Apolipoprotein E and Alzheimer Disease: Genotype-Specific Risks by Age and Sex

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Summary

The distribution of apolipoprotein E (APOE) genotypes as a function of age and sex has been examined in a French population of 417 Alzheimer disease (AD) patients and 1,030 control subjects. When compared to the APOE ε3 allele, an increased risk associated with the APOE ε4 allele (odds ratio [OR] [ε4] = 2.7 with 95% confidence interval [CI] = 2.0–3.6; $P < .001$) and a protective effect of the APOE ε2 allele (OR[ε2] = 0.5 with 95% CI = 0.3–0.98; $P = .012$) were retrieved. An effect of the ε4 allele dosage on susceptibility was confirmed (OR[ε4/ε4] vs. the ε3/ε3 genotype = 11.2 [95% CI = 4.0–31.6]; OR[ε3/ε4] vs. the ε3/ε3 genotype = 2.2 [95% CI = 1.5–3.5]). The frequency of the ε4 allele was lower in male cases than in female cases, but, since a similar difference was found in controls, this does not lead to a difference in OR between sex. ORs for the ε4 allele versus the ε3 allele, OR(ε4), were not equal in all age classes: OR(ε4) in the extreme groups with onset at <60 years or >79 years were significantly lower than those from the age groups 60–79 years. In ε3/ε4 individuals, sex-specific lifetime risk estimates by age 85 years (i.e., sex-specific penetrances by age 85 years) were 0.14 (95% CI 0.04–0.30) for men and 0.17 (95% CI 0.09–0.28) for women.

Received February 8, 1996; accepted for publication November 15, 1996.

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0002-9297/97/6002-0024$02.00

Introduction

Following the initial report of an association between late-onset Alzheimer disease (LOAD) and the ε4 allele of the apolipoprotein E (APOE) gene (Saunders et al. 1993), this finding has been confirmed by numerous studies worldwide and extended to early-onset Alzheimer disease (EOAD) (Dai et al. 1994; Van Duijn et al. 1994; Pérez-Tur et al. 1995; St. Clair et al. 1995). It has been suggested that patient's survival was influenced by APOE genotype and that disease duration was longer in ε4 carriers than in non-ε4 carriers (Frisoni et al. 1995). As a result, the association found in cross-sectional studies could, at least in part, be accounted for by this phenomenon and not by a true risk conferred by the ε4 allele. The data of Corder et al. (1995*a*) and Norrman et al. (1995), which show that the progression of AD is not related to ε4 gene dose, strongly argue against this hypothesis. Several lines of evidence (Corder et al. 1994; Tsuda et al. 1994; Locke et al. 1995) suggest that the ε4 allele itself (and not a genetic factor in linkage disequilibrium with the APOE ε4 allele) is the risk factor for developing Alzheimer disease (AD). Although the mechanism by which APOE ε4 participates in the pathogenesis is still unknown, several hypotheses based on APOE binding to Aβ peptide and Tau have been developed (for review, see Strittmatter and Roses 1995). A protective role for the APOE ε2 allele has also been reported (Chartier-Harlin et al. 1994; Corder et al. 1994; Smith et al. 1994; Talbot et al. 1994; Locke et al. 1995), although in early-onset patients the ε2 allele could confer a risk for AD (Sorbi et al. 1994; Van Duijn et al. 1995). In a recent statement, a consensus group (American College of Medical Genetics/American Society of Human Genetics [ACMG/ASHG] Working Group 1995) has stressed that a sample of cases and controls much larger than any of those found in any single study

