Effects of Age, Sex, and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease

A Meta-analysis

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Objective.—To examine more closely the association between apolipoprotein E (*APOE*) genotype and Alzheimer disease (AD) by age and sex in populations of various ethnic and racial denominations.

Data Sources.—Forty research teams contributed data on *APOE* genotype, sex, age at disease onset, and ethnic background for 5930 patients who met criteria for probable or definite AD and 8607 controls without dementia who were recruited from clinical, community, and brain bank sources.

Main Outcome Measures.—Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age and study and stratified by major ethnic group (Caucasian, African American, Hispanic, and Japanese) and source, were computed for *APOE* genotypes ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε4, and ε4/ε4 relative to the ε3/ε3 group. The influence of age and sex on the OR for each genotype was assessed using logistic regression procedures.

Results.—Among Caucasian subjects from clinic- or autopsy-based studies, the risk of AD was significantly increased for people with genotypes ε2/ε4 (OR=2.6, 95% CI=1.6-4.0), ε3/ε4 (OR=3.2, 95% CI=2.8-3.8), and ε4/ε4 (OR=14.9, 95% CI=10.8-20.6); whereas, the ORs were decreased for people with genotypes ε2/ε2 (OR=0.6, 95% CI=0.2-2.0) and ε2/ε3 (OR=0.6, 95% CI=0.5-0.8). The *APOE* ε4–AD association was weaker among African Americans and Hispanics, but there was significant heterogeneity in ORs among studies of African Americans ($P<.03$). The *APOE* ε4–AD association in Japanese subjects was stronger than in Caucasian subjects (ε3/ε4: OR=5.6, 95% CI=3.9-8.0; ε4/ε4: OR=33.1, 95% CI=13.6-80.5). The ε2/ε3 genotype appears equally protective across ethnic groups. We also found that among Caucasians, *APOE* genotype distributions are similar in groups of patients with AD whose diagnoses were determined clinically or by autopsy. In addition, we found that the *APOE* ε4 effect is evident at all ages between 40 and 90 years but diminishes after age 70 years and that the risk of AD associated with a given genotype varies with sex.

Conclusions.—The *APOE* ε4 allele represents a major risk factor for AD in all ethnic groups studied, across all ages between 40 and 90 years, and in both men and women. The association between *APOE* ε4 and AD in African Americans requires clarification, and the attenuated effect of *APOE* ε4 in Hispanics should be investigated further.

JAMA. 1997;278:1349-1356

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A complete listing of the APOE and Alzheimer Disease Meta Analysis Consortium appears at the end of this article.

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EPIDEMIOLOGIC and molecular evidence suggests there are multiple causes for Alzheimer disease (AD). Most of the known genetic causes—including defects in the amyloid precursor protein (*APP*) gene and the presenilin 1 and presenilin 2 genes[1-3]—are rare and account for less than 2% of cases.[4] These mutations behave as classic autosomal dominant traits. Simply put, with rare exceptions, persons inheriting one of these defects will develop AD unless they die prematurely from other causes.

Apolipoprotein E (*APOE* indicates the gene and APOE indicates the protein), a plasma protein involved in cholesterol transport and encoded by a gene on chromosome 19,[5] is the fourth genetic factor implicated in the risk of developing AD. There are 3 common alleles (ε2, ε3, and ε4) corresponding to 6 phenotypes, each with different isoelectric points. In typical Caucasian populations, ε3 is the most common allele, occurring on more than 75% of chromosomes. The average frequencies of ε2 and ε4 are 8% and 15%, respectively.[5] Initial reports demonstrated that the *APOE* ε4 allele is disproportionately represented among patients with late-onset AD (symptoms occurring after age 65 years),[6-8] but this association was soon extended to patients with early-onset AD.[9-11] Subsequent confirmations in numerous ethnic populations have established the *APOE* genotype as perhaps the most important biological marker for susceptibility for AD identified thus far, accounting for 45% to 60% of its genetic component.[12,13] Individuals who are heterozygous for the ε4 allele have an odds ratio (OR) between 2.2 and 4.4 of developing AD compared with persons who have the ε3/ε3 genotype, while those who are homozygous for ε4 have an OR ranging from 5.1 to 34.3.[4,14] In contrast, the ε2 allele may confer a protective effect because it is slightly underrepresented in patients

with AD,[15] but this is unclear in some populations.[16,17] The *APOE* genotype also is associated with the age at onset of AD in a dose-dependent fashion such that the more ε4 alleles there are, the younger the age at disease onset tends to be, and age at onset tends to be older among persons with the ε2/ε3 genotype.[15,18,19]

Several biological explanations of this association have been offered,[20-23] but none adequately explain why at least one third of patients with AD lack ε4[4,6] and why as many as 50% of people who have a double dose of ε4 and survive to age 80 years do not develop AD.[24,25] Factors such as family history, sex, serious head injury, smoking, cholesterol level, and estrogen may modify the *APOE*-related risk.[11,26-31] The risk of AD attributable to the *APOE* ε4 allele is significant at both ends of the age spectrum,[9-11,32] but there is disagreement regarding the strength of the association among the very elderly.[32-34] It is unclear whether variability in *APOE* genotype–specific risks of AD across populations is in part the result of bias in the recruitment or diagnostic incongruities of case patients or controls or the modifying effects of different genetic and environmental backgrounds. For example, the *APOE* ε4–AD association in African Americans is controversial[35,36] and apparently absent in a Nigerian sample of patients with AD and controls.[37]

The detection of small effects, such as the protective effect of the *APOE* ε2 allele or effects among relatively small strata of the population (eg, nonagenarians or ethnic or racial minorities), requires a subject population much larger than that obtainable for most individual research studies. To this end, we have assembled a data set containing diagnostic, demographic, and *APOE* genotype information on more than 15 000 patients with AD and controls contributed by 40 research teams from many different parts of the world. In this article, we report a meta-analysis using raw data that further strengthens the association of *APOE* ε4 with AD in Caucasian and Japanese subjects and defines more precisely than in other studies the *APOE* ε4–AD association in African-American and Hispanic subjects.

