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Evidence for Major Gene Inheritance of Alzheimer Disease in Families of Patients With and Without Apolipoprotein E ε4

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Summary

Apolipoprotein E (APOE) genotype is the single most important determinant to the common form of Alzheimer disease (AD) yet identified. Several studies show that family history of AD is not entirely accounted for by APOE genotype. Also, there is evidence for an interaction between APOE genotype and gender. We carried out a complex segregation analysis in 636 nuclear families of consecutively ascertained and rigorously diagnosed probands in the Multi-Institutional Research in Alzheimer Genetic Epidemiology study in order to derive models of disease transmission which account for the influences of APOE genotype of the proband and gender. In the total group of families, models postulating sporadic occurrence, no major gene effect, random environmental transmission, and Mendelian inheritance were rejected. Transmission of AD in families of probands with at least one ε4 allele best fit a dominant model. Moreover, single gene inheritance best explained clustering of the disorder in families of probands lacking ε4, but a more complex genetic model or multiple genetic models may ultimately account for risk in this group of families. Our results also suggest that susceptibility to AD differs between men and women regardless of the proband's APOE status. Assuming a dominant model, AD appears to be completely penetrant in women, whereas only 62%–65% of men with predisposing genotypes develop AD. However, parameter estimates from the arbitrary major gene model suggests that AD is expressed dominantly in women and additively in men. These observations, taken together with epidemiologic data, are consistent with the hypothesis of an interaction between genes and other biological factors affecting disease susceptibility.

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Introduction

Molecular genetics studies have demonstrated that at least some cases of Alzheimer disease (AD) are caused by heritable defects (Goate et al. 1991; Levy-Lahad et al. 1995; Sherrington et al. 1995). Most of these patients belong to rare families in which the disorder usually manifests before the age of 65 years and aggregates in an autosomal dominant pattern. However, in most cases a singular cause of AD is not evident. Risk of AD to first-degree relatives of patients ascertained consecutively in AD specialty clinics or community samples is substantially higher than the risk to relatives of nondemented persons (Breitner et al. 1988; Huff et al. 1988; Martin et al. 1988; Farrer et al. 1989; Mayeux et al. 1991; van Duijn et al. 1993; Hirst et al. 1994; Silverman et al. 1994; Lautenschlager et al. 1996). However, studies comprised of >100 families consistently show that the lifetime risk is significantly less than 50%, the risk predicted if all cases were explained by autosomal dominant inheritance. These findings suggest that the genetic component is not present in all affected individuals or is more complex than dominant inheritance. Early attempts to elucidate mechanisms of AD transmission by complex segregation analysis using the mixed model approach (Morton and MacLean 1974) implemented in the POINTER computer program (Lalouel and Morton 1981) concurred that there is a major dominantly transmitted susceptibility gene for AD (Farrer et al. 1991; van Duijn et al. 1993), but not all of the parameter estimates from the best-fitting models were easily interpretable. Refinements to the genetic model and evidence for heterogeneity in transmission of AD were provided by Rao et al., (1994) who carried out segregation analyses in >400 families by using logistic regressive models (Bonney 1984, 1986).

Genetic linkage and linkage disequilibrium studies identified the ε4 allele of apolipoprotein E (APOE) as a risk factor for AD (Pericak-Vance et al. 1991; Saunders et al. 1993; Strittmatter et al. 1993). Subsequent con-

firmations have established the APOE genotype to be the single most important genetic determinant of susceptibility to AD (Roses 1994), with an attributable risk estimated to be 50%–60% (Nalbantoglu et al. 1994). Individuals heterozygous for the ε4 allele have an odds ratio between 2.2 and 4.4 of developing AD, compared to persons who have the ε3/ε3 genotype, while ε4 homozygotes have an odds ratio ranging from 5.1 to 30.1 (Corder et al. 1993; Lucotte et al. 1993; Brousseau et al. 1994; Liddell et al. 1994; Mahieux et al. 1994; Nalbantoglu et al. 1994; Tsai et al. 1994; van Duijn et al. 1994*b;* Maestre et al. 1995; Myers et al. 1996). In contrast, the ε2 allele may confer a protective effect (Chartier-Harlin et al. 1994; Corder et al. 1994; Talbot et al. 1994), but this effect is unclear in some populations (Sorbi et al. 1994; van Duijn et al. 1995*a*). In spite of the remarkable dose-dependent effect of ε4 on risk and age at onset of AD (Borgaonkar et al. 1993; Corder et al. 1993), the predictive value of APOE genotype is relatively modest (van Gool and Hijdra 1994; Farrer et al. 1995*a;* Mayeux and Schupf 1995).