must be evaluated before reliable conclusion about the protective effect of the ε2 allele can be made. In numerous studies, the odds ratios (ORs) associated with homozygosity for the APOE ε4 allele versus absence of the ε4 allele were found to be greater than those associated with heterozygosity, suggesting an effect of the ε4 allele dosage on susceptibility. Some studies demonstrated a difference between the OR of ε4/ε4 versus ε3/ε3 and the OR of ε3/ε4 versus ε3/ε3 (Yoshizawa et al. 1994; St. Clair et al. 1995; Jarvik et al. 1996; Myers et al. 1996). In many other studies, the large and overlapping confidence intervals (CIs) did not allow one to establish this conclusion firmly (Mayeux et al. 1993; Dai et al. 1994; Kuusisto et al. 1994; Van Duijn et al. 1994; Lehtovirta et al. 1995; Tang et al. 1996). It has also been reported that the dose of the ε4 allele may influence the age at onset. In limited series of AD patients, a decrease in average ages at onset ranging from 8 years (Poirier et al. 1993; Tsai et al. 1994) to 16 years (Corder et al. 1993) was found when the number of ε4 allele increased from zero to two. Finally, a gender difference in APOE-associated risk for AD has been assessed, yielding conflicting results (Corder et al. 1995*b*; Payami et al. 1994, 1996). Because of ascertainment biases and severe truncation of data in the different samples so far studied, the distribution of the APOE genotypes as a function of age at onset in the AD population is not precisely known. It has been stressed that epidemiologically based distributions will be needed before risks associated with the different APOE genotypes can be estimated in the general population (Roses 1995). In the present report, APOE genotype–specific ORs by age and sex are estimated in a large sample of French patients and controls. Genotype-specific AD incidences and AD lifetime risks (LTRs) are also calculated.

Subjects and Methods

Subjects

All subjects enrolled in this study were Caucasian living in France. Informed consent was obtained for each subject either directly or from a legal tutor. Control subjects <60 years of age (n = 87) were either patient's spouses or healthy blood donors. Control subjects of older ages consisted of a sample of individuals randomly selected from residential facility lists of 30 retirement homes located in the north of the France (Amouyel et al. 1994). A single trained physician interviewed each subject and performed blood sampling. One thousand forty-three subjects, representing 78.9% of the eligible subjects, were included. Drop out was due to the absence of the person at the time of the interview (9.9%) or refusal (11.2%). Information concerning mentally incompetent subjects was supplied by their next of kin. Age, gender, years of school, and profession were recorded, and a medical examination was performed. Mini–mental state examination (Folstein et al. 1975) was administrated to all subjects. Among these 1,043 individuals, 100 cases of dementia were clinically diagnosed according to DSM 3 criteria (American Psychiatric Association [APA] 1987). Stratifying these cases by age and comparing the observed prevalence rates with those found in the EURODEM epidemiological study (Rocca et al. 1991) did not reveal any significant difference. The affected individuals were excluded from the control group. Thus, the total control group consisted of 1,030 individuals.

Unrelated AD patients were ascertained in a 3-year period through consecutive admissions in several hospitals or nursing homes located mainly in northwestern France. All patients fulfilled the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer Disease and Related Disorders Association (McKhann et al. 1984) criteria for probable AD. The mean duration of illness at inclusion was 5 years. Age at onset for all AD patients were assessed by interviewing a next of a kin. Age at onset was defined as the age at which the patient or his family first noticed the symptoms required for the diagnosis. Eighteen patients belonging to families characterized by autosomal dominant inheritance of an AD gene with complete penetrance by age 60 years were excluded from the analysis, since we have shown that in this particular subgroup of patients the APOE ε4 allele does not play any etiological role (Pérez-Tur et al. 1995; Martinez et al. 1996). Four hundred seventeen patients were finally included.

APOE Genotypes

Genomic DNA was amplified by PCR using amplification conditions and primers described by Hixson and Verrier (1990). PCR products were digested with *Hha*I restriction enzyme and subjected to electrophoresis on polyacrylamide gels.

