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Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease

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Gene dosage of the apolipoprotein E (APOE) ε4 allele is a major risk factor for familial Alzheimer disease (AD) of late onset (after age 60). Here we studied a large series of 115 AD case subjects and 243 controls as well as 150 affected and 197 unaffected members of 66 AD families. Our data demonstrate a protective effect of the ε2 allele, in addition to the dose effect of the ε4 allele in sporadic AD. Although a substantial proportion (65%) of AD is attributable to the presence of ε4 alleles, risk of AD is lowest in subjects with the ε2/ε3 genotype, with an additional 23% of AD attributable to the absence of an ε2 allele. The opposite actions of the ε2 and ε4 alleles further support the direct involvement of APOE in the pathogenesis of AD.

Alzheimer disease (AD) is the leading cause of dementia in the elderly[1,2]. Family studies in the past five years have localized most forms of AD with onset before age 60 to either chromosome 14 (refs 3–5) or 21 (refs 6, 7). Analysis of the familial clustering of late-onset AD (after age 60)[8–15], however, did not show linkage to these regions[4,16]. Using established diagnostic criteria[17,18], we recently investigated a series of late onset AD families for genetic linkage[19], and found evidence for the involvement of a region of chromosome 19 near the apolipoprotein E locus (*APOE*) in late onset AD[19]. Subsequent reports showed an increased frequency of the *APOE* ε4 allele in late-onset AD patients, familial and sporadic, and a gene-dose effect on risk for the ε4 allele in AD families[20–28], implicating this allele as a major risk factor for AD.

APOE has three major allelic variants, ε3 (77%), ε4 (15%) and ε2 (8%)[29], which encode the three isoforms of apolipoprotein E (apoE), a 34 kilodalton protein that mediates the clearance of several plasma lipoproteins[30]. ApoE isoforms differ by single amino acid substitutions at positions 112 and 158: E3 (Cys112, Arg158), E4 (Cys112Arg), and E2 (Arg158Cys).

We now demonstrate the protective effect of the ε2 allele in late-onset AD, as well as expand our studies on the ε4 dose-related increase in AD risk to include sporadic as well as familial AD. We also calculate the proportion of AD attributable to the *APOE* locus, and show it is a major determinant of AD.

Study subjects

We studied 115 unrelated AD case subjects and 243 unrelated controls as well as 150 affected and 197 unaffected members of 66 AD families, all age 60 or older at the onset of the symptoms of AD or when examined and found to be unaffected (Table 1; see Methodology).

Genotypic frequencies

Genotypes containing the ε2 allele were less frequent than expected in the AD patients. Most notably, just 1% of the case subjects and 2% of the affected members of the AD families had the ε2/ε3 genotype compared to 16% of the controls (Table 2). Conversely, genotypes containing the ε4 allele were more common than expected in the AD patients, especially the affected members of the AD families. The frequency of the ε3/ε4 genotype was 22% in the control subjects, 49% in the case subjects, and 58% in the affected members of the AD families. The frequency of the ε4/ε4 genotype was 2% in the control subjects, 16% in the case subjects, and 23% in the affected members of the AD families.

Frequency of the ε2 allele

It can be seen directly that the frequency of the ε2 allele is reduced in AD patients, even after allowing for the increased frequency of ε4 in AD patients. Specifically, looking only at ε2 and ε3 alleles, among the case subjects only 6 of 113 alleles (4.5%) were ε2 versus 52 out of 413 alleles (12.6%) in the controls ($\chi^2 = 6.05$, $p<0.025$). The patients from the AD families displayed a similar trend. Four out of 144 alleles (2.8%) in affected members were ε2, compared to 19 out of 291 alleles (6.5%) for their unaffected relatives ($\chi^2 = 2.0$, n.s.).

