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No Genetic Effect of α_1-Antichymotrypsin in Alzheimer Disease

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Alzheimer disease (AD) is the most common neurodegenerative disorder for individuals over the age of 40. AD has a complex etiology, and it is likely that multiple genes, acting independently and/or interacting, affect the risk of developing AD. Several genes involved with AD have been described already, but only the APOE gene on chromosome 19q has been shown to affect the risk of the common late onset form of AD. α_1-Antichymotrypsin (AACT) is a major component of the amyloid plaques found in the brains of AD patients, and an allele in its gene has been proposed to increase the risk of developing AD when also associated with the APOE-4 allele. We have examined the role of this AACT polymorphism in a large set of families and sporadic cases, and do not see any effect, either alone or in combination with the APOE-4 allele.

INTRODUCTION

Alzheimer disease (AD) is the most common neurodegenerative disorder of the elderly, affecting over 4 million individuals in the United States (Max, 1993). Onset is variable, and is generally divided into early (<65) and late (>65) onset forms. AD is also genetically complex. Autosomal dominant mutations in the APP gene (on chromosome 21) (Goate *et al.*, 1991), in the presenilin I gene (on chromosome 14) (Sherrington, *et al.*, 1995), and in the presenilin II gene (on chromosome 1) (Levy-Lehad *et al.*, 1995; Rogaev *et al.*, 1995) have been demonstrated in early onset AD, and account for perhaps 10% of all AD cases.

In 1993, an association between AD and the apolipoprotein E (APOE)-4 allele was reported in both familial (Strittmatter *et al.*, 1993; Corder *et al.*, 1993), and sporadic late onset AD (Saunders *et al.*, 1993). The APOE-4 allele acts in a dose-dependent fashion, with risk increasing (and age-at-onset decreasing) with the number of APOE-4 alleles (Corder *et al.*, 1993), a result confirmed in many different populations (Roses *et al.*, 1995). Although the APOE-4 allele is involved in half of all AD cases (Roses *et al.*, 1996), a substantial number of individuals with the APOE-4 allele escape AD, and a substantial number of AD cases have no APOE-4 allele. This result suggests that other genes may modify the effect of the APOE-4 allele.

α_1-Antichymotrypsin [AACT] has been suspected to play a role in AD since it was first shown to bind the β-amyloid peptide in AD brains (Abraham *et al.*, 1988; Abraham *et al.*, 1990). The AACT gene resides on chromosome 14, some 30 cM from the presenilin I gene and thus is not involved in the early-onset AD linked to chromosome 14. A signal peptide polymorphism (Kamboh *et al.*, 1995) has been described for the AACT gene, and has two alleles with nearly equal frequencies in the general population. AACT is a natural candidate gene to examine for additional genetic effects on AD, and potentially for interaction with the APOE effect. Recently, (Kamboh *et al.*, 1995) reported that in individuals carrying an APOE-4 allele, the AACT-TT genotype appeared to be protective, while the AACT-AA genotype appeared to confer a two- to three fold increased risk.

MATERIALS AND METHODS

Patient samples. The 67 AD families used in this study were identified through the Duke University Alzheimer Disease Research Center (Duke-ADRC), the Indiana Alzheimer Disease Center National Cell Repository, the UCLA Neuropsychiatric Institute, and the UCLA Alzheimer Disease Research Center as part of long term familial studies of AD. To be part of these studies, families must have two or more sampled affected individuals meeting the standard clinical criteria for AD (McKhann *et al.*, 1984). These families contain 154 genotyped affected individuals and 250 unaffected, at-risk geno-

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TABLE 1

Distribution of AACT in APOE-4 Carriers and Non-APOE-4 Carriers

	Non-APOE-4				APOE-4				Total			
	AD		Control		AD		Control		AD		Control	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
AACT genotypes												
AA	43	0.24	95	0.25	114	0.29	35	0.23	157	0.27	130	0.24
AT	94	0.53	207	0.54	196	0.49	80	0.53	290	0.50	287	0.54
TT	42	0.23	81	0.21	87	0.22	37	0.24	129	0.22	118	0.22
Total	179		383		397		152		576		535	
AACT alleles												
A	180	0.50	397	0.52	424	0.53	150	0.49	604	0.52	547	0.51
T	178	0.50	369	0.48	370	0.47	154	0.51	548	0.48	523	0.49
Total	358		766		794		304		1152		1070	

Note. No significant differences in the AACT allele frequencies are observed between APOE-4 allele carriers and non-APOE-4 allele carriers. The AACT alleles are in Hardy-Weinberg equilibrium.

typed individuals. The average age-at-onset for the affected individuals was 69.9 (±7.4) years, 40% were male, and virtually all families were Caucasian in origin.

