No Association between α1-Antichymotrypsin and Familial Alzheimer's Disease[a]

JONATHAN L. HAINES,[b,c]
MEREDYTH L. PRITCHARD,[e] ANN M. SAUNDERS,[e]
JOELLEN M. SCHILDKRAUT,[f] JOHN H. GROWDON,[d]
PETER C. GASKELL,[e] LINDSAY A. FARRER,[g]
SANFORD A. AUERBACH,[g] JAMES F. GUSELLA,[c]
PATRICIA A. LOCKE,[c] BARBARA L. ROSI,[e]
LARRY YAMAOKA,[e] GARY W. SMALL,[h]
P. MICHAEL CONNEALLY,[i] ALLEN D. ROSES,[e] AND
MARGARET PERICAK-VANCE[e]

[c]*Molecular Neurogenetics Unit*
Massachusetts General Hospital
Charlestown, Massachusetts 02129
[d]*Department of Neurology*
Massachusetts General Hospital
Boston, Massachusetts 02114
[e]*Division of Neurology*
[f]*Duke Comprehensive Cancer Center*
Duke University Medical Center
Durham, North Carolina 27710
[g]*Department of Neurology*
Boston University Medical Center
Boston, Massachusetts 02118
[h]*Department of Psychiatry and Biobehavioral Sciences*
UCLA School of Medicine
Los Angeles, California 90024
[i]*Department of Medical and Molecular Genetics*
Indiana University Medical Center
Indianapolis, Indiana 46202

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[b]Address for correspondence: Dr. Jonathan L. Haines, Molecular Neurogenetics Unit, Massachusetts General Hospital, Building 149, 6th floor, 13th St., Charlestown, MA 02129; Fax (617) 726-5736.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly, affecting over 4 million individuals in the United States.[1] As the population continues to age, it will become an increasing health burden to the U.S. population. Onset after age 65 is the most common, although 10 to 20% of cases occur before the age of 65 and can occur as early as the third decade. Numerous studies have shown that AD has a strong genetic component, but one that cannot be explained by invoking the effects of a single gene.[2] Molecular studies have demonstrated that mutations in the APP gene (on chromosome 21),[3] in the presenilin I gene (on chromosome 14),[4] and in the presenilin II gene (on chromosome 1)[5,6] can cause AD in some cases. These three genes all cause early-onset AD (onset before the age of 65) and demonstrate an autosomal dominant mode of inheritance. Although community-based epidemiological studies have not yet been performed, these three genes likely explain most of early-onset familial AD, less than half of all early-onset AD, and less than 15% of all AD (both early and late-onset).

In 1991, linkage and/or association was observed between families with late-onset AD and markers on chromosome 19q.[7] This was confirmed by the finding of a strong association between the APOE ϵ4 allele and both familial[8,9] and sporadic late-onset AD.[10] APOE ϵ4 acts in a dose-dependent fashion, with risk increasing (and age at onset decreasing) with the number of APOE ϵ4 alleles.[9] This has been confirmed in many different populations.[11] In addition, the APOE ϵ2 allele appears to be protective, at least in some populations.[12] Recently, we have shown that APOE explains approximately half of the genetic effect in late-onset AD,[13] suggesting that other genes are involved in the etiology of late-onset AD. This suggestion is further supported by the fact that the APOE ϵ4 effect is neither specific nor sensitive. That is, there are many individuals with the APOE ϵ4 allele who do not develop AD, even in very old age, and there are many late-onset AD patients who do not carry the APOE ϵ4 allele.

That α1-antichymotrypsin (ACT) plays a role in AD has been suspected since it was first shown to exist in the amyloid plaques found in AD brains.[14,15] A signal peptide polymorphism has been described in the ACT gene that has two alleles with nearly equal frequencies in the general population.[16] Thus, ACT 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.*[16] reported an interaction between ACT and APOE. In persons carrying an APOE ϵ4 allele, they showed that the ACT TT genotype appeared as protective, while the ACT AA genotype appeared to confer a two- to threefold increased risk.

MATERIALS AND METHODS

Patient Samples

The primary focus of this study was to test the potential interaction of ACT and APOE in 67 AD families identified through the Massachusetts

General Hospital Alzheimer Diseases Research Center (MGH-ADRC), the Duke University ADRC (Duke-ADRC), the UCLA Neuropsychiatric Institute and UCLA-ADRC, and the Indiana Alzheimer Disease Cell Bank. Each family must have two or more sampled affected individuals meeting the standard clinical criteria for AD.[17] These families contain 154 genotyped affected individuals and 250 unaffected genotyped individuals.

Five hundred seventy-six additional patients with no apparent family history (isolated cases) and 535 (295 spouse) controls were identified through the MGH-ADRC, the Duke-ADRC, or the Alzheimer Disease Clinic at Boston University (BU-ADC). All patients received a clinical diagnosis of Alzheimer's disease in accordance with standardized criteria.[17] Our experience indicates that over 95% of clinically diagnosed AD patients will be confirmed upon autopsy.[7] Controls had no evidence of dementia upon initial contact.

Genotyping

Blood samples were obtained from all subjects after appropriate informed consent. DNA was obtained using standard techniques either from direct extraction or from lymphoblast cultures. APOE and ACT genotypes were determined as previously described.[16,18] All resulting gels and autorads were visually scored, and data entered into computerized database systems.