METHODS

Sample Recruitment

A committee comprising individuals with expertise in genetics and diagnosis of AD, epidemiology, and statistics was constituted to set the goals of this study and identify pertinent data to be collected. A MEDLINE search was used to assist in the identification of data sets. Letters of invitation to participate in this project were sent to representatives of groups who had published or made known to the committee the existence of data on the *APOE* ε4–AD association prior to July 1, 1995. Of the 48 groups sent invitations, 44 agreed to participate, 1 declined, and 3 did not respond. Data were received from 40 of the 44 positive responders. For each subject the following information was sought: a unique identifier, diagnostic status, sex, censoring age (ie, age at last examination or death), age at disease onset (if affected), ethnicity, family history, and *APOE* genotype. The data set included case patients who had a provisional or final diagnosis of AD, AD-like dementia, and other neuropsychiatric illness and controls. Subjects with dementia who did not meet criteria for definite or probable AD and subjects with other neuropsychiatric illnesses were not considered. Case patients were diagnosed as having definite or probable AD, and controls were free of neurodegenerative and neuropsychiatric illnesses. Case patients with known mutations in the *APP* or presenilin genes or coexisting neuropathological findings (eg, Lewy bodies, Parkinson disease changes) were excluded. Selected characteristics of the patients with AD and controls at each of the 40 participating centers are listed in Table 1.[39-68]

Pooling Criteria

Ascertainment.—Participating centers used a variety of sampling strategies such as case-control, cohort, and cross-sectional samples and mixtures thereof. Some centers enrolled subjects with AD only. Review of the designs at each center suggested a trichotomous classification scheme based on one of the following recruitment settings: community/population, clinic/hospital, or autopsy/brain bank. Several centers ascertained subjects under multiple designs (Table 1). Subjects who were enrolled in a clinic-based setting and whose diagnosis was confirmed at autopsy were assigned as clinic ascertainment.

Ethnicity.—Subjects were categorized by a variety of ethnic or racial designations, as follows: Caucasian (6264), Anglo-Saxon (667), French (704), French Canadian (792), Scandinavian (77), Finnish (259), Italian (280), Dutch (1848), German (270), Ashkenazi Jewish (201), Sephardic Jewish (5), African American (475), American Indian (2), Hispanic (528), Asian (10), Japanese (2313), Chinese (1), other (7), and unknown (18). Japanese, Hispanic, and African-American subjects were classified into separate groups because of evidence indicating *APOE* allele frequency differences, distinct patterns of association with AD, or both.[10,35] The 38 subjects labeled as American Indian, Chinese, Asian, and other or unknown ethnicity were dropped from the analysis. The distribution of *APOE* genotypes was similar among the remaining Caucasian groups (data not shown), and these groups were pooled into a single Caucasian group.

Diagnostic Categories.—The diagnoses of definite (ie, autopsy-confirmed) and probable AD were established at all sites using standardized research criteria.[38,69] The enormity of the sample enabled detection of a statistically significant difference in the distribution of *APOE* genotypes between Caucasian case patients with definite and probable AD (χ^2=12.76, df=5, P=.03), but this does not appear to be meaningful because the difference in the ε4 allele frequency was only 1.4%. It was therefore deemed appropriate to pool case patients with probable and definite AD in subsequent analyses. Non-Caucasian case patients with definite and probable AD were combined in each of the other ethnic groups because there were so few diagnosed as having definite AD.

APOE Isotyping Methods

Investigators determined APOE isoforms using 1 of 2 basic approaches. In a few studies, APOE phenotypes were determined from plasma very low-density lipoproteins after ultracentrifugation. The isolated very low-density lipoprotein particles were lyophilized, delipidated, and subjected to isoelectric focusing within a pH range of 4 to 6.5, using previously described methods.[70] Most studies conducted *APOE* genotyping by a polymerase chain reaction (PCR)–based approach[71] using DNA isolated from blood or autopsy tissue. The *APOE* gene was amplified according to conditions developed in the individual laboratories. The PCR product was digested with either *HhaI* or *CfoI* following a standard procedure,[72] and fragments were separated on either an agarose or standard, nondenaturing 6% polyacrylamide gel. Alternatively, biotinylated PCR products were analyzed using a reverse DNA hybridization test.[48]

Statistical Methods

A χ^2 test was used to compare *APOE* allele frequencies by diagnostic category and ethnic group. Subjects were analyzed within ascertainment and ethnic strata previously described. In each stratum, an age- and study-adjusted OR for AD according to presence or absence of at least 1 ε4 allele was estimated using Mantel-Haenszel statistics.[73] For the Caucasian groups, age was adjusted by the use of 10 classes (0-49 years, 50-54 years, 55-59 years, 60-64 years, 65-69 years, 70-74 years, 75-79 years, 80-84 years, 85-89 years, and ≥90 years). Age classes were collapsed into fewer categories for the