Several studies suggest that susceptibility to AD is determined by a combination of APOE with other factors such as serious head injury, smoking, and cholesterol level (van Duijn et al. 1994*a,* 1995*b;* Mayeux et al. 1995; Jarvik et al. 1995). Case reports showing apparent nonpenetrance of the disorder among persons possessing a causative mutation in the APP gene and the APOE ε2 allele support the idea that expression of disease (or lack thereof) may be governed by synergistic or epistatic action of multiple genes (Hardy et al. 1993; St George-Hyslop et al. 1994). We investigated this possibility in ~4,000 first-degree relatives of 549 AD probands whose APOE genotypes were known. The lifetime risk of AD in relatives was compared with the estimated proportion of ε4 carriers among the relatives in this group of families (Farrer et al. 1995*b*). The risk of AD in relatives increased significantly with the number of APOE ε4 alleles in the proband. However, among relatives in the ε3/ε3 group, the lifetime risk for AD by age 90 years was three times greater than expected proportion of ε4 carriers, suggesting that other familial factors contribute to AD susceptibility. Moreover, this study showed that among male relatives, the risk for AD in the ε3/ε4 group was similar to that for the ε3/ε3 group, whereas, among female relatives, the risk for the ε3/ε4 group was nearly twice that for the ε3/ε3 group and identical to the risk for the ε4/ε4 group. This finding, which is consistent with evidence in other studies (Payami et al. 1994; Duara et al. 1996) suggests that gender may modify the risk of AD in ε4 carriers.

The aims of the current study were to determine whether there exists a residual familial component to AD and whether it is genetic. To accomplish these goals we used complex segregation analysis to evaluate models of disease transmission that incorporate the influences of APOE genotype and gender.

Subjects, Material, and Methods

Subjects

Diagnostic and genealogical data on 549 AD patients and their first-degree relatives reported by Farrer et al. (1995*b*) were incorporated into this study. This sample, including 378 families from seven centers in the Multi-Institutional Research in Alzheimer Genetic Epidemiology (MIRAGE) study and 171 families from a Dutch population-based study of early-onset AD, was augmented by an additional 88 MIRAGE families subsequently ascertained in the same manner at these centers. Our previous studies had shown that lifetime risk and mode of transmission of illness in these families (van Duijn et al. 1993) were similar to results obtained from studies of a subset of MIRAGE families (Farrer et al. 1989, 1991). The distribution of APOE alleles in the Dutch probands is similar to that of late-onset patients (van Duijn et al. 1994*b*). Diagnosis of AD was established in all probands by using accepted research criteria (McKhann et al. 1984; Khachaturian 1985). Diagnoses of first-degree relatives were assigned using the MIRAGE AD Rating Scale (Farrer et al. 1994) on the basis of the information obtained from interview of multiple informants, medical records (including autopsy reports where available), death certificates, and nursing home records. Individuals meeting criteria for possible, probable, or definite AD were considered to be affected. One family from the original set of 549 was excluded because it was learned that the proband who recently came to autopsy had Creutzfeldt-Jakob disease. Thus, the final sample comprised 636 families in which 84 probands (13.2%) met criteria for definite AD and 552 probands (86.8%) met criteria for probable AD. The breakdown of families by center is as follows: Boston University, 60; Bedford, MA, 75; Massachusetts General Hospital, 173; University of Southern California, 39; Emory University, 20; University of Miami, 20; Technical University of Munich, 78; Rotterdam, 171.

APOE genotypes for AD probands were determined using PCR (Wenham et al. 1991) in a manner described elsewhere (van Duijn et al. 1994*b;* Farrer et al. 1995*b*). Genotypes for relatives were not determined.

