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Review

Apolipoprotein E and Alzheimer disease

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ABSTRACT **Inheritance of specific apolipoprotein E (apoE) alleles determines, in large part, the risk and mean age of onset of late-onset familial and sporadic Alzheimer disease. The mechanism by which the apoE isoforms differentially contribute to disease expression is, however, unknown. Isoform-specific differences have been identified in the binding of apoE to the microtubule-associated protein τ, which forms the paired helical filament and neurofibrillary tangles, and to amyloid β peptide, a major component of the neuritic plaque. These and other isoform-specific interactions of apoE give rise to testable hypotheses for the mechanism(s) of pathogenesis of Alzheimer disease. An unresolved issue of increasing importance is the relationship between the structural pathological lesions and the cellular pathogenesis responsible for the clinical disease phenotype, progressive dementia. The identification of apoE in the cytoplasm of human neurons and the characterization of isoform-specific binding of apoE to the microtubule-associated proteins τ and MAP-2 present the possibility that apoE may affect microtubule function in the Alzheimer brain.**

Alzheimer disease (AD) occurs in the later decades of life and is characterized by progressive dementia, the diffuse deterioration of mental function. Thought and memory processes are primarily affected; affective and behavioral changes may follow. The patient with AD experiences gradually increasing forgetfulness, decreasing attention span, and alterations in mood, often with frustration and agitation. As the disease progresses the patient ultimately cannot care for the simplest needs and becomes bedridden, totally dependent on caregivers. The interval between initial diagnosis and death can vary considerably, usually between 3 and 15 years.

In 1984, the work group of the National Institute of Communicative Diseases and Stroke and the Alzheimer's Disease and Related Disorders Association published criteria for the *clinical* diagnosis of AD that were based on features of the patient's history, physical and neurological examination, and laboratory investigations including brain imaging (1). These diagnostic criteria require the exclusion of other causes of memory loss and impaired cognitive function, such as multiple infarcts, intracranial mass lesions, infections, and toxic and metabolic disorders. Fulfilling these clinical criteria permits the diagnosis of *possible* or *probable* AD.

By the defined diagnostic criteria, the diagnosis of *definite* AD can be made only by microscopic examination of brain tissue, at either biopsy or, more commonly, autopsy. The neuropathologic criteria require the presence of neuritic plaques and neurofibrillary tangles (NFT), at specified densities (2). The diagnosis of definite AD has therefore been defined by phenotypic neuropathological findings. The relationship between the neuritic plaques and NFT and the mechanism causing Alzheimer dementia is an unknown and controversial subject. Discovering the relationship between AD neuropathology and the pathogenesis of the disease is a central issue in developing and testing hypotheses to uncover the molecular and cellular mechanisms that ultimately result in the dementia.

APOE and AD

The delineation of the apolipoprotein E gene (*APOE*, gene; apoE, protein) in familial and sporadic AD can be viewed from two different vantage points: the emerging revolution of molecular genetics and the existing science of AD. The identification of *APOE* as a relevant genetic locus affecting the age of onset of AD represents an evolution of gene mapping technology developed over the past decade. This linkage involved the first application of nonparametric techniques to identify interesting chromosomal regions for susceptibility loci (3).

There are three common alleles of *APOE*. *APOE3* is the most common allele representing ≈78% of all chromosomes; *APOE4*, 15%; and *APOE2*, 7%. The proportion of different *APOE* alleles varies between racial and ethnic groups, particularly with regard to the relative proportions of *APOE2* and *APOE4* (4–7). In 1993 Strittmatter *et al.* (8) reported the association of *APOE4* with late-onset familial AD. Saunders *et al.* (9) extended the *APOE4* association to 176 autopsy-verified sporadic AD patients and found that although the United States Caucasian control population allele frequency of *APOE4* was 0.16, that of the AD patients was 0.40. These data have been confirmed and extended to AD groups in populations around the world. In Japan, for example, multiple investigators have demonstrated a lower *APOE4* allele frequency (<0.09) in the control population and have uniformly confirmed an increased *APOE4* frequency to >0.25 in AD patients (6, 7, 10, 11). Similar racial variations in the control African-American and Hispanic *APOE4* allele frequency have been confirmed, as has its association with AD (5).

