- Neurology 1989;39:125-126.
4. American Medical Association Council on Scientific Affairs. Persistent vegetative state and the decision to withdraw support. *JAMA* 1990;263:426-430.
 5. Tresch DD, Farrol HS, Duthie EH, Goldstein MD, Lane PS. Clinical characteristics of patients in the persistent vegetative state. *Arch Intern Med* 1991;151:930-932.
 6. Hays WL. Statistics for psychologists. New York: Holt, Rinehart and Winston, 1963:585-586.
 7. Sheskin D. Statistical tests and experimental design: a casebook. New York: Gardner Press, 1984:23-24.
 8. Plum F, Posner JB. The diagnosis of stupor and coma. Philadelphia: FA Davis, 1980:1-11.
 9. Jennett B, Plum F. Persistent vegetative state after brain damage: a syndrome in search of a name. *Lancet* 1972;1:734-737.
 10. New "Baby Doe" rules tested. *Am Med News* 1986;1:45.
 11. Teasdale G, Jennett B. Assessment of coma and impaired consciousness: a practical scale. *Lancet* 1974;2:81-83.
 12. Sazbon L. Prolonged coma. *Prog Clin Neurosci* 1985;2:65-81.
 13. Sazbon L, Groswasser Z. Outcome in 134 patients with prolonged posttraumatic unawareness. Part 1: parameters determining late recovery of consciousness. *J Neurosurg* 1990;72:75-80.
 14. Bricolo A, Turazzi S, Feriotti G. Prolonged posttraumatic unconsciousness: therapeutic assets and liabilities. *J Neurosurg* 1980;52:625-634.
 15. Rappaport M, Dougherty AM, Kelting DL. Evaluation of coma and vegetative states. *Arch Phys Med Rehabil* 1992;73:628-634.
 16. Hagen C. Language disorders secondary to closed head injury: diagnosis and treatment. *Top Lang Dis* 1981;1:73-87.
 17. Alexander MP. Traumatic brain injury. In: Benson DF, Blumer D, eds. *Psychiatric aspects of neurologic disease*, vol. 2. New York: Grune & Stratton, 1982:219-247.
 18. Giacino JT, Kezmarsky MA, DeLuca J, Cicerone KD. Monitoring rate of recovery to predict outcome in minimally responsive patients. *Arch Phys Med Rehabil* 1991;72:897-901.
 19. Whyte J, Glenn MB. Management of the patient in a persistent vegetative state: current status and needed research. In: Bach-y-Rita P, ed. *Comprehensive neurologic rehabilitation*, vol. 2: traumatic brain injury. New York: Demos, 1989:13-29.
 20. Lehman LB. The persistent vegetative state. *Postgrad Med* 1990;88:150,152.
 21. Berrol S. Considerations for management of the persistent vegetative state. *Arch Phys Med Rehabil* 1986;67:283-285.

Association of apolipoprotein E allele $\epsilon 4$ with late-onset familial and sporadic Alzheimer's disease

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Article abstract—Apolipoprotein E, type $\epsilon 4$ allele (APOE $\epsilon 4$), is associated with late-onset familial Alzheimer's disease (AD). There is high avidity and specific binding of amyloid β -peptide with the protein ApoE. To test the hypothesis that late-onset familial AD may represent the clustering of sporadic AD in families large enough to be studied, we extended the analyses of APOE alleles to several series of sporadic AD patients. APOE $\epsilon 4$ is significantly associated with a series of probable sporadic AD patients (0.36 ± 0.042 , AD, versus 0.16 ± 0.027 , controls [allele frequency estimate $\pm$ standard error], $p = 0.00031$). Spouse controls did not differ from CEPH grandparent controls from the Centre d'Etude du Polymorphisme Humain (CEPH) or from literature controls. A large combined series of autopsy-documented sporadic AD patients also demonstrated highly significant association with the APOE $\epsilon 4$ allele (0.40 ± 0.026 , $p \leq 0.00001$). These data support the involvement of ApoE $\epsilon 4$ in the pathogenesis of late-onset familial and sporadic AD. ApoE isoforms may play an important role in the metabolism of β -peptide, and APOE $\epsilon 4$ may operate as a susceptibility gene (risk factor) for the clinical expression of AD.

