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Apolipoprotein E Affects the Rate of Alzheimer Disease Expression: β-Amyloid Burden Is a Secondary Consequence Dependent on APOE Genotype and Duration of Disease

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"... a walled city ... bones of contention and matters of disagreement ... an investigator ... tends to be explaining the known by the unknown, the specific by the unspecific. His identification of the normative principles may be so vague as to be universally useful, i.e. anything and everything becomes explicable. ..."

Robert M. Pirsig in *Lila, An Inquiry into Morals* (29)

Background

The present forum for simultaneous publication of apparently conflicting hypotheses about the pathogenesis of Alzheimer disease (AD) provides the opportunity to clarify data, beliefs, and preferences in a manner usually unavailable in the academic literature and frequently inaccurate and misleading in the lay literature. Specific genotypes of apolipoprotein E (APOE, gene; apoE, protein) are associated with the rate of disease expression of AD (1, 2). The genetics of APOE implies nothing about the molecular pathogenesis of AD except that the inheritance of specific APOE alleles is genetically relevant to the biology of disease expression. How apoE interacts phenotypically is at the level of hypotheses, with the knowledge that these hypotheses are biologically relevant to the age of onset distributions in the population. With the exception of less than 20 families of early-onset AD with amyloid precursor protein (APP) mutations, the deposition of β-amyloid (Aβ) in AD may be viewed as a common phenotypic expression, but one that is not genetically relevant (3, 4). As will be discussed, even in patients with APP mutations it is also a great leap of faith to ascribe increased Aβ deposition as the proximal cause of AD. This contribution will discuss AD in the mainstream of modern molecular genetics and assess current definitions, data, and hypotheses related to APOE and Aβ.

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Definitions as a Source of Semantic Controversy

Senile plaques (SP), Aβ deposition, paired helical filaments (PHF), neurofibrillary tangles (NFT), and granulovacuolar degeneration are pathological manifestations associated with a consistent clinical pattern of signs and symptoms that we call AD. The most striking pathological finding is seen at postmortem examination when the weight of the brain can be reduced by one-third or more in AD patients. Figure 1 illustrates cellular differences between control and the shrunken, simplified granule cells of the hippocampus observed commonly in AD patients (5).

The clinical definition of possible or probable AD was agreed to by a group of experienced physician-scientists to provide a common working definition for research purposes (6). A second working group defined criteria for the neuropathological diagnosis of definite AD (7). These criteria established that a certain number of senile or neuritic plaques per high power field, relative to patient age, was necessary for the diagnosis of definite AD. This automatically resulted in the ascendancy of neuritic plaques as the gold standard for diagnosis, even though there has been a better correlation of NFT with degree of cognitive impairment (8, 9). Although hypotheses incorporating plaques or tangles may be reasonable and appropriate for research purposes, there are no compelling *a priori* implications for causation of either phenotypic marker.

The Amyloid Hypotheses through the Eyes of a Non-believer

Statements such as "accelerated beta A4 deposition is an early and critical event" (10) and that "beta A4 amyloid protein is now understood to play a pivotal role in the development of Alzheimer's disease" (11) are common. The possibility that Aβ deposition may be a common bystander, not an agent provocateur, is not popular. In logic, a tautology is true by virtue of the statement itself. Amyloid deposition has been tautologically defined as relevant to AD (10–12). Because all definite AD patients have amyloid plaques by definition, it is therefore concluded that "the central pathological event in Alzheimer's disease is the deposition of Aβ as amyloid fibrils within the senile plaques and cerebral blood vessels" (12). It is commonly stated that amyloid deposition causes AD. The term "cause" is used too casually as a verb

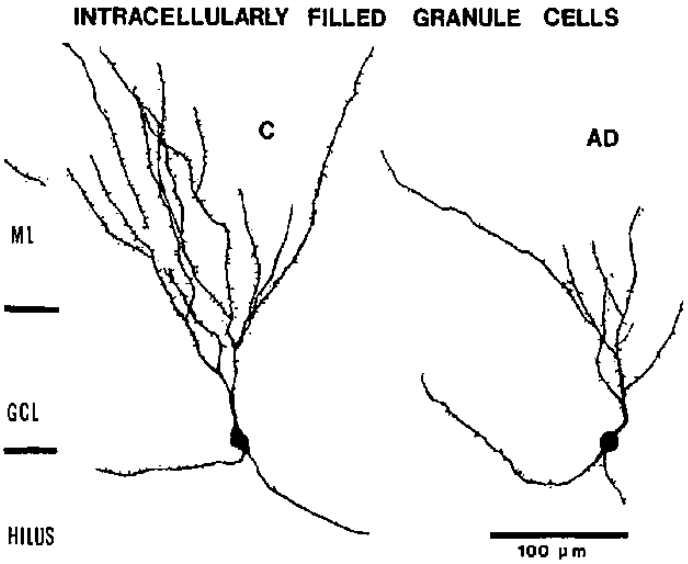


Fig. 1. Camera lucida drawings of Lucifer yellow-filled granule cells from a 66 year old man with cancer and no neuropsychological evidence of AD (C) and a 71 year old woman with AD (AD). Note how the dendritic tree of the granule cell in the control is much fuller, longer, and bears more spines than the granule cell in the AD patient. ML = molecular layer, GCL = granule cell layer. Reproduced from Einstein et al (5) with permission.

in medicine, especially when biochemical and physiological mechanisms are unknown. It might better be said that because all definite AD patients are defined by numbers of SP, SP are associated with all patients meeting diagnostic criteria.

