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The Apolipoprotein E/CI/CII Gene Cluster and Late-Onset Alzheimer Disease

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Summary

The chromosome 19 apolipoprotein E/CI/CII gene cluster was examined for evidence of linkage to a familial Alzheimer disease (FAD) locus. The family groups studied were Volga German (VG), early-onset non-VG (ENVG; mean age at onset <60 years), and late-onset families. A genetic association was observed between apolipoprotein E (ApoE) allele ε4 and FAD in late-onset families; the ε4 allele frequency was .51 in affected subjects, .37 in at-risk subjects, .11 in spouses, and .19 in unrelated controls. The differences between the ε4 frequencies in affected subjects versus controls and in at-risk subjects versus controls were highly significant (standard normal deviate [Z_{SND}]) = 7.37, $P < 10^{-9}$; and Z_{SND} = 4.07, $P < .00005$, respectively). No association between the ε4 allele and FAD was observed in the ENVG or VG groups. A statistically significant allelic association between ε4 and AD was also observed in a group of unrelated subjects; the ε4 frequency was .26 in affected subjects, versus .19 in controls (Z_{SND} = 2.20, $P < .03$). Evidence of linkage of ApoE and ApoCII to FAD was examined by maximum-likelihood methods, using three models and assuming autosomal dominant inheritance: (1) age-dependent penetrance, (2) extremely low (1%) penetrance, and (3) age-dependent penetrance corrected for sporadic Alzheimer disease (AD). For ApoCII in late-onset families, results for close linkage were negative, and only small positive lod-score-statistic (Z) values were obtained (model 1, maximum Z [Z_{max}] = 0.61, recombination fraction [θ] = .30; model 2, Z_{max} = 0.47, θ = .20). For ApoE in late-onset kindreds, positive Z values were obtained when either allele frequencies from controls (model 1, Z_{max} = 2.02, θ = .15; model 2, Z_{max} = 3.42, θ = .05) or allele frequencies from the families (model 1, Z_{max} = 1.43, θ = .15; model 2, Z_{max} = 1.70, θ = .05) were used. When linkage disequilibrium was incorporated into the analysis, the Z values increased (model 1, Z_{max} = 3.17, θ = .23; model 3, Z_{max} = 1.85, θ = .20). For the ENVG group, results for ApoE and ApoCII were uniformly negative. Affected-pedigree-member analysis gave significant results for the late-onset kindreds, for ApoE (Z_{SND} = 3.003, P = .003) and ApoCII (Z_{SND} = 2.319, P = .016), when control allele frequencies were used but not when allele frequencies were derived from the families.

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Introduction

Familial Alzheimer disease (FAD) is genetically heterogeneous. Two loci for early-onset autosomal dominant FAD have been identified. One is the amyloid precursor protein (APP) gene on chromosome 21 (Goate et al.

1991). A second early-onset locus was recently identified by linkage analysis (Schellenberg et al. 1992*a*) in families whose mean onset ages were 42–52 years. APP mutations account for Alzheimer disease (AD) in approximately 5%–10% of early-onset kindreds, and the chromosome 14 locus accounts for AD in most other early-onset families (Mullan et al. 1992; St George-Hyslop et al. 1992; Schellenberg et al. 1992*a*; Van Broeckhoven et al. 1992; Nechiporuk et al. 1993).

Neither the APP gene (Schellenberg et al. 1991*a*; Kamino et al. 1992; Tanzi et al. 1992) nor the chromosome 14 FAD locus (Schellenberg et al. 1993) appears to be a major contributor to late-onset AD. The genetics of late-onset FAD (mean family onset age > 60 years) is difficult to resolve, for the following reasons: First, it is unclear what fraction of late-onset AD is the result of inherited gene defects. Epidemiologic (e.g., see van Duijn et al. 1991) and twin studies (Bergem et al. 1992; Breitner et al. 1992) clearly indicate that inheritance is important in late-onset AD, and some have proposed that all AD could be genetic (Mohs et al. 1987). However, a substantial portion of late-onset disease could be the result of nongenetic environmental factors (i.e., "sporadic" AD). Second, the mode of inheritance of late-onset FAD is unknown. Third, late-onset AD is common. Prevalence rate estimates for the 75–84-year-old age group range from 4.1% (Bachman et al. 1992) to 18.7% (Evans et al. 1989). Thus clustering of cases in a family could be the result of chance, a mixture of genetic and "sporadic" cases, or a mixture of genetic cases entering a family through different lines of descent. Fourth, because the disease is late onset, the quality of family material is limited; subjects often die of other causes (age censoring) prior to reaching the age of maximum risk for AD, and typically only a single generation is available for sampling. Fifth, because the clinical diagnosis of AD is one of exclusion, and because the elderly have a high prevalence of conditions confounding the diagnosis of AD, the correct specification of the disease phenotype is problematic. Autopsy documentation is critical in AD studies.