Familial aggregation

The ε2 allele was significantly less common in the unaffected members of the AD families (5%) than in the

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Table 1 Descriptive statistics for study subjects

Unrelated subjects	Age at onset Sporadic cases (*n*=115)[a]				Age at examination Control subjects (*n*=243)[a]			
	n	Mean	(s.d.)	[Range]	*n*	Mean	(s.d.)	[Range]
Men	34	71.3	(6.0)	[60–84]	65	70.3	(5.6)	[60–86]
Women	69	71.6	(7.8)	[60–90]	87	70.3	(5.0)	[61–86]
AD families	**Age at onset Affected members (n=150)**				**Age at examination Unaffected members (n=197)**			
	n	Mean	(s.d.)	[Range]	*n*	Mean	(s.d.)	[Range]
Men	55	69.7	(6.2)	[60–87]	86	72.3	(8.3)	[60–92]
Women	95	71.5	(6.4)	[60–91]	111	70.7	(7.5)	[60–90]

[a]Age information was not available for all subjects.

control subjects (11%, p=0.001). The ε2 allele was also less common in the affected members of AD families (1.3%) than in the case subjects (2.6%, p=0.23). The ε4 allele was significantly more common in the unaffected members of the AD families (26%) than in the control subjects (15%, p < 0.0001), and also more common in the affected members of AD families (52%) than in the case subjects (42%, p=0.03).

Risk of Alzheimer disease

Logistic models were constructed to quantify risk of AD in relation to APOE genotype (Table 3). In the AD cases and controls, the occurrence of an ε2 allele was protective, decreasing risk by a factor of four (OR=0.25, 95% CI 0.10–0.62) while each occurrence of an ε4 allele increased risk by a factor of more than four (OR=4.39, 95% CI 2.88–6.71). Thus, risk was lowest in subjects with the ε2/ε3 genotype (the one subject with the ε2/ε2 genotype was combined with the ε2/ε3 subjects). In the AD families, each occurrence of an ε4 allele was also associated with increased risk of AD by a factor of four (OR=3.94, 95% CI 2.62–5.94). However, the protective effect of the ε2 allele, infrequent in AD families, was not statistically significant (OR=0.48, 95% CI 0.15–1.51).

To determine whether risk varied with age, separate models were made for subjects aged 60–66, 67–74, and 75+ (Table 3). We found that benefit related to the ε2 allele and risk related to the ε4 allele waned with increasing age. The absence of ε2 alleles among affected members of AD families aged 60–66 did not allow evaluation of the ε2 allele in this age group.

Two conditional logistic models were made to evaluate whether or not the risk of AD conferred by the ε4 allele was different in men and women. Both models used the number of ε4 alleles, the occurrence of an ε2 allele, and gender as predictors of AD. Both controlled for age by specifying three-year risk sets. One model had a gender-ε4 dose interaction term. This model was intended to see whether the dose-wise increase in risk was the same in men and in women. The other model had a gender-ε4 heterozygous interaction term as the high percentage of men and women with the ε4/ε4 genotype affected with AD suggested that any difference in risk between men and women involving ε4 might be limited to persons with one copy of ε4. No difference in risk was seen for men and women in relation to the number of ε4 alleles (p=0.53 unrelated subjects; p=0.22 AD families) or for men and women having a single ε4 allele (p=0.80 unrelated subjects; p=0.12 AD families).

Attributable proportions

Risk estimates were used to calculate the proportion of AD that might be avoided if all subjects had lower-risk *APOE* genotypes (Table 4). We used two reference genotypes in the calculations; first, the common ε3/ε3 genotype, and second, the less common lower risk ε2/ε3 genotype. We found that 77% of cases with the ε3/ε4 genotype and 95% of those with the ε4/ε4 genotype would not have become affected if risk in subjects with these genotypes had been the same as for those with the common ε3/ε3 genotype. Applying the frequencies of the ε3/ε4 and ε4/ε4 genotypes in the case subjects to the attributable proportions (AP), we found that 53% of late-onset sporadic AD was attributable to the occurrence of the ε4 allele.