It is here that a major logical fuzziness has occurred: the blurring of the distinction between the terms "senile plaques" and "amyloid plaques" that permeates the literature. The neuropathological definition, known as the Khachaturian criteria, was established for the purpose of consistency in diagnosis. These criteria state that a certain number of SP per high power field per age group was necessary to achieve the pathological diagnosis of definite AD (7). The criteria were "intended to start a process for establishing consistent and accurate pathologic diagnosis. The criteria were meant to be re-examined and modified as more data became available. They were never intended to be a lead standard, let alone a gold standard" (Khachaturian Z., personal communication, 20 April 1994). Despite the intent, all definite AD patients are currently classified by SP. Even though SP without amyloid cores are frequently observed, the **senile plaque** definition has been subtly obfuscated to state that all AD patients have **amyloid plaques**—therefore **amyloid** must cause AD. Many authors have implicitly or explicitly stated and recorded such views (10–12).

At the time that the Khachaturian criteria were adopted, Aβ had not yet been isolated nor sequenced. The amyloid precursor protein and its gene (APP) were unknown and antibodies to Aβ did not exist. The original diagnostic criteria were based on silver staining methods that defined degenerating neurites. It is now well known that there can be many different component proteins associated with these plaques, only one of which is the Aβ peptide. Somewhere along the line the common usage of the terms "senile" or "neuritic" plaques became interchangeable with amyloid plaques and Aβ deposition. The incorporation of positive staining of amyloids with Thioflavin S into the more recent CERAD criteria contributed to the confused impression that SP and amyloid plaques are equivalent (13). Figure 2 demonstrates that this is not always the case, illustrating pathological data from two genetically well-defined siblings with early-onset AD due to inheritance of the APP717val-ile mutation (4). In particular, the patient with a paucity of Aβ deposition illustrates two important points: 1) SP density can be quite distinct from Aβ plaque density; and 2) neither the disease course nor the presence of the APP mutation were directly related to the amount of Aβ deposition (see below). Conversely, the APP670,671 "Swedish" mutation has been demonstrated to produce more Aβ in *in vitro* experiments in which human neuroblastoma cells were transfected with constructs expressing the mutant APP. To date there have been no data to support overexpression of APP or increased Aβ release from tissue culture cells derived from APP717 or other APP mutation patients, or any mechanism other than increased Aβ deposition proposed (14).

It is well recognized that extreme variations of Aβ deposition can commonly be observed in different patients and in non-demented individuals meeting the pathological criteria for AD. Comparisons of immunoreactivity using Aβ antibodies demonstrate that there can be large variations between patients in the size and density of plaques (15–21). Most authors rationalized the variations with reference to unknown genetic and/or environmental factors. Hyman et al (15) suggested that there could be a dynamic balance between deposition and resolution. The amyloid burden can now be re-examined by comparing patients with defined APOE genotypes. However, clinical series would be biased because of differences in the relative proportion of each genotype in sporadic AD groups. Studies comparing equal numbers of each APOE genotype to measure amyloid burden and duration as genotype-specific variables would be useful to evaluate worldwide (1, 21). Figure 3 illustrates ten brain samples from patients with two genotypes, APOE3/3 and APOE4/4, with highly variable Aβ immunoreactivity (21). If these samples were randomly mixed the variability of Aβ deposition would be indecipherable. However, the samples are paired for duration of disease with the left hand column from APOE3/3 patients and the right hand column from APOE4/4 cases. The amyloid burden can be correlated with APOE genotype and duration of disease.