Statistical Analysis

Allele and genotype frequencies for both AD cases and controls were estimated by counting alleles and genotypes and calculating sample proportions. For the calculation of the OR of a factor F, we used the following notation. Let a, b, c, and d denote the number of cases observed with F, of cases without F, of controls with F, and of controls without F, respectively. For each age by sex category, values of ORs were estimated by the maximum-likelihood estimate ad/bc and 95% CIs were computed using logit confidence limits (Breslow and Day 1980). The OR approximates the relative risk.

Homogeneity of ORs across age and sex groups and the significance of the overall OR were tested by appropriate χ^2 tests (Fleiss 1991). Estimates of the overall OR for several or all strata were computed with the Mantel-

Haenszel estimate (Mantel and Haenszel 1959; Breslow and Day 1980) using appropriate CIs (Robins et al. 1986; Breslow 1996). The Mantel-Haenszel estimate is not affected by zero cell entries in some of the individual fourfold tables for the age and sex strata. Since tests of homogeneity across several fourfold tables are often not very powerful, homogeneity of ORs was also considered separately for age and for sex by decomposing the χ^2 statistic for homogeneity into "within sex" and "between sex" components and likewise for age. This allows to test for homogeneity of ORs for sex while allowing for heterogeneity across age and vice versa. Multiple testing is carried out using the Bonferroni correction, for which the significance level α is adjusted to $\alpha_{adj} = \alpha/n$, where n is the number of tests to consider. In this article, we take $\alpha = .05$.

The probability of being affected with AD with age at onset in age group i, i = 1, 2, 3, or 4, given age (i.e., age at onset for cases and age at exam for controls), sex, and APOE genotype can be calculated with age- and sex-specific incidences and the probabilities for the APOE genotype, given cases or controls from the general population for each age and sex group. Let r, s, and t denote, respectively, $r = P$(affected with age at onset in age group i | age group i, sex), $s = P$(APOE genotype | affected with age at onset in age group i, sex), and $t = P$(APOE genotype | age group i, sex). Then $P = P$(affected with age at onset in age group i | age group i, sex, APOE genotype) $= rs/t$.

Estimates r' of r along with the corresponding sample size nr are available from the Rochester epidemiological study (Schoenberg et al. 1987). Estimates s' of s are the age- and sex-specific genotype frequencies for cases as stated above (with the sample sizes ns). Age- and sex-specific genotype frequencies for controls (with the sample size nt) are taken to be approximate estimates t' of t. The distribution of P has been found by simulation, to take into account the uncertainty in r', s', and t'. For each simulation j, P_j has been calculated as $P_j = r_j s_j / t_j$, where r_j, s_j, and t_j are sampled from the appropriate binomial distributions with parameters (r', nr), (s', ns), and (t', nt). (Some special cases had to be considered for $t_j = 0$). Fifty thousand simulations have been shown to be sufficient for stability of the estimate of P (median of the simulated distribution per 10,000 person years) and of the 95% CI for P (P_l and P_u representing the lower and upper bound values, respectively, of P). Necessary sample sizes for a desired length of CIs were also determined by simulation.

The sex- and APOE genotype–specific LTRs by the

Table 1

Genotype Frequencies and ε4-Allele Frequencies for AD Cases and Controls Stratified by Age and Sex