Corder *et al.* (12) demonstrated a dose effect of the inheritance of *APOE4* on the distribution of age of onset in familial AD. Each *APOE4* allele inherited increases risk and lowers the distribution of the age of onset. In a series of familial AD families, sporadic AD patients, and case controls, Corder *et al.* (13) also showed that the inheritance of an *APOE2* allele decreases the risk and increases the mean age of onset. The mean age of onset of AD for individuals inheriting the *APOE4/4* genotype (≈2% of the age-matched population) is <70 years, while the mean age of onset for *APOE2/3* individuals (≈10% of the age-matched population) is >90 years. (*APOE2/2* represents <0.05% of the population and insufficient data were available for the onset distribution curve.) Thus the *APOE* genotype differences account for more than two decades' difference in the rate of disease expression.

Inheritance of alleles at the *APOE* locus therefore influences the rate of clinical expression of AD dementia (12–14). Differences in the distribution of age of onset of AD as a function of the *APOE* genotype provide the basis for the common acceptance of age as a risk factor. These data represent the biology of AD and will allow predictions regarding the risk of AD within populations based on different allele frequencies of *APOE* once proper epidemiological studies are completed for different races and ethnic populations. However, while the role of *APOE* in AD has been widely confirmed, the genetic

Abbreviations: AD, Alzheimer disease; apoE, apolipoprotein E; Aβ, amyloid β; NFT, neurofibrillary tangles.

data do not explain the mechanism(s) of pathogenesis. Hypotheses relating *APOE* allele-specific mechanisms in AD can now be tested.

Pathology and Pathogenesis of AD

Since 1907, when Alzheimer first described this clinical phenotype, investigators have attempted to understand the pathogenic mechanism at the cellular and molecular levels. A variety of recognizable neuropathological structures, including neuritic plaques, sometimes associated with amyloid, NFT, and paired helical filaments, are commonly observed in the AD brain. These neuropathologic structures, described later, are frequently used to set the limits for diagnosis and for investigating the pathogenesis of the disease. These neuropathological phenomena are phenotypic manifestations associated with the expression of disease, but their role in the pathogenesis of the disease is unknown.

Neuritic Plaques as a Phenotype

Neuritic plaques are required, by definition, for the diagnosis of definite AD (2, 15). Neuritic plaques are extracellular structures with complex molecular and cellular constituents. Plaques contain amyloid β (Aβ), a peptide of 39–43 amino acids, that is produced by proteolytic cleavage of the amyloid precursor protein (APP) in its normal metabolism (for review see ref. 16). β-pleated sheet fibrils of Aβ interact with Congo red dye or thioflavin silver stains to produce the defining amyloid appearance. Aβ peptide aggregates in these structures. Other proteins found in the neuritic plaque include apoE (8, 17, 18), APP, α-1-antichymotrypsin, IgG, several complement proteins, amyloid P, and glycosaminoglycans and Sp40,40 (19). The complete molecular composition of the fibrillar structures in the plaque, the mechanism of assembly, and their role in the disease are unknown. Neuropathological diagnosis of AD requires a certain number of neuritic plaque counts per microscopic field to meet established criteria for definite disease (2). These criteria do not consider the density or size of plaques.

Some individuals may present clinically as probable AD but may lack sufficient plaques for a definite diagnosis. A variant "tangle-only" AD has been described with the clinical phenotype of AD, with NFT, but with no neuritic plaques. Other patients with progressive dementia have other pathological features such as Lewy or Pick bodies and are given other neuropathological diagnoses. Overlap cases with both Lewy bodies and amyloid plaques have also been described (20). The classification of disease(s) with similar clinical manifestations, but with a variety of neuropathologic phenotypes, is a source of much current discussion and confusion in the literature.

The *APOE* genotype affects the risk of AD and the mean age of onset, acting as a susceptibility gene (21). The effects of *APOE* genotype are on the rate of disease expression, clinical dementia, and neuropathological markers. Aggregate data compiled in many laboratories during the past year demonstrate that *APOE4* is related to the earlier presence and greater density of amyloid plaques in patients meeting criteria for AD (22). The *APOE4* allele frequency is increased in categories of dementia where the plaque counts are insufficient for the diagnosis of definite AD. For example, the *APOE4* allele frequency is only slightly less than that observed in AD in the overlap syndrome of Lewy body dementia (23, 24), which has sparse amyloid plaques.

If AD patients homozygous for *APOE4* or *APOE3* are compared by selecting patients with the same age of onset of dementia, survival is a function of the age of onset, not amyloid load. Although patients homozygous for *APOE4* have denser and larger amyloid plaques, their survival is not different from patients homozygous for *APOE3*. Deposition of Aβ relates to the *APOE4* allele as a phenotypic variable that is independent of the course of AD.