NEUROLOGY 1993;43:1467-1472

Alzheimer's disease is the leading cause of dementia in the elderly. Most patients encountered in practice have no obvious family history and are

classified as sporadic. However, genetic factors in early- and late-onset familial Alzheimer's disease (FAD) are now well documented. Early genetic

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Supported by NIA LEAD Award AG-07922, NIA Alzheimer's Disease Center AG-05128 to A.D.R.; NIH General Clinical Research Centers Program M01-RR-30, and NINDS Center for Neurogenetic Diseases NS26330 to M.A.P.-V.; and NIA Center for Aging AG-09463 (Harvey J. Cohen, principal investigator), the Medical Research Council of Canada, the Alzheimer's Disease and Related Disorders Association, the Alzheimer's Association of Ontario, the American Health Assistance Foundation, and numerous contributions, less than \$100, to the Joseph and Kathleen Bryan Alzheimer's Disease Research Center.

Received December 11, 1992. Accepted for publication in final form February 8, 1993.

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linkage studies suggested a locus for early-onset FAD on chromosome 21.¹ Subsequently, investigators identified a rare mutation of the amyloid precursor protein (APP717Val→) on chromosome 21 in 11 early-onset FAD kindreds.²⁻⁷ Two groups described several other very rare mutations of the APP gene in families segregating dementia of the Alzheimer type.^{8,9} Schellenberg et al¹⁰ and St. George-Hyslop et al¹¹ localized most of the early-onset FAD kindreds showing support for linkage to chromosome 21 to a region on chromosome 14q. Another study reported¹² possible linkage of late-onset FAD to the proximal long arm of chromosome 19.

Several independent lines of evidence led us to examine apolipoprotein E (ApoE, protein; APOE, gene) in late-onset FAD.¹³ We observed several proteins in CSF that bound to immobilized amyloid β -peptide with high affinity. Microsequencing and Western blotting techniques identified ApoE as one of these proteins. APOE was known to be localized to the region of chromosome 19,¹⁴ which in previous studies had shown possible linkage to late-onset FAD.^{12,15} Furthermore, antisera to ApoE stained senile plaques, neurofibrillary tangles, and cerebral vessel amyloid deposits in AD brains.^{13,16,17} In this communication, we present data supporting the association of the ϵ 4 allele of APOE with late-onset sporadic AD, including clinical (probable) AD cases and autopsy-confirmed AD cases.

Methods. Families. Blood samples for the genomic DNA studies were obtained from families described previously.^{5,7,11,12,18} All sampled individuals diagnosed with probable AD were examined by a neurologist and associated diagnostic personnel of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) Memory Disorders Clinic at Duke University, the Centre for Neurodegenerative Diseases at the University of Toronto, or the Departments of Neurology at Massachusetts General Hospital and the Harvard Medical School. The clinical diagnosis was made according to the NINCDS-ADRDA criteria.¹⁹ The Duke University pedigrees were primarily late-onset AD families with an average age of 66.1 ± 10.3 years in the 35 families. Three of the families could be classified as early-onset (mean, <60 years) AD families. One family segregates with the APP717Val→Ile mutation,² a second segregates with the APP717Val→Phe mutation,⁵ and the other is linked to chromosome 14 markers with a maximum lod score of 3.5.^{10,11} The Toronto pedigrees were classified as primarily early-onset families (13 of the 17 families). Five of the families were linked to chromosome 14 with lod scores greater than 3.0 in each family,¹¹ and a sixth pedigree had the APP717Val→Ile mutation.⁷ The family and genotypic data were processed via the PEDI-GENE system.²⁰

Genomic DNA from some patients diagnosed as having sporadic cases of probable AD at Duke, Toronto, and Boston have been banked over the past 6 years. Sporadic AD patients were defined as those without a known family history of AD or dementia. The sporadic, probable AD patients represented all the banked DNAs in the Toronto and Duke banks as of November 1992 except for an ongoing prospective series begun in August 1992 at the Bryan