	Age (Years)										
	Men				Women						
	≤59	60–69	70–79	≥80	≤59	60–69	70–79	≥80	Men	Women	Total
APOE	Cases										
n	52	38	35	9	82	84	75	42	134	283	417
ε4/ε4	.12	.16	.14	.11	.10	.26	.04	.07	.13	.13	.13
ε3/ε4	.33	.34	.43	.33	.41	.46	.51	.29	.36	.43	.41
ε3/ε3	.52	.47	.43	.44	.44	.25	.35	.55	.48	.37	.41
ε2/ε4	.00	.00	.00	.00	.00	.01	.07	.05	.00	.03	.02
ε2/ε3	.04	.03	.00	.00	.05	.01	.04	.05	.02	.04	.03
ε2/ε2	.00	.00	.00	.11	.00	.00	.00	.00	.01	.00	.00
ε4	.28	.33	.36	.26	.30	.50	.33	.24	.31	.36	.34
	Controls										
n	40	93	80	103	47	75	143	449	316	714	1030
ε4/ε4	.00	.00	.00	.01	.04	.03	.01	.02	.00	.02	.01
ε3/ε4	.28	.17	.16	.20	.30	.31	.29	.25	.19	.27	.24
ε3/ε3	.62	.70	.71	.64	.51	.52	.56	.56	.67	.55	.59
ε2/ε4	.02	.00	.02	.03	.00	.03	.01	.04	.02	.03	.03
ε2/ε3	.08	.12	.10	.11	.15	.11	.12	.13	.10	.13	.12
ε2/ε2	.00	.01	.00	.01	.00	.01	.00	.01	.01	.01	.01
ε4	.15	.09	.09	.13	.19	.19	.17	.16	.11	.17	.15

NOTE.—Age for cases is age at onset. n = number of observations.

Table 2

Mantel-Haenszel Estimates of Combined ORs of APOE Genotypes and Alleles with Respect to ε3/ε3 or ε3, Respectively, for Men and Women for Each Age Group Separately and across Ages with 95% CI

	AGE (years)				
GENOTYPE	≤59	60–69	70–79	≥80	ALL
ε4/ε4	5.2 (.6–44.3)	29.1 (3.6–239.5)	...	...	11.2 (4.0–31.6)
ε3/ε4	1.5 (.6–3.6)	3.1 (1.4–6.9)	3.2 (1.5–6.6)	1.3 (.5–3.4)	2.2 (1.5–3.5)
ε2/ε4	...	...	...	...	1.6 (.5–5.5)
ε2/ε3	.4 (.1–2.1)	.3 (.0–2.3)	.4 (.1–2.3)	.3 (.0–2.6)	.4 (.1–.9)
ALLELE					
ε4	1.9 (.96–3.7)	4.1 (2.3–7.5)	3.0 (1.7–5.2)	1.7 (.9–3.4)	2.7 (2.0–3.6)
ε2	.4 (.1–1.6)	.2 (.0–1.3)	.7 (.2–2.2)	.7 (.2–2.4)	.5 (.3–.98)

NOTE.—OR(ε2/ε2) for every age and some OR (...) could not be determined, because of low cell counts. Ages for cases are ages at onset.

middle of age group i was computed by the method described by Thompson and Weissman (1981). LTRs and extreme bounds on the 95% CI of LTRs were obtained using P, P_l, and P_u values for individual age groups.

Results

For each class/sex stratum, the age distributions (i.e., age at onset for cases and age at exam for controls) were compared by the median test. This test revealed that in three age/sex classes (70–79 years for men and women and ≥80 years for men) controls were slightly older than cases. However, comparing the distribution of APOE genotypes for controls below and above the median age at exam within each of those groups by Fisher's exact test revealed no differences.

APOE genotype frequencies for both cases and controls, stratified by age group and sex, are given in table 1. The frequencies of the APOE alleles ε2, ε3, and ε4 in the overall control population are 8.0%, 77.1%, and 14.9%, respectively. It should be emphasized that in controls the ε4 allele frequency is significantly lower in men than in women (10.9% in men; 16.7% in women; $\chi^2 = 11.42$; df = 1; $P = .0007$). For each sex, no heterogeneity across age classes in the ε4 frequency estimates was observed. The lower frequency in males is observed in the four age classes.

For AD cases the overall observed allele frequencies across age and sex are 34.4% for ε4, 62.8% for ε3, and 2.8% for ε2. The ε4 allele frequency in AD cases is 31.3% in men and 35.6% in women.