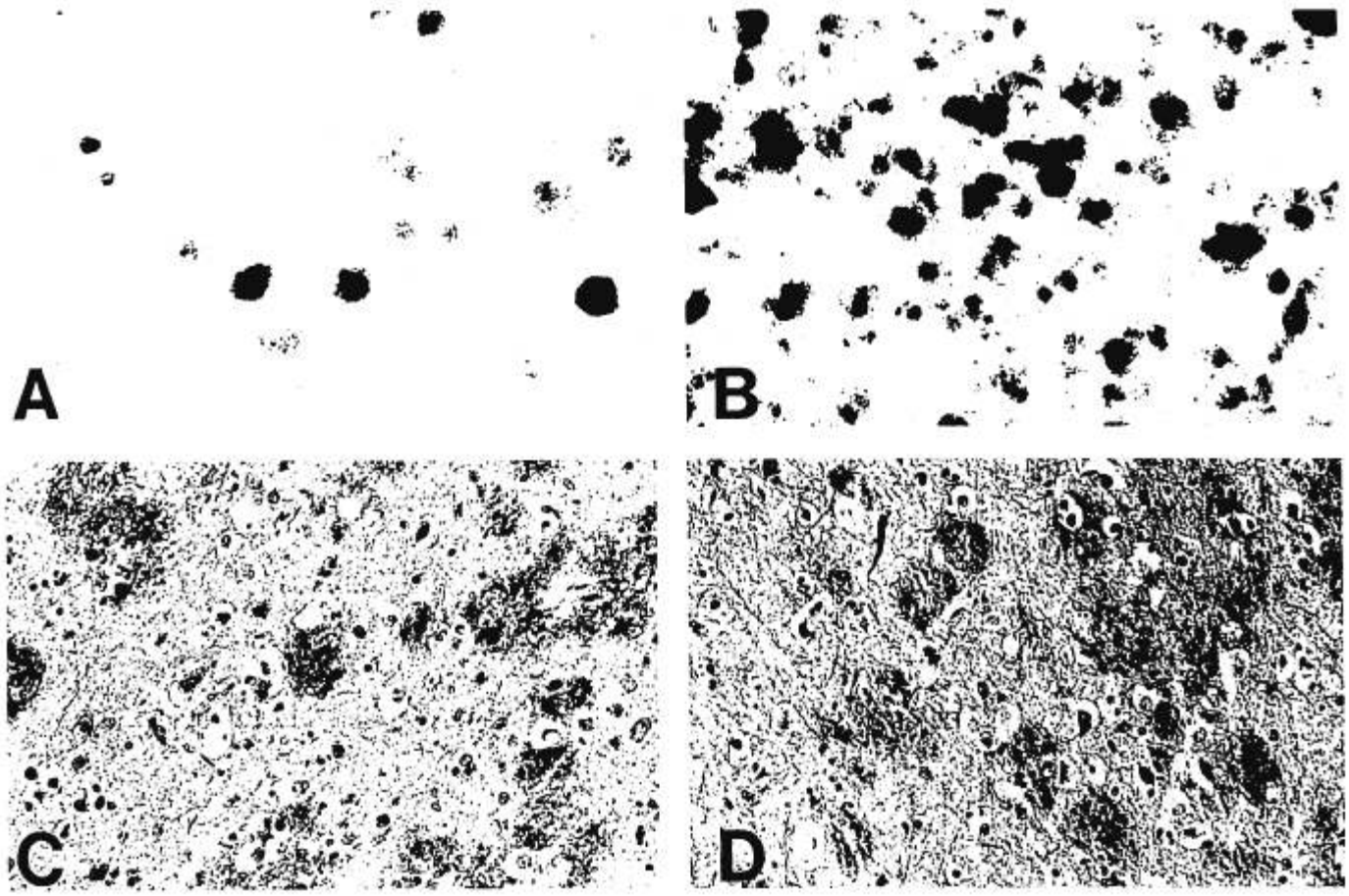
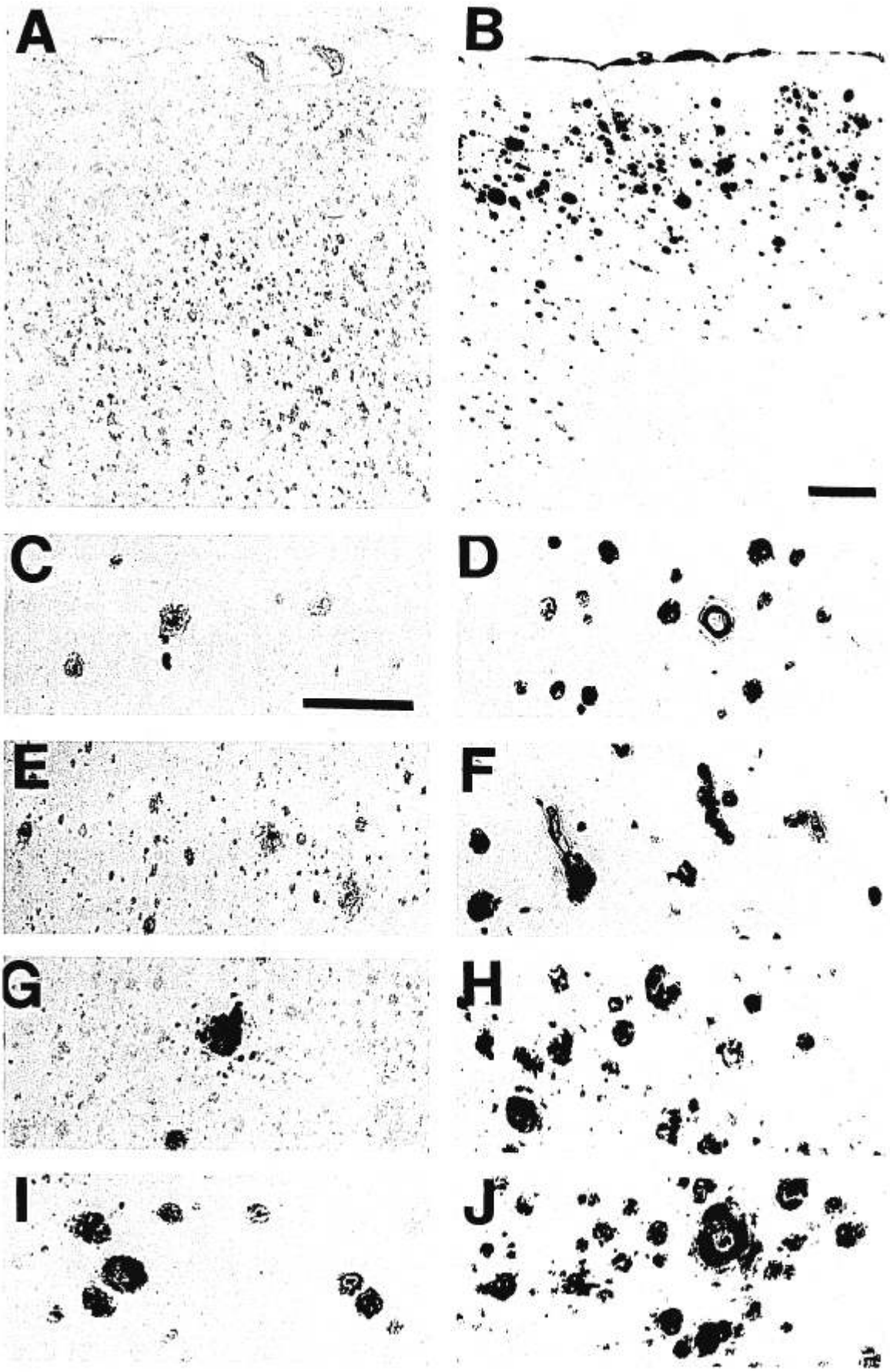


Fig. 2. Silver staining and immunocytochemistry for Aβ in brains of female siblings with early-onset AD, both of whom inherited the rare APP717val-ile mutation. Striking differences between the Aβ immunostained sections of hippocampus (4) and frontal cortex (A, B) are observed in the patient 372-0106 compared to the other sibling, 372-0102. Aβ immunoreactivity is extremely sparse in patient 106 while quite dense in 102. Both had a similar density of neuritic plaques stained with silver stains (C, D). Despite the difference in Aβ deposition, the age of onset at 49 years was identical and the clinical courses were similar. Both patients have an APOE2/4 genotype.

