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No association between the alpha 1-antichymotrypsin A allele and Alzheimer's disease

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Article abstract—The alpha 1-antichymotrypsin (ACT) A allele was recently associated with Alzheimer's disease (AD), and the ACT AA genotype was reported to be more frequent in AD subjects with the apolipoprotein E (APOE) ε4 allele. We examined ACT and APOE genotypes in a sample of 160 subjects with probable AD and in 102 elderly control subjects. ACT A allele frequencies were similar in AD subjects (0.503) and elderly controls (0.519). In addition, we found no evidence that in AD the AA genotype is more frequent in subjects with the APOE ε4 allele than in those without it. Our results do not support an association between the ACT A allele and AD.

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Kamboh et al.[1] recently reported that the alpha 1-antichymotrypsin (ACT) A allele is overrepresented in subjects with Alzheimer's disease (AD). Among AD subjects, 35.1% were homozygous for the A allele, whereas among controls only 27.0% had the AA genotype. The same study found that among AD subjects with the apolipoprotein E (APOE) ε4 allele, a demonstrated risk factor for AD,[2] 37.1% had the ACT AA genotype, whereas among AD subjects without the ε4 allele, only 31.7% had the AA genotype. However, a subsequent study did not confirm these findings, reporting no significant differences between AD cases and controls in the distribution of ACT genotypes and no association between the ACT A allele and the APOE ε4 allele.[3]

The ACT gene is located on the long arm of chromosome 14[4] but is physically and genetically distinct from the presenilin-1 gene, also on chromosome 14.[5-7] Mutations in the presenilin-1 gene are deterministic for early-onset familial AD,[7] as are rare mutations in the β-amyloid precursor protein gene, located on chromosome 21.[8] The APOE gene is located on the long arm of chromosome 19, a region linked to late-onset familial AD,[9] and the APOE ε4 allele is associated with both familial and sporadic AD.[10]

The ACT A allele differs from the alternative form of the gene, called the T allele, in that it codes for an alanine rather than a threonine in the signal peptide region of the molecule.[11] Polymorphisms in the ACT gene that could alter protein function are of considerable interest because ACT accumulates with the β-amyloid peptide (βAP) in diffuse and neuritic plaques in AD[12,13] and in the diffuse βAP deposits in young persons with Down's syndrome.[14] Further,

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ACT promotes assembly of βAP into filaments,[15] which could facilitate βAP deposition in AD.

In the present study, we examined the purported association between the ACT A allele and AD in a sample of 160 subjects with antemortem diagnoses of probable AD and in 102 elderly control subjects, all of whom had been screened for cognitive impairment, as well as in 40 additional younger controls. We also compared the relationship between the APOE ε4 allele and the ACT A allele in our sample of AD and control subjects.

Methods. AD subjects were recruited through the Stanford/Veterans Affairs NIMH Center for the Study of Senile Dementia in Palo Alto, CA (Stanford/VA) (n = 126) and from the University of California, Davis, Alzheimer's Disease Center in Sacramento, CA, and Berkeley, CA (UCD) (n = 34). All AD subjects underwent complete medical, psychiatric, neurologic, and neuropsychologic evaluations and met NINCDS-ADRDA criteria[16] for probable AD at entry into the study cohort. Evaluations of AD subjects at the Stanford/VA and the UCD sites are standardized as part of an Alzheimer's Disease Program sponsored by the State of California. Of the 160 AD subjects, 70 were female and 90 were male. Mean age at onset was 65.8 years (SD = 8.0; range, 42–86). Mean age at time of this study (or age at death for deceased subjects) was 74.4 years (SD = 8.1; range, 47–96).

Fifty-one of the AD subjects had complete gross and microscopic neuropathologic evaluations. Of these, 48 had AD alone or in combination with Parkinson's disease, Lewy bodies, or cerebral infarcts. Two of the remaining three subjects had Lewy body dementia, and one had Pick's disease.

Controls were obtained from three sources. Forty-nine controls were elderly (55 years of age or older) caregivers of AD subjects enrolled at the Stanford/VA center. These were drawn from a group of 54 caregivers, five of whom were excluded because they scored below the 25th percentile for age on the Folstein Mini-Mental State Exam (MMSE) according to the criteria of Bleecker et al.[17] Of the remaining 49 subjects, 35 had MMSE scores of 30, 11 had scores of 29, and three had scores of 28. Fifteen controls were elderly caregivers of AD subjects at the UCD center. These subjects had complete neurologic and medical examinations and were free of significant illness. A detailed battery of neuropsychological tests with age-adjusted norms was used to exclude dementia. Finally, 78 control subjects ranging in age from 22 to 87 years of age were recruited from Palo Alto, CA, and surrounding communities through newspaper advertisements and word of mouth. These subjects underwent complete medical, neurologic, psychiatric, and neuroimaging screening to exclude any neurologic or psychiatric illness, as well as cognitive screening using the MMSE. We divided this group of controls into those 55 years of age or older (n = 38) and those younger than 55 years of age (n = 40). We grouped all the caregiver controls and the community volunteers over age 55 and designated them as elderly controls (n = 102; 33 males and 69 females; mean age, 69.3 years; SD = 7.6; range, 56–87 years). We also considered the entire mixed-age control group in our analysis (n = 142; 57 males and 85 females; mean age, 60.0 years; SD = 17.1; range, 20–87 years). The majority of control and AD subjects were white. All AD and control subjects or their designated representatives provided written informed consent.