ADRC Memory Disorders Clinic. The DNA from those individuals had been collected randomly prior to any interest in APOE isotyping. Not all sporadic AD patients evaluated in these clinics were banked routinely. The diagnosis of probable AD in this group can be expected to be in the 80% to 90% accuracy range that is observed in most specialized AD clinics. Brain DNA was obtained from autopsy-confirmed, white cases of AD that had been banked in the Kathleen Bryan Brain Bank at Duke, the University of Toronto, and the Harvard Medical School. Six black or Native American autopsied subjects were eliminated from the series of sporadic AD autopsies because the association analyses are sensitive to the control group.

Two sets of controls were used in the study. The first set was 91 unrelated grandparents from the Centre d'Etude du Polymorphisme Humain (CEPH) reference families.²¹ These families were collected for human gene mapping and are characterized by having grandparents, parents, and many grandchildren available for DNA mapping. The grandparents represent a random group of white, aged controls of European and American background, similar to the late-onset FAD families and the autopsy-confirmed sporadic AD population. Twenty-one white spouses of patients participating in an ongoing prospective analysis of probable AD patients and spouses were also used as a control group.

Genomic DNA. High-molecular-weight DNA was obtained from transformed lymphoblasts according to a method described elsewhere^{22,23} or to the Genepure 341 nucleic acid extractor's supplied protocol (Applied Biosystems). Genomic DNA from brain tissue was isolated by pulverizing approximately 300 mg of frozen brain tissue under liquid nitrogen, adding 4 ml of lysis buffer (Applied Biosystems) and 1 mg of proteinase K (Applied Biosystems), and gently rocking overnight at 37 °C before extracting on the Genepure 341.

Amplification and restriction isotyping of APOE. Genomic DNA was amplified by polymerase chain reaction (PCR) in a Techne MW-2 thermocycler using HI-TEMP 96-well plates (Techne) and the primers described by Wenham et al.²⁴ The PCR protocol was based on those described by Wenham et al.²⁴ and Hixson and Vernier.²⁵ Each amplification reaction contained 20 ng genomic DNA, 1.0 pmol/ μ l of each primer, 10% dimethylsulfoxide (Sigma), 200 μ M each dNTP (Pharmacia), 1.0 μ Ci (α -³²P) dCTP (800 Ci/mol in 10 mM Tricine; NEN Research Products), 0.05 U/ μ l *Taq* DNA polymerase, and supplied 1 $\times$ buffer (Boehringer Mannheim) in a final volume of 15 μ l. An initial denaturation at 94 °C for 5 minutes was followed by 35 cycles of annealing at 65 °C for 0.5 minutes, extension at 70 °C for 1.5 minutes, denaturation at 94 °C for 0.5 minutes, and a final extension at 70 °C for 10 minutes. After amplification, 5 U of *Hha* I (Gibco) was directly added to each well, and the plates were incubated at least 3 hours at 37 °C. Fifteen μ l of 2 $\times$ type III stop dye²⁶ was added to each well, and 3 μ l of each reaction was loaded on a 6% nondenaturing polyacrylamide gel (0.4 mm thick $\times$ 43 cm long) and electrophoresed for 1 hour under constant current (45 mA). After electrophoresis, the gel was transferred to Whatman 3M chromatography paper, dried, and autoradiographed for 1 hour using Kodak XAR-5 film. Each autoradiograph was read independently by two different observers.

Statistical analysis. We estimated allele frequencies for the control and AD groups by counting alleles and calculating sample proportions. Allele frequency esti-