The q13 region of chromosome 19 has been implicated in late-onset AD. We reported a genetic association between FAD and an allele of a polymorphism at the apolipoprotein CII (ApoCII) gene (Schellenberg et al. 1987, 1992*b*). Subsequently, Pericak-Vance et al. (1991) reported positive evidence for linkage of AD to the same region, using both maximum-likelihood and affected-pedigree-member (APM) analysis methods. Recently, an association study of the common ApoE polymorphisms identified the ε4 allele as a potential risk factor for late-onset FAD (Strittmatter et al. 1993). The ApoE results are consistent with the prior ApoCII association results, since these two genes are part of an apolipoprotein gene cluster and are separated by only 40 kb (Houlston et al. 1989).

In this study, we examine the role of the ApoE/CI/CII gene cluster in both late-onset FAD families and a population of unrelated consecutive newly diagnosed late-onset AD subjects. Results from a variety of analytic techniques and genetic models are compared.

Subjects and Methods

FAD Families, Unrelated AD Subjects, and Controls

The families studied (table 1) have been described elsewhere (Bird et al. 1988, 1989; Schellenberg et al. 1991*b*, 1993). The diagnosis of AD was assigned, and autopsies were performed as described elsewhere (McKhann et al. 1984; Bird et al. 1988, 1989; Schellenberg et al. 1991*b*). Autopsy documentation of AD was available for all of the Volga German (VG) kindreds, 11 of the 12 early-onset non-Volga German (ENVG) kindreds, and for 39 of the 53 late-onset families.

The unrelated AD group consisted of 164 consecutive newly diagnosed AD subjects meeting the NINCDS criteria for probable AD (mean age at onset = 76.39 years, SD = 6.49 years), ascertained through a Seattle-area HMO. The HMO controls (237 subjects) were from the same HMO and were age matched to the cases (mean age = 78.0 years, SD = 6.08 years). Controls had Mini-Mental State Exam scores ≥28 (≥27 if they were >80 years of age), no indication of dementia on either the Mattis Dementia Rating Scale or the Blessed Dementia Rating scale, and no history of dementia determined by interview or medical record review.

Genotyping

The dinucleotide repeat polymorphism at the ApoCII locus (Weber and May 1989) was genotyped as described elsewhere (Schellenberg et al. 1992*b*). ApoE genotypes were determined by a dot-blot method using the primers and PCR conditions described by Emi et al. (1988). Genotypes for 96 individuals were determined by both the dot-blot method and by digestion of PCR amplification products by *Hha*I (Hixson and Vernier 1990); genotypes from both methods were identical.

Allele Frequency Estimation in FAD Families

Allele frequencies for unrelated AD subjects and controls were estimated by allele counting. For the

Table 1

Characteristics of FAD Families

Family Group	Total No. of Families	Total No. Affected[a]	Total No. Autopsied[b]	No. of Affecteds Sampled[a]	No. of Subjects Sampled	Range of Family Mean Age at Onset (years)
ENVG	12	125 (1)	38	34	176	41–51
VG	7	86	16	23	111	52–65
Late onset	53	278 (27)	54	137 (2)	290	62–80
Total	72	489 (28)	108	194 (4)	577	

[a] Values in parentheses indicate number of family members with the diagnosis of possible AD or dementia, by family history.
[b] Includes two brain biopsies and three unaffected subjects autopsied.

FAD groups, allele frequencies were initially estimated by allele counting of all sampled individuals. This produces unbiased estimates of allele frequencies but underestimates the standard error. Therefore, two other approaches were taken. First, allele frequencies and standard errors of these frequencies were estimated by a maximum-likelihood pedigree analysis method, which correctly takes relationships between family members into account (Schellenberg et al. 1987; Boehnke 1991). Second, generalized estimating-equation (GEE) methodology (Olson, in press), which adjusts for the correlations between family members, was used to estimate allele frequencies and to determine empirical standard errors of those estimates. Comparisons of allele frequencies between family members of different AD phenotypes (affected, at risk, and spouses) were made by computing an estimate of the covariance between group status (e.g., affected vs. at risk) and ApoE allele status. This covariance estimate, when standardized by its GEE variance estimate, has, for samples containing a sufficiently large number of families, a standard normal distribution and thus produces a test of marker-disease association (Olson, in press). Since the behavior of the test for samples containing small numbers of families is at present not well understood, caution is suggested in interpreting results for the ENVG and VG groups.