Table 1.—Characteristics of Case Patients With Alzheimer Disease (AD) and Controls*

Study†	Case Patients With AD: No. (% Female)	Case Patients With AD: Mean (SD) Onset Age	Controls: No. (% Female)	Controls: Mean (SD) Examination Age	Ethnicity‡	Ascertainment Scheme§
Benjamin et al[39]	139 (55.4)	76.0 (7.2)‖	89 (42.7)	64.2 (16.0)	C	A
Benjamin et al[40]	61 (70.5)	79.7 (9.7)	14 (57.1)	82.4 (8.5)	C	A
Betard et al[41]	122 (69.7)	71.1 (8.8)	4 (0)	80.8 (4.9)	C	A
Betard et al[41]	38 (65.8)	81.1 (10.8)	246 (45.9)	81.0 (5.7)	C	P
Blacker et al[42]¶	310 (69.0)	71.4 (8.6)	0 (. . .)	. . .	95% C; 5% AA; 0.3% H	C
Chartier-Harlin et al[9]	45 (84.4)	70.0 (6.7)	45 (73.3)	72.5 (10.8)	C	P
Chartier-Harlin et al[9]	115 (60.9)	83.2 (6.1)	37 (56.8)	82.5 (8.4)	C	C
Corder et al[15]	190 (58.4)	67.2 (9.1)	100 (59.0)	69.1 (7.3)	93% C; 7% AA	C
Duara et al[43]	196 (73.5)	75.0 (8.1)	46 (67.4)	71.8 (11.1)	64% C; 4% AA; 32% H	C
Duara et al[43]	23 (43.5)	77.4 (4.7)	150 (66.0)	72.1 (7.8)	73% C; 12% AA; 15% H	P
Fallin et al[44]	130 (67.7)	70.0 (10.0)	137 (72.3)	79.0 (8.0)	83% C; 2% AA; 15% H	C
Farrer et al[26]	441 (50.3)	68.3 (9.1)	166 (56.6)	70.8 (10.8)	99.7% C; 0.3% J	C
Frisoni et al[45]	157 (76.4)	69.3 (8.3)	120 (58.3)	69.4 (11.2)	C	C
Galasko et al[46]	175 (43.4)	68.8 (7.7)	91 (52.8)	73.8 (8.2)	91% C; 3% AA; 7% H	C
Gearing et al[47]	41 (46.3)	68.1 (11.1)	1 (0)	64	95% C; 5% AA	A
Harrington et al[48]	173 (58.4)	71.1 (10.4)	138 (45.6)	76.7 (14.3)	C	A
Hendrie et al[36]	24 (66.7)	79.0 (4.0)	54 (59.3)	78.2 (6.1)	AA	P
Houlden et al[49]	125 (59.2)	65.0 (13.1)	119 (48.7)	73.9 (9.3)	C	C
Kawamata et al[50]	53 (81.1)	72.7 (11.3)	20 (30.0)	46.3 (25.7)	J	C
Kukull et al[14]	242 (64.0)	76.1 (6.9)	340 (64.1)	81.6 (6.7)	94.5% C; 5.0% AA; 0.5% H	P
Kurz et al[51]	190 (49.0)	67.7 (9.6)	80 (0)	69.5 (12.0)	C	C
Kuusisto et al[52]	46 (69.6)	74.1 (2.6)#	985 (64.5)	72.9 (2.9)	C	P
Lehtimaki et al[53]	95 (55.8)	66.6 (7.4)	74 (59.5)	63.5 (7.6)	C	C
(A. Levey, MD, PhD, unpublished data, Sept 1995)	61 (62.3)	69.7 (8.0)	41 (61.0)	70.7 (10.2)	79% C; 21% AA	C
Lehtovirta et al[54]	204 (70.1)	70.6 (8.5)	55 (63.6)	73.5 (6.2)	C	C
Lippa et al[55]	19 (21.0)	67.3 (12.9)	9 (44.4)	68.3 (15.0)	93% C; 7% H	A
Lucotte et al[56]	128 (69.5)	75.6 (5.9)	0 (. . .)	. . .	C	C
Maestre et al[35]	305 (75.7)	79.7 (7.6)	485 (67.0)	73.0 (6.6)	22% C; 33% AA; 45% H	P
Mahieux et al[57]	112 (67.9)	73.4 (7.5)	466 (36.0)	37.2 (10.6)	99.6% C; 0.4% H	C
Martins et al[58]	141 (57.4)	71.9 (9.8)	78 (25.6)	66.2 (16.0)	C	P
Nalbantoglu et al[12]	93 (49.5)	76.3 (9.2)#	73 (30.1)	71.1 (14.7)	C	A
Noguchi et al[59]	38 (81.6)	79.7 (8.3)#	584 (27.4)	55.8 (11.5)	J	C/P
Okuizumi et al[10]	83 (61.4)	66.8 (10.3)	149 (45.6)	49.1 (21.3)	J	C
Pickering-Brown et al[60]	61 (60.7)	65.1 (11.1)	0 (. . .)	. . .	C	C
Poirier et al[8]	90 (66.7)	64.0 (9.1)	74 (60.8)	75.9 (9.7)	C	C
Rebeck et al[20]	213 (62.4)	72.9 (11.0)	128 (59.4)	79.4 (13.5)	C	C
Smith et al[61]	27 (48.2)	65.0 (6.4)	130 (56.9)	71.8 (12.4)	C	P
Smith et al[62]	74 (62.2)	73.2 (8.2)	10 (40.0)	77.5 (7.7)	C	A
Sorbi et al[16]	135 (61.5)	58.5 (10.4)	196 (57.6)	54.8 (28.5)	C	C
St Clair et al[63]	154 (69.5)	68.1 (13.1)#	47 (38.3)	77.1 (7.0)	C	C
Talbot et al[64]	157 (53.5)	70.6 (8.6)	112 (56.2)	78.5 (9.6)	98% C; 2% AA	C
Tsai et al[65]	115 (74.8)	78.2 (9.0)	268 (69.8)	82.5 (7.7)	C	P
Ueki et al[66]	77 (62.3)	71.0 (8.3)	616 (50.5)	48.4 (20.3)	J	C
van Duijn et al[17]	348 (44.0)	69.3 (13.4)	1500 (43.6)	67.4 (8.3)	C	P
Yoshizawa et al[67]	83 (77.1)	65.6 (11.8)	608 (0)	47.6 (7.8)	J	C/P
Zubenko et al[68]	0 (. . .)	. . .	38 (63.2)	56.3 (9.6)	C	C
Zubenko et al[68]	86 (47.8)	68.7 (7.8)	23 (60.5)	67.1 (11.9)	97% C; 3% AA	A