Data Analysis

Lod scores were calculated using the LINKAGE package,[19] assuming an autosomal dominant mode of inheritance for AD. An age-dependent penetrance function was used,[7,18] with penetrance varying from 0.004 at age 40 to 0.99 after age 90. Affected-Pedigree-Member (APM) analysis[20] was also performed on these families using the $f(p)=1/\mathrm{sqrt}(p)$ weighting function. This function has been suggested as the most robust function to use.[20]

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

In the isolated cases, odds ratios were calculated using standard logistic regression, controlling for age and sex, as previously described.[21]

RESULTS

Familial AD

We examined the potential effect of ACT in 67 AD families. Lod scores were calculated assuming an autosomal dominant mode of inheritance (TABLE

TABLE 1. Lod Score between ACT and AD

Model	Recombination Fraction						
	0.00	0.05	0.10	0.15	0.20	0.30	0.40
1 All individuals	−9.63	−3.87	−2.23	−1.30	−0.72	−0.16	−0.02
2 Affected persons only	−7.72	−3.25	−1.99	−1.27	−0.80	−0.28	−0.06

1). Two different models were tested. The first model used phenotype information on every individual, applying an age-at-onset penetrance function to the unaffected at-risk individuals.[7,18] The second model used the genotypic information on everyone, but phenotypic information on only the affected individuals. Model 1 excluded linkage for 11 cM around the ACT locus, whereas model 2 excluded linkage for 9 cM around the ACT locus.

We also examined for linkage and/or association by using the APM method.[20] The scores for the three given allele frequency weighting functions are −2.00 (p = 0.98), −2.01 (p = 0.98), and −2.02 (p = 0.98) for the f(p) = 1, F(p) = 1/sqrt(p), and f(p) = 1/p, respectively.

To see if there was any association between the ACT A or T allele and AD in the families, we examined the allele frequencies by APOE genotype (TABLE 2). The genotypes are in Hardy-Weinberg equilibrium, and there are no significant differences in allele frequencies, either in the entire sample or broken down by APOE ϵ4 status.

TABLE 2. Distribution of ACT in APOE ϵ4 Carrier and Non-APOE ϵ4 Carrier Families

ACT Genotypes	Non-APOE ϵ4				APOE ϵ4			
	AD		Control		AD		Control	
	n	%	*n*	%	*n*	%	*n*	%
AA	4	0.22	24	0.37	45	0.36	17	0.32
AT	8	0.44	26	0.41	69	0.44	30	0.42
TT	6	0.33	14	0.22	31	0.19	14	0.26
Total	**18**		**64**		**72**		**38**	
ACT Alleles								
A	8	0.44	37	0.58	42	0.58	20	0.53
T	10	0.56	27	0.42	30	0.42	18	0.47

TABLE 3. Distribution of ACT in APOE ϵ4 Carrier and Non-APOE ϵ4 Carrier Isolated Cases

ACT Genotypes	Non-APOE ϵ4				APOE ϵ4			
	AD		Control		AD		Control	
	n	%	*n*	%	*n*	%	*n*	%
AA	43	0.24	95	0.25	114	0.29	35	0.23
AT	94	0.53	207	0.54	196	0.49	80	0.53
TT	42	0.23	81	0.21	87	0.22	37	0.24
Total	**179**		**383**		**397**		**152**	
ACT Alleles								
A	180	0.50	397	0.52	424	0.53	150	0.49
T	178	0.50	369	0.48	370	0.47	154	0.51

Sporadic Cases

To see if perhaps the effect of ACT was only in isolated cases, we also tested a set of 576 isolated cases and 535 controls.[21] TABLE 3 presents the allele frequencies in these cases. As with the familial patients, no effect of APOE is seen.

DISCUSSION

The ACT A allele has been proposed as a modifying factor for the APOE effect.[16] This study focused on isolated cases, with no indication of familial background. Thus we undertook our study to determine if any effect of ACT, whether alone or interacting with APOE, could be seen in our large sample of families. In our familial sample, no independent effect of the ACT polymorphism was seen, whereas the effect of the APOE allele was, as expected, strong. We could find no evidence of linkage via lod scores, or linkage/association via APM analysis. In addition, there were no significant differences in allele frequencies between familial affected persons and controls. We also looked for an effect of ACT in a large sample of sporadic patients, which is described in more detail elsewhere.[21] No effect was seen in that sample either, looking at ACT alone, or interactively with APOE.

How can we explain the difference between our study and the Kamboh *et al.* study? One obvious possibility is that there is a difference between familial and sporadic AD in the effect. However, since we have not observed any effect in our isolated cases, this may not be the best explanation. Another possibility is that the positive result was a chance false-positive result. Such findings are inherent in the use of statistical analysis and point out the need

for replication in additional sample populations. A final possibility is some difference in the populations being studied. This could arise either in the affected or normal populations being studied. Both studies used standard criteria for AD diagnosis, and both populations were drawn from a heterogeneous U.S. Caucasian population. Thus if this possibility is true, it may be more likely that the control populations differ in some unknown way.

SUMMARY

Alzheimer's disease (AD) is the most common mid to late age-of-onset neurodegenerative disorder. AD has a strong and complex genetic etiology, and multiple genes, acting independently and/or interacting, likely affect the risk of developing AD. Several genes involved with AD already have been described, but only the APOE gene on chromosome 19q has been shown to affect the risk of the most common form of AD, occurring with onset over the age of 65. Because a substantial portion of late-onset AD is not explained by APOE, other genes affecting late-onset AD likely occur. These could act either independently or perhaps interact with APOE. α1-Antichymotrypsin (ACT) is a major component of the amyloid plaques found in the brains of AD patients and may play a role in the pathophysiology of AD. It has been proposed that a specific polymorphism within the ACT gene interacts with APOE to increase the risk of developing AD. Our results do not confirm this finding.

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