Figure. Example of APOE alleles from 12 different people. The three major alleles of APOE differ by single nucleotide substitutions within the amino acid codons at positions 112 (Cys→Arg; $\epsilon 3 \rightarrow \epsilon 4$) and 158 (Arg→Cys; $\epsilon 3 \rightarrow \epsilon 2$). These sequence differences can be demonstrated by using PCR to amplify DNA obtained from blood samples. After the alleles are amplified from blood DNA, a restriction enzyme can cut the PCR product DNA so that the substitutions can be recognized on an autoradiogram following gel electrophoresis. This enzyme, Hha I, cuts the PCR product of $\epsilon 3$ to generate 91-bp and 48-bp fragments (lane 1, $\epsilon 3$ homozygote). The fragment sizes in base pairs (bp) are shown to the left. Fragments of 72-bp and 48-bp are produced in $\epsilon 4$ homozygotes (lane 6). The rare allele, $\epsilon 2$, produces a 91-bp and 83-bp doublet, and is illustrated in heterozygotes in lanes 8 and 12. The presence of the 83-bp fragment makes $\epsilon 2$ recognition easier. The absence of the 72-bp fragment in lane 12 distinguishes the $\epsilon 2/\epsilon 3$ genotype from the $\epsilon 2/\epsilon 4$ genotype in lane 8. In this assay, the 244-bp PCR fragment also yields constant, smaller Hha I cleavage fragments that are irrelevant to the genotyping and are not illustrated.

mates for the early- and late-onset FAD families were calculated using one randomly selected affected patient from each family. We calculated the Z statistic for comparing two proportions.^{27,28} An extreme value of Z compared with the probabilities for the standard normal distribution would suggest rejecting the null hypothesis that the allele frequencies in the two groups are equal. To compare the APOE allele frequencies in the different populations, ie, AD versus controls, and between control groups, we made the following comparisons: (1) CEPH controls versus spouse controls; (2) CEPH controls versus literature controls; (3) CEPH controls versus late-onset FAD; (4) CEPH controls versus early-onset FAD; (5) CEPH controls versus clinical (probable) sporadic AD; and (6) CEPH controls versus autopsy-confirmed sporadic AD. Affected-pedigree-member (APM) linkage analysis for APOE was analyzed as previously described.^{12,29} Two-point lod scores were calculated using the computer program LINKAGE Program Package (version 5.0).^{30,31} The age-of-onset and disease parameters used in the lod score calculations were as formerly outlined.¹²

Results. The figure illustrates an autoradiograph from individuals with several different APOE genotypes. These restriction isotyping methods have been used in many laboratories for several years, especially in studies relating to atherosclerosis and heart disease.^{24,25}

The table illustrates the APOE $\epsilon 4$ allele frequency estimates in three control populations: (1) 91 grandparents from the CEPH reference families, (2) 21 spouses from an ongoing prospective study examining consecutive sporadic, probable AD

Table. APOE $\epsilon 4$ allele frequency estimates

Population*	$\epsilon 4$ allele*	Z†	p Value
Normal controls			
CEPH‡ (182)	0.16 ± 0.027		
Spouses§ (42)	0.10 ± 0.046	0.59	0.56
Menzel ³² (2,000)	0.14 ± 0.008	0.71	0.48
Alzheimer's			
LOAD¶ (72)	0.42 ± 0.058	4.30	0.000017
EOAD# (32)	0.19 ± 0.069	0.40	0.69
Clinical sporadic AD** (138)	0.36 ± 0.042	4.17	0.00031
Autopsy sporadic AD†† (352)	0.40 ± 0.026	6.49	<0.00001

* Number of chromosomes counted is presented in parentheses; allele frequency estimates ± SE.
† Z values are versus the CEPH control group.
‡ Ninety-one unrelated grandparents from the Centre d'Etude du Polymorphisme Humain (CEPH).
§ Spouse controls in the Bryan ADRC Memory Disorders Clinic, Duke University.
¶ One randomly selected affected individual from each of 32 Duke and four Toronto/Boston late-onset AD families.
One randomly selected affected individual from each of 13 Toronto/Boston and three Duke early-onset AD families.
** Thirty-nine Duke and 30 Toronto/Boston sporadic (probable) AD patients.
†† One hundred forty-three Duke and 33 Toronto/Boston autopsy-confirmed sporadic AD subjects.

patients, and (3) a representative control series from a similar population in the literature.³² Also illustrated are the APOE $\epsilon 4$ allele frequency estimates for several different Alzheimer's disease

groups: (1) one randomly selected affected individual in the combined Duke and Toronto/Boston late-onset FAD series; (2) one randomly selected affected individual in the combined Toronto/Boston and Duke early-onset FAD families; (3) banked DNA samples from sporadic patients carrying the diagnosis of probable AD from the Duke and Toronto/Boston clinics; and (4) DNA from 176 autopsy-confirmed sporadic AD patients from Duke and Toronto/Boston.