It is important to incorporate survival in the analysis of Aβ deposition. At first glance the increased Aβ deposition in homozygous APOE4 patients with a younger age of onset distribution would seem to support a theory of more rapid pathogenesis based on Aβ toxicity. Our data clearly demonstrate that survival is related to the age of onset, not to the inheritance of the APOE4 allele: the earlier the onset, the longer the duration of illness (as well as the anticipated life expectancy; Corder et al., submitted for publication). Thus, although risk and age of onset distribution are related to the inheritance of the APOE4 allele, survival (duration of disease after onset) is not. Despite the fact that APOE4/4 patients have more amyloid burden earlier, survival is not affected by the Aβ load because they live as long as APOE3/3 patients with onset at the same age. Aβ deposition is a consequence of disease expressed as a function of APOE genotype and time, not causally related to either age of onset or duration of disease. These analyses illustrate how the use of the relevant genetic variable, APOE genotype, can make sense of otherwise complex Aβ deposition data, allowing Aβ deposition to be analyzed as a dependent variable (21, 22). As other genetic factors that may be involved in the pathogenesis of AD or the phenomenon of amyloid deposition are delineated, the mechanism of disease and the resulting scars will be further clarified.

The hypothesis that Aβ deposition causes AD has been the subject of intense testing for many years. Some elegant experimental data have been generated to study the putative effects of Aβ in cell systems. For instance, the formation of Aβ fibrils may kill cells in tissue culture, as will many other chemical additives (23, 24). The phenomena demonstrated in tissue culture experiments have yet to be relevantly connected to the pathogenesis of AD in patients.

One of the most prominently published views was termed the "amyloid cascade hypothesis" (25–28). Many similar views had existed in the literature but this ter-



minology was prompted by the demonstration that mutations of APP associated with early-onset AD flanked the coding region for the β-peptide fragment (10–12). In its initial form it stated that, "recent genetic evidence clearly shows that deposition of β amyloid is the primary event in the pathological cascade for Alzheimer's disease" (26). Thus the tautology was restated with genetic credentials. The description of the APP717 mutations was never shown to be related to "deposition of β amyloid" as a primary or even secondary event. The association of the inheritance of a particular mutation does not predict the mechanism by which the disease is expressed, only that certain APP mutations are associated with the autosomal dominant inheritance of an early-onset form of AD. The genetic analyses provide no support one way or another to Aβ deposition as a pathogenic mechanism. The inferences included in the "framing β-amyloid" theory are reasonable hypotheses, but require experimental support (27). To date, such mechanistic hypotheses have been suitably vague and inclusive, frequently "so vague as to be universally useful, i.e. anything and everything becomes explicable" (29). For example, it was stated that the "anatomical cascade hypothesis . . . is an attempt to integrate [putative] biochemical processes with the observation that . . . [Alzheimer] . . . disease has a precise neuroanatomy" (28). The hypothesis presents the opportunity to design experimental tests combining biochemistry, neuroanatomy and cell biology. How does the deposition of Aβ kill neurons? What are the relevant cellular mechanisms to account for the "cascade"? Is there anatomical evidence for such a cascade? The hypotheses are stated and repeated, but without compelling experimental support over the intervening years.

In 1991, in a *Nature* "News and Views" essay, it was stated that, "circumstantial evidence for the central involvement of the amyloid β/A4 protein . . . has been long building, but this year has witnessed two developments which provide 'smoking guns' for the amyloid hypothesis. First, missense mutations at codon 717 of the gene encoding . . . . Second, studies of transgenic mice overexpressing some or all of the human β-APP molecule have started to bear fruit" (30). The role of Aβ deposition as causative in APP717 AD remains "circumstantial." With regard to the "fruit" borne from transgenic experiments, overproduction of APP or APP717 has not led to an animal model of AD and, over the intervening years, has disappointed many investigators. The transgenic excitement of *Nature* 1991 was largely based on a paper that was fraudulent (31, 32). The unbridled enthusiasm in the statement: "The results from transgenic mice . . . indicate that amyloidogenic processing of β-APP can precede all other manifestations of Alzheimer's disease and thus be truly causative of the disorder" was unfortunately premature (30). The "smoking gun" evidence should be discarded. The evidence disappears, yet the pronouncements linger on. Suffice it to say that Aβ causation has been a popular hypothesis, but hypotheses are meant to be tested, not simply advocated or voted the most likely to succeed (33). To test the other "smoking gun" we can examine the neuropathological data derived directly from patients with APP mutations.

APP Mutations and AD

Certain specific mutations of the APP gene are present in individuals inheriting an autosomal dominant form of early-onset AD and have been used to support the amyloid hypothesis that Aβ deposition causes AD. However, even in patients with APP mutations, there are no data to support a causal relationship between Aβ deposition and AD. Figure 2 illustrates the silver staining and Aβ immunopathology of adjacent sections of the hippocampus from two female siblings from a large family segregating the APP717val-ile mutation. Both inherited the APP mutation and the same APOE-ϵ4/2 genotype, both had onset of symptoms at age 49 years, and both had similar disease progression over 7 years leading to incapacity and eventual death (4, Roses et al, unpublished data). The number of SP using silver stain was comparable in both patients. However, despite the diagnostic density of silver-stained plaques in both cases, there were marked differences in the number and density of Aβ immunostained plaques in all parts of the brain examined, including frontal cortex and hippocampus. This comparison of neuritic plaques and immunostained Aβ plaques in nearby sections demonstrates that significant variations in Aβ deposition were unrelated to disease onset or course, or to the inheritance of the APP717 mutation. Of course, these mutations are rare etiologies for AD and these data come from only two siblings. Yet patients with APP mutations should be the best examples to test the hypothesis that Aβ deposition causes AD.