Table 1 ACT genotypes and allele frequencies

	Elderly controls (n = 102)	Full control sample (n = 142)	AD (n = 160)
ACT genotypes			
AA	30	41	41
	29.4%	28.9%	25.6%
AT	46	66	79
	45.1%	46.5%	49.4%
TT	26	35	40
	25.5%	24.6%	25.0%
ACT allele frequencies			
ACT* A	0.519	0.521	0.503
	(106)	(148)	(161)
ACT* T	0.480	0.479	0.497
	(98)	(136)	(159)

ACT = alpha 1-antichymotrypsin; AD = Alzheimer's disease.

Genomic DNA was extracted from frozen whole blood, frozen blood clots from serum separator tubes, buccal epithelial cell samples, frozen brain, or formalin-fixed paraffin-embedded histologic sections. DNA was extracted from whole blood samples or from blood clots using the method of Lahiri and Nurnberger.[18] Buccal mucosa samples were obtained using a cytology brush, and DNA was extracted using the protocol of Richards et al.[19] DNA from frozen brain samples was extracted using the TurboGen kit (Invitrogen, San Diego, CA), following the manufacturer's instructions. DNA extraction from histologic sections was performed according to the protocol of Greer et al.[20] ACT genotyping was performed according to the protocol of Kamboh et al.,[1] and APOE genotyping was performed according to the method of Hixson and Vernier.[21] To enhance signal intensity when genotyping from buccal mucosa samples and paraffin-embedded material, 0.5 μl α-^{32}P-dCTP (10 mCi/ml, 3000 Ci/mmol; New England Nuclear, Boston, MA) was added to the 50 μl polymerase chain reaction (PCR) mixture, and restriction digested PCR products were visualized using autoradiograms of 6 to 8% polyacrylamide gels. Statistical analyses were performed using χ^2 tests.

Results. Table 1 (presented in the same format as that of Kamboh et al.[1]) shows ACT genotypes and allele frequencies for AD and control subjects. ACT genotypes were in Hardy-Weinberg equilibrium for both AD and control subjects. There was no evidence of an association between the three ACT genotypes and diagnosis (AD vs. elderly control group: $\chi^2 = 0.57$; d.f. = 2; $p > 0.05$; AD vs. full control group: $\chi^2 = 0.43$; d.f. = 2; $p > 0.05$).

Table 2 gives APOE allele frequencies among the three ACT genotypes for AD and control subjects. Because of the small number of ε2 alleles, we combined ε2 and ε3 alleles and compared their distribution with that of ε4 alleles in a χ^2 analysis with ACT genotype as the second dimension.

Table 2 APOE allele frequencies among ACT genotypes

	AA	AT	TT	Total
AD subjects (n = 160)				
E*2	6/82	4/158	2/80	12/320
	0.073	0.025	0.025	0.038
E*3	43/82	82/158	52/80	177/320
	0.524	0.519	0.65	0.553
E*4	33/82	72/158	26/80	131/320
	0.402	0.456	0.325	0.409
Elderly controls (n = 102)				
	AA	AT	TT	Total
E*2	4/60	3/92	6/52	13/204
	0.067	0.033	0.115	0.064
E*3	46/60	82/92	44/52	172/204
	0.767	0.891	0.846	0.843
E*4	10/60	7/92	2/52	19/204
	0.167	0.076	0.038	0.093
Full control sample (n = 142)				
	AA	AT	TT	Total
E*2	5/82	7/132	6/70	18/284
	0.061	0.053	0.086	0.063
E*3	66/82	115/132	57/70	238/284
	0.805	0.871	0.814	0.838
E*4	11/82	10/132	7/70	28/284
	0.134	0.076	0.100	0.099

APOE = apolipoprotein E; ACT = alpha 1-antichymotrypsin; AD = Alzheimer's disease.

There was no evidence of an association between the ACT genotype and the ε4 allele among the AD subjects (χ^2 = 3.8; d.f. = 2; $p > 0.05$). In the elderly control group, there were relatively few ε4 alleles, but the number associated with the TT genotype was less than for the AA genotype, opposite to the findings in the Kamboh et al.[1] control group. However, in our combined control group of older and younger subjects, which included nine additional ε4 alleles, ε4 alleles were distributed more evenly among ACT genotypes.