Statistical Methods

Preliminary analyses revealed a birth cohort effect on disease outcome among sibs but not parents. Specifically, the observed proportion of affected parents was the same among birth cohorts stratified at the median year of 1890 (15.5% vs. 18.8%, Fisher's exact test = .20), whereas the proportion of affected sibs born before 1920 (11.2%) was four times greater than the proportion of affected sibs born after 1920 (2.7%, Fisher's exact test = 8.0×10^{-14}). Lifetime risks of AD (to age 74) estimated using survival analysis methods (Cupples et al. 1991) in the four birth cohorts of par-

ents and sibs were the same, suggesting that the under-ascertainment of affected sibs born after 1920 is unlikely explained by diagnostic differences or secular changes in the incidence of AD. In contrast to both parental cohorts and the older sib cohort in which most subjects have been censored at their age at death, a substantial proportion of sibs in the younger birth cohort are still living and may still develop AD. Because this ascertainment bias (i.e., paucity of affected sibs among younger probands) cannot be corrected sufficiently by an age-dependent penetrance function, birth year of each member was included as a covariate in the segregation analyses.

Segregation analysis was performed following the logistic regressive approach of Bonney (1984, 1986) for family data implemented in the REGTL program of SAGE (Bailey-Wilson and Elston 1987). In this approach, AD was treated as a dichotomous trait with age-dependent penetrance, and the major gene component was modeled as a diallelic locus. Since diagnosis of AD and estimation of age at onset among individuals beyond first-degree relatives are relatively inaccurate, the study was limited to nuclear families only. All variation among sibs was measured through the major locus component only by fixing the regressive familial components to zero. Age at onset was assumed to follow a logistic distribution with age coefficient α and baseline parameter β and constrained to cumulative incidence values of 0.2 for women and 0.11 for men by the age of 102 years (the oldest age in the sample), which were extrapolated from population incidence data (Schoenberg et al. 1987; Kokmen et al. 1988). We assumed that β is the same for all genotypes and that risk to AD is modified through the sex-specific genotype susceptibilities (γ's). Because significant improvement in likelihoods was not observed when models were allowed for sex dependence on α and/or β, all models were derived assuming no sex dependence among age-at-onset parameters. Under Mendelian inheritance, the transmission probability (τ) is defined as the probability that an offspring inherits the AD allele (A) and takes on the values of 1, $\frac{1}{2}$, and 0 for parental genotypes of AA, AB, and BB, respectively. Additional details and the efficacy of this approach and possible alternatives are described elsewhere (Rao et al. 1994).

Several genetic and nongenetic models—namely, dominant, recessive, additive, and arbitrary major gene, no major gene, sporadic, random environmental—as well as two general transmission (i.e., unrestricted) models were fit to the family data. Sex- and genotype-dependent susceptibilities were estimated under each model. Likelihoods were calculated following the hybrid maximization technique of Atwood et al. (1992). Hypotheses were tested hierarchically using the large sample approximation of χ^2 with df equal to the difference in number of independent parameters of the two models in the comparison. Confidence in parameter estimates is reported as the standard error.

APOE Considerations

The ideal strategy for testing the influence of APOE on the mode of inheritance of AD would be to adjust for each individual's genotype in the segregation analysis. The regressive models in SAGE are well suited for this approach because APOE genotype can be treated as a covariate. However, because this method requires that every member in the pedigree be typed for APOE, and most of the critical individuals (i.e., parents and sibs) in retrospective studies are deceased, this study design was not feasible. In the absence of APOE data for relatives, we stratified the families according to the proband's APOE genotype. Despite the relatively large number of families in this study, several APOE genotype groups, notably $\varepsilon2/\varepsilon2$, $\varepsilon2/\varepsilon3$, and $\varepsilon2/\varepsilon4$, had <40 probands (table 1). The 101 probands homozygous for the $\varepsilon4$ allele were also too few to permit meaningful segregation analyses in this group of families separately. In our experience, $\geqslant200$ nuclear families are required to distinguish models in segregation analysis (Rao et al. 1994). Therefore, families were classified into two groups, those with and without $\varepsilon4$, to evaluate the relationship between the proband's APOE genotype and transmission of AD. We demonstrated elsewhere that the frequency of the $\varepsilon4$ allele in first-degree relatives of probands lacking $\varepsilon4$ is more than four times less than among relatives of probands having at least one $\varepsilon4$ allele (Farrer et al. 1995*b*), suggesting that patterns of familial aggregation might differ between the two groups of families. In order to test whether this stratification rendered a better fit to the data than no stratification, the likelihoods were compared in the following manner: $L = -2 \ln L_{\text{total}} - (-2 \ln L_{\varepsilon4(+)} - 2 \ln L_{\varepsilon4(-)})$, which is assumed to follow a χ^2 distribution asymptotically with $\text{df} = \text{df}_{\varepsilon4(+)} + \text{df}_{\varepsilon4(-)} - \text{df}_{\text{total}}$. One additional df was added to adjust for the stratification parameter when testing the significance of the L statistic.