At this time, Aβ deposition and AD with APP717 mutations may be viewed as separate phenomena. The observed variations in Aβ deposition may also be due to the effects of other factors independent of disease expression. APOE genotype may be one factor but, in the

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Fig. 3. Formic acid-treated paraffin sections of middle frontal gyrus from five AD patients with APOE3/3 genotype (A, C, E, G, I) and five APOE4/4 patients (B, D, F, H, J). Pairs are matched for duration of disease from age of onset to death. Aβ immunoreactivity in APOE4/4 patients is more intense and extensive than in APOE3/3 patients with similar duration of disease, with numerous darkly stained plaques, vessels and subpial deposits. (A, B 25×; C–J 100×; Bar 500 μm in B and 200 μm in C.) Reproduced from Schmechel et al (21) with permission.

TABLE 1
Allele Frequency of APOE4 in AD Groups

Population	E4 frequency	#	p
Sporadic autopsy series	0.40 ± 0.026	352	<0.00001
CEPH grandparents, controls	0.16 ± 0.027	182	—
1st affected twin	0.40 ± 0.044	124	<0.00001
CH 14 or APP mutation families	0.19 ± 0.069	32	NS
Probable/possible AD diagnosis	0.41 ± 0.039	160	<0.00001
Spouse controls in clinic	0.14 ± 0.032	118	—

Reproduced from Saunders et al (63) with permission.

examples illustrated, both had APOE2/4 genotypes so another variable involved in Aβ deposition is suggested. With regard to determining the pathogenesis of APP717 AD, studying the metabolism and intracellular trafficking of APP may be the appropriate field of research. That the site of the mutations predicts the role of Aβ deposition in disease pathogenesis is a hypothesis that awaits experimental and neuropathological testing (25–28).

Genetics Defines the Relevance of APOE

The gene for apolipoprotein E is located on chromosome 19. There are three common polymorphisms present in ethnic and racial groups in slightly varying proportions, called APOE-ε2, -ε3, and -ε4. Using genetic linkage techniques APOE4 has been associated with late-onset familial and sporadic AD in populations all over the world (1, 34–47; Table 1). APOE4 is not a genetic mutation causing AD, but rather a susceptibility gene or risk factor for earlier onset (2; Fig. 4). The inheritance of APOE4 increases the risk and lowers the distribution of the ages of onset of AD in a dose-dependent manner (2). The inheritance of APOE2 decreases the risk and increases the age of onset distribution (48). The critical point is that there is a genetically determined, biological relationship to the distributions of age of onset of AD and the APOE genotype inherited.

ApoE has played a relatively obscure role in the neuropathology of AD. Namba et al (49) found that antibodies to apoE stained plaques, tangles and vascular amyloid. Diedrich et al (50) reported increased expression of apoE in astrocytes in AD, but no neuronal expression was observed. Wisniewski and Frangione (51) found a "possible role for apoE and the other amyloid associated proteins such as P component, glucosaminoglycans and antichymotrypsin" and demonstrated that apoE was bound to the amyloids of several different amyloid-producing diseases. They have referred to apoE, as well as the other proteins, as "pathological chaperones," associated with amyloid deposition in each of the diseases. Their definition of "pathological chaperones" as a "group of unrelated proteins that mediate β-pleated amyloid formation

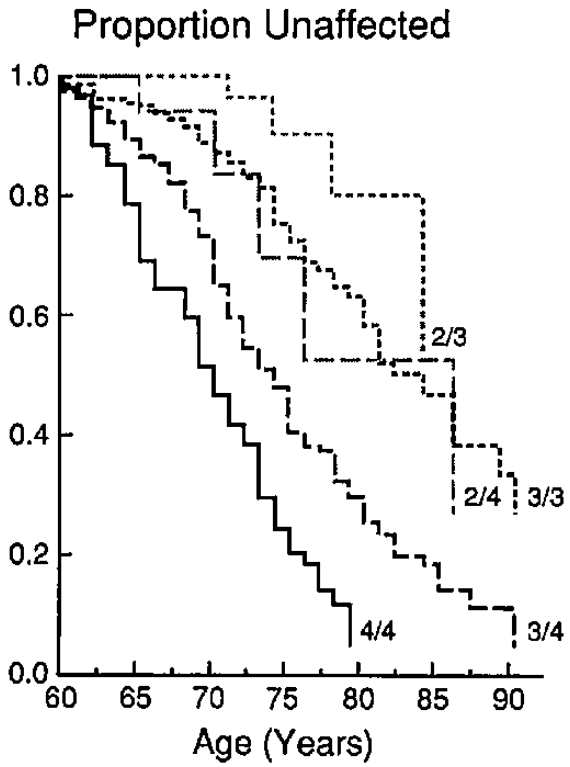

Fig. 4. Age of onset for subjects in 72 late-onset AD families (158 affected and 220 unaffected) and additional sporadic subjects and controls (103 autopsy-confirmed sporadic AD cases and 198 controls with age information from two sources: the Duke Memory Disorders Clinic and the Duke Established Populations for Epidemiologic Studies of the Elderly). The age of onset is scored as a function of the individual's genotype. Onset curves were estimated by Kaplan-Meier product limit distributions. More than 2 decades differentiate the mean age of onset distribution for APOE4/4 individuals (approximately 68 years old) compared to APOE2/3 individuals (more than 90 years old). Note also that individuals who are APOE3/3 are very similar to those who are APOE2/4. (48, 64). **Note added in proof**: Van Duijn et al (65) and Okuizumi et al (66) have demonstrated that the inheritance of APOE is also associated with populations of early-onset AD, so that this figure would be extended slightly before the age of 60 years if it reflected the epidemiology of populations.

of polypeptide fragments" did not suggest a distinctive role for apoE in AD. Strittmatter et al (4) had also described apoE immunoreactivity in plaques, vascular amyloid, and NFT. They also found that apoE immunostained neuronal cells specifically, some of which contained NFT (4). However, once genetic relevance was established, the presence of apoE as a phenotypic constituent in the neuropathology of AD provided added support for a genetically relevant metabolic role in AD expression.