Table 3 shows ACT genotypes and allele frequencies for AD subjects and controls according to ε4 carrier status. Unlike Kamboh et al.,[1] we did not find that among ε4 carriers AD subjects were more likely to have the AA genotype than were elderly control subjects (χ^2 = 5.6; d.f. = 2; $p > 0.05$). Among AD subjects there was no evidence that ACT genotype frequencies were different between ε4 carriers and non-ε4 carriers (χ^2 = 0.58; d.f. = 2; $p > 0.05$). In the elderly control ε4 carriers, there was a reduction in the number of subjects with the TT genotype, a trend not seen in the elderly control subjects with no ε4 alleles. This difference was marginally statistically significant (χ^2 = 6.7; d.f. = 2; $p < 0.04$). In the full control group of younger and older subjects, however, there was no difference between ε4 carriers and non-ε4 carriers in the distribution of ACT genotypes (χ^2 = 2.4; d.f. = 2; $p > 0.05$). Examination of APOE allele frequencies and ACT genotypes by AD and control subgroups revealed no significant effects (data not shown).

Discussion. These results do not support the hypothesis that the ACT A allele is associated with AD. Likewise, we found no evidence of an association between the ACT A allele and the APOE ε4 allele in AD subjects. These findings are contrary to those of Kamboh et al.[1] but support those of Haines et al.[3] We detected a marginally significant reduction in the number of subjects with the TT genotype among elderly control subjects with the ε4 allele. However, the elderly control group contained relatively few ε4 alleles, and we found no such effect in the full control group, which contained more ε4 alleles. Because of the small number of elderly controls in our sample who carried the APOE ε4 allele, it is difficult to interpret the meaning, if any, of the relatively low number of TT genotypes in this subgroup.

Several features of our study should be noted in comparison with others. First, all the subjects had antemortem diagnoses of NINCDS-ADRDA probable AD. This group is thus representative of living patients typically seen by clinicians in AD long-term care facilities and clinics. On the other hand, because neuropathologic confirmation was not required for inclusion in our sample, some subjects had additional or alternate neuropathologic changes besides

*Table 3 ACT genotypes among non-APOE*4 carriers and APOE*4 carriers*

	Non-APOE*4 carriers			APOE*4 carriers		
	AD cases (n = 60) n(%)	Elderly controls (n = 83) n(%)	Full control sample (n = 114) n(%)	AD cases (n = 100) n(%)	Elderly controls (n = 19) n(%)	Full control sample (n = 28) n(%)
ACT genotypes						
AA	15 (25.0)	20 (24.1)	30 (26.3)	26 (26.0)	10 (52.6)	11 (39.3)
AT	28 (46.7)	39 (47.0)	56 (49.1)	51 (51.0)	7 (36.8)	10 (35.7)
TT	17 (28.3)	24 (28.9)	28 (24.6)	23 (23.0)	2 (10.5)	7 (25.0)
ACT allele frequencies						
ACT*A	0.483	0.476	0.509	0.515	0.711	0.571
ACT*T	0.517	0.524	0.491	0.485	0.289	0.429

ACT = alpha 1-antichymotrypsin; APOE = apolipoprotein E; AD = Alzheimer's disease.

AD. Among our 51 autopsied cases, the three with insufficient neuropathologic changes for a diagnosis of AD (two with Lewy body dementia and one with Pick's disease) all had AT genotypes; thus, exclusion of these subjects did not affect ACT allele frequencies in the AD sample.

We recruited control subjects from geographic and socioeconomic populations similar to those of our AD subjects. However, they were heterogeneous in that they contained spouses as well as volunteers recruited through newspaper advertisements. Further, there were more females than males in the control group, whereas the opposite was true for the AD subjects. However, in our sample, there was no association between gender and ACT genotype among the AD subjects or among the controls (data not shown).

We screened all controls for cognitive impairment, and a large proportion underwent a rigorous search for signs and symptoms of medical, neurologic, and psychiatric disease. This may have contributed to the relatively low frequency of the APOE ε4 allele in our elderly control sample, because the ε4 allele is associated with mild cognitive impairment and memory complaints in nondemented subjects.[22] The Kamboh et al.[1] control sample included some subjects who had been screened for cognitive impairment, but 82% of them are only described as "healthy," which may not have included screening for dementia. Another difference between our study and that of Kamboh et al.[1] is that their full control sample had a younger mean age (52.8 years) than did ours (60.0 years). The younger control group in Kamboh et al.'s[1] study is likely to have contained individuals destined to develop AD, but who had not reached the age at which the disease manifests. Thus, our control group would be expected to contain fewer undetected or incipient AD cases than that of Kamboh et al.,[1] which would hypothetically provide greater power in detecting a difference from AD subjects in ACT allele frequencies. Yet we found no such difference in our data.

In summary, we found no statistically significant evidence for an association between AD and the ACT A allele, nor did we find that in AD subjects the ACT A allele was associated with the APOE ε4 allele. There are many possible reasons why our results differ from those of Kamboh et al.,[1] but differences in sampling techniques and differences in allele frequencies among populations sampled are likely to be important. Additional studies using other samples will resolve this issue.

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