As previously reported,¹³ the $\epsilon 4$ allele frequency of the randomly selected affected patients in the predominantly late-onset FAD families was significantly different from that of the CEPH controls: 0.50 ± 0.06 versus 0.16 ± 0.027 (allele frequency estimate $\pm$ SE), $Z = 2.44$, $p = 0.014$. Likewise, the combined Duke and Toronto/Boston late-onset FAD series presented here is significantly different from the CEPH controls ($p = 0.000017$). The $\epsilon 4$ frequency of the combined early-onset FAD series from Toronto/Boston and Duke (0.19 ± 0.069) did not differ significantly ($p = 0.69$) from the frequency in the CEPH controls. Statistical analyses demonstrated highly significant differences in the $\epsilon 4$ allele frequencies in both sporadic (probable) AD ($p = 0.00031$) and autopsy-confirmed sporadic AD ($p < 0.00001$) when compared with the CEPH controls.

We also examined the association of the 3.5-kb *Taq* I restriction fragment length polymorphism of APOCII with FAD in the same randomly chosen affected individuals from the Duke pedigrees who previously were shown to have a significantly higher frequency of the $\epsilon 4$ allele.¹³ We did not find a statistically significant association of the 3.5-kb allele with FAD in these families (allele frequency = 0.52 ± 0.069 ; $Z = 1.46$, $p = 0.14$) when compared with the frequency in a literature control population (0.41 ± 0.03).^{28,33}

Discussion. We previously demonstrated a statistically significant association between the APOE $\epsilon 4$ allele and late-onset FAD¹³ and have now extended this association to sporadic AD. The initial data were derived from a randomly selected affected individual in each of 30 families with FAD¹³; that series contained late-onset FAD families used for association/linkage studies of chromosome 19 markers as well as two early-onset FAD families in our series. One of the early-onset FAD families had been linked to chromosome 14^{10,11}; the other was reported in the original description of the APP717Val $\rightarrow$ Ile mutation.²

Several points are illustrated in the table. First, the CEPH grandparent control group reflects the APOE $\epsilon 4$ allele frequency observed in other published populations of the same race and ethnicity.^{32,34-36} Second, the $\epsilon 4$ allele frequency in the ongoing series of spouse controls from the Bryan ADRC Clinic is not significantly different from that of the CEPH controls even though the number of chromosomes counted is relatively small. There was a

highly significant association of APOE $\epsilon 4$ in each of the late-onset AD groups, including the combined Duke/Toronto/Boston families, the clinical sporadic AD patients, and the combined sporadic AD autopsy groups. In contrast, there was no significant difference from controls in the combined early-onset FAD groups.

The Toronto/Boston series of FAD families represents a primarily early-onset group that has been robustly linked to a locus on chromosome 14q.¹¹ The early-onset families linked to chromosome 14 are not associated with an increase of the $\epsilon 4$ allele, which suggests a genetic locus on chromosome 14 that is sufficient for expression of the AD phenotype at an early age and that segregates as a typical autosomal dominant trait. Schellenberg et al²⁸ reported an association of an APOCII 3.5-kb *Taq* I polymorphism with predominantly early-onset FAD families. APOCII is also on chromosome 19,¹⁴ within 50 kb of APOE.³⁷ We found no significant association of this polymorphism with our predominantly late-onset FAD families. The significance of the Schellenberg et al²⁸ data in their families remains to be interpreted.

Published data using the APM method of linkage analysis found a highly significant association of multiple contiguous chromosome 19q markers with late-onset FAD.¹² However, it could not be determined at that time whether these results indicated true linkage or association; APOE data were not included in the original analyses. Standard likelihood analysis of these data, assuming a single-gene autosomal dominant model of inheritance, was suggestive but could not firmly establish linkage to this region. APM analysis of APOE and the FAD in this communication also resulted in highly significant results ($p < 0.001$) for linkage/association between APOE and AD. Lod score analysis, although suggestive, is not significant ($Z = 2.4$ at a maximum recombination fraction of 0.10).

