis of AD can be differentiated clinically and with brain imaging studies (45, 46). Longitudinal studies have clearly demonstrated that there is a rapid acceleration of clinical dementia associated with structural brain atrophy (47). Figure 2 illustrates the width of the anterior temporal lobe as measured on serial computerized tomography images of the same individuals over several years (48). It is clear that an age-related clinical acceleration occurs, beginning in different patients at varying ages dependent on genetic and environmental factors. Besides age, which is a function of APOE genotype, the ε4 allele is associated with less favorable outcomes as a result of head injury, anesthesia, and/or cardiac by-pass, and recovery from other stresses such as intracerebral hemorrhage (49–52).
FOUR STAGES OF AD

Etiology (Anticipating Metabolism)

The pathogenesis of AD is triggered by genetic and/or environmental factors, with an earlier age of onset in those individuals with particular genetic constitutions. The initial stages are subclinical, with recent evidence suggesting altered brain metabolism at least two decades before the predicted onset of disease (45). Subjects with mild memory complaints but normal cognitive performance on neuropsychologic tests, and who had at least two relatives...
with AD, were studied using positron emission tomography to examine cerebral glucose metabolism (45). Parietal metabolism was significantly lower and left-right parietal asymmetry was significantly higher in at-risk subjects with ε4 compared with those without an ε4 allele. Thus, metabolic abnormalities can be observed in nondemented ε4 relatives at risk who would not be expected to develop AD for another 20 years (see Figure 1, ε3/4 curve).

Staging of the development of Alzheimer-type neuropathology over decades is also consistent with a slow progression and development of tau-related neurofibrillary changes in relevant brain areas. The Braak stages are derived from carefully studied autopsies from the general population characterized as a function of age. Initial changes in the entorhinal cortex of the hippocampus can be observed in the 40- to 50-year age group. Recently, the earlier appearance of neurofibrillary Alzheimer-type pathology was associated with carriers of the ε4 allele (53). The ε4 etiology, as well as each of the autosomal dominant missense mutations, may be viewed as long smoldering triggers, whereas autocatalytic events no longer dependent on the triggers may account for the acceleration of disease.

Clinical Age of Onset (Trigger)

The age of onset of a slowly dementing disease is a notoriously difficult point to establish. The generally used ADRDA-NINCDS clinical criteria do not allow the diagnosis of probable AD until there is evidence of progressive cognitive impairment involving several neuropsychologic areas (14). In practice, many individuals present to physicians for memory problems or mild cognitive difficulties before a diagnosis can be made. Previously, there were no good predictors to identify which patients would develop probable AD. However, in a recent study of patients presenting with mild cognitive difficulties and followed over 54 months, the presence of an ε4 allele was highly statistically associated with those individuals who progressed to dementia (46).

A diagnosis of possible AD usually progresses to probable AD within a year of follow-up. Therefore the age of onset, although not exact, is a surprisingly reasonable measure to use in population studies. In Figure 1, the mean age of onset for each of the APOE genotypes varies by 7–10 years. If the age of onset for each individual is off by a year or so, the variation is too small to affect the shape of these curves significantly, especially since the same difficulty exists for most patients.

By the time a patient meets the criteria for the diagnosis of probable AD, there is usually brain atrophy observed on imaging studies such as computed tomography scanning or magnetic resonance imaging (47, 48) (Figure 2). The use of imaging studies in patients presenting with memory disorders or cognitive impairment for confirmation of brain atrophy, or identifying structural
lesions such as tumors, had been the best evidence that the disease has been active for some undetermined period before “onset.”

DIFFERENTIAL DIAGNOSIS The literature on diagnostic testing can be quite confusing. The differential diagnosis of early cognitive difficulties and dementia is quite distinct from the specificity of certain neuropathologic definitions for AD observed at autopsy in patients who followed a long-term clinical course consistent with probable AD. Differential diagnosis requires the testing for known reversible causes of dementia and the establishing of the diagnoses of those diseases for which effective treatment is available. Physicians commonly use diagnostic instruments for uncommon forms of reversible dementias, such as vitamin B12 deficiency or thyroid disease. Perhaps the most common form of reversible cognitive impairment is that associated with the use of medications in the elderly. Confusion, memory problems, and abnormal behaviors can often be treated by eliminating medications.

The use of APOE testing may only be a major factor in the differential diagnosis of certain symptomatic individuals, especially those with the ε4/ε4 genotype, or 15–20% of AD patients. The operational difference between using APOE genotyping in differential diagnosis from the application of autosomal dominant traits, such as the triplet repeat size of the huntingtin gene in Huntington disease, is that the absence of the disease-associated marker does not rule out the disease. A patient with early dementia and the ε3/ε3 genotype not only could still have AD, but statistically probably does. However, finding the ε4/ε4 genotype in a patient with early dementia raises the odds of AD from the 60–70% a priori risk to 94–98%, increasing the odds with the age of presentation (54). The more common ε3/ε4 genotype (40–50% of AD) raises the odds over 80%, increasing the odds with the age of presentation. APOE genotyping cannot rule out AD. Since the age of risk extends over a 40- to 50-year period, APOE genotyping cannot be used to predict age of onset in asymptomatic individuals until and unless there are supporting ethnic-, racial-, and gender-specific epidemiologic data available (54). Unlike the long history of heart disease epidemiology, these studies are now beginning in AD.