Results

The 636 probands had a mean age at onset of 65.4 $\pm$ 9.3 years (range 35–94 years) and 3,684 first-degree relatives, of whom 9.9% were affected (table 1). Affected status of 156 family members (4.2% of the relatives) was unknown. A significantly higher proportion of affected first-degree relatives was observed among families in which the proband had at least one $\varepsilon4$ allele than among families of families lacking $\varepsilon4$ ($\chi^2_1 = 20.4$, $P < .0001$). Only 1 of 27 relatives of the $\varepsilon2/\varepsilon2$ probands was affected.

The results of segregation analyses performed on the total group of families are presented in table 2. All of

Table 1

Characteristics of Subjects, by APOE Genotype of Probands

Proband's APOE Genotype	Probands			First-Degree Relatives			
				Affected		Unaffected	
	No. of Males	No. of Females	Onset age (years) (mean ± SD)	No.	%	No.	%
22	3	1	62.5 ± 9.3	1	3.7	26	96.3
23	18	21	63.0 ± 8.6	14	6.0	214	92.2
33	88	128	66.2 ± 10.6	94	7.5	1113	88.5
24	5	6	68.3 ± 8.3	5	8.9	45	80.4
34	112	153	65.5 ± 8.6	174	11.5	1281	84.5
44	41	60	64.2 ± 8.6	75	12.6	486	81.7
22/23/33	109	150	65.6 ± 10.3	109	7.2	1353	89.2
24/34/44	158	219	65.3 ± 8.6	254	11.7	1812	83.6
All	267	369	65.4 ± 9.3	363	9.9	3165	85.9

the non-Mendelian models (3–5) and the arbitrary major gene model (6) were rejected in favor of the general transmission model ($P < .0001$ for models 3–5 and $P < .04$ for model 6), indicating that either the mode of inheritance of AD is more complex than any of these models or this sample of families is heterogeneous. The arbitrary major gene model was rejected primarily because transmission of the AD allele from the heterozygote (τ_{AB}) was much less than the expected value of .5 ($\tau_{AB} = 0.26 \pm 0.0035$ and 0.24 ± 0.0033 for models 1 and 2, respectively).

There was evidence for a major gene for AD in families where the proband had at least one APOE ε4 allele (table 3), because the arbitrary major gene model (6) was not rejected in favor of the general model ($\chi^2_3 = 5.53$; $P = .17$). This conclusion is supported by the rejection ($P < .0001$) of all nongenetic models (3–5). Further comparison of the genetic models (7–9) to the arbitrary major gene model led to the rejection of both recessive ($\chi^2_2 = 26.93$) and additive ($\chi^2_2 = 37.20$) models ($P < .0001$), but not to the rejection of the dominant model ($\chi^2_2 = 4.09$; $P = .16$). According to the dominant model, the frequency of the AD allele in this group of families is ~8.8% ± 0.4%. After adjustment for age, penetrance appears to be complete in women but only 62% ± 0.3% in men. The proportion of phenocopies (i.e., persons with the nonsusceptibility genotype who are expected to develop AD) was estimated to be 5.0% ± 0.1% in women and 1.0% ± 0.06% in men.

There was also evidence for a major AD susceptibility gene in families where the proband did not have an APOE ε4 allele. In comparison to the general model in table 4, the arbitrary major gene model was not rejected ($\chi^2_3 = 6.46$; $P = .09$), whereas the no-major-gene, sporadic, and random environmental models were soundly rejected ($P < .0001$). As for the specific genetic models (7–9), only the additive model was rejected in comparison to the arbitrary major gene model ($\chi^2_2 = 6.11$; $P < .05$). Although the dominant model was the best fit to the data in this group of families, the recessive model was equally likely. Under the assumption of dominant inheritance, penetrance of AD in this group of families is nearly identical to that in families of ε4 probands, that is, 100% in women and 65% ± 0.8% in men. In contrast to families of ε4 probands, the frequency of the AD susceptibility allele was 3.3% lower (at 5.5% ± 0.1%) and the proportion of phenocopies was 2.7 times higher in women (at 12.0% ± 0.2%) and 5 times higher in men (at 5.4% ± 0.1%).