Linkage Analysis

Lod-score-statistic (Z) values were calculated under the assumption of linkage equilibrium between FAD and ApoE, using the computer program LIPED modified to handle ≤ 20 alleles and with the capacity to handle different alleles for each family. LIPED was also modified to incorporate a cumulative normal sporadic AD age-at-onset function (model 3; see below), by using the approach suggested by Margaritte et al. (1992). Z values were also calculated with the LINKAGE package (Lathrop et al. 1984), under the assumption of linkage disequilibrium between FAD and ApoE. The disease allele frequencies used were .001 for the VG and ENVG family groups and .01 for the late-onset families. The lower allele frequency was used for the early-onset groups because early-onset FAD is a rarer disease than late-onset FAD. Z values were computed under three different models, all assuming autosomal dominant inheritance. Model 1 assumed age-dependent penetrance with a cumulative normal age-at-onset correction (Schellenberg et al. 1991*b*). The age curve was constructed using family-specific means and overall SD of 7.18 years for late-onset families, 8.62 years for VG families, and 5.60 years for ENVG families. For model 2, Z values were calculated by setting the penetrance of the AD genotype to a fixed value of 1% (also referred to as "affected-only analysis"), which effectively results in ignoring the AD phenotypes of the unaffected but at-risk subjects but uses their marker genotypes in the likelihood computations. Model 3 was the same as model 1, with the addition of a cumulative normal sporadic age-at-onset function with a mean of 110 years and an SD of 14 years. This model effectively corrects for sporadic AD patients in the families by altering the probability that an affected individual is a sporadic versus a FAD gene carrier; as a subject's age at onset increases, the probability that the subject is a sporadic subject increases (Margaritte et al. 1992). Since misspecification of marker allele frequencies can have dramatic effects on the results of the analysis (Wijsman 1993), analyses were performed both with published marker allele frequencies and with frequencies estimated from

the families. For the analysis that assumed the presence of linkage disequilibrium between ApoE and FAD, the disequilibrium coefficient and allele frequencies used were derived from the control population.

APM Method

Families in which genotypes from two or more affected subjects could be either directly typed or completely inferred were analyzed by the APM method of Weeks and Lange (1988), using a computer program provided by D. Weeks. The APM method tests the null hypothesis of independent segregation of the disease with marker alleles. The distribution of the test statistic is approximately normal; a one-sided test is used to determine the significance of the results. The test statistic was computed for three weighing schemes: $f(p) = 1$, $f(p) = 1/\sqrt{p}$, and $f(p) = 1/p$, where p is the allele frequency of the test marker. The first scheme gives no weight to allele frequencies, whereas the second and third make the sharing of a rare allele a more significant event than the sharing of a common allele. The third scheme, however, usually leads to non-normality of the test statistics. The intermediate function is therefore a good compromise for incorporating an allele frequency function and generating a normal distribution. When the test statistic was >1.645 (providing a nominal significance level of 5%), the segregation of the marker alleles through the pedigrees was simulated to determine the actual significance level; 5,000 iterations were performed, and the mean, the variance, and the upper 95th and 99th percentiles were determined empirically. APM analysis is sensitive to allele frequencies; sharing of rare alleles is counted as a more significant event than sharing a common allele. Also, APM can confound linkage and genetic association. Therefore, for the APM analysis, we used both population allele frequencies and frequencies derived from the FAD families.

Results

Three groups of FAD kindreds were studied (table 1): ENVG, VG, and late-onset families. These family groups have been analyzed for linkage to the APP gene on chromosome 21, and each group yielded significant negative Z values for close linkage (Kamino et al. 1992). Also, the ENVG and VG kindreds and most of the late-onset families were screened for mutations in exons 16 and 17 of the APP gene, and no FAD mutations were found (Schellenberg et al. 1991*a*; Kamino et al. 1992). Linkage analysis of chromosome 14q24.3 markers gave strong positive evidence of linkage for the ENVG group and gave negative results for the VG kindreds (Schellenberg et al. 1992*a*) and the late-onset family group (Schellenberg et al. 1993).