Clinicians routinely accept the contributions of multiple factors in the development of a disease. Words such as "susceptibility" and "predisposition" are used to describe why some individuals develop disease when others do not. In the field of atherosclerosis, the contribution of multiple loci to the development of a particular phenotype, such as coronary artery disease, is well defined, and atherosclerosis accepted as a complex genetic disease.³⁸ In fact, locus heterogeneity of FAD is now well documented.^{2,10-12,18} Our data support the view that late-onset AD can also be viewed as a complex genetic disease, clustered in families that segregate the APOE $\epsilon 4$ allele. The extension of the data from the late-onset FAD families to the population of apparently sporadic AD patients suggests an important functional role for the $\epsilon 4$ allele in the pathogenesis of AD. The $\epsilon 4$ allele may be viewed as a susceptibility gene or risk factor for AD that can be tested in properly constructed epidemiologic studies.

Multiple genes can apparently contribute to sim-

ilar phenotypic expression in AD. Some, like APOE $\epsilon 4$, may contribute to increased susceptibility, while others may be sufficient to express the disease. In the presence of the APP mutations, the rate of pathogenesis of the disease in these families is apparently increased, leading to an earlier age of onset. In these families, the disease segregates as a classic autosomal dominant trait. The finding of linkage for early-onset FAD on chromosome 14q will presumably lead to the identification of another locus sufficient to accelerate the rate of disease expression. We continue to screen the late-onset FAD pedigrees for other associations and expect that other susceptibility loci will be identified.

Association of late-onset FAD with a contiguous series of polymorphic markers on chromosome 19 suggested that evaluation of potential candidate genes from this region might be a productive research strategy. Antisera to ApoE demonstrate the presence of ApoE in plaques, neurofibrillary tangles, and vascular amyloid in AD.^{13,16,17} ApoE immunoreactivity is also present in the plaques of Creutzfeldt-Jakob disease, scrapie, Down's syndrome, and other cerebral and systemic amyloidoses,^{16,17} and ApoE fragments are associated with human amyloid A protein.³⁹ These data suggest a broad role for ApoE in diseases in which amyloids are deposited. With the recent finding that amyloid β -peptide may be a normal cellular product,^{40,41} the metabolism of normally produced β -peptide becomes important. The deposition of β -amyloid in AD can be viewed as a pathologic sign that is used to define AD. Thus, faulty mechanisms of clearance or sequestration of β -amyloid may occur during the development of AD. Studies with purified ApoE-E3/3 and ApoE-E4/4 have demonstrated a difference in the rate of binding to amyloid β -peptide.⁴² Bridging the gap between genetic data and relevant pathogenetic interactions may dissect the interactions that can be targeted for rational therapeutic interventions.

Late-onset FAD can be recognized only in families that are large enough to ascertain and test. In contrast, the diagnosis of "sporadic AD" is used when there is no family history. This distinction is confounded by family size, age of onset of disease, and age at death of family members. If, in fact, discrimination between late-onset FAD and sporadic AD is a function of family size and longevity, then data developed in the late-onset FAD subgroup should be applicable to sporadic AD patients. The association with APOE $\epsilon 4$ supports this view and provides a rationale for using the late-onset FAD pedigrees to search for other associated genetic regions.

ApoE is the major apolipoprotein in brain.⁴³ While its functional role with lipid transport is well described,³⁵ we have proposed a broader role for ApoE in the metabolism, transport, and targeting of amyloid β -peptide. Differential metabolism of ApoE-E3 and ApoE-E4 isoforms may lead to differences in β -peptide sequestration, particularly in

the brain parenchyma and cerebral blood vessels.^{44,45} Studies of the specific molecular interactions of amyloid β -peptide with ApoE-E3/3 and ApoE-E4/4 isoforms are currently in progress. The combined weight of linkage/association, immunochemical pathology, and binding data in late-onset FAD and sporadic AD, as well as the published immunochemical studies in scrapie and Creutzfeldt-Jakob disease, should provide an impetus to examine the functional roles of APOE isoforms in late-onset AD.