Separation of families into ε4 and non-ε4 groups gave a better fit than the total group of families for every model tested (e.g., general model: $\chi^2_{12} = 32.86$; $P < .005$; arbitrary major gene model: $\chi^2_9 = 29.37$; $P < .005$). These results indicate that although there is evidence for a major AD susceptibility gene in families of probands with and without ε4, the transmission models may not be identical. In the families of ε4 probands, a dominant model is clearly preferable over other single gene models, whereas dominant and recessive models were equally likely explanations for transmission of AD among families of probands lacking ε4. Moreover, comparison of the dominant model between families with and without ε4 resulted in significant differences between the γ's ($P < .0001$) and AD allele frequencies ($P < .001$).

Discussion

In this large multicenter sample of 636 families, transmission of AD has a major gene component in both families of probands having at least one APOE ε4 allele and families of probands lacking ε4. In agreement with our previous segregation analysis of AD in 400 families from one center (Rao et al. 1994), transmission of the disorder in the total group of families cannot be fully explained by any simple genetic or nongenetic model, suggesting that susceptibility to AD in the population of families represented in our study is heterogeneous.

Table 2

Segregation Analysis of AD in the Total Group of Families

Model	q_A	τ_{AA}	τ_{AB}	τ_{BB}	β	♀-γ_{AA}	♀-γ_{AB}	♀-γ_{BB}	♂-γ_{AA}	♂-γ_{AB}	♂-γ_{BB}	$-2\ln L$	No. of Estimated Parameters
General:													
1. Unrestricted	.1038	{1}	.2609	.0151	−25.33	{1}	{1}	.0119	{1}	.5521	{0}	3642.48	11
2. Unrestricted (τ_{AA} and τ_{BB} fixed)	.0959	[1]	.2373	[0]	−31.83	{1}	{1}	.0288	{1}	.5436	.0121	3643.03	9
Non-Mendelian:													
3. No major gene	...	...	...	...	−44.53	.2017	...	...	.1109	...	...	3852.43	3
4. Sporadic[a]	.0420	.1708	.1708	.1708	−1.56	.9646	.7313	.1565	{1}	.6420	.0642	3819.09	9
5. Environmental[b]	.0465	.0465	.0465	.0465	−44.17	.9891	.7212	.1492	.9990	.1412	.1059	3852.43	8
Mendelian:													
6. Arbitrary major gene	.0734	[1]	[.5]	[0]	−14.92	{1}	{1}	.0766	{1}	.5880	.0333	3650.98	8
7. Dominant[c]	.0684	[1]	[.5]	[0]	−14.95	{1}	{1}	.0862	.6444	.6444	.0331	3654.04	6
8. Recessive[d]	.4297	[1]	[.5]	[0]	−10.43	{1}	.0286	.0286	.6195	{0}	{0}	3656.91	6
9. Additive	.1453	[1]	[.5]	[0]	−14.76	{1}	.5354	.0707	.7788	.3894	{0}	3678.42	6

NOTE.—Data in curly braces indicate that parameter went to a boundary; and data in square brackets indicate parameter fixed at the value.

[a] $\tau_{AA} = \tau_{AB} = \tau_{BB}$.

[b] $q_A = \tau_{AA} = \tau_{AB} = \tau_{BB}$.

[c] $\gamma_{AA} = \gamma_{AB}$.

[d] $\gamma_{AB} = \gamma_{BB}$.