A larger proportion (88%) of late-onset AD would be prevented if risk could be reduced to the level found in subjects with the less common but lower risk ε2/ε3 genotype as the reference genotype. We found that 75% of cases with the ε3/ε3 genotype, 77% with the ε2/ε4 genotype, 94% with the ε3/ε4 genotype and 99% with the ε4/ε4 genotype would have remained unaffected if subjects with these genotypes enjoyed the lower

Table 2 Frequency of each *APOE* genotype[a]

Unrelated subjects APOE Genotype	Case subjects Men		Women		All		Control subjects Men		Women		All	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
ε2/ε2	0	0%	0	0%	0	0%	0	0%	1	1%	1	0%
ε2/ε3	1	3%	0	0%	1	1%	22	20%	16	12%	38	16%
ε2/ε4	1	3%	4	5%	5	4%	6	5%	6	5%	12	5%
ε3/ε3	12	32%	23	30%	35	30%	59	53%	76	58%	135	56%
ε3/ε4	17	45%	39	51%	56	49%	22	20%	31	23%	53	22%
ε4/ε4	7	18%	11	14%	18	16%	2	2%	2	2%	4	2%
Sum	38		77		115		111		132		243	

AD families APOE Genotype	Affected members Men		Women		All		Unaffected members Men		Women		All	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
ε2/ε2	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
ε2/ε3	2	4%	1	1%	3	2%	5	6%	9	8%	14	7%
ε2/ε4	1	2%	0	0%	1	1%	1	1%	4	4%	5	3%
ε3/ε3	6	11%	19	20%	25	17%	39	45%	46	41%	85	43%
ε3/ε4	27	49%	60	63%	87	58%	39	45%	49	44%	88	45%
ε4/ε4	19	35%	15	16%	34	23%	2	2%	3	3%	5	3%
Sum	55		95		150		86		111		197	

[a]Column percentages sum to 100% ± 1% depending on rounding error. The allelic variants result in six genotypes and expected frequencies given Hardy-Weinberg equilibrium: ε2/ε2 (1%), ε2/ε3 (12%), ε2/ε4 (2%), ε3/ε3 (59%), ε3/ε4 (23%) and ε4/ε4 (2%).

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Table 3 The odds of AD and *APOE* genotype[a]

	APOE Genotype				
	ε2/ε3	ε3/ε3	ε2/ε4	ε3/ε4	ε4/ε4
Unrelated subjects					
All	0.3[b]	1.0	1.1	4.4[b]	19.3[b]
Age (years)					
60 to 66	0.1	1.0	1.2	11.1[b]	123.8[b]
67 to 74	0.3	1.0	1.1	4.6[b]	20.8[b]
75 to 92	0.5	1.0	1.6	3.2[b]	10.0[b]
AD families					
All	0.5	1.0	1.9	3.9[b]	15.6[b]
Age (years)					
60 to 66[c]	–	1.0	–	7.4[b]	55.4[b]
67 to 74	0.5	1.0	2.2	4.4[b]	19.7[b]
75 to 92	0.7	1.0	1.8	2.7[b]	7.1[b]

[a]The ε3/ε3 genotype was the referent genotype.
[b]The 95% CI does not overlap the reference value 1.
[c]No ε2 alleles in affected subjects.

level of risk associated with the ε2/ε3 genotype. Applying the frequencies of these genotypes in the case subjects to the AP, we found that 65% of late-onset sporadic AD was attributable to the occurrence of the ε4 allele and an additional 23% was attributable to the absence of ε2 in subjects with the common, but not lowest risk, ε3/ε3 genotype.

Discussion

ε2 allele. Our analysis of the allele frequencies, the odds ratios, and attributable proportions are consistent in demonstrating that the *APOE* ε2 allele has a protective effect in late-onset AD. Further support for the protective effect of the ε2 allele comes from the observations of Hardy *et al.*[31] who noted a later age of onset for two ε2/ε3 heterozygotes in early-onset families segregating an amyloid precursor protein mutation, and from Locke *et al.* (manuscript submitted) who have recently confirmed a decrease in the frequency of the *APOE* ε2 allele in a set of late-onset AD families. The pattern of risk in relationship to *APOE* genotype was common to familial and sporadic late-onset AD, making *APOE* genotype a major determinant of risk regardless of family history.