Genetic Association Analysis

The ApoE locus was analyzed for genetic association. The ApoE gene is polymorphic at codons for amino acids 112 and 158. Three haplotypes are commonly observed: ε2 (Cys_{112}-Cys_{158}), ε3(Cys_{112}-Arg_{158}), and ε4 (Arg_{112}-Arg_{158}). For Caucasians, published allele frequencies are .080, .769, and .150 for ε2, ε3, and ε4, respectively (Davignon et al. 1988*b*). Similar frequencies were obtained in the present study (table 2), using 237 elderly healthy Caucasians ascertained from a local HMO as age-matched controls for the HMO AD patients. Maximum-likelihood estimates of Apo E allele frequencies suggested that there was an excess of ε4 allele in affected and at-risk subjects in the late-onset kindreds (table 2); the ε4 frequencies were .510 in affected subjects, .367 in at-risk subjects, and .111 in spouses. At-risk subjects are defined as unaffected blood relatives of affected subjects, and spouses are individuals who married into the family. The ε4 allele frequencies were compared with that of the HMO controls. (The HMO controls were used as the source of the more conservative value, since the ε4 frequencies were slightly higher than published values.) For the late-onset families, the ε4 allele frequencies were significantly different for both the affected ($P < 10^{-9}$) and at-risk subjects ($P < .00005$) (table 2), compared with the HMO controls. This association was also observed when the late-onset families were further subdivided into earlier (family mean onset age 60–70 years) and late (family mean onset age >70 years). For the ENVG group, ε4 frequencies in affected subjects (.149), at-risk subjects (.112), and spouses (.083) were comparable with those in controls. For the VG group, ε4 allele frequencies were higher than those in controls, for all three classes of subjects—.310, .229, and .265 for affected subjects, at-risk subjects, and spouses, respectively. However, these frequencies were not significantly different from those in controls. The GEE method gave similar estimates of allele frequencies and somewhat larger standard errors; the level of significance was somewhat reduced (table 2).

Examination of the pedigrees revealed that, in 35 of 53 late-onset kindreds, (*a*) all affecteds sampled were

Table 2

Maximum-Likelihood Allele Frequency Estimates

Group and Status	No. Analyzed	Allele Frequency Estimates ± Standard Error			Z_{SND}[a]	P[a]
		ε2	ε3	ε4		
Late onset:						
Affected	137	.039 ± .015	.450 ± .039	.510 ± .040	7.37	$<10^{-9}$
At risk	133	.065 ± .020	.568 ± .041	.367 ± .040	4.07	<.00005
Spouses	18	.083 ± .046	.806 ± .066	.111 ± .052	−1.42	.16
Age 60–70 years:						
Affected	77	.037 ± .018	.403 ± .050	.560 ± .051	6.87	$<10^{-9}$
At risk	88	.069 ± .025	.574 ± .051	.357 ± .049	3.20	<.002
Spouses	17	.059 ± .040	.824 ± .065	.118 ± .056	−1.24	.21
Age >70 years:						
Affected	60	.043 ± .024	.521 ± .062	.435 ± .062	3.82	<.0002
At risk	45	.056 ± .068	.558 ± .068	.386 ± .067	2.83	<.005
Spouses	1	.500 ± .353	.500 ± .353	.00 ± .000		
VG:						
Affected	24	.028 ± .028	.707 ± .081	.265 ± .079	.93	.35
At risk	73	.040 ± .023	.752 ± .055	.209 ± .052	.34	.73
Spouses	17	.059 ± .040	.677 ± .080	.265 ± .076	.96	.34
ENVG:						
Affected	34	.041 ± .028	.810 ± .057	.149 ± .052	−.74	.46
At risk	114	.064 ± .025	.824 ± .039	.112 ± .033	−2.07	<.04
Spouses	24	.042 ± .029	.875 ± .048	.083 ± .040	−2.43	<.02
Sporadic AD:[b]						
Affected	164	.061 ± .013	.68 ± .026	.26 ± .024	2.20	<.03
HMO controls:[b]						
Normal	237	.074 ± .012	.736 ± .020	.190 ± .018		
Published controls:[c]						
Normal	5,805	.080	.769	.150		

[a] For comparison of ApoE ε4 allele frequencies in FAD groups vs. those in the HMO control group.

[b] Controls and unrelated AD subjects from the HMO group. Allele frequencies were estimated by simple allele counting.

[c] From Davignon et al. (1988*b*).

[d] When the late-onset group was analyzed by the GEE method, allele frequency estimates (± standard error) for the ε4 allele in affected subjects, at-risk subjects, and spouse subjects were .526 ± .035, .356 ± .051, and .111 ± .056, respectively. The ε4 frequency in affected subjects and at-risk subjects was significantly different from that in the HMO controls (Z_{SND} = 8.58, $P < .000005$; and Z_{SND} = 3.15, P = .0016, respectively).

either heterozygous or homozygous for the ε4 allele, (*b*) 13 pedigrees had both affected subjects with the ε4 allele and affected subjects without the ε4 allele, and (*c*) in 5 pedigrees all affected subjects lacked the ε4 allele. Of 137 affected subjects, 113 had at least one ε4 allele.