*Ellipses indicate not applicable.
†Additional data were used to supplement the data from many of the studies listed in this table.
‡For ethnicity, C indicates Caucasian; AA, African American; H, Hispanic; and J, Japanese.
§For ascertainment, C indicates clinic/hospital; P, population/community; and A, autopsy/brain bank.
‖N=23.
¶Data set comprises affected sibling pairs; 1 sibling from each pair was randomly selected.
#Age at examination.

smaller African-American and Hispanic groups (0-69 years, 70-79 years, and ≥80 years) and the Japanese group (0-59, 60-69, 70-79, and ≥80 years). Odds ratios adjusted for age and study were also computed for *APOE* genotypes ε2/ε2, ε2/ε3, 2/ε4, ε3/ε4, and ε4/ε4 relative to the ε3/ε3 group. In addition, a χ^2 statistic recommended by Breslow and Day[74] was computed to assess whether data sets in each stratum could be pooled. The non-Caucasian groups could not be evaluated by ascertainment scheme because there were too few observations.

The influence of *APOE* genotype, age, and sex on the odds of developing AD was assessed using logistic regression.[75] To accommodate the polychotomous classification of *APOE* genotype in the regression analysis, 4 indicator variables were made to represent the genotype classes ε2/ε2 or ε2/ε3, ε2/ε4, ε3/ε4, and ε4/ε4. These variables took on the value of 1 if the subject had the corresponding genotype; otherwise, the value was 0. The ε3/ε3 genotype was the referent. Age at onset of AD among case patients and age at last ex-

Table 2.—Apolipoprotein E (*APOE*) Genotype and Allele Distributions in Case Patients With Alzheimer Disease (AD) and Controls by Ethnic Group

Ethnic Group	No.	*APOE* Genotype Frequency, %						*APOE* Allele Frequency, %		
		ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4
Caucasian										
Case patients	5107	0.2	4.8	2.6	36.4	41.1	14.8	3.9	59.4	36.7
Controls	6262	0.8	12.7	2.6	60.9	21.3	1.8	8.4	77.9	13.7
African American										
Case patients	235	1.7	9.8	2.1	36.2	37.9	12.3	7.7	59.1	32.2
Controls	240	0.8	12.9	2.1	50.4	31.8	2.1	8.3	72.7	19.0
Hispanic										
Case patients	261	0.4	9.6	2.3	54.4	30.7	2.7	6.3	74.5	19.2
Controls	267	0.4	12.0	0.8	67.4	17.6	1.9	6.7	82.3	11.0
Japanese										
Case patients	336	0.3	3.9	0.9	49.1	36.9	8.9	2.7	69.5	27.8
Controls	1977	0.4	6.9	0.8	75.7	15.5	0.8	4.2	86.9	8.9

amination among controls were assigned to the age variable. Nonlinear effects of age were considered by use of an with age-squared term. Interaction between *APOE*, age, and sex was evaluated by deriving product terms for each genotype with age, age-squared, and sex terms. Models were evaluated using the LOGISTIC procedure in SAS.[76] The relative fit values of the hierarchal models were determined by computing the differences in the −2-ln likelihoods for the models, and these differences follow a χ^2 distribution.

RESULTS

Ethnic Patterns

The relative increase in the frequency of *APOE* ε4 in case patients with AD compared with controls is substantially less in African Americans (32.2%/19.0%=1.7) than Caucasians (36.7%/13.6%=2.7) (Table 2). The frequency of the ε4/ε4 genotype among case patients and controls is comparable across these groups, although the ε3/ε4 genotype is more frequent in Caucasian than in African-American case patients and less frequent in Caucasian than in African-American controls. There was less of a difference between Hispanic case patients and controls in ε4 frequency (19.2%/11.0%=1.7), largely because of a paucity of ε4/ε4 homozygotes relative to ε3/ε4 heterozygotes. The ε4 allele is less prevalent in Japanese case patients and controls, but the ratio appears similar to that in Caucasians (27.8%/8.9%=3.1). The ε2 allele is more than twice as frequent in controls than case patients among Caucasians. A similar trend was noted in the Japanese controls and case patients, although ε2 was equally frequent in African-American and Hispanic case patients and controls. Analysis of the allele frequency data revealed that all the control populations are in Hardy-Weinberg equilibrium (Caucasians: $\chi^2=2.65$, $df=3$, $P=.5$; African Americans: $\chi^2=4.35$, $df=3$, $P=.3$; Hispanics: $\chi^2=2.02$, $df=3$, $P=.6$; Japanese: $\chi^2=6.39$, $df=3$, $P=.1$), suggesting that ethnic differences in the pattern of association are not attributable to recent admixture of populations with different distributions of *APOE* genotypes.