Table 2 shows, for each age, the Mantel-Haenszel estimate of the ORs for men and women combined for the association of AD with a particular genotype versus the genotype ε3/ε3 or with a particular allele versus the allele ε3. The Mantel-Haenszel estimate of the overall OR for allele ε4 is significantly different from 1: OR(ε4) = 2.7 with 95% CI = 2.0–3.6. The Mantel-Haenszel estimates of the overall OR(ε4/ε4) and OR(ε3/ε4) are 11.2 with 95% CI = 4.0–31.6 and 2.2 with 95% CI = 1.5–3.5, respectively. OR(ε4/ε4) is greater than OR(ε3/ε4), as can be seen from the nonoverlapping CIs. The overall OR estimate of ε4/ε4 versus ε3/ε4 is 5.2 with 95% CI = 1.8–15.0 (results not shown in table 2).

The overall χ^2 test statistic for homogeneity across age and sex of the OR for allele ε4 is 13.9 (df = 7; $P = .052$). Although this test does not give formal evidence for heterogeneity of OR(ε4) across age and sex, it gives a suggestion. Since the test for homogeneity is not very powerful, homogeneity of OR(ε4) has been considered separately for sex and age. There is no evidence for heterogeneity of OR(ε4) between sex (while allowing for heterogeneity between age) ($\chi^2 = 3.82$; df = 1; $P = .051$; $\alpha_{adj} = .05/2 = .025$). However, there is evidence for heterogeneity of OR(ε4) between age groups (while allowing for heterogeneity between sex) ($\chi^2 = 9.98$; df = 3; $P = .019$; $\alpha_{adj} = .025$). In addition, the extreme age groups of <60 years and ≥80 years are significantly different from the age groups of 60–79 years, even after adjusting for multiple testing ($\chi^2 = 8.56$; df = 1; $P = .003$; $\alpha_{adj} = .05/7 = .007$). There are four possible tests to assess whether one group is significantly different from the other three groups and three possible tests to assess whether two groups are different from the other two groups. Thus, there are seven possible tests for comparing the four age groups.

The frequency of the ε2 allele among ε4 noncarriers is 4.2% for cases and 9.4% for controls. This difference is highly significant ($\chi^2 = 14.81$; df = 1; $P = .0001$). The Mantel-Haenszel estimate of the overall OR for allele ε2

Table 3

Age- and Sex-Specific AD Incidences *r'* per 10,000 Person-Year (Schoenberg et al. 1987) Compared to the Corresponding APOE Genotype–Specific AD Incidence Estimates *P*

	AGE (years)			
SEX AND APOE	≤59	60–69	70–79	≥80
Men:				
Overall	0 (0–2)	15 (7–29)	56 (34–87)	105 (55–185)
ε4/ε4	0 (0–1)	15 (7–25)	57 (30–82)	132 (0–3,221)
ε3/ε4	0 (0–2)	15 (7–25)	57 (30–82)	161 (0–453)
ε3/ε3	0 (0–1)	10 (4–19)	33 (16–58)	69 (17–156)
ε2/ε3	0 (0–1)	3 (0–16)	0 (0–0)	0 (0–0)
Women:				
Overall	1 (0–2)	6 (2–13)	51 (34–74)	160 (115–218)
ε4/ε4	1 (0–6)	43 (3–189)	108 (0–546)	700 (0–2,786)
ε3/ε4	1 (0–2)	8 (2–20)	88 (50–142)	181 (90–312)
ε3/ε3	1 (0–1)	3 (1–6)	31 (18–50)	156 (97–229)
ε2/ε3	0 (0–1)	0 (0–3)	16 (0–49)	54 (0–161)

NOTE.—Estimates for ε2/ε4 and ε2/ε2 are not given, since there were no observations for cases and controls in some instances. Ninety-five percent CIs are in brackets.

