

# Alpha-1-Antichymotrypsin Gene Polymorphism and Risk for Sporadic Alzheimer's Disease in a German Population

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## Key Words

$\alpha_1$ -Antichymotrypsin · Apolipoprotein E · Alzheimer's disease · Association

## Abstract

The A allele of a common A-T polymorphism in the signal peptide of  $\alpha_1$ -antichymotrypsin gene (ACT) has been reported to contribute a two- to threefold increased risk to Alzheimer's disease (AD) patients who carry the apolipoprotein E  $\epsilon_4$  (APOE  $\epsilon_4$ ) genotype. Since the ACT expression in AD brains is enhanced in particular in areas that develop amyloid plaques, the ACT polymorphism is considered to be a good candidate gene. We have analyzed this polymorphism in 102 AD patients and 191 matched controls, all originating from Western Germany. No statistically significant differences in allele frequencies and in genotype distribution of ACT could be shown between AD patients and controls. When we analyzed the polymorphism in APOE  $\epsilon_4$  carriers, no overrepresentation in our AD group could be shown for the ACT\*AA genotype carriers.

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## Introduction

Alzheimer's disease (AD) is a genetically heterogeneous neurodegenerative disorder that is the most frequent cause of dementia in the elderly. It is well established that early-onset forms of AD often show an autosomal dominant pattern of inheritance which is mainly due to mutations in the amyloid precursor protein (APP), presenilin 1 (PS1) or presenilin 2 gene (PS2) [1]. In contrast, the more common late-onset and sporadic forms of AD exhibit a more complex pattern of inheritance. In the last 5 years apolipoprotein E  $\epsilon_4$  (APOE  $\epsilon_4$ ) has been well established as a major risk factor [1]. Most recently it has been suggested that  $\alpha_2$ -macroglobulin is associated with late-onset AD [2]. In addition, there may be other genes acting independently or interactively in the pathogenesis of the disease. One candidate gene that might play a role in the development of the disease is  $\alpha_1$ -antichymotrypsin (ACT). In the filamentous deposits found in brains of AD patients ACT binds to  $\beta$ -amyloid peptide with high affinity. Thus it is thought that ACT serves as a strong activating factor in the polymerization of  $\beta$ -amyloid peptide into amyloid filaments [3, 4].

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**Table 1.** Comparison of ACT genotype and allele frequency in AD patients and controls

Genotype <sup>1</sup>	AD patients (n = 102)	Controls (n = 191)
ACT*AT	52 (51%)	115 (60.2%)
ACT*AA	30 (29.4%)	46 (24.1%)
ACT*TT	20 (19.6%)	30 (15.7%)
Allele frequency		
ACT*A	0.549	0.542
ACT*T	0.451	0.458

<sup>1</sup>  $\chi^2 = 2.3$ ; d.f. = 2;  $p = 0.32$ .

**Table 2.** ACT genotypes dependent on APOE  $\epsilon 4$  genotypes in AD patients and controls

Genotype	AD patients		Controls	
	n	%	n	%
APO $\epsilon 4$ noncarrier <sup>1</sup>				
ACT*AT	24	60.0	84	59.2
ACT*AA	13	32.5	36	25.4
ACT*TT	3	7.5	22	15.4
APOE $\epsilon 4$ carrier <sup>2</sup>				
ACT*AT	28	45.2	31	63.3
ACT*AA	17	27.4	10	20.4
ACT*TT	17	27.4	8	16.3

APOE  $\epsilon 4$  noncarriers: 40 AD patients, 142 controls. APOE  $\epsilon 4$  carriers: 62 AD patients, 49 controls.

<sup>1</sup>  $\chi^2 = 2.1$ ; d.f. = 2;  $p = 0.36$ .

<sup>2</sup>  $\chi^2 = 3.7$ ; d.f. = 2;  $p = 0.16$ .

Kamboh et al. [5] were the first to report an association between a common polymorphism in the signal peptide of ACT and APOE  $\epsilon 4$  carriers with AD. On the background of controversial results on association of ACT concerning the German population, it was our intention to analyze this polymorphism together with the APOE polymorphism in a German sample of 102 sporadic, late-onset AD patients and 191 controls. Patients and controls were recruited from the general population. No association between ACT\*A allele neither in APOE  $\epsilon 4$  carriers nor in our total AD group was detected.