The 576 additional patients with no apparent family history (sporadic cases) were identified through the MGH-ADRC, the Duke-ADRC, or the Alzheimer Disease Clinic at Boston University. All patients received a clinical diagnosis of Alzheimer disease in accordance with standardized criteria (McKhann *et al.*, 1984). Our experience indicates that over 95% of clinically diagnosed AD patients will be confirmed upon autopsy (Pericak-Vance *et al.*, 1991). The average age-at-onset was 69.1 (±8.6) years, 37% of the patients were male, and virtually all were Caucasian. The 535 controls were also ascertained and sampled. These included 295 spouses of AD patients (mean age of examination was 70) and 240 unrelated controls of all ages. All controls had no evidence of dementia upon initial contact.

Genotyping. Blood samples were obtained, after appropriate informed consent, from all subjects, and DNA was obtained using standard techniques either by direct extraction or from lymphoblast cultures. APOE and AACT genotypes were determined as previously described (Locke *et al.*, 1995; Kamboh *et al.*, 1995). All resulting gels and autoradiograms were visually scored, and the data were entered into computerized database systems.

Data analysis. Lod scores were calculated using the LINKAGE package (Lathrop *et al.*, 1984) assuming an autosomal dominant mode of inheritance for AD with an age-dependent penetrance as previously described (Pericak-Vance *et al.*, 1991; Locke *et al.*, 1995). The penetrance varied from 0.4% at age 40 to 99% after age 90. No phenocopy rate was assumed. Affected-Pedigree-Member (APM) analysis (Weeks and Lange, 1988) was also performed on these families using the $f(p) = 1/\mathrm{sqrt}(p)$ weighing function. This function has been suggested as the most robust function to use (Weeks and Lange, 1988).

Tests of differences in allele and genotype frequencies were performed using either the χ^2 test statistic or deviations from the binomial distribution.

Odds ratios were calculated using standard logistic regression controlling for age and sex. Two models were tested, one including only APOE and AACT main effects and a second allowing for interaction between loci. The differences between models was tested using the likelihood ratio criterion.

RESULTS

A total of 576 sporadic patients and 535 controls were examined for both APOE and AACT genotypes. Table

TABLE 2

Joint Distribution of APOE and AACT Genotypes

	AACT genotypes													
	AA				AT				TT					
	AD		Control		AD		Control		AD		Control		Total	
APOE genotypes	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	AD	Control
22	0	0.00	1	0.25	1	1.00	1	0.25	0	0.00	2	0.50	1	4
23	3	0.21	14	0.24	7	0.50	32	0.54	4	0.29	13	0.22	14	59
24	7	0.41	4	0.31	6	0.35	7	0.54	4	0.24	2	0.15	17	13
33	40	0.24	80	0.25	86	0.52	174	0.54	38	0.23	66	0.21	164	320
34	83	0.29	29	0.23	142	0.50	63	0.50	61	0.21	33	0.26	286	125
44	24	0.26	2	0.14	48	0.51	10	0.71	22	0.23	2	0.14	94	14
Total	157		130		290		287		129		118		576	535

Note. In each subject group the percentages reflect the percentage of the APOE genotypes for each of the three AACT genotypes. No significant differences are observed. All genotypes are in Hardy–Weinberg equilibrium.

