No association of $\alpha_1$-antichymotrypsin flanking region polymorphism and Alzheimer disease risk in early- and late-onset Alzheimer disease patients

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Abstract

The $\alpha_1$-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. © 1998 Elsevier Science Ireland Ltd. All rights reserved

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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 $\beta$-amyloid (A$\beta$), with apolipoprotein E (apoE) and $\alpha_1$-antichymotrypsin (AACT) present as well. Both have been shown to accelerate A$\beta$ 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 A$\beta$ 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.
[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-onset (≥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 equal in age and sex to the cases. 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 equal in age and sex to the cases. Spouses were sampled from the spouses of AD patients. Spouses were used in an effort to have a sample which was roughly equal in age and sex to the cases.

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 described previously [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].

AACT and APOE allele and genotype frequencies were compared between cases and controls using the χ² 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 χ² 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 (χ² = 69.89, df = 2, P < 0.0001 for the alleles; χ² = 64.12, df = 2, P < 0.0001 for the genotypes). No difference was seen between cases and controls for the AACT allele distribution (χ² = 4.76, df = 5, P = 0.45), nor was there a difference

### Table 1

<table>
<thead>
<tr>
<th>AACT alleles</th>
<th>Affecteds (n = 250)</th>
<th>Controls (n = 164)</th>
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<tbody>
<tr>
<td>155</td>
<td>248 (49.6)</td>
<td>148 (45.1)</td>
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<tr>
<td>157</td>
<td>16 (3.2)</td>
<td>9 (2.7)</td>
</tr>
<tr>
<td>159</td>
<td>99 (19.8)</td>
<td>85 (25.9)</td>
</tr>
<tr>
<td>161</td>
<td>67 (13.4)</td>
<td>39 (11.9)</td>
</tr>
<tr>
<td>171</td>
<td>48 (9.6)</td>
<td>31 (9.5)</td>
</tr>
<tr>
<td>Other</td>
<td>22 (4.4)</td>
<td>16 (4.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AACT genotypes</th>
<th>Affecteds (n = 250)</th>
<th>Controls (n = 164)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>69 (27.6)</td>
<td>49 (29.9)</td>
</tr>
<tr>
<td>155/X</td>
<td>114 (45.6)</td>
<td>82 (50.0)</td>
</tr>
<tr>
<td>155/155</td>
<td>67 (26.8)</td>
<td>33 (20.1)</td>
</tr>
</tbody>
</table>

*Other, AACT 147, 149, 151, 153, 163, 167, 169, 173, 175, 177 alleles. †Two alleles per case/control.
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.

We wish to thank all the individuals who participated in this study, since they are the people who make Alzheimer disease research possible. We would also like to thank the personnel of the Joseph and Kathleen Bryan Alzheimer Disease Research Center (ADRC). This work was supported by the following grants: NS31153, AG05812 and funds from the Alzheimer’s Association. The authors would like to thank Ms. Valorie Roberts for her help in preparing this manuscript for publication.

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<tr>
<th>Table 4</th>
<th>Ages at onset compared for presence or absence of AACT-155, APOE-4, and interaction of both</th>
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<tbody>
<tr>
<td>AACT only n Mean AAO (SD)</td>
<td>APOE only n Mean AAO (SD)</td>
</tr>
<tr>
<td>AACT-155</td>
<td>Y 178 68.0 (8.5)</td>
</tr>
<tr>
<td>APOE only</td>
<td>N 91 65.9 (8.5)</td>
</tr>
</tbody>
</table>