Table 3

Segregation Analysis of AD in Families of Probands Having at Least One APOE ε4 Allele

Model	q_A	τ_{AA}	τ_{AB}	τ_{BB}	β	♀-γ_{AA}	♀-γ_{AB}	♀-γ_{BB}	♂-γ_{AA}	♂-γ_{AB}	♂-γ_{BB}	$-2\ln L$	No. of Estimated Parameters
General:													
1. Unrestricted	.1068	{1}	.2840	.0025	−16.04	{1}	{1}	.0034	{1}	.5309	{0}	2486.18	11
2. Unrestricted (τ_{AA} and τ_{BB} fixed)	.1064	[1]	.2772	[0]	−16.06	{1}	{1}	.0043	{1}	.5284	.0011	2486.29	9
Non-Mendelian:													
3. No major gene	...	...	...	...	−31.24	.2012	...	...	.1107	...	...	2671.99	3
4. Sporadic[a]	.0872	.2230	.2230	.2230	−.69	.8399	.9061	.0625	.9556	.6546	{0}	2633.81	9
5. Environmental[b]	.1005	.1005	.1005	.1005	−29.94	.9557	.9496	.0245	.9746	.5438	.0031	2672.07	8
Mendelian:													
6. Arbitrary major gene	.0966	[1]	[.5]	[0]	−.21	{1}	{1}	.0273	{1}	.5440	.0112	2491.71	8
7. Dominant[c]	.0881	[1]	[.5]	[0]	−.29	{1}	{1}	.0449	.6181	.6181	.0109	2495.80	6
8. Recessive[d]	.3735	[1]	[.5]	[0]	−.21	{1}	.0778	.0778	.8140	{0}	{0}	2518.64	6
9. Additive	.1120	[1]	[.5]	[0]	−.29	{1}	.5516	.1032	{1}	.5000	{0}	2528.91	6

NOTE.—Data in curly braces indicate that parameter went to a boundary; and data in square brackets indicate parameter fixed at the value.

[a] $\tau_{AA} = \tau_{AB} = \tau_{BB}$.

[b] $q_A\ \tau_{AA} = \tau_{AB} = \tau_{BB}$.

[c] $\gamma_{AA} = \gamma_{AB}$.

[d] $\gamma_{AB} = \gamma_{BB}$.

Table 4

Segregation Analysis of AD in Families of Probands Lacking an APOE ε4 Allele

Model	q_A	τ_{AA}	τ_{AB}	τ_{BB}	β	♀-γ_{AA}	♀-γ_{AB}	♀-γ_{BB}	♂-γ_{AA}	♂-γ_{AB}	♂-γ_{BB}	$-2\ln L$	No. of Estimated Parameters
General:													
1. Unrestricted	.1035	.6647	.0060	{0}	−66.82	{1}	{1}	.0150	.6567	.5348	.0103	1123.44	11
2. Unrestricted (τ_{AA} and τ_{BB} fixed)	.1178	[1]	.0138	[0]	−33.24	{1}	.8663	.0257	.6531	.4596	.0168	1124.80	9
Non-Mendelian:													
3. No major gene	...	...	...	...	−58.39	.2044	...	...	.1124	...	...	1168.94	3
4. Sporadic[a]	.0073	.1482	.1482	.1482	−35.29	.5893	.3161	.2054	{1}	.3824	.1099	1166.09	9
5. Environmental[b]	.0449	.0449	.0449	.0449	−54.44	.9396	.6185	.1642	.9962	.6006	.0648	1169.09	8
Mendelian:													
6. Arbitrary major gene	.0554	[1]	[.5]	[0]	−34.63	{1}	{1}	.1187	{1}	.6248	.0550	1129.90	8
7. Dominant[c]	.0550	[1]	[.5]	[0]	−30.29	{1}	{1}	.1202	.6480	.6480	.0544	1130.15	6
8. Recessive[d]	.4241	[1]	[.5]	[0]	−36.23	{1}	.0438	.0438	.5731	.0191	.0191	1131.47	6
9. Additive	.1800	[1]	[.5]	[0]	−34.68	{1}	.5206	.0412	.6193	.3134	.0074	1136.01	6

NOTE.—Data in curly braces indicate that parameter went to a boundary; and data in square brackets indicate parameter fixed at the value.

[a] $\tau_{AA} = \tau_{AB} = \tau_{BB}$.

[b] $q_A = \tau_{AA} = \tau_{AB} = \tau_{BB}$.

[c] $\gamma_{AA} = \gamma_{AB}$.

[d] $\gamma_{AB} = \gamma_{BB}$.