TABLE 3

Odds Ratios for Developing AD for Both the APOE and the AACT Genotypes

		Independent model		Interaction model	
		OR	95% C.I.	OR	95% C.I.
Genotype					
APOE	X/X	1.00	Referent group	1.00	Referent group
	4/X	4.56	3.41–6.11	3.53	1.94–6.41
	4/4	17.89	9.24–34.66	66.24	7.28–602.90
AACT	T/T	1.00	Referent group	1.00	Referent group
	A/T	0.93	0.65–1.33	0.90	0.56–1.46
	A/A	1.01	0.67–1.52	0.81	0.46–1.41
	X/X, T/T		NA	1.00	Referent group
	4/X, A/T		NA	1.28	0.62–2.63
	4/X, A/A		NA	1.70	0.74–3.94
	4/4, A/T		NA	0.16	0.02–1.61
	4/4, A/A		NA	0.91	0.04–18.79
Likelihood		312.61, 6 *df*		319.82, 10 *df*	

Note. The two models are not significantly different from each other ($\chi^2 = 7.21$, 4 *df*, $P = 0.12$). NA, not applicable; X, APOE-2 or APOE-3 allele.

1 presents the allele frequencies of both APOE and AACT. As expected, the APOE-4 allele is over-represented in the AD patients vs. the controls (0.43 vs. 0.16, $P > 0.0001$), while the control frequency is not significantly different from those reported elsewhere. For AACT, there is no significant difference in the frequency of the T allele between the affecteds and the controls (0.48 vs. 0.49, $P = 0.54$), and the control frequency is not different from those reported elsewhere (Kamboh *et al.*, 1995). Table 2 presents the joint APOE and AACT genotypes. The distributions of the APOE and AACT genotypes are in Hardy–Weinberg equilibrium for both the controls and the affected populations.

To examine for potential interactions between APOE and AACT alleles, we performed logistic regression to calculate odds ratios for the various genotype combinations. We first calculated a main model examining the independent effects of the APOE-4 allele and the AACT-A allele. The dose-dependent effect of the APOE-4 allele was confirmed, while no effect of the AACT-A allele was seen (Table 3). A second logistic regression was performed to examine specifically for interactive effects. No significant additional effects were seen (Table 3), and the two models were not significantly different from each other ($\chi^2 = 7.21$, 4*df*, $P = 0.12$).

We also examined the potential effect of AACT in 67 families segregating AD. Lod scores were calculated assuming an autosomal dominant mode of inheritance. Two different models were tested. The first used the full information from both affecteds and their unaffected relatives. This model excluded linkage to 11 cM around the AACT gene, with no positive lod scores at any recombination fraction. The second used phenotypic information from only the affected individuals (an affecteds-only analysis). This analysis excluded linkage to 9 cM around the AACT gene, with no positive lod scores. We also examined for linkage and/or association by using the APM (Weeks and Lange 1988) method of analysis and no evidence of linkage or association was found ($P > 0.90$). Although the numbers are small, there was no evidence of any interaction between APOE-4 and the AACT-A allele in the families.

DISCUSSION

Our dataset combines and expands upon two previously published datasets (Saunders *et al.*, 1993; Locke *et al.*, 1995) and thus refines the estimated odds ratios for the dose-dependent effect of APOE-4. Given that the risk related to the APOE-4 allele is neither specific nor sensitive, it is reasonable to look for other factors, both environmental and genetic, that may modify this APOE-4-related risk. The AACT-A allele has recently been proposed as such a modifying factor (Kamboh *et al.*, 1995). In the initial study (Kamboh *et al.*, 1995), no independent effect of the AACT polymorphism was seen, while the effect of the APOE-4 allele was strong. In a joint analysis the odds ratios for having AD were doubled in heterozygous APOE-4 individuals who also had the AACT-AA genotype, and tripled in the double homozygotes APOE-44, AACT-AA. Our data do not support this finding. The AACT-A allele has no effect either independently or interactively. This was true even using various subsets of control samples (data not shown). We also designed our study with internal replication. Approximately 1/2 of the overall sporadic patient data were generated on a dataset independently collected and analyzed at each site (MGH and Duke). The results of each subset were virtually identical.

The difference between the initial (Kamboh *et al.*, 1995) study and our study could arise for several rea-

sons. One possibility is that the study (Kamboh et al., 1995) was a chance positive result. Another possibility is some difference in either the patient or the control populations being sampled. Apparent differences can arise if patients and control populations are not representative of the same population.

In diseases of complex genetic etiology such as AD, identifying underlying susceptibility genes is a difficult process. Thus replication is a key element in determining which genes have substantial and important effects. Our internal, independent replication design helps to address this problem, and we would advocate its use in dissecting complex genetic etiologies.

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