Comparability Across Studies

The odds of AD for subjects with at least 1 ε4 allele compared with subjects without ε4 were calculated for each study, stratified by ethnic group (data available on request). The variability in the OR (ranging from 2.1 to 8.1) across 22 clinic- or hospital-based studies of Caucasians was not significant. Preliminary analysis of the ORs computed for Caucasians in autopsy-based studies revealed significant heterogeneity (Breslow-Day $P=.02$). However, examination of the subject recruitment procedures in these data sets revealed that the controls in the group from Nalbantoglu et al[12] were selected on the because they had few plaques and tangles. The proportion of 73 controls in the data set from Nalbantoglu et al[12] who had at least 1 ε4 allele (5.5%) was significantly lower ($P<.001$) than the proportion among 287 controls from other autopsy studies (26.8%); whereas, the proportions of case patients from these studies who had at least 1 ε4 allele were the same (60.2% and 62.3%, respectively; $P=.7$). Because of the substantially lower ε4 frequency and the unique selection criteria for the Nalbantoglu et al[12] controls, these 73 subjects were excluded. Reevaluation of the data from the autopsy studies suggested that the ORs were not different. The test of the variability among clinic- and autopsy- studies combined was not significant (combined OR=4.2, Breslow-Day $P=.2$), suggesting that these data could be pooled for subsequent analysis. By contrast, the *APOE* ε4–AD association in the population-based studies was somewhat weaker and significantly variable ($P=.004$); however, with the exception of one of the smaller studies yielding an OR of 7.7, the range of ORs was relatively narrow (2.0-4.2).

Table 3.—Odds Ratios for Developing Alzheimer Disease (AD) According to Apolipoprotein E (*APOE*) Genotype and Ethnic Group, Adjusted for Age and Study*

APOE Genotype	No.	Odds Ratio (95% Confidence Interval)	Breslow-Day *P* Value†
Caucasians: Clinic/Autopsy Studies			
ε3/ε3	2854	1.0 (Referent)	. . .
ε2/ε2	21	0.6 (0.2-2.0)	.41
ε2/ε3	447	0.6 (0.5-0.8)	.37
ε2/ε4	141	2.6 (1.6-4.0)	.36
ε3/ε4	2171	3.2 (2.8-3.8)	.21
ε4/ε4	671	14.9 (10.8-20.6)	.88
Caucasians: Population-based Studies			
ε3/ε3	2683	1.0 (Referent)	. . .
ε2/ε2	36	0.9 (0.3-2.8)	.94
ε2/ε3	568	0.6 (0.5-0.9)	.93
ε2/ε4	152	1.2 (0.8-2.0)	<.01
ε3/ε4	1226	2.7 (2.2-3.2)	.15
ε4/ε4	193	12.5 (8.8-17.7)	.03
African Americans			
ε3/ε3	206	1.0 (Referent)	. . .
ε2/ε2	6	2.4 (0.3-22.7)	.35
ε2/ε3	54	0.6 (0.4-1.7)	.09
ε2/ε4	10	1.8 (0.4-8.1)	.27
ε3/ε4	164	1.1 (0.7-1.8)	.03
ε4/ε4	34	5.7 (2.3-14.1)	.01
Hispanics			
ε3/ε3	322	1.0 (Referent)	. . .
ε2/ε2	2	2.6 (0.2-33.3)	.14
ε2/ε3	57	0.6 (0.3-1.3)	.60
ε2/ε4	8	3.2 (0.9-11.6)	.05
ε3/ε4	127	2.2 (1.3-3.4)	.93
ε4/ε4	12	2.2 (0.7-6.7)	.12
Japanese			
ε3/ε3	1661	1.0 (Referent)	. . .
ε2/ε2	9	1.1 (0.1-17.2)	.52
ε2/ε3	149	0.9 (0.4-2.5)	.84
ε2/ε4	19	2.4 (0.4-15.4)	.80
ε3/ε4	430	5.6 (3.9-8.0)	.11
ε4/ε4	45	33.1 (13.6-80.5)	.62

*Odds ratios for *APOE* genotypes derived assuming a reference odds ratio of 1 for *APOE* ε3/ε3 genotype.
†These *P* values are a test for heterogeneity of odds ratios for genotype among data sets.