These results extend our previous survival analyses showing that risk of AD among first-degree relatives of probands lacking ε4 is substantially higher than what would have been expected if the genetic component to disease susceptibility is the APOE genotype alone (Farrer et al. 1995*b*). The results in table 4 suggest that there is a major gene for AD in these families, but presumably it is not APOE, since the probands lack ε4 and the expected frequency of ε4 in their first-degree relatives is no higher than the frequency for the general population of ~13% (Farrer et al. 1995*b*). The inability to distinguish between the dominant and recessive models may be due to a limitation of sample size. On the other hand, this finding may reflect heterogeneity within this group of families (i.e., the existence of both dominant and recessive forms of AD) or indicate a more complex genetic model for AD (e.g., oligogenic). Phenocopy rates (as measured by the estimate of γ_{BB}) of 5.4% in men and 12% in women in these families suggest that environmental or other genetic factors may independently or synergistically contribute to susceptibility.

AD is most likely transmitted in an autosomal dominant fashion in families of probands having at least one ε4 allele. Although the dominant model was the only Mendelian model not rejected in comparison with the arbitrary major gene model, careful inspection of the γ's in the latter model suggest that AD is fully penetrant in women inheriting one or two copies of the AD allele (the expectation for dominant inheritance), whereas, in men, penetrance is complete among homozygotes and 54% in heterozygotes (a finding consistent with an additive model). Previously, we found that among male relatives, lifetime risk of AD in the ε3/ε4 proband group was similar to that for the ε3/ε3 proband group and significantly less than the risk for the ε4/ε4 proband group (Farrer et al. 1995*b*). In contrast, among female relatives the lifetime risk for the ε3/ε4 proband group was nearly twice that for the ε3/ε3 proband group and identical to that for the ε4/ε4 proband group. Taken together, the observations from the survival and segregation analyses support the idea that a single major gene, that is, APOE, having different penetrance in men and women, is associated with transmission of AD in families of probands with at least one ε4 allele. A dose effect of the ε4 allele on risk has been suggested (Corder et al. 1993) and even observed within families with autosomal dominant AD (Borgaonkar et al. 1993). However, it is unclear whether APOE alone accounts for transmission in this group of families. The estimated frequency of the AD susceptibility allele in the arbitrary gene model was 9.7% ± 0.07%, which is significantly less than the 14%–16% frequency of the ε4 allele in the general population (Menzel et al. 1983; Ordovas et al. 1987), suggesting that AD may not manifest in as many as one-third of families segregating the ε4 allele. Penetrance as low as 50% in male ε4 heterozygotes would not explain entirely the observation that the lifetime risk to age 93 years of AD in first-degree relatives of ε4/ε4 probands is only half the expected frequency of the ε4 allele (Farrer et al. 1995*b*).

Separate effects of APOE genotype and family history on risk of AD have been demonstrated in several population-based and clinic-based samples (Jarvik and Wijsman 1994; van Duijn et al. 1994*b*; Farrer et al. 1995*b*; St. Clair et al. 1995), suggesting the involvement of other genetic loci. Recently, Jarvik et al. (1996) carried out a complex segregation analysis of AD in 204 families ascertained through a health maintenance organization using an approach similar to ours and the same computer program (REGTL). All of the Mendelian and environmental models tested separately within families of persons with and without APOE ε4 were rejected. The authors concluded that failure to resolve a genetic model in the presence of a known transmissible major factor (i.e. APOE) is evidence for other disease mechanisms including multiple genetic factors. There are several possible factors which may have affected the ability of Jarvik et al. to detect a genetic factor by segregation analysis, despite evidence from logistic regression analyses supporting the existence of a familial effect independent of APOE. First, their sample of families, which was less than one-third the size of our sample, may have been too small to discriminate a genetic model. Second, initially, we were also unable to obtain meaningful results from our analyses until we adjusted for the birth cohort effect. Third, their study apparently did not adjust for a gender effect on susceptibility. Fourth, in contrast to our study, Jarvik et al. assumed that the major gene influences are mitigated through age at onset, rather than through susceptibility to the disease. Finally, it is noteworthy that in their general model corrected for ascertainment the frequency of the AD allele in the total sample of families was estimated to be .96. This value is nearly five times greater than the cumulative incidence of AD in the general population (Kokmen et al. 1988). Although Mendelian inheritance was not evident in any subgroup in the Seattle study, their results and those presented in this report suggest that transmission of AD differs among families of ε4+ and ε4− probands and implicate genetic factors other than APOE genotype in AD susceptibility.