is .5 with 95% CI = .3–.98. The χ^2 test statistic for the overall test of association is 6.28 (df = 1; P = .01). Thus, the overall OR is significantly <1. There is evidence for a protective effect of the ε2 allele. Tests of homogeneity are not carried out, because of the small number of ε2 alleles in individual age and sex strata. The estimates of the overall OR for the ε2 genotypes are OR(ε2/ε3) = .4 with 95% CI = .1–.9 (χ^2 = 7.60; df = 1; P = .006) and OR(ε2/ε4) = 1.6 with 95% CI = .5–5.5 (χ^2 = .57; df = 1; P = .5). Thus, there is evidence for a protective effect in OR(ε2/ε3). For the ε2/ε4 genotype, no effect is found, presumably because of the counteractive effects of ε2 and ε4, but maybe also because of a lack in power. Note that for the genotypes ε2/ε2 and ε2/ε4 individual ORs for most age and sex strata are not possible, because of small observed cell counts.

Table 3 shows the APOE genotype–, age-, and sex-specific AD incidences P compared to the corresponding age- and sex-specific incidence estimates r' (drawn from the Rochester study, Schoenberg et al. 1987). In general, P estimates associated with ε3/ε4 and ε4/ε4 genotypes were higher and estimates associated with ε3/ε3 and ε2/ε3 genotypes were lower than r', but a large overlap was found between CIs for r' and P. In ε3/ε4 individuals, sex-specific LTRs by age 85 years (i.e., penetrances of the corresponding genotype) were .14 (95% CI .04–.30) for men and 0.17 (95% CI .09–.28) for women. (fig. 1). In APOE ε4 homozygotes, the large CIs precluded any reasonable estimation of LTRs.

Discussion

Since the discovery of an association between APOE ε4 and AD, curves that reflect the probability of remaining unaffected for a given age as a function of APOE genotype have been produced (Corder et al. 1993). These curves are based on the empirical distribution of AD onsets in an arbitrary collection of families. They are useful to reveal a dose effect in APOE genotype, where each additional ε4 allele shifts the onset to an earlier age. However, as correctly pointed out by Corder et al., they do not provide an accurate estimate of the age- and sex-specific risk associated with each APOE genotype (see discussion in Bird 1995 and Roses 1995). Indeed, OR estimates in family studies as estimates of OR in the general population may be biased in the sense that, for example, OR (ε4) might be increased. In the

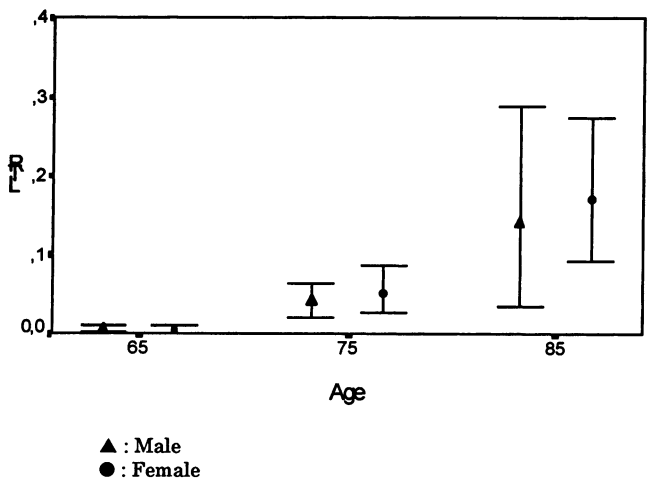

Figure 1 AD LTR by age i = 65, 75, and 85 years, for APOE ε4 heterozygotes. Point estimates and extreme bounds for 95% CIs.

present report, we compared a population of AD patients ascertained through consecutive admissions in several hospitals with a control population, including a sample of unaffected subjects randomly selected from retirement homes (control subjects >60 years of age) and a sample of patient's spouses or healthy blood donors (control subjects <60 years of age). This control group is likely to approximately reflect the general non-AD population of the same age classes.