The higher frequency of ε4 and lower frequency of ε2 in the AD families compared to the unrelated subjects suggests that their relative frequencies in the parents contribute to the familial aggregation of AD by conferring a higher level of risk to their children. Our estimate of the proportion of AD that might be prevented by lowering risk to the level found in the most common ε3/ε3 genotype, 53%, is smaller than that estimated by Payami *et al.*[22], 78% (95% CI 61%–90%) for familial AD who used unrelated control subjects as their comparison group. This is an expected result if ε4 contributes to the familial aggregation of AD.

Age effects and gender. Risk and benefit related to the *APOE* locus diminished with age. Thus, actual attributable proportions are likely to be higher from age 60 to 66 and lower after age 75. The genotypic composition of affected subjects depends on age, so risk assessment must consider age and genotype. Thus, preventive strategies need to consider the population as a whole, especially the large proportion of persons with the ε3/ε4 genotype at moderate risk. We found no evidence that risk in relation to *APOE* ε4 differs in men and women.

Role of *APOE* in AD. Several lines of circumstantial evidence suggest that the E4 protein may be directly involved in the pathogenesis of late-onset AD. ApoE immunoreactivity is present in the senile plaques and neurofibrillary tangles that define the AD phenotype[21] and E4 has higher avidity *in vitro* for β amyloid protein than E3[32]. Homozygous ε4 subjects exhibit more amyloid β-peptide immunoreactivity at autopsy than other AD patients[33]. *APOE* ε4 frequency is not elevated in subjects with other neurologic amyloidopathies[34], but apoE is present within neurons in both normal subjects and AD patients (S.-H. Han *et al.*, manuscript submitted)[21,35]. The strength of the association in both sporadic and familial AD and its confirmation in several populations also

Table 4 Attributable proportion of AD and APOE genotype

APOE Genotype	Frequency in unrelated AD cases	Reference genotype ε3/ε3 AP[a] 95% CI	ε3/ε3 Weighted AP[b] 95% CI	ε2/ε3 AP[a] 95% CI	ε2/ε3 Weighted AP[b] 95% CI
ε2/ε3	0.01 –	– –	– –	0.00 –	0.00
ε3/ε3	0.30 –	0.00 –	0.00 –	0.75 0.38-0.90	0.23 0.11-0.27
ε2/ε4	0.04	0.09 n.s.	0.00 n.s.	0.77 0.65-0.85	0.03 0.03-0.03
ε3/ε4	0.49	0.77 0.65-0.85	0.38 0.32-0.42	0.94 0.84-0.98	0.46 0.41-0.48
ε4/ε4	0.16	0.95 0.88-0.98	0.15 0.14-0.16	0.99 0.96-1.00	0.16 0.15-0.16
Sum			0.53		0.88

[a]The AP are the attributable proportions of sporadic late-onset AD for each APOE genotype. Each AP indicates the proportion of AD that would not have occurred in subjects with a particular APOE genotype if risk was reduced to the level in subjects with the reference genotype. AP= [OR(–/–) – OR(–/3)] / OR(–/–) where –/– represents an APOE genotype and -/3 represents a reference genotype, either ε3/ε3 or ε2/ε3. Odds ratios were estimated from logistic models in the unrelated subjects.
[b]The weighted AP indicates the proportion of all late-onset sporadic AD that would have been avoided if the specified genotype had been replaced by the reference genotype.

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support a causal relationship between the number of *APOE* ε4 alleles and AD.

Despite this evidence, genetic linkage disequilibrium of an unknown AD gene physically next to the APOE locus could have accounted for the allelic association between ε4 and late onset AD. Genetic linkage disequilibrium arises when two loci are so close together that recombination very rarely occurs. Thus, specific alleles may be passed through many generations in *cis* orientation, leading to an increase in the *cis* allele in affected subjects, despite the fact that it has no biological role in increasing risk. A previous report[36] found an allelic association of an APOC2 *TaqI* polymorphism in predominantly early-onset "Volga German" AD families which lies within 50 kilobases of the *APOE* loci[37]. We have not seen an increased frequency of this polymorphism in affected subjects in our late-onset AD families. Explanation of both the ε2 and ε4 allelic associations would require that the ε4 allele be in disequilibrium with a disease-causing allele at the nearby locus and that the ε2 allele be in disequilibrium with a disease-preventing allele at (presumably) the same locus. Thus, two specific mutations would have to have occurred, each in different chromosomes carrying rare alleles at *APOE*.