Maximum-Likelihood Linkage Analysis

In order to investigate the possibility that the ApoE association indicates the presence of a susceptibility locus in the region, the apolipoprotein gene cluster was also evaluated for linkage to FAD, by the maximum-likelihood lod-score method, using a dinucleotide repeat polymorphism adjacent to the ApoCII gene (Weber and May 1989) and the ApoE gene polymorphism described above. Results for the three different models analyzed are given in table 3. For the ENVG group, all Z values for both markers were negative at all values of the recombination fraction (θ) tested; significant evidence against linkage to ApoCII was obtained for $\theta \leq .15$. For the VG group, Z values for linkage of ApoCII to FAD were negative at all values of θ, and significant evidence against linkage was obtained for $\theta \leq .15$ (model 1) and $\theta \leq .05$ (model 2). For linkage of ApoE to FAD in the VG group, small positive values of $Z_{max} = 0.25$ ($\theta = .20$) and $Z_{max} = 0.46$ ($\theta = .10$) were obtained for models 1 and 2, respectively. For late-onset kindreds, significantly positive Z values ($Z_{max} \geq 3.0$) for linkage of FAD to ApoE were obtained under some

Table 3

Two-Point Lod-Score Values for Linkage of Late-Onset FAD to ApoE and ApoCII

Locus and Family Group	Model[a]	Source of Allele Frequencies[b]	Z at θ = .001	.05	.10	.15	.20	.30	.40
ApoE:									
Late onset	1	Controls	−.65	1.59	2.01	2.02	1.83	1.11	.36
Late onset	1	Family	−1.36	.89	1.35	1.43	1.32	.80	.25
ENVG	1	Controls	−1.65	−1.44	−1.17	−.89	−.64	−.29	−.09
VG	1	Controls	−3.72	−.88	−.21	.11	.25	.24	.10
Late onset	2	Controls	3.19	3.42	3.16	2.71	2.19	1.13	.33
Late onset	2	Family	1.25	1.70	1.67	1.48	1.21	.63	.18
ENVG	2	Controls	−.79	−.69	−.55	−.42	−.30	−.14	−.05
VG	2	Controls	−.44	.36	.46	.44	.38	.20	.05
Late onset	3	Controls	−.77	−.45	−.23	−.08	.00	.06	.03
Late onset	3	Family	−.58	−.32	−.15	−.04	.02	.06	.02
ApoCII:									
Late onset	1	Controls	−13.47	−4.52	−1.66	−.26	.41	.61	.26
ENVG	1	Controls	−36.24	−13.04	−8.22	−5.46	−3.61	−1.38	−.32
VG	1	Controls	−12.71	−6.32	−3.98	−2.53	−1.60	−.57	−.15
Late onset	2	Controls	−5.15	−1.49	−.27	.27	.47	.39	.14
ENVG	2	Controls	−14.17	−6.65	−4.05	−2.56	−1.61	−.54	−.10
VG	2	Controls	−4.72	−2.01	−1.27	−.87	−.62	−.32	−.15
Late onset	3	Controls	−3.44	−2.11	−1.26	−.72	−.37	−.07	.03

[a] Model 1, age-corrected penetrance; model 2, 1% penetrance; and model 3, age-dependent penetrance corrected for a sporadic AD rate.
[b] ApoE allele frequencies used in Z calculations were either from the HMO controls (Controls) or from all the individuals in the late-onset FAD families (Family). The ApoCII allele frequencies are from Weber and May (1989).

conditions. When published allele frequencies were used, the Z_{max} values obtained were 2.02 (θ = .15), 3.42 (θ = .05), and 0.06 (θ = .30) for models 1, 2, and 3, respectively. When linkage disequilibrium was incorporated into the analysis (table 4), the Z values increased (model 1, Z_{max} = 3.17, θ = .23; model 3, Z_{max} = 1.85, θ = .20). However, when ApoE allele frequencies were derived from the late-onset families, evidence of linkage for the late-onset families was reduced (table 3). For ApoCII, significant evidence against close linkage of AD to late-onset FAD was obtained under all models, and no Z value >0.61 was observed.