Acknowledgments

We are grateful to Dr. James E. Hixson, Southwest Foundation for Biomedical Research, San Antonio, TX, for supplying APOE isoform control DNA samples; to K. Dole, E. Hanson, O. Yorgba, P. Pate, H. Harbett, C. Haynes, J. Rimmler, and S. Margaux for technical assistance; and to Drs. Amaducci, Bergamini, Bruni, Drachman, Foncin, Polinsky, Frommelt, Growden, and Pollen for their contributions to the development of the early-onset FAD families.

References

1. St. George-Hyslop PH, Tanzi RE, Polinsky RJ, et al. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* 1987;235:885-890.
2. Goate A, Chartier-Harlin MC, Mullan M, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 1991;349:704-706.
3. Naruse S, Igarashi S, Aoki K, et al. Mis-sense mutation Val $\rightarrow$ Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. *Lancet* 1991;337:978-979.
4. Chartier-Harlin M-C, Crawford F, Houlden H, et al. Early-onset Alzheimer's disease caused by mutations at codon 717 of the β -amyloid precursor protein gene. *Nature* 1991;353:844-846.
5. Murrell J, Farlow M, Ghetti B, Benson MD. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science* 1991;254:97-99.
6. Yoshioka K, Miki T, Katsuya T, Ogiwara T, Sakaki Y. The ⁷¹⁷Val $\rightarrow$ Ile substitution in amyloid precursor protein is associated with familial Alzheimer's disease regardless of ethnic groups. *Biochem Biophys Res Commun* 1991;178:1141-1146.
7. Karlinsky H, Vaula G, Haines JL, et al. Molecular and prospective phenotypic characterization of a pedigree with familial Alzheimer's disease and a missense mutation in codon 717 of the β -amyloid precursor protein gene. *Neurology* 1992;42:1445-1453.
8. Hendriks L, van Duijn CM, Cras P, et al. Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β -amyloid precursor protein. *Nature Genet* 1992;1:218-221.
9. Mullan M, Crawford F, Axelman K, et al. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β -amyloid. *Nature Genet* 1992;1:345-347.
10. Schellenberg GD, Bird TD, Wijsman EM, et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* 1992;258:668-671.
11. St. George-Hyslop P, Haines J, Rogaev E, et al. Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. *Nature Genet* 1992;2:330-334.
12. Pericak-Vance MA, Bebout JL, Gaskell PC, et al. Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. *Am J Hum Genet* 1991;48:1034-1050.
13. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high affinity binding to β A amyloid and

