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No association of α_1-antichymotrypsin flanking region polymorphism and Alzheimer disease risk in early- and late-onset Alzheimer disease patients

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Abstract

The α_1-antichymotrypsin (AACT)-155 allele was found elsewhere to have a significant effect on Alzheimer disease (AD) risk in individuals with at least one APOE-4 allele. We compared AACT genotypes of 284 cases of sporadic AD and 172 controls. The frequency of the AACT-155 allele did not differ significantly between cases and controls, either overall or when restricted to subjects with at least one APOE-4 allele. Logistic regression controlling for age and sex failed to show an effect due to AACT either alone or acting with APOE. There was no evidence of an interaction between APOE-4 and the AACT-155 allele to reduce age at onset. Thus, our data do not support an association of AACT-155 with risk or age at onset in AD.

Keywords: α_1-Antichymotrypsin; Alzheimer disease; Candidate gene; Polymorphism; Association study

Alzheimer disease (AD) is characterized pathologically by the presence of neurofibrillary tangles and senile plaques in the brain which impede proper neuronal function. The plaques are composed primarily of β-amyloid (Aβ), with apolipoprotein E (apoE) and α_1-antichymotrypsin (AACT) present as well. Both have been shown to accelerate Aβ fibril formation in vitro [11]. The 4 allele of the APOE gene has been shown to be an important risk factor for AD in both early- and late-onset sporadic and late-onset familial AD cases [1,19]. However, it is neither necessary nor sufficient to cause the disease. In addition to APOE, three other genes have been identified which contribute only to early onset autosomal dominant AD. These are the amyloid precursor protein (APP) [6] and the presenilin I [20] and II [10,16] loci (PS1, PS2). Collectively, these loci account for approximately 50% of the total genetic effect in AD, with APOE-4 contributing greater than 45% of this effect [4,17]. Therefore, researchers are still searching for other genetic and environmental AD risk factors which may act alone or in conjunction with APOE or other genes.

Since AACT is also found in senile plaques and because it has been shown to promote Aβ fibril formation, it has been proposed as a candidate gene for AD. Genetic association studies assessing the risk of AD conferred by this gene have reported mixed results. In a study published in 1995, Kamboh et al. reported an increased risk associated with a common allele of a polymorphism found in the AACT signal peptide coding region. This finding was later replicated with an expanded data set by the same researchers [2]. A number of studies have failed to confirm these results

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[3,7,14,21]. Talbot et al. (1996) did not find an increase in risk of AD, but did find an indication that the same common allele may be associated with a decrease in age at onset in APOE-4 carriers. In 1997, Morgan et al. reported a significant increase in AD risk, after stratification by APOE genotype, associated with the AACT-155 (reported as A10) allele of a polymorphism of no known function in the 5′-flanking sequence of the AACT gene in a data set composed of early- and late-onset sporadic AD patients [13]. Given these recent reports, we re-examined our data set for evidence of an association between the newly-identified AACT polymorphism and an increased risk and/or age at onset in AD.

Two hundred and eighty-four Caucasian early- (<60 years) and late- (≥ 60 years) onset AD patients with no known family history of AD or dementia (sporadic cases) participated in this study. Family history of dementia was assessed by personal interviews of primary care giver and/or other family members. These patients were identified through Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) at Duke University. All subjects were clinically diagnosed with AD in accordance with the standardized NINDS-ADRDA criteria [12]. The mean age at onset for this group was 67.1 ± 8.5 years (ranging from 40 to 85). The mean age at examination was 72.1 ± 7.9 years (ranging from 42 to 93). Forty-three percent of cases were male.

A sample of 172 Caucasian controls was also ascertained and sampled from the spouses of AD patients. Spouses were used in an effort to have a sample which was roughly matched on age. The mean age of examination for the controls was 69.3 ± 7.9 years (ranging from 37 to 86). Forty-four percent of controls were male.

Blood samples were obtained, after appropriate informed consent, from all subjects. DNA was obtained using standard techniques, either by direct extraction or from lymphoblast cultures. APOE genotypes were determined as previously described [19]. AACT genotyping was performed using a semi-automated genotyping system [15,22]. All data were entered and maintained in the PEDIGENE® database System [8].

Table 1
AACT allele and genotype distributions

	Affecteds ($n = 250$[b])	Controls ($n = 164$[b])
AACT alleles		
155	248 (49.6)	148 (45.1)
157	16 (3.2)	9 (2.7)
159	99 (19.8)	85 (25.9)
161	67 (13.4)	39 (11.9)
171	48 (9.6)	31 (9.5)
Other[a]	22 (4.4)	16 (4.9)
AACT genotypes		
X/X	69 (27.6)	49 (29.9)
155/X	114 (45.6)	82 (50.0)
155/155	67 (26.8)	33 (20.1)

[a]Other, AACT 147, 149, 151, 153, 163, 167, 169, 173, 175, 177 alleles. [b]Two alleles per case/control.