In summary, the ε2 allele at the *APOE* locus confers substantial protection for late-onset AD while the ε4 allele confers substantial risk in a dose-related fashion. The pattern of risk in relation to *APOE* genotype is common to familial and sporadic AD, although the many ε4 alleles and few ε2 alleles in the AD families suggests that *APOE* genotype contributed to the familial aggregation of AD. Over 80% of late-onset AD can be attributed to variation from the lowest risk ε2/ε3 genotype indicating that *APOE* is the most important biological risk factor yet identified for AD.

Note added in proof: St. George-Hyslop *et al.* published another APP mutation family in which one individual with the APP mutation and the 2/3 genotype had no evidence of disease at age greater than 2 standard deviations above the family mean age at onset (*Science* **263**, 537 (1994)).

Methodology

Study subjects. 115 autopsy-confirmed late-onset AD cases with no family history of AD or histopathologic evidence of other neurologic disorders were identified and sampled. These included 103 cases where interviews with surviving family members indicated that onset of AD was after age 60. Age at death was > 75 years for the remaining 12 subjects consistent with onset of AD after age 60[20]. Control subjects were derived from three sources and combined: 92 subjects in the Duke Established Populations for Epidemiologic Studies of the Elderly[38] (age > 65 years), 60 unaffected spouses of AD patients seen at the Duke Memory Disorders Clinic[20,21,35] (age > 60 years), and 91 grandparents in Centre d'Etude du Polymorphisme Humain (CEPH) pedigrees[39] (ages not available). CEPH subjects were used in all analyses except for those that required age.

The second subject group consisted of 150 affected and 197 unaffected members > 60 years of age in 66 late-onset AD families (that is, mean age at onset > 60 years) who were genotyped for APOE as previously described[16,19,20,28]. These families were ascertained because at least two first or second degree relatives were affected with AD. Uniform diagnostic criteria were used to identify affected family members[18]. Autopsy confirmation was made for at least one member in 37 families. Informed consent was obtained from each participating individual or, when necessary, their legal guardian. All study protocols were approved by the Duke University Medical Center Institutional Review Board or the Institutional Review Board at the institution which ascertained the subject.

Hypothesis tests. Tests are two-sided and significance was declared at the α=0.05 level. Allele frequencies were compared with Pearson χ^2 tests. The risks of AD for each *APOE* genotype were estimated by construction of logistic models in which the number of ε4 alleles and the occurrence of an ε2 allele were used to predict affected status[40,41]. Parameter estimates and standard errors were exponentiated to arrive at the odds of AD and 95% confidence intervals (CI) for subjects with each genotype compared to subjects with the ε3/ε3 genotype (OR).

Attributable proportions (AP) were estimated from the OR's. Preliminary analyses indicated that survival following onset in affected subjects was unrelated to genotype. This finding supported the use of a prevalence OR to approximate the incidence rate ratio usually used to estimate AP. First, the AP of AD was estimated for each high-risk genotype (–/–) compared to a lower-risk genotype (–/3), either ε3/ε3 or ε2/ε3: AP(–/–)=[OR(–/–) – OR(–/3)] / OR(–/–)[42]. Each AP represents the proportion of affected subjects (with the specified genotype) who would not have developed AD if risk for the genotype could be reduced to the level found in subjects with the lower-risk, comparison, genotype. Next, each AP was multiplied by the frequency of the higher-risk genotype in the cases. This product represents the proportion of AD that would have been avoided by reducing risk to the level found in the lower-risk referent genotype; 95% confidence intervals for the AP were calculated using the exponentiated parameter standard errors. Additional logistic models were constructed for subjects near the mean onset age (67–74 years of age), for younger subjects (60–66 years of age), and for older subjects (75+ years of age). To compare genotype-specific risk in men and women, conditional logistic models were constructed[43]. Three-year age intervals (60–62 etc.) defined the risk sets. AD was predicted by the number of ε4 alleles, the presence or absence of an ε2 allele, sex and an ε4-sex interaction term (either ε4 dose-sex or ε4 heterozygote-sex).

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