Because apoE is genetically associated with the expression of AD and Aβ is not, it is difficult to consider apoE as the "unrelated protein" interacting with Aβ (51). Rather Aβ may be viewed as the "chaperone" or unre-

lated escort of apoE. (We prefer the term "escort" to "chaperone" since the latter term refers to molecular chaperones that help newly formed polypeptides fold into their functional three-dimensional conformations. That function was not implied by Wisniewski and Frangione's use of the term as a nonspecific amyloid binding protein.) ApoE binds to the low density lipoprotein-related receptors (LRP) and low density lipoprotein (LDL) receptors present on neurons, particularly on retracting synapses in neuritic plaques (37). The amount and density of Aβ binding and deposition is related to the type of apoE inherited and the apparent duration of AD from age of onset to autopsy (21, 22). Since several components of SP are known, there is no more special rationale for a central pathogenetic role for Aβ in AD than there would be for any of the other plaque constituents, except apoE (51–54). Aβ deposition could appropriately be viewed as a phenotypically associated consequence of AD expression, rather than implicating Aβ fibril formation or Aβ deposition as the proximal cause of the disease.

Genetics establishes etiology and relevance. Most of the disease genes that have been discovered as a result of the gene-mapping efforts of the past decade had not been described previously. We do not yet understand the role or the function of the variable trinucleotide repeats inherited in Huntington disease, Kennedy disease, spinocerebellar ataxia type 1, or dentatorubral pallidoluysian atrophy (55). But one fact is universally understood: No matter what phenotypic data, associations, theories or inspirations existed before the discovery of the inherited genetic locus, all existing data must now be explained relevant to that locus. A pre-existing theory of pathogenesis can now be tested to determine whether it is relevant to the gene defect, not vice versa. How apoE relates to Aβ addresses the question in reverse. That the APOE gene and its protein products have been so well studied provided a great advantage to recent studies of its role in AD (56).

If the only data available were genetic, the single overriding fact would be that there is a biological relationship between inheritance of specific APOE alleles and the development of AD. Relevance flows from that starting point. It is reasonable and understandable that researchers would want to test the role of apoE with respect to prior scientific contributions by incorporating apoE into previously described hypotheses. However, the critical role of apoE in AD may redefine the data: Is apoE an escort to Aβ, or is Aβ an escort to apoE? Which is the driver and which is along for the ride? In genetic terms, the role of apoE is primary. Aβ deposition is an associated phenotypic manifestation of the disease process. To ascribe a causative role for Aβ deposition, perhaps regulated by apoE, requires better data than simply "being there" (57). In fact, the literature for a closer phenotypic association with the presence of NFT is far more compelling (8, 9, 15).

An Alternative Hypothesis to Explain How the Inheritance of apoE Isoforms Affects the Rate of AD Expression

A single amino acid difference between apoE3 and apoE4 produces a dramatic biological effect on the expression of AD. The mean age of onset difference between inheriting two ε4 alleles and no ε4 alleles is approximately 15 years. With the effect of inheritance of the ε2 allele, the distribution of the mean age of onset of AD can be over 2 decades. How do small changes in the apoE molecule affect the rate of AD expression by up to 20 years? ApoE can bind Aβ and also binds lipid particles, LDL and LRP receptors, and other proteins. Which of these phenomena, if any, are relevant to AD expression remains to be proven.

The immunoreactivity of apoE antibodies with NFT inside neurons was as striking as that of Aβ deposition in plaques and blood vessels (34, 49). Neurofibrillary tangle staining implied an intraneuronal role. We have proposed a hypothesis that involves the differential metabolic interactions of apoE3 (and apoE2) and apoE4 with microtubule-associated proteins (4). We have suggested that apoE2 and apoE3 sequester or protect tau (and MAP2c), so that the formation of PHF and associated hyperphosphorylation of tau near the soma are fractionally slowed over many years (4). Apparently MAP2c does not form NFT. There is a remarkable *in vitro* difference in the binding of apoE3 and apoE4 to tau (4, 35). ApoE3 binds to the microtubule binding domains of tau and MAP2c, the microtubule-associated proteins in axons and dendrites, respectively. The binding occurs with very low concentrations of apoE, consistent with concentrations that may be present in the cytoplasm of neurons (Strittmatter et al, unpublished data). The implications are that: 1) the binding of apoE to microtubule-associated proteins may protect a small, but critical, population of tau molecules involved with intracellular maintenance and repair of microtubules from forming PHF; 2) this effect may precede the formation of PHF and/or hyperphosphorylation of tau; and 3) over many years fractionally more tau may be available to stabilize microtubules than to form PHF. Thus, in this hypothesis, NFT may not be the "cause" of cell death, but a phenotypic consequence of altered apoE metabolism. Whether the interference with microtubule stability and function or the clogging of neurons with NFT functionally kills the cells is unclear, but the role of apoE in protecting tau and MAP2c may be critical to the rate at which the process occurs (4).