Table 2
Affecteds versus controls for AACT-155 allele, stratified by APOE-4

	APOE-4 negative		APOE-4 positive	
AACT Genotype	Affecteds *n* (%)	Controls *n* (%)	Affecteds *n* (%)	Controls *n* (%)
155/155	22 (26.5)	19 (16.4)	44 (27.2)	13 (27.7)
155/X	39 (47.0)	59 (50.0)	73 (45.1)	24 (51.1)
X/X	22 (26.5)	39 (33.6)	45 (27.8)	10 (21.3)

Table 3
Odds ratios for and 95% confidence intervals for AACT genotypes

AACT	Overall	APOE-4 neg	APOE-4 pos
Genotype	OR (95% CI)	OR (95% CI)	OR (95% CI)
X/X	1.00 (referent)	1.00 (referent)	1.00 (referent)
155/X	0.99 (0.6, 1.6)	1.19 (0.6, 2.3)	0.68 (0.3, 1.5)
155/155	1.44 (0.8, 2.5)	2.05 (0.9, 4.6)	0.75 (0.3, 1.9)

OR, odds ratio; CI, confidence interval.

AACT and APOE allele and genotype frequencies were compared between cases and controls using the χ^2 statistic. Odds ratios were calculated to estimate risk of AD due to specified exposures, where these exposures included presence or absence of AACT-155 allele, presence or absence of APOE-4 allele, and the interaction of the two alleles. Odds ratios were also obtained using logistic regression [5] via the SAS/STAT software package [18] to estimate risk of AD, thereby being able to control for age and sex as well as testing for an interaction between APOE-4 and AACT-155. Models were fitted forcing each independent variable to be added to the model in decreasing order of effect. The most significant variables (as determined by SAS/STAT) were included first, followed by variables with smaller effect in the model of AD risk. Models were then compared using goodness-of-fit χ^2 statistics to determine the most parsimonious model.

Differences in age at onset were tested for AACT-155 positive and negative AD cases and for APOE-4 positive and negative AD cases. An interaction was also tested by comparing onset for AACT-155 positive and negative cases within a subset of APOE-4 positive AD affected patients. All differences were tested using the non-parametric Wilcoxon score statistic to control for the possibility of non-normality in the data upon stratification by genotype.

Allele and genotype frequencies were evaluated for APOE and confirmed the previously described APOE-4 association with AD in these data ($\chi^2 = 69.89$, $df = 2$, $P < 0.0001$ for the alleles; $\chi^2 = 64.12$, $df = 2$, $P < 0.0001$ for the genotypes). No difference was seen between cases and controls for the AACT allele distribution ($\chi^2 = 4.76$, $df = 5$, $P = 0.45$), nor was there a difference

Table 4

Ages at onset compared for presence or absence of AACT-155, APOE-4, and interaction of both

AACT only			APOE only			APOE-4 positive		
AACT-155	*n*	Mean AAO (SD)	APOE-4	*n*	Mean AAO (SD)	AACT-155	*n*	Mean AAO (SD)
Y	178	68.0 (8.5)	Y	178	67.6 (7.8)	Y	117	67.7 (7.9)
N	91	65.9 (8.5)	N	91	66.7 (9.9)	N	61	67.2 (7.8)

for the distribution of genotypes ($\chi^2 = 2.41$, $df = 2$, $P = 0.30$) (Table 1). Examination of the AACT-155 allele when stratified by APOE genotype showed no difference between cases and controls based on AACT genotypes ($\chi^2 = 3.3$, $df = 2$, $P = 0.19$, APOE-4 negative subjects; $\chi^2 = 0.87$, $df = 2$, $P = 0.65$, APOE-4 positive subjects) (Table 2).

Odds ratios were also calculated, estimating the risk of AD for the various AACT-155 genotypes. No significant increase in risk was found either in the data set as a whole or when subdividing based on presence of APOE-4 (Table 3). Analyses on the data set indicate 80% power to detect an odds ratio of 2.65 or higher, a smaller effect size than that reported by Morgan et al. in 1997. Therefore it is unlikely that the absence of a detectable interaction between the AACT-155 allele and APOE-4 was due to a lack of statistical power in this study.

Age at onset was compared using the Wilcoxon score statistic for AACT-155 positive cases versus AACT-155 negative cases. No difference was found between the two groups ($P = 0.12$). APOE-4 positive and negative cases were compared similarly. Again, no difference was found between the two groups ($P = 0.68$). In addition, testing for an interaction between APOE-4 and AACT-155 failed to show any differences in age at onset ($P = 0.97$, Table 4).

We were unable to find an association between the risk of AD and the presence of a 155-allele in the 456 subjects in this study. Our study consisted of 284 clinically-diagnosed sporadic cases of AD and 172 spouse controls who showed no evidence of dementia. No significant differences were found between the AACT-155 allele or genotype distributions for cases and controls ($P = 0.45$, alleles; $P = 0.30$, genotypes). Because previously-published results [2,9] indicated an interaction between the two genes, we stratified the data set based on presence of APOE-4. There were still no differences between cases and controls. Logistic regression confirmed that there was no significant effect due to AACT either alone or in conjunction with the APOE-4 allele. Estimates of the odds ratios for these data, while not significantly different from the referent group, trended toward a decrease in risk of AD with the presence of the AACT-155 allele and an APOE-4 allele (Table 3) a result opposite that previously reported.

Since one earlier report regarding the AACT signal peptide coding region polymorphism indicated a lower age at onset associated with AACT-A homozygotes [21], we also looked at the age at onset distribution for the polymorphism in the 5′ flanking region of the gene. There was no difference found in age at onset for cases with or without an AACT-155 allele ($P = 0.12$), or with or without an APOE-4 allele ($P = 0.68$). There was also no indication of earlier age at onset when looking for an interaction between the two alleles ($P = 0.97$). Based on these results and those obtained in an earlier study [7] we conclude that any effect of the variation in the AACT gene must be very small, if it exists at all.

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