NFT as a Phenotype

NFT are dense bundles of long unbranched filaments in the cytoplasm of some neurons. These filamentous structures are paired helical filaments. Each filament is 10 nm in width, and two filaments are helically twisted about each other with a periodic full twist every 160 nm. NFT may be so dense they distort the neuronal cell body and displace the nucleus. Paired helical filaments may also be found in neurites undergoing degeneration. The filaments consist primarily, and probably exclusively, of the microtubule-associated protein τ. For review see ref. 25.

τ normally binds and stabilizes microtubules and promotes the assembly of microtubules by polymerizing tubulin. Microtubules are necessary for neurite extension and maintenance and for the transport of materials along the axon and dendrites in both orthograde and retrograde directions. In AD, τ becomes abnormally phosphorylated and self-assembles into the pathological paired-helical filaments forming NFT (26–28).

The topology and abundance of NFT in the brains of patients who meet neuritic plaque criteria for AD relate better to the dementia than do the plaques (29, 30). This observation has fueled debate, particularly during the past decade, when plaques became the defined currency of diagnosis. Some forms of dementia have tangles but insufficient plaques for the diagnosis of AD. NFT are also present in neurons in other neurodegenerative diseases. NFT may represent a phenotype common to several neurodegenerative diseases and, rather than being nonspecific, provide clues to a common pathogenesis.

An important question is whether NFT themselves cause neuronal death or are simply phenotypic manifestations of dying cells. Arguments that NFT kill cells are aided by the rather obvious presence of this collection of intrusive material within the neurons. This association, however, does not provide evidence of pathogenesis. Recent studies have shown that apoE exists in many neurons in the hippocampus, only some of which contain NFT (31). Since it is necessary to explain the primary role of a susceptibility gene, *APOE*, in AD, the apoE-containing neurons may represent a stage in the life of the neuron, with fully formed NFT representing one of the end points shortly before death. The age of onset of clinical disease is the variable most associated with inheritance of different *APOE* alleles (12, 13). The number of NFT at the time of death did not differ in AD patients with *APOE4/4* or *APOE3/3* genotypes (22, 32).

Testing of One Hypothesis

The effect of the inheritance of *APOE* genotypes has been widely and rapidly confirmed in >60 laboratories around the world. These confirmations do not, however, provide any additional data concerning the pathogenesis of disease. Multiple hypotheses can, and will, be generated. We hope that the testing of these hypotheses will provide insight into the mechanism of disease expression and opportunities to interdict with safe and effective therapies.

ApoE may perform multiple metabolic functions in the brain. ApoE is in the extracellular space as a free and as a bound protein. It is also found within some neurons, both in the cytoplasmic space and in the intravesicular space of endosomes, lysosomes, and peroxisomes (31, 33). This multitude of sites suggests multiple metabolic functions. Not all of the functions of apoE may be relevant to its role in AD. Interactions that are qualitatively or quantitatively different involving the apoE isoforms are candidates for developing hypotheses of how the various *APOE* alleles differentially regulate disease expression. Several isoform-specific functions of apoE have already been characterized. These isoform-specific interactions include binding with the low density lipoprotein receptor, with Aβ peptide (34), and with the microtubule-associated proteins τ (35) and MAP-2 (36). The apoE

isoforms differentially determine neurite extension in cultured neurons (37). Other isoform-specific interactions and functions of apoE in the brain will be identified.

The isoform-specific interactions of apoE with the microtubule-associated proteins τ and MAP2c form the basis of a testable hypothesis (38). These microtubule-associated proteins assist in the assembly and maintenance of microtubules in the neuronal cell body, axon, and dendrite. *In vitro*, apoE2 and apoE3, but not apoE4, bind to the microtubule binding domains of τ and MAP2c. Binding of these microtubule-associated proteins by apoE2 and apoE3 may stabilize their interactions with β-tubulin and thereby stabilize the microtubule. Binding of apoE2 and apoE3 to τ may also inhibit the ability of τ to self-associate in the formation of paired helical filaments. Time-dependent failure of microtubule structure and function can lead to loss of normal transport functions, withdrawal and simplification of synapses, accelerated death of neurons, appearance of NFT in the sickest cells, and neuritic plaques in sites of distal dendritic atrophy. Because of an independent difference in the interaction of the apoE isoforms with Aβ, differences in plaque density are also observed as another phenotypic consequence.

Summary

Considerable genetic evidence supports the role of *APOE* in the distribution of age of onset of AD. Testing of hypothetical mechanisms is just beginning. The examination of apoE isoform-specific biology and biochemistry will shed considerable light on the mechanisms of pathogenesis of AD. By using a genetic framework, phenotypic manifestations associated with disease expression can also be explained. Testing mechanisms of pathogenesis will lead to the identification of putative targets for therapeutic intervention.

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