APM Analysis

For late-onset families, the significant positive Z values observed under some conditions but not others could indicate that genetic and etiologic heterogeneity exists or that the genetic model assumed is incorrect. Therefore, we also used nonparametric methods to evaluate evidence for linkage of FAD to ApoE and ApoCII. For ApoE, results for the late-onset group were significant when population controls were used but not when the frequencies used were derived from the FAD families (table 5). The results from the ApoCII analysis also differed depending on the allele frequen-

Table 4

Two-Point Lod-Score Values Computed Assuming Linkage Disequilibrium for Linkage of ApoE to Late-Onset FAD

Model	Z at θ = .0	.05	.10	.15	.20	.30	.40	Z_{max} (θ)
1	−14.14	−1.42	1.26	2.55	3.09	2.82	1.51	3.17 (.23)
3	.02	1.09	1.61	1.83	1.85	1.48	.77	

Table 5

APM Analysis Test Statistics

	ApoE		ApoCII		
FAMILY GROUP	HMO Controls	FAD Families	Published	HMO Controls	FAD Families
Late onset	3.003 (P = .003)	.252 (ns)	2.319 (P = .016)	1.619 (ns)	.863 (ns)
Age 60–70 years	3.596 (P = .001)	1.107 (ns)	3.155 (P = .003)	2.322 (P = .017)	1.504 (ns)
Age >70 years	.704 (ns)	−.718	.163 (ns)	−.047 (ns)	−.295 (ns)
VG	.784 (ns)	.643 (ns)	−1.539 (ns)	−1.267 (ns)	−1.353 (ns)
ENVG	−.276 (ns)	.448 (ns)	.029 (ns)	−.100 (ns)	−.391 (ns)

NOTE.—Test statistics are for the function $f(P) = 1/\sqrt{p}$. P values (given in parentheses) were determined empirically through 5,000 iterations. ApoCII allele frequencies are given in table 6, and ApoE allele frequencies from the HMO controls are given in table 2. ApoE allele frequencies from the FAD families (ENVG+VG+late onset) were .043, .659, and .299 for ε2, ε3, and ε4, respectively, as estimated from the complete family data. Published frequencies (table 6) are from Weber and May (1989). Five individuals had alleles 161 and/or 163, which were not observed in the study by Weber and May (1989) and thus were excluded from the analysis using the published frequencies. When frequencies for these alleles were set at .001 and when (*a*) the remaining frequencies taken from Weber and May (1989) and (*b*) the five subjects were returned to the analysis, the results became insignificant for the total late-onset group but were still significant for the 60–70-year age group (Z_{SND} = 2.412, P = .016).

cies used (tables 5 and 6). Marginally significant results for the late-onset families were obtained when published frequencies (Weber and May 1989) were used but not when the HMO controls' or FAD families' frequencies were used.

ApoE Allele Frequencies in Unrelated AD Subjects

The ENVG, VG, and late-onset families were selected as kindreds having multiple affected subjects in several generations, and thus ascertainment was not random. ApoE allele frequencies were also examined in unrelated Caucasian AD subjects ascertained as newly diagnosed cases from a local HMO. The probands were not selected for family history, although some subjects do have first-degree relatives with dementia consistent with AD. In this group, ε4 frequencies were .26, compared with .19 for age- and sex-matched healthy controls from the same HMO (table 2). These frequencies were significantly different (standard normal deviate [Z_{SND}] = 2.34, $P < .01$).

Discussion

The above work provides strong evidence for a genetic association between the ε4 allele of the ApoE locus and late-onset FAD. This finding is consistent with earlier association studies with an ApoCII polymorphism (Schellenberg et al. 1987, 1992*b*) and ApoE (Strittmatter et al. 1993). The ApoE ε4 allele does not appear to be associated with FAD in the ENVG or VG groups. Allelic associations can be confounded by, e.g., population admixture; thus the source of control allele frequencies is critical. For ApoE, allele frequencies have been determined for a large number of Caucasian subjects (Davignon et al. 1988*b*; Hallman et al. 1991; de Knijff et al. 1992); ε4 frequencies for Caucasians are typically .13–.16, with an overall average frequency of $p_{\varepsilon 4}$ = .150 (n = 5,805; Davignon et al. 1988*b*). Swedes ($p_{\varepsilon 4}$ = .206; de Knijff et al. 1992) and Finns ($p_{\varepsilon 4}$ = .244; Haleman et al. 1991) have the highest reported frequencies. The ε4 frequency used for comparison in the present study ($p_{\varepsilon 4}$ = .19; table 2) is within the range of published Caucasian values, although it is somewhat higher than the frequency ($p_{\varepsilon 4}$ = .150) obtained in the comprehensive study by Davignon et al. (1988*b*); this is possibly explained by the large number of individuals of Scandinavian ancestry who are in the study population. However, the somewhat high $p_{\varepsilon 4}$ value used in this study is unlikely to be the source of spurious results, since, if anything, it would tend to obscure the reported association. The conclusion that there is an association is supported by the fact that the spouses (n = 18) in the late-onset families have an ε4 frequency of .111, which is similar to that in controls. Also, at-risk family members have an ε4 frequency (.356) intermediate between those in affected subjects and those in spouses. However, population admixture as a source of the apparent genetic association cannot be absolutely excluded.