There are now several early reports that effective treatment of AD with specific compounds may be related to APOE genotype. Tacrin, the only FDA-approved medication for AD, had appeared to be effective in a small proportion of patients during clinical testing. A significant range of side effects limits its enthusiastic use in most AD patients. However, recent data have found that the drug-responsive individuals are among those without an ε4 allele (55). In another clinical trial, with a different drug, the responsive individuals seemed to be among those with an ε4 allele (56). The selection of patients for therapeutic benefit may depend on their biological risk factor, APOE genotype. This will be particularly important if drugs have significant side effects. Use of
APOE testing for disease management may supersede questions of use for differential diagnosis of symptomatic patients. Not only will APOE genotyping be useful as a diagnostic adjunct, treatment planning may require APOE testing as standard care.

**Pathogenesis (Progression)**

apoE is immunoreactive with extracellular neuritic plaques, intraneuronal neurofibrillary tangles (NFTs), vascular amyloid deposits, and in some neurons that do not have NFTs (57–60). The interaction of apoE with the major microscopic, phenotypic markers of AD, plaques and tangles, has generated considerable experimental activity. The literature involving the interaction of apoE with Aβ (of plaques) or tau (the major fibrillary component of tangles) is growing rapidly (24, 53, 57, 58). Although it is clear that apoE binds to neuritic plaques and NFTs, there is no compelling evidence to assume that the interactions cause disease rather than being relatively characteristic consequences.

Plaques and tangles are observed at autopsy, usually many years after the clinical onset of symptoms. Brain atrophy, however, is observed around the time of diagnosis in most cases (47, 48). The plaques and tangles are relatively specific markers and are used for accurate diagnosis at the time of autopsy (15, 16). The neuropathologic changes are rarely viewed during the early course of disease. In fact, the density of Aβ deposition in plaques is a function of APOE genotype and time of survival from recognized onset to death (duration of disease). Alzheimer patients who had the ε4/ε4 genotype demonstrated more Aβ deposition for similar duration than patients who had the ε3/ε3 genotype (24, 58). On the other hand, now that many more control autopsy series are defined by normal cognitive functioning prior to death (not simply the presence or absence of amyloid in brains of untested individuals), the association of significant immunoreactive Aβ deposition in cognitively intact individuals has been well recognized (61). The intensity of Aβ deposition is directly related to inheritance of an ε4 allele. Survival is related to the age of onset, not APOE genotype, so that ε4/ε4, ε3/ε3, ε3/ε4, and ε2/ε3 patients with onset at the same age have similar duration of illness (62). Thus, the heavier Aβ deposition observed in the ε4/ε4 and ε3/ε4 patients is not related to survival.

Strong arguments and data exist to demonstrate the gradual accumulation of phosphorylated tau and neurofibrillary accumulations in neurons decades before the onset of disease. Recent data confirm that neurofibrillary changes are associated with the ε4 allele in cognitively intact individuals who died before they were old enough to express late-onset AD (53). The complex phosphorylation of tau protein has been extensively examined, primarily within a hypothetical network that relates the state of tau phosphorylation to the pathogenesis of the disease (63).
The metabolic events leading to the neuropathologic markers of AD are undoubtedly triggered by etiologic genetic factors. Longitudinal clinical and imaging data support the concept of accelerating disease. Proponents of hypotheses involving amyloid deposition or neurofibrillary tangle formation as the causative factors in AD (as opposed to consequences) had invoked cascades of interactions. These autocatalytic, accelerating cascades may well play a role in pathogenesis in the clinical years of the disease. The initiating factors, however, are genetic and involve metabolic interactions of missense mutations of membrane-associated proteins or polymorphisms of apoE.

Terminal Neuropathology (Neuropathologic Diagnosis)

At the conclusion of many years of illness, probable AD patients may be examined neuropathologically. At this time, approximately 10–15% of patients do not manifest the pathology defined by convention for the diagnosis of AD (15, 16). The specificity of the diagnosis is based on the presence of amyloid plaques, whereas other pathologies are defined by description of other recognized lesions. Lewy body disease, Lewy body variant (LBV), Pick disease, uncommon cases of vascular dementia, and other pathologies are categorized at autopsy. It is important to point out for clinicians that neuropathologic diagnosis at the time of autopsy defines a different group of patients then the specificity issues involved with APOE testing at the time of early cognitive impairment and dementia. The ε4 association is also present in LBV, although the LBV patients look like probable AD during life and are treated as AD patients. From a practical point of view, use of APOE testing as a risk factor early in diagnostic evaluations is useful for more than 90% of patients who eventually die from AD and LBV. In fact, it remains to be determined whether the other neuropathologic descriptions may be enriched in ε2 and ε3, thus without prominent Aβ plaques, and are additional phenotypes influenced by the APOE locus and other susceptibility loci.

THERAPEUTIC IMPLICATIONS FOR DRUG TARGETING

Ultimately, the utility of any hypothesis is that it provides the foundation for the development of therapeutic targets. If pathogenic mechanisms involving the protection of tau and MAP2 by ε3 or ε2 are valid, compounds that can enter the central nervous system and mimic their effect may delay the onset of AD (64–66). Compounds that perform this function might also have collateral effects, like interfering with Aβ deposition or reducing the formation of NFTs. Theories of pathogenesis directly suggest the development of in vitro screens for effective compounds. In the case of AD, a relatively modest effect that could functionally protect microtubule-associated proteins from forming paired helical filaments and increase their efficiency for microtubule stabiliza-
tion extending efficient dendritic integrity for additional years or decades. Compounds could be tested for safety in animals and effectiveness in apoE-deficient mice as an animal model for protecting synaptic loss.

**Literature Cited**