This hypothesis is consistent with the loss of synapses in AD (58). Synapse loss has been correlated with cognitive severity (59). Early data from aged mice in which

the APOE gene had been knocked out has revealed a statistically significant loss of synapses (Masliah E, personal communication). Thus, processes leading to the slow strangulation of the microtubule system, perhaps from disturbances of microtubule maintenance and repair at the proximal ends, can be responsible for the simplification of neurons, loss of synapses, and slightly increased rate of formation of PHF and NFT over many years (5, 58, 59).

Of course there are several obvious problems with this hypothesis that must be investigated. Neurons do not express APOE mRNA, so that any apoE entering into the neuron would presumably be associated with lipid particles transported in endosomes into the cell body. The cellular trafficking of apoE in neurons is totally unstudied, particularly because apoE had not previously been identified in the cytoplasm of neurons. Our data demonstrate that apoE can be localized to specific sets of neurons in AD, Parkinson disease, other neurodegenerative diseases, and some aged controls (4, 34, 49, 60). Immunoelectron microscopic studies of neurons in humans and prosimians has localized intracellular apoE to the cytoplasm (61).

The data implicating apoE in metabolism associated with the rate of expression of AD are only beginning to be examined experimentally in many laboratories. There are, of course, other pathogenic hypotheses involving apoE that can be generated and tested experimentally. A critical role for apoE in neuronal metabolism might involve membrane trafficking, effects on cation transport, involvement in lipid metabolism, interactions with intracellular APP metabolism, processes involved with cellular responses to degeneration/regeneration or inflammation, and many other scenarios. The range of testable hypotheses are only limited by the imaginations of investigators. One can safely assume that time and experiments will test apoE theories in many laboratories. It is reasonable to expect that scientific light will dispel some of the heat of initial skepticism (62).

We believe that focusing research on apoE metabolic interactions and intracellular trafficking in neurons will add new relevant data to the mechanisms of pathogenesis of AD and other neurodegenerative diseases. It may also include some answers to the role of APP717 and other AD associated mutations. We fully expect that new data from many laboratories will rapidly clarify neuronal metabolic and intracellular trafficking principles. Metabolic differences produced by a small change in a small molecule (one amino acid in a 299 amino acid protein) leading to disease onset distributions varying over 2 decades must be explained.

REFERENCES

1. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467–72
2. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer disease in late onset families. Science 1993;261:921–3
3. Roses AD. The Alzheimer diseases. In: Appel SH, ed. Current neurology. Vol. 14. Chicago: Mosby, 1994 (in press)
4. Strittmatter WJ, Weisgraber KH, Goedert M, et al. Hypothesis: Microtubule instability and paired helical filament formation in the Alzheimer disease brain as a function of apolipoprotein E genotype. Exp Neurol 1994;125:163–71
5. Einstein G, Farris D, Crain BJ. Investigating single neurons in human autopsy material. In: Meredith G, Arbuthnott G, eds. Morphological investigations of single neurons in vitro. Sussex: John Wiley and Sons, 1993:105–27
6. McKhann C, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44
7. Khachaturian ZS. Diagnosis of Alzheimer's disease. Arch Neurol 1985;42:1097–1105
8. McKee AC, Kosik KS, Kowall NW. Neuritic pathology and dementia in Alzheimer's disease. Ann Neurol 1991;30:156–65
9. Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW. The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cerebral Cortex 1991;1:103–16
10. Joachim CL, Selkoe DJ. The seminal role of beta-amyloid in the pathogenesis of Alzheimer's disease. Alzheimer Dis Assoc Disord 1992;6:7–34
11. Bush AI, Beyreuther K, Masters CL. Beta A4 amyloid protein and its precursor in Alzheimer's disease. Pharmacol Ther 1992;56:97–117
12. Wisniewski T, Golabek A, Matsubara E, et al. Apolipoprotein E: Binding to soluble Alzheimer's β-amyloid. Biochem Biophys Res Commun 1993;192:359–65
13. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–86
14. Cai XD, Golde TE, Younkin SG. Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science 1993;259:514–16
15. Hyman BT, Marzloff K, Arriagada PV. The lack of accumulation of senile plaques or amyloid burden in Alzheimer's disease suggests a dynamic balance between amyloid deposition and resolution. J Neuropathol Exp Neurol 1993;52:594–600
16. Arriagada PV, Growden JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 1992;42:631–9
17. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30:572–80
18. Morris JC, McKeel DW, Storandt M, et al. Very mild Alzheimer's disease: Informant-based clinical psychometric and pathologic distinction from normal aging. Neurology 1991;41:469–78
19. Katzman R, Terry R, De Teresa R, et al. Clinical, pathological, and neurochemical changes in dementia: A subgroup with preserved mental status and numerous neocortical plaques. Ann Neurol 1988;23:138–44
20. Price DL, Davis PB, Morris JC, White DL. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. Neurobiol Aging 1991;12:295–312
21. Schmechel DE, Saunders AM, Strittmatter WJ, et al. Increased amyloid β-peptide deposition in cerebral cortex as a consequence of

apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:9649–53
22. Strittmatter WJ, Weisgraber KH, Huang D, et al. Binding of human apolipoprotein E to synthetic amyloid β peptide: Isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:8098–102
23. Yankner BA, Dawes LR, Fisher S, et al. Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. Science 1989;245:417–20
24. Yankner BA, Caceres GA, Duffy LK. Nerve growth factor potentiates the neurotoxicity of β amyloid. Proc Natl Acad Sci USA 1990;87:9020–3
25. Hardy JA, Higgins GA. The amyloid cascade hypothesis. Science 1992;256:184–5
26. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 1991;12: 383–8
27. Hardy J. Framing β-amyloid. Nature Genet 1992;1:233–4
28. Hardy J. An 'anatomical cascade hypothesis' for Alzheimer's disease. Trends Neurosci 1992;15:201–2
29. Pirsig RM. Lila: An inquiry into morals. New York: Bantam, 1992
30. Selkoe DJ. Alzheimer's disease: In the beginning ... Nature 1991;354:432–3
31. Kawabata S, Higgins GA, Gordon WW. Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein. Nature 1991;354:476–8
32. Kawabata S, Higgins GA, Gordon WW. Retractions. Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein. Nature 1992;356:23 and 265
33. Hardy J. ApoE, amyloid, and Alzheimer's disease. (Letter) Science 1994;263:454
34. Strittmatter WJ, Saunders AM, Schmechel DE, et al. Apolipoprotein E: High affinity binding to βA amyloid and increased frequency of type 4 allele in familial Alzheimer's. Proc Natl Acad Sci USA 1993;90:1977–81
35. Strittmatter WJ, Saunders AM, Goedert M, et al. Isoform-specific interactions of apolipoprotein E with tau and phosphorylated tau: Implications for Alzheimer disease. Proc. Natl Acad Sci USA 1994 (in press)
36. Poirier J, Davignon J, Bouthillier D, et al. Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 1993;342:697–9
37. Rebeck GW, Reiter JS, Strickland DK, Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: Allelic variation and receptor interactions. Neuron 1993;11:575–80
38. Borgaonkar DS, Schmidt LC, Martin SE, et al. Linkage of late-onset Alzheimer disease with apolipoprotein E type 4 on chromosome 19 confirmed by family studies. Lancet 1993;342:625
39. Amouyel P, Brousseau T, Fruchart J-C, et al. Apolipoprotein E-4 and Alzheimer's disease. Lancet 1993:342:1309
40. Anwar N, Lovestone S, Cheetham ME, et al. Apolipoprotein E-4 and Alzheimer's disease. Lancet 1993:342:1308–9
41. Brousseau T, Legrain S, Berr C, et al. Confirmation of the e4 allele of the apolipoprotein E gene as a risk factor for late-onset Alzheimer's disease. Neurology 1994;44:342–4
42. Czech C, Monning U, Tienari PJ, et al. Apolipoprotein E-4 and Alzheimer's disease. Lancet 1993;342:1309–10
43. Lucotte G, David F, Visvikis S, et al. Apolipoprotein E-4 and Alzheimer's disease. Lancet 1993:342:1309
44. Mayeax R, Stern Y, Ottman R, et al. The apolipoprotein E4 allele as a risk factor for Alzheimer's disease. Ann Neurol 1993;34:752–5
45. Noguchi S, Murakami K, Yamada N. Apolipoprotein E genotype and Alzheimer's disease. Lancet 1993;342:737
46. Payami H, Kaye J, Heston LL, Bird TD, Schellenberg GD. Apolipoprotein E genotype and Alzheimer's disease. Lancet 1993;342: 738
47. Peacock ML, Fink JR. ApoE e4 allelic association with Alzheimer's disease: Independent confirmation using denaturing gradient gel electrophoresis. Neurology 1994;44:339–41
48. Corder EH, Saunders AM, Risch NJ, et al. Apolipoprotein E type 2 allele decreases the risk of late-onset Alzheimer's disease. Nature Genet 1994;7:180–3
49. Namba Y, Tomonaga M, Kawasaki H, et al. Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res 1991;541:163–6
50. Diedrich JF, Minnigan H, Carp RI, et al. Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. J Virol 1991;65:4759–68
51. Wisniewski T, Frangione B. Apolipoprotein E: A pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 1992;135:235–8
52. Beyreuther K, Masters CL. Amyloid precursor protein and βA4 amyloid in the etiology of Alzheimer's disease: Precursor–product relationships in the derangement of neuronal function. Brain Pathol 1991;1:241–51
53. Ueda K, Fukushima H, Masliah E, et al. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci USA 1993;90:11282–6
54. Snow AD, Wight TN. Proteoglycans in the pathogenesis of Alzheimer's disease and other amyloidoses. Neurobiol Aging 1989;10: 481–97
55. Richards RI, Sutherland GR. Simple repeat DNA is not replicated simply. Nature Genet 1994;6:114–16
56. Weisgraber KH. Apolipoprotein E: Structure–function relationships. Adv Prot Chem 1994 (in press)
57. Einstein G, Buranosky R, Crain BJ. Dendritic pathology of granule cells in Alzheimer's disease is unrelated to neuritic plaques. J Neurosci 1994 (in press)
58. Masliah E, Terry RD, DeTeresa RM, Hansen LA. Immunohistochemical quantification of the synapse related protein synaptophysin in Alzheimer's disease. Neurosci Lett 1989;103:234–9
59. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. Ann Neurol 1990;27:457–64
60. Han S-H, Hulette C, Saunders AM, et al. Immuno-histochemical localization of apolipoprotein E to neurons in late-onset Alzheimer disease. Exp Neurol 1994 (in press)
61. Han S-H, Einstein G, Weisgraber KH, et al. Apolipoprotein E is localized to the cytoplasm of human cortical neurons: A light and electron microscopic study. J Neuropathol Exp Neurol 1994;53: 535–44
62. Pennisi E. A molecular whodunit: New twists in the Alzheimer's mystery. Science News 1994;145:8–11
63. Saunders AM, Schmader K, Breitner JCS, et al. Apolipoprotein E4 allele association is specific to late-onset Alzheimer disease. Lancet 1993;342:710–11
64. Roses AD, Strittmatter WJ, Pericak-Vance MA, et al. Clinical applications of apolipoprotein E genotyping to Alzheimer's disease. Lancet 1994;343:1564–5
65. van Duijn CM, de Knijff P, Cruts M, et al. Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. Nature Genet 1994;7:74–8
66. Okuizumi K, Onodera O, Tanaka H, et al. APOE-ε4 and early-onset Alzheimer's. Nature Genet 1994;7:10–11