The age-adjusted odds of AD for *APOE* genotypes that had at least 1 ε2 or ε4 allele relative to the ε3/ε3 genotype are shown in Table 3. People with the ε3/ε4 genotype had increased odds of AD (ranging from 2.2 in Hispanics to 5.6 in Japanese). African Americans were an exception (OR=1.1), but there was significant heterogeneity among studies in this group. Odds of AD were also increased between 1.8 and 3.2 times among ε2/ε4 subjects in all ethnic groups, except in the subset of Caucasians from population-based studies who were heterogeneous with respect to risk associated with this genotype. The odds of AD associated with homozygosity of ε4 were very high in Caucasians (12.5-14.9) and Japanese (33.1), but in Hispanics the odds were identical to those for the ε3/ε4 group (2.2). The OR for ε4/ε4 African Americans (5.7) was intermediate to the ORs for Caucasians or Japanese and Hispanics, but that may be deceptive because of the significant variability among studies of African Americans. The ε2/ε3 geno-

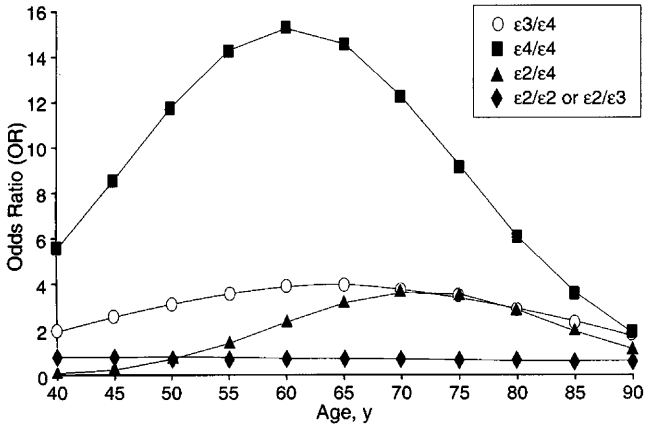

Figure 1.—Relative odds of Alzheimer disease according to apolipoprotein E (*APOE*) genotype and age among Caucasian subjects ascertained in clinic- and autopsy-based studies. These odds were generated from a logistic regression model for subjects with genotypes *APOE* ε2/ε2 or ε2/ε3, ε2/ε4, ε3/ε4, and ε4/ε4 relative to the ε3/ε3 group. Curves were derived from a model that included *APOE* dummy variables, age, age-squared, and second-order interaction terms involving *APOE* variables and age.

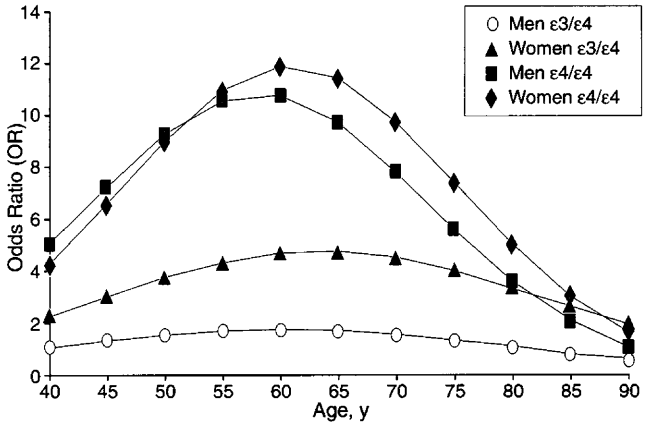

Figure 2.—Relative odds of Alzheimer disease according to apolipoprotein E (*APOE*) genotypes ε3/ε4 and ε4/ε4, age, and sex among Caucasian subjects ascertained in clinic- and autopsy-based studies. Curves were derived from a model including *APOE* dummy variables, age, age-squared, sex, and second-order interaction terms involving *APOE* variables, age, and sex.

type is protective and had a similar OR in all ethnic groups, although ORs were significant in the larger Caucasian groups only. There were too few ε2/ε2 subjects to demonstrate a significantly different risk of AD from that for ε3/ε3 subjects. However, among the 2 groups of studies of Caucasians, in which more than 20 ε2/ε2 subjects participated, the odds of AD associated with this genotype were similar to the odds for the ε2/ε3 genotype. Overall, the results (Table 3) indicate that pooling is inappropriate among studies in the Caucasian population-based and African-American groups.

Age and Sex Effects

Because evidence for heterogeneity among the Caucasian population-based and African-American studies indicates that pooling of data sets within these strata is not justified, age- and sex-specific ORs for AD were derived for *APOE* genotypes ε2/ε2 or ε2/ε3, ε2/ε4, ε3/ε4, and ε4/ε4 relative to the ε3/ε3 genotype among Caucasian (clinic- or autopsy-based), Japanese, and Hispanic groups only. Among Caucasians, the odds of AD were significantly increased between ages 40 and 95 years among ε3/ε4 subjects and between ages 50 and 85 years among ε4/ε4 subjects. In these groups, risk increased steadily between ages 40 and 60 years but declined with age thereafter (Figure 1). A similar pattern was demonstrated for ε2/ε4 subjects, but the highest odds were observed at age 70 years. The apparent protective effect of this genotype in subjects younger than age 50 years was not significant owing to the paucity of ε2/ε4 subjects (0.98% of case patients and 1.24% of controls) in this age range. Age-related changes in the OR were much more dramatic among ε4 homozygotes than ε4 heterozygotes. The increased risk associated with ε4 was still evident at age 90 years but could not be assessed reliably after age 95 years. The protective effect associated with the ε2/ε2 and ε2/ε3 genotypes was unaffected by age (OR=0.6 across all ages). The model allowing for an interaction between *APOE* and sex was significant (χ^2=13.43, *df*=4, *P*=.01), suggesting that the sex effect is not uniform across all *APOE* genotypes. At most ages and across all genotypes, women are more likely than men to develop AD (Figures 2 and 3). In comparison with ε3/ε3 individuals, the sexual dimorphic character in AD risk was 1.5 times greater in ε3/ε4 individuals (*P*=.01). Sex differences in odds of AD among persons with other genotypes were not significantly different from those seen among ε3/ε3 persons. Lack of significance for the ε2/ε4 group that showed a relative increase in female-male odds of 1.8 is probably related to small sample size.