- increased frequency of type 4 allele in familial Alzheimer's. *Proc Natl Acad Sci USA* 1993;90:1977-1981.
14. Ropers HH, Pericak-Vance MA. Report of the committee on the genetic constitution of chromosome 19. *Human Gene Mapping 11* (1991). *Cytogenet Cell Genet* 1991;58:751-784.
 15. Haines JL, St. George-Hyslop PH, Rimmer JB, et al. Inheritance of multiple loci in familial Alzheimer disease. *Neurobiol Aging* 1992;13:S65(254).
 16. Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K. Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeld-Jakob disease. *Brain Res* 1991;541:163-166.
 17. Wisniewski T, Frangione B. Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci Lett* 1992;135:235-238.
 18. St. George-Hyslop PH, Haines JL, Farrer LA, et al. Genetic linkage studies suggest that Alzheimer's disease is not a single homogeneous disorder. *Nature* 1990;347:194-197.
 19. McKhann G, 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 Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984;34:939-944.
 20. Haynes C, Pericak-Vance M, Dawson D. Analysis of Huntington disease linkage and age-of-onset distribution. *Genet Epidemiol* 1986;3(suppl 1):235-239.
 21. Dausett J, Cann H, Cohen D, Lathrop M, Lalouel JM, White R. Centre D'Etude du Polymorphisme Humain (CEPH): collaborative genetic mapping of the human genome. *Genomics* 1990;6:575-577.
 22. Pericak-Vance MA, Yamaoka LH, Assinder RIF, et al. Tight linkage of apolipoprotein C2 to myotonic dystrophy on chromosome 19. *Neurology* 1986;36:1418-1423.
 23. Pericak-Vance MA, Yamaoka LH, Haynes CS, et al. Genetic linkage studies in Alzheimer's disease families. *Exp Neurol* 1988;102:271-279.
 24. Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. *Lancet* 1991;337:1158-1159.
 25. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. *J Lipid Res* 1990;31:545-548.
 26. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989:B.24.
 27. Elston RC, Johnson WD. *Essentials of biostatistics*. Philadelphia: FA Davis, 1987.
 28. Schellenberg GD, Deeb SS, Boehnke M, et al. Association of an apolipoprotein CII allele with familial dementia of the Alzheimer type. *J Neurogenet* 1987;4:97-108.
 29. Weeks DE, Lange K. The affected-pedigree-member method of linkage analysis. *Am J Hum Genet* 1988;42:315-326.
 30. Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;36:460-465.
 31. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-3446.
 32. Menzel H-J, Kladetzky R-G, Assmann G. Apolipoprotein E polymorphism and coronary artery disease. *Arteriosclerosis* 1983;3:310-315.
 33. Wallis SC, Donald JA, Forrest LA, Williamson R, Humphries SE. The isolation of a genomic clone containing the apolipoprotein CII gene and the detection of linkage disequilibrium between two common DNA polymorphisms around the gene. *Hum Genet* 1984;68:286-289.
 34. Ehnholm C, Lukka M, Kuusi T, Nikkila E, Utermann G. Apolipoprotein E polymorphisms in Finland. In: Fidge NH, Nestel PJ, eds. *Excerpta medica: Atherosclerosis VII*. Amsterdam: Elsevier, 1986:325-328.
 35. Mahley RW, Rall SC. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic basis of inherited disease*. 6th ed. New York: McGraw-Hill, 1989:1195-1213.
 36. Hallman DM, Boerwinkle E, Saha N, et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. *Am J Hum Genet* 1991;49:338-349.
 37. Myklebost O, Rognes S. A physical map of the apolipoprotein gene cluster on human chromosome 19. *Hum Genet* 1988;78:244-247.
 38. Kraus RM. The tangled web of coronary risk factors. *Am J Med* 1991;90(suppl 2A):36S-41S.
 39. Prelli F, Pras M, Strasburg S, Frangione B. Characterization of high molecular weight amyloid A proteins. *Scand J Immunol* 1991;33:783-786.
 40. Haass C, Schlossmacher MG, Hung AY, et al. Amyloid β -peptide is produced by cultured cells during normal metabolism. *Nature* 1992;359:322-325.
 41. Seubert P, Vigo-Pelfry C, Esch F, et al. Isolation and quantification of soluble Alzheimer's β -peptide from biological fluids. *Nature* 1992;359:325-327.
 42. Roses AD, Pericak-Vance MA, Saunders AM, Schmechel D, Goldgaber D, Strittmatter W. Complex genetic disease: can genetic strategies in Alzheimer's disease and new genetic mechanisms be applied to epilepsy? *Epilepsia* (in press).
 43. Elshourbagy NA, Liao WS, Mahley RW, Taylor JM. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other tissues of rats and marmosets. *Proc Natl Acad Sci USA* 1985;82:203-207.
 44. Goldgaber D, Schwarzman AI, Bhasin R, et al. Sequestration of amyloid β -peptide. In: Nitsch R, Growdon JH, Corkin S, Wurman RJ, eds. *Alzheimer's disease: amyloid precursor proteins, signal transduction, and neuronal transplantation*. Cambridge, MA: Center for Brain Sciences and Metabolism Charitable Trust, 1993:279-284.
 45. 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's disease. *Proc Natl Acad Sci USA* (in press).

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Neurology 1993;43;1467

DOI 10.1212/WNL.43.8.1467

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