The ApoE/CI/CII gene cluster was tested for evidence of linkage to an FAD locus, by lod-score analysis, by the nonparametric APM method, and by testing for

Table 6

ApoCII Allele Frequency Estimates

Allele (bp)	Allele Frequency Estimates[a]						
					Late-Onset families		
	Published Controls (n = 150)	HMO Controls (n = 472)	FAD Families[b] (n = 1,040)	HMO Affecteds (n = 336)	Affected (n = 260)	At Risk (n = 222)	Spouses (n = 30)
129	.12	.142	.133	.128	.112	.090	.100
135	0	0	.003	.003	0	0	0
137	.11	.083	.066	.063	.046	.072	.033
145	.01	.034	.055	.048	.027	.050	.067
147	.02	.013	.013	.006	.015	.036	0
149	.03	.032	.021	.024	.027	.018	0
151	.34	.246	.285	.274	.300	.270	.333
153	.15	.193	.143	.182	.138	.104	.133
155	.15	.163	.171	.161	.177	.248	.20
157	.04	.064	.072	.051	.092	.063	.133
159	.03	.019	.029	.024	.054	.041	0
161	0	.006	.005	.006	.004	.005	0
163	0	.004	.004	.018	.008	.005	0
165	.01	0	.001	.003	0	0	0
167	0	0	0	.009	0	0	0
171	0	0	0	.003	0	0	0

[a] Published allele frequencies are from Weber and May (1989). n = number of chromosomes sampled.
[b] Data are allele counts from all family members sampled in all family groups.

a genetic association between ApoE and FAD. Despite the fact that ApoE association was highly significant, lod-score analysis gave significant evidence for linkage under only some of the conditions used, and it gave no significant Z values with ApoCII, a locus within 40 kb of ApoE. The APM method also gave significant results under only some of the conditions tested. The accuracy of both of these methods is dependent on marker allele frequencies used in the analysis when there are missing genotypes in deceased individuals (Ott 1992; Wijsman 1993), and relatively small changes in the frequencies of rare alleles can dramatically alter the results.

There are a number of possible explanations that could account for the apparent discrepancy between the positive results obtained with linkage and association analysis with ApoE and the negative linkage results obtained with ApoCII. First, the inability to detect linkage with the ApoCII marker by the lod-score method could indicate that the genetic model used was inappropriate. This could involve misspecification of either mode of inheritance or specific model parameters such as disease allele frequencies. Studies by Greenberg and Hodge (1989) and others (Clerget-Darpoux et al. 1986) indicate that linkage analysis is likely to give erroneous negative results when the true mode of inheritance is recessive but a dominant model is assumed, or vice versa. Some studies suggest that late-onset FAD may be codominant (Corder et al. 1993) or recessive (Payami et al., submitted). Also, if parameter values used, including phenocopy rate or late-onset FAD gene frequency, are badly misspecified, false recombinants could obscure the true linkage relationship. Epistatic interaction between ApoE and an as-yet-unidentified locus elsewhere could also compromise the ability of the lod-score method to detect linkage. Alternatively, the ApoE-associated disease allele could be a susceptibility allele that is neither necessary nor sufficient for the development of AD; Greenberg (1993) has shown that linkage may be difficult to detect if the relative risk associated with the susceptibility allele is <10. While model or parameter misspecification might explain why the linkage analysis with ApoCII could produce false-negative results, it does not explain why the analysis with ApoE, with the same model and parameter values, produced apparently positive results. A second explanation is that the ApoE genetic association observed is spurious, although this seems unlikely in light of the fact that others have reported the same association between

ApoE ε4 and AD (Noguchi et al. 1993; Saunders et al. 1993; Strittmatter et al. 1993).

One additional explanation for the discrepancy between the results of the linkage analyses with ApoCII and those with ApoE is that the linkage disequilibrium between ApoE and late-onset AD may have introduced ascertainment bias into the analysis. Linkage analyses are not usually affected by pedigree ascertainment. As long as pedigrees are chosen on the basis of genotypes or phenotypes at only one of the two loci in the linkage analysis, neither bias in the estimated θ nor inflation of the Z value is expected (Morton 1955; Ott 1991, pp. 218–219). However, inflation of the Z value may occur if ascertainment is through both the disease and marker phenotype or genotype (Gershon and Matthysse 1977). Although, in the linkage analysis of late-onset FAD with ApoE, there is no conscious attempt to exclude families with certain ApoE genotypes, the presence of linkage disequilibrium between ApoE and late-onset FAD means that, by selecting pedigrees with multiple cases of AD, we also will have selected pedigrees with a disproportionate number of ε4 alleles. Unfortunately, we cannot appropriately adjust for the disequilibrium simply by incorporating an estimate of the disequilibrium into the analysis, as would be possible for a linkage analysis of two loci neither of which was involved in the ascertainment of the pedigrees. Thus we inadvertently introduce a joint-selection criterion on both the disease and marker locus, which violates one of the assumptions behind the analysis. The analysis could then produce inflated Z values, which may be interpreted as providing positive evidence of linkage. The ApoCII locus used in these analyses does not demonstrate evidence of being in disequilibrium with late-onset AD, so there should be no bias introduced into the linkage analysis with ApoCII.