The results of our study, as well as other studies relying on amnestic information obtained from family members, need to be interpreted very cautiously. Among living probands, diagnostic accuracy is ~90% (Joachim et al. 1988; Rao et al. 1994), and this rate is much higher than among relatives who are not subjected to the same rigorous evaluation. To improve diagnostic certainty and standardize classification across centers, we used a rating scale that incorporates existing research diagnostic criteria and has been shown to be reliable across MIRAGE centers (Farrer et al. 1994). In order to mini-

mize misclassification of relatives, we used multiple informants and reviewed medical records when available which have been proven to be very effective in correctly diagnosing secondary cases of AD (Silverman et al. 1986; Rao et al. 1994).

Our results may have been biased by heterogeneity with respect to patterns of familial aggregation of AD among patients recruited under different ascertainment schemes. This concern is lessened by evidence suggesting similar transmission models for clinic-based and community-based samples (Farrer et al. 1991; van Duijn et al. 1993). To further investigate this possibility, we computed for each family the probability that AD was transmitted in an autosomal dominant pattern using the method of Farrer and Cupples (1994). We found that the variability in probabilities among families across centers was not significantly greater than the variability among families within centers, suggesting that familial patterns of AD do not vary between clinic and community based families (results not shown).

Our finding of reduced penetrance in males after age adjustment may reflect a confounding relationship between cardiovascular disease (CVD) and the APOE ε4 allele (Cumming and Robertson 1984; Davignon et al. 1988; Kuusi et al. 1989), particularly among men (van Bockxmeer and Momotte 1992). Arguably, ε4 men are selectively removed from the population by succumbing to CVD at ages before they would have developed AD. This effect is not evident in women because they tend to develop CVD later in life, i.e., during the critical risk period for AD. While this phenomenon may have an impact on the age specific risk of AD, our data do not support this explanation for the evidence of decreased penetrance of an AD susceptibility gene in men. If this hypothesis were true, penetrance should be higher in male relatives from ε4 families than non-ε4 families. Penetrance estimates for these groups of men were 62% and 65%, respectively. To investigate this relationship more directly, we performed a proportional hazards regression (done separately in relatives of probands with and without ε4) in which the outcome variable was onset age of AD and the predictors were gender and CVD death. In both sets of relatives, we found that women had a significantly higher risk of AD after adjusting for the higher incidence of CVD deaths among men (ε4 families: odds ratio = 1.78, $P < .02$; non-ε4 families: odds ratio = 2.39, $P < .05$).

In summary, the results presented here extend our previous finding of a familial effect on risk of AD (Farrer et al. 1995b) in several important ways. First, transmission of AD in families of probands with at least one ε4 allele fits a dominant inheritance model. Second, single gene inheritance also best explains clustering of the disorder in families of probands lacking ε4, but a more complex genetic model or multiple genetic models may ultimately account for risk in this group of families. Regardless, transmission of AD differs significantly in families of APOE ε4 carriers from families of probands without the ε4 allele. Third, susceptibility to AD differs between men and women. Adjusting for survival patterns among men and women, and assuming a dominant model, AD appears to be completely penetrant in women, whereas only 62%–65% of men with predisposing genotypes develop AD. However, parameter estimates from the arbitrary major gene model suggests that AD is expressed dominantly in women and additively in men. In other words, a single AD susceptibility allele is sufficient to cause disease in women, but men having only one such allele have a markedly reduced risk. Estrogen is one gender specific factor that may modify genetic influences in this manner (Paganini-Hill et al. 1994).

Future genetic modeling studies need to consider the joint effects of APOE genotype and other loci. Association studies indicate that α_1-antichymotrypsin (AACT), low-density lipoprotein receptor, and PS-1 genotypes may modulate the influence of APOE genotype (Kamboh et al. 1995; Okuizumi et al. 1995; Wragg et al. 1996), but the these findings are controversial (Haines et al. 1996; W. K. Scott, L. H. Yamaoka, P. A. Locke, B. L. Rosi, P. C. Gaskell, A. M. Saunders, P. M. Coneally, et al., unpublished information). Furthermore, although our study ruled out environmental factors alone as responsible for transmission of AD in these families, evidence for joint effects of genes and environment for risk of AD is emerging (Mayeux et al. 1995; van Duijn et al. 1995). Elucidation of the various genetic and nongenetic components to AD risk may ultimately require the development of genetic epidemiological profiles on a large group of patients and their relatives.

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