Age-related trends in the risk of AD among Japanese ε3/ε4 subjects paralleled that of Caucasian ε3/ε4 subjects, although age did not influence risk among Hispanic ε3/ε4 subjects (Figure 4). The effect of age on the OR for the other genotypes could not be assessed reliably in these ethnic groups because of an insufficient number of persons, particularly at the extremes of the age distribution. The effect of sex on AD risk in Japanese and Hispanics was the same across genotypes, although the sex-related trends among Hispanics were similar to those among Caucasians.

COMMENT

Our meta-analysis of 40 studies representing nearly 30 000 *APOE* alleles demonstrates a significant association between the *APOE* ε4 allele and AD in Caucasians, African Americans, Hispanics, and Japanese. The results of this study were based on analyses of raw data and included investigators from the original studies and the evaluation of several covariates.

The goals of this study were to assess the variability in the association between *APOE* ε4 and AD and the degree to which differences in published studies were attributable to demographic characteristics and study design. Variability in ethnic or racial background, age and sex distributions, and certainty of diagnosis could have affected the *APOE* ε4–AD association. Sample size variability is important as 10 of the 47 independent data sets had fewer than 100 total subjects; one third of these studies had fewer than 50 controls.

Our results suggest that the distribution of *APOE* alleles is the same in populations of patients with a clinical diagnosis of probable AD and those meeting autopsy criteria for AD. Therefore, it was deemed appropriate to pool these subjects for *APOE* association studies. We also observed that within the Caucasian, Hispanic, and Japanese groups, differences in the *APOE* ε4–AD association were entirely attributable to sampling variance and were not statistically significant. However, the apparent heterogeneity among studies in the Caucasian community-based and African-American groups could not be resolved. Heterogeneity among community-based Caucasian studies may reflect biases of prospective vs prevalent case sampling. The relative risk (RR) of AD for ε4 subjects in the Rotterdam study[17] (OR=2.0) may be lower than that for ε4

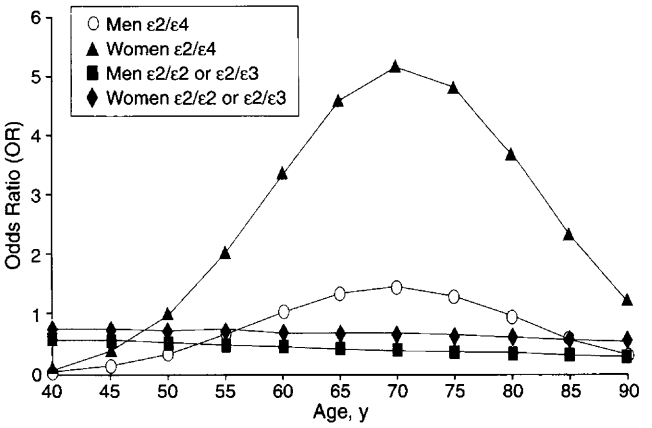

Figure 3.—Relative odds of Alzheimer disease according to apolipoprotein E (*APOE*) genotypes ε2/ε2 or ε2/ε3 and ε2/ε4, age, and sex among Caucasian subjects ascertained in clinic- and autopsy-based studies. Curves were derived from a model that included *APOE* dummy variables, age, age-squared, sex, and second-order interaction terms involving *APOE* variables, age, and sex.

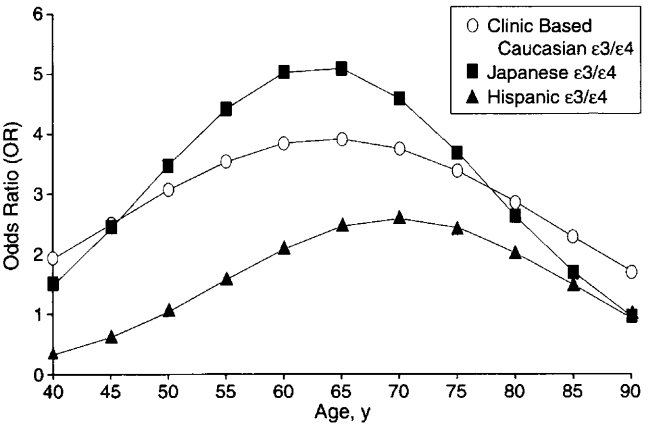

Figure 4.—Relative odds of Alzheimer disease according to age among Caucasian, Japanese, and Hispanic apolipoprotein E (*APOE*) ε3/ε4 subjects. Curves were derived from the model described in Figure 1 for each ethnic group.

subjects in the 9 other community-based Caucasian studies (ORs between 3.0 and 7.7) because it included a large number of subjects younger than 65 years.

An elevated frequency of *APOE* ε4 among case patients was detected in every group, but the *APOE* ε4–AD association was weaker among African Americans (ORs of 1.1 and 5.7 in ε3/ε4 and ε4/ε4 subjects, respectively) than among Caucasians (ORs of 2.7-3.2 and 12.5-14.9 in ε3/ε4 and ε4/ε4 subjects, respectively). However, the largest data set of African-American subjects was ascertained as a community sample[35] and was different from the other studies of African Americans in clinical populations.[14,15,36,43] Larger and more diverse groups of African Americans need to be studied. The odds of AD were also lower among Hispanics than among Caucasians, which is attributed largely to a paucity of ε4 homozygous Hispanic patients. The ε3/ε4 genotype confers a significantly increased risk of AD in Hispanics, but the ε4/ε4 genotype does not (Table 3). In contrast, the *APOE* ε4–AD association among Japanese subjects was substantially stronger (ORs of 5.6 and 33.1 for ε3/ε4 and ε4/ε4 genotypes, respectively) than among Caucasians.