The first result from this study is that, in the French population of elderly non-AD subjects, the ε4 allele is less frequent in males than in females. This finding might result from an early selection against ε4 male carriers. It has indeed been shown that inheritance of ε4 allele confers risk of ischemic heart disease in middle-aged men (Cumming and Robertson 1984; Lenzen et al. 1986; Laakso et al. 1991; Van Bockxmeer et al. 1992) but not in elderly subjects (Kuusisto et al. 1995). Second, no sex difference in OR(ε4) is detected. This is in accordance with data of Corder et al. (1995*b*) but not with those of Payami et al. (1995, 1996), which suggested a gender difference in APOE associated risk for AD. However, Payami et al. (1996) based their results on family data and point out that gender may be a risk factor only in familial AD. In addition, their sample includes very few non-AD men >60 years of age. In contrast, because of our study design, a large sample of non-AD men >60 years of age are included in the present investigation. Putting together the two basic findings of this study, we suggest that the co-occurrence of lower ε4 frequency in elderly male controls and of similar OR(ε4) in males and females could explain, at least in part, the higher age-specific prevalence of AD in women reported in the literature (Breteler et al. 1992). Third, the ORs are significantly >1 in the middle two of the four age classes, but it should be stressed that the ORs(ε4) are not equal in all age classes. Our data suggest that the APOE genotype does not exert its influence with the same magnitude within the whole period at risk for AD, but mainly in the 60-79-years interval. Fourth, there is no overlap in overall OR between subjects with the ε3/ε4 genotype and those with the ε4/ε4 genotype, thus confirming the ε4 dose effect. Fifth, a protective effect of the ε2 allele is clearly found. OR(ε2) estimates are <1, whatever the age class, with a significant overall effect. OR(ε2/ε3) is significantly <1. The estimate of OR(ε2/ε4) is smaller than the estimate of OR(ε3/ε4), even though this cannot be confirmed, because of overlapping CIs. Our results do not confirm the recent report (Sorbi et al. 1994; Van Duijn et al. 1995) that the APOE 2 allele is associated with an increased risk of EOAD.

The APOE genotype–specific incidences are close to the corresponding age- and sex-specific incidences, with a tendency for higher rates in individuals with ε3/ε4 and ε4/ε4 genotypes and lower rates in individuals without an ε4 allele. Incidence rates for genotype ε4/ε4 are not uniformly larger than those for genotype ε3/ ε4. Previous studies reported a shift to earlier onset with each additional ε4 allele. This would mean that incidences should be higher for ε4 homozygotes than for ε3/ε4 heterozygotes in earlier ages and probably vice versa in older ages. No such tendency could be detected. This failure could reflect lack of statistical power. CIs are large, mainly because of large CIs for age- and sex-specific incidence rates; CIs for ε4 homozygotes with age ⩾80 years are intolerably large. However, increasing the sample sizes for cases and controls in this category does not help much to reduce the variability. Our estimates are based on $ns = 9$ cases and $nt = 103$ controls for men and $ns = 42$ cases and $nt = 449$ controls for women. To reduce by 50% the length of the 95% CI for women an increase in sample size to 800 cases and 800 controls would be necessary. To obtain such a size for the AD group would be very hard, since it took three years to constitute the present sample in a collaborative study including seven hospitals.

The same problem is compounded when computing cumulative incidences. As a result, the AD LTRs for ε4 homozygotes cannot be assessed with sufficient precision. For ε3/ε4 heterozygotes, it is interesting to note that the upper bound of the CI estimate reaches only 30% in men and 28% in women by age 85 years. We conclude that the APOE-specific incidence estimates and the corresponding age-dependent penetrance values of APOE genotypes cannot be improved unless better estimates of age- and sex-specific incidence rates are provided.

Acknowledgments

We are grateful to Dr. Fanon, Dr. Avenel, and all our colleagues who helped to identify patients for this study and to M. J. Dupire for technical assistance and B. Prum for statistical advices. This study was supported by INSERM (network grant 4R004A) and the Mutuelle Générale de l'Education Nationale (grant 704045).

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