## Material and Methods

### Patients

AD patients were recruited from the outpatient memory disorder clinic of the Psychiatric Department of the University of Bonn. Diagnosis of probable AD was performed by standard clinical evaluation according to NINCDS-ADRDA criteria. Medical and family history, general medical and neurological examination, psychiatric interview, neurological testing, blood and cerebrospinal fluid studies as well as CT scans were carried out to exclude other forms of dementia. Altogether 102 AD patients were included in this study. The mean age was 74.4 years (range 51–101 years), 68 of them were female and 34 male patients. Patients with familial (autosomal-dominant) AD were not included.

### Controls

Supported by the local Census Bureau and the regional Board of Data Protection (Nordrhein-Westfalen, Germany) a random sample of subjects over 50 years was selected from the general population. The control group comprised 191 nondemented subjects. They were evaluated based upon a psychiatric interview and neurological testing. Mean age was 70.6 years (range 50–100 years), 99 were female and 92 male subjects. Informed consent was obtained for all patients and controls.

### Genotyping

DNA was extracted from whole blood using Quiagen Blood DNA Kit (Quiagen, Hilden, Germany). The APOE polymorphism was analyzed by a PCR-based method as described by Hixon and Vernier [6]. The ACT polymorphism also was analyzed by a PCR-based method as described by Kamboh et al. [5].

### Statistical Analysis

$\chi^2$  distribution and Fisher's exact test were used for analysis of the data. Power calculations were carried out using arcsine transformations of percentages as proposed by Lipsey [7].

## Results

Altogether 102 AD patients and 191 matched controls were genotyped for the ACT polymorphism and the APOE polymorphism. Hardy-Weinberg equilibrium could be demonstrated for both polymorphisms in patients and controls. ACT was first analyzed neglecting the APOE genotype. Kamboh et al. [5] reported an increased risk for carriers of the ACT\*AA genotype. In our analysis the ACT\*AA genotype was detected in 30 patients (29.4%) and in 46 controls (24.1%); 20 AD patients (19.6%) and 30 controls (15.7%) carried the ACT\*TT genotype. This difference was statistically not significant ( $\chi^2 = 2.3$ ; d.f. = 2,  $p = 0.32$ , table 1). Breakdown of the samples according to age and sex had no influence on this distribution (data not shown).

Since an increased risk was reported for ACT\*AA carriers that in addition carry the APOE  $\epsilon 4$  genotype we

grouped the AD patients and controls in individuals carrying the APOE  $\epsilon 4$  genotype or not. Thirteen patients (32.5%) and 36 controls (25.4%) without APOE  $\epsilon 4$  allele were homozygous for ACT\*A, 3 patients (7.5%) and 22 controls (15.4%) were homozygous for ACT\*T. Seventeen patients (27.4%) and 10 controls (20.4%) among the carriers of the APOE  $\epsilon 4$  allele had an ACT\*AA genotype while 17 AD patients (27.4%) and 8 controls (16.3%) showed an ACT\*TT genotype. These data did not reach any statistical significance when Fisher's exact test was applied. For the APOE  $\epsilon 4$  noncarriers a p value of 0.36 and for the APOE  $\epsilon 4$  carriers, a p value of 0.16 was obtained (table 2).

Power calculations revealed a statistical power of 72% to detect an effect of the size originally reported by Kamboh et al. [5].

## Discussion

In the present study we could not observe a significant influence of the ACT genotype on the risk of AD. After the first report on a modification of an APOE  $\epsilon 4$ -associated risk for AD by ACT [5] a number of contradictory results have been published. Yoshiiwa et al. [8] replicated the original finding in a Japanese population, Thome et al. [9] in a German population, Ezquerra et al. [10], working with a Spanish population, could only replicate the finding in non-APOE  $\epsilon 4$  carriers. Nacmias et al. [11] obtained some hint that ACT may interact with APOE in

their late-onset familial AD cases. In 1997, Kamboh et al. [12] also reported a gender-specific effect. However, most groups could not replicate the finding even if they break down their samples according to sex and age [13–23].

Power calculations showed that the statistical power of our sample to detect the originally reported differences in the distribution of the ACT polymorphism between AD patients and healthy subjects stratified by the presence or absence of the APOE  $\epsilon 4$  allele was 72%. In context with the other negative results reported in the literature [13–23] we conclude that the ACT genotype might have no or only a minor effect on AD risk in our population.

Moreover, results of association studies using a case-control design are explicitly sensitive to stratification. In AD it is almost impossible to perform family-based association studies. In order to circumvent stratification problems, we used a sample that was carefully matched for age, gender and ethnicity.

When we assume genetic heterogeneity between different populations, we cannot exclude that ACT may have an effect on AD risk in some populations. However, regarding all the contradictory results, this effect, if present, might be a very minor one.

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