A protective effect of the ε2 allele was observed, but it was limited to the ε2/ε3 genotype and possibly the ε2/ε2 genotype. In comparison with the variable effect of the ε3/ε4 and ε4/ε4 genotypes across ethnic groups, the ε2/ε3 genotype appears to be equally protective (OR=0.6) in all populations represented in our sample. The influence of the rare ε2/ε2 genotype on AD risk could not be discerned even in this very large sample. A novel finding of our study is that, among Caucasians and possibly other groups, genotypes ε2/ε4 and ε3/ε3 are not equivalent in terms of AD risk.

Although the *APOE* ε4–AD association has been previously documented in persons younger than 65 years,[8-11,51,77] our study is the first to demonstrate increased risk in persons as young as 40 years. Among Caucasians recruited from clinical settings, the ORs for AD associated with the ε4 allele increased until age 60 years in ε3/ε4 and ε4/ε4 persons and age 70 years in ε2/ε4 persons, although it diminished thereafter. Age did not influence the benefit of the ε2/ε3 genotype. Using a similar analytic strategy, Corder et al[15] also observed substantial decrement in risk in ε3/ε4 and ε4/ε4 persons aged 60 years and older in a smaller sample from the United States. Bickeböller et al[78] also observed lower ORs for the ε4 allele in the youngest (<60 years) and oldest (≥80 years) age groups and ORs below 1 for ε2/ε3 persons across all age groups.[78] In a study of affected relative pairs, Blacker et al[42] showed a weaker *APOE* ε4–AD association after age 70 years.

Life-table studies suggest that the higher risk of AD in women than men is not because of greater longevity in women.[79] Sex effects have been attributed to sex-specific susceptibility among ε3/ε4 heterozygotes,[27] but this is controversial.[78,80] Poirier et al[8] observed a stronger *APOE* ε4–AD association in women than men, which is primarily attributable to a higher proportion of ε3/ε4 among female case patients but not controls compared with men. After adjusting for age, our meta-analysis confirms the presence of a sex effect on risk of *APOE* ε3/ε4 among Caucasians and possibly Hispanics but not among Japanese. The present study and genetic modeling studies[81] suggest that women may have higher susceptibility to AD than men regardless of *APOE* genotype. It is likely that other factors such as estrogen, independently or in concert with certain *APOE* genotypes, also account for some of these sex differences in risk of AD.[31,82]

Reporting bias is unlikely to have greatly influenced our results. Once the *APOE* ε4–AD association was established[15,18] and confirmed in independent data sets,[4] negative findings[37,83] showing different patterns of association[17,32,35] were equally or perhaps more likely to attract the attention of reviewers for scientific and medical journals. Nearly all these studies were included.

Our meta-analysis has shown that odds of AD among ε4 carriers relative to those lacking ε4 initially increase but eventually decrease with age. Among Caucasians, the *APOE* ε4–AD association is not uniform across sexes, and ε4 is a weaker risk factor for AD among African Americans and Hispanics than among Caucasians and Japanese. Even though a large proportion of the data in this study were derived from self-selected case patient and control groups, conclusions about genotype RR are robust because subjects were not selected on the basis of *APOE* status. However, these results do not address the efficacy of *APOE* as a diagnostic test because antemortem data were unavailable for case patients who had undergone autopsy. The predictive value of *APOE* genotype for diagnostic purposes has been estimated in relatively small samples of case patients with probable AD who were followed up to autopsy.[62,84,85] The genotype-specific ORs presented in the figures are relative odds, and thus are also not appropriate for AD risk assessment in cognitively normal persons. Use of *APOE* genotype in predictive test situations requires absolute risk information from prospective

cohort studies. A number of prospective studies are under way, but most of the subjects in these studies have not been followed up long enough or have not undergone sufficient diagnostic scrutiny to derive accurate and precise risk estimates beyond age 80 years, particularly for the rarer genotypes like ε2. Because ORs computed in the meta-analysis approximate RRs, we anticipate that RRs estimated in prospective studies will be comparable with our findings by age, sex, and ethnic group.

This study was supported in part by contributions from the Alzheimer Association, Chicago, Ill, Athena Diagnostics, Inc, Worcester, Mass, and by grant AG-09029 from the National Institutes of Health, Bethesda, Md.

We are indebted to numerous colleagues who contributed to the clinical evaluation of subjects, collection of tissue specimens, *APOE* genotyping, and preparation of data sets. We are especially grateful to Michael Watson, PhD, Washington University, St Louis, Mo, and the American College of Medical Genetics, Bethesda, Md, for help in initiating the study. The data set provided by Marilyn Albert, PhD, Massachusetts General Hospital, Boston, Mass, was established as part of the National Institute of Mental Health's Genetics Initiative for Alzheimer Disease. The contribution of unpublished data by Sandro Sorbi, MD, Università di Firenze, Firenze, Italy, was supported by CNR PF Aging and Telethon-Italia E 482.

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