It is possible that the ApoE ε4 allele (or some as-yet-unidentified variant in disequilibrium with ApoE) is a modifier locus that lowers the age at onset in a dose-dependent fashion (Corder et al. 1993) but that by itself is neither necessary nor sufficient to cause AD. If this is the case, then, because AD is a late-onset disease, subjects and families with the early-onset modifier allele (ApoE ε4) would be more likely to be ascertained than families without the modifier allele, because of age-dependent censoring by other causes of death. This might produce an association between the ApoE ε4 allele and the disease in the population, as observed, but attempts to demonstrate cosegregation with an adjacent marker (in this case the dinucleotide repeat polymorphism at ApoCII) would fail, since the major disease-causing locus is somewhere else in the genome. This model, though consistent with available data, cannot be proved at this time.

The ε4 allele frequency in the unrelated AD subjects from the HMO was also significantly elevated compared with that in age-matched HMO controls. The ε4 frequency in the HMO AD subjects was lower (.26) than that in the late-onset FAD patients (.526; table 2). The difference may reflect how the two groups were ascertained; the late-onset FAD families were selected for the presence of a strong family history of AD, while the HMO cases were essentially randomly ascertained with respect to family history. Thus the late-onset families should be more heavily loaded with genetic cases than are the HMO cases, and the frequency of a susceptibility locus (such as ApoE) would be expected to be higher. However, evaluation of the HMO results is confounded by the following observations: First, the ε4 allele is a risk factor for coronary artery disease and thus may influence longevity. This may be particularly true in women, since cohorts of older women may have lower ε4 frequency values than do younger women (Davignon et al. 1988*a*; Cauley et al. 1993). Women may also be at a higher risk for AD (e.g., see Rocca et al. 1991). Finally, the ε4 frequencies in both the HMO group and the AD group decline with age (authors' unpublished data). Therefore, the difference between ε4 frequencies in the control group versus the HMO group must be interpreted with caution. Additional work needs to be performed to determine whether the elevated ε4 frequencies observed in the unrelated (HMO-group) cases are associated with an increased risk of AD.

The most likely explanation for the genetic association is that (1) the ε4 allele itself is a risk factor for late-onset FAD or (2) the ApoE polymorphism studied here is in linkage disequilibrium with an AD susceptibility mutation either in ApoE or in a gene in the ApoE/CI/CII region. At present, the genetic data cannot distinguish between these two possibilities. However, several lines of evidence implicate the ApoE gene in AD. Namba et al. (1991) and others (Wisniewski and Frangione 1992; Strittmatter et al. 1993) showed that anti-ApoE antibody stains AD plaques, extracellular tangles, and vascular amyloid. However, cerebral and systemic amyloid deposits in numerous other amyloid diseases, including kuru plaques in Creutzfeldt-Jakob disease, are also ApoE positive (Wisniewski and Frangione 1992). Thus the association of ApoE with amyloid is not specific to the Aβ-peptide amyloid seen in AD. ApoE binds to glucosaminoglycans (Mahley et al.

1979), which are present in most types of amyloid deposits (Snow et al. 1987); thus the ubiquitous presence of ApoE in different types of amyloid plaques may reflect its binding to glycosaminoglycans. Other work suggests that ApoE binds directly to the Aβ peptide amino acids 12–28 (Strittmatter et al. 1993), which is the major component of AD amyloid. Wisniewski and Frangione (1992) have suggested that ApoE acts as a "pathologic molecular chaperone," which binds to amyloidgenic peptides and facilitates β-pleated sheet amyloid structures. Another potential role for ApoE in AD is as a neuronal injury response protein, possibly involved in the redistribution of lipid (in particular cholesterol) during neuronal repair (Mahley 1988; Goodrum 1991). AD could be the result of chronic injury that is inappropriately repaired when the ApoE ε4 allele is present. However, the role of ApoE in nerve repair is presently unclear, since no deficiency in sciatic nerve repair was observed in mice lacking a functional ApoE gene (ApoE knockout mice; Popko et al. 1993). Therefore, additional work is needed to determine whether ApoE or a neighboring gene is a risk factor for late-onset FAD.

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