American Journal of Medical Genetics 91:355–358 (2000)

No Association Between Alpha-1-Antichymotrypsin Polymorphism and Alzheimer's Disease in Koreans

K.W. Kim,[1] J.H. Jhoo,[2] K.U. Lee,[1] D.Y. Lee,[2] J.H. Lee,[3] J.Y. Youn,[2] B.J. Lee,[4] S.H. Han,[5] and J.I. Woo[2]*

[1]*Neuroscience Research Institute of Medical Research Center and Clinical Research Institute, Seoul National University Hospital, Seoul, Korea*

[2]*Department of Neuropsychiatry, Seoul National University, College of Medicine and Seoul National University Hospital, Seoul, Korea*

[3]*Research Institute of Aging and Physical Culture of Medical Research Center, Seoul National University, College of Medicine, Seoul, Korea*

[4]*Institute of Molecular Biology and Genetics, Seoul National University, Seoul, Korea*

[5]*Department of Neurology, Chungbuk National University, College of Medicine, Korea*

To examine the possible involvement of the alpha-1-antichymotrypsin gene (*ACT*) polymorphism in the manifestation of Alzheimer's disease (AD), we analyzed genotypes of the *ACT* and apolipoprotein E gene (*APOE*) among 110 Korean patients with probable AD and 209 nondemented controls. No significant difference was obtained in genotypic (χ^2=1.98, df=2, *P*>0.1) and allelic frequencies (χ^2=1.61, df=1, *P*>0.1) of *ACT* between the AD and control groups. No overexpression of the *ACT* A/A genotype and *ACT* A allele was found when we analyzed the late-onset AD patients and the early-onset AD patients, separately. Then we stratified the *ACT* genotypes based on the presence or absence of the APOE ε4 allele to evaluate the possible interaction between them. In the APOE ε4-negative subjects, although the *ACT* A allele tended to be overexpressed in the AD group, the differences in the frequencies of the *ACT* A allele (χ^2=2.79, df=1, *P*>0.1) and *ACT* A/A genotype (χ^2=0.16, df=1, *P*>0.1) were not statistically significant. No significant overrepresentations of the *ACT* A allele (χ^2=0.02, df=1, *P*>0.1) and *ACT* A/A genotype (χ^2=0.17, df=1, *P*>0.1) were found in the APOE ε4-positive subjects, either. In addition, the status of the *ACT* genotype did not influence the age-at-onset of AD (F=0.03, df=2, *P*>0.1). Therefore, the *ACT* polymorphism does not contribute to the development of AD independently or interactively with the APOE ε4 allele in Koreans. Am. J. Med. Genet. 91:355–358, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: Alzheimer's disease; alpha-1-antichymotrypsin; apolipoprotein E; allele frequency; polymorphism; risk factor; age-at-onset

INTRODUCTION

Although the apolipoprotein E gene (*APOE*) ε4 allele is a well-established genetic risk factor for Alzheimer's disease (AD), it is neither necessary nor sufficient to cause AD [Farrer et al., 1997], and thus additional genetic or nongenetic factors might be involved in the manifestation of AD. Alpha-1-antichymotrypsin (ACT) needs to be tested as such an additional genetic risk factor for AD, since it has been reported not only to play an important role in the pathogenesis of AD, but also to bind to the position 1-12 of amyloid beta protein, which is adjacent to the APOE binding position 12-28 [Abraham et al., 1988; Ma et al., 1994].

Kamboh et al. [1995] reported that the A/A genotype of the ACT gene (*ACT*) increased the APOE ε4-conferred risk for AD twofold in heterozygotes for the APOE ε4 allele and threefold in homozygotes for the allele beyond the risk conferred to the APOE ε4 allele alone, suggesting that the *ACT* polymorphism might be a probable additional genetic risk factor for AD. However, in a series of successive studies, an association between the *ACT* polymorphism and AD has not been consistently replicated [Didierjean et al., 1997; Haines et al., 1996; Helisalmi et al., 1997; Itabashi et al., 1998; Muller et al., 1996; Nacmias et al., 1996; Yamanaka et al., 1998; Yoshizawa et al., 1997]. The allele frequencies of the *ACT* polymorphism differed among ethnic

Contract grant sponsor: Biotech 2000, the Ministry of Science and Technology of Korea; Contract grant numbers: 97-N1-02-03-A-12 and 98-N1-02-03-A-12

*Correspondence to: Dr. Jong Inn Woo, Department of Neuropsychiatry, Seoul National University, College of Medicine and Seoul National University Hospital, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea. E-mail: jiwoomd@plaza.snu.ac.kr

Received 19 July 1999; Accepted 6 January 2000

groups: the frequencies of the *ACT* A allele and A/A genotype were significantly higher in Nigerian Blacks and lower in the Japanese than those in the Caucasians [Kamboh et al., 1997; Yoshizawa et al., 1997].

The purpose of this study is to know whether the *ACT* polymorphism contributes to the development of AD and whether it influences on the age-at-onset of AD in Koreans.

MATERIALS AND METHODS

All the AD patients and nondemented controls used in the present study were unrelated Koreans. We administered the standardized CERAD Clinical Assessment Battery [Morris et al., 1989] and the modified Hachinski Ischemic Score (MHIS) [Hachinski et al., 1974]. Following this evaluation, a consensus committee meeting was held to diagnose each subject. Diagnosis for dementia was made according to *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria [American Psychiatric Association, 1994] and that for AD according to the NINCDS-ADRDA criteria [McKhann et al., 1984]. The subjects who were diagnosed as cognitively normal and got 4 or fewer on the MHIS were included in the normal control group. Informed consent was obtained for each subject.

Genomic DNA was extracted from peripheral blood leukocytes from the subjects. Genotyping for the *APOE* gene was done according to Wenham et al. [1991], and that for *ACT* according to Kamboh et al. [1995]. Allele and genotype frequencies of *ACT* and *APOE* were estimated by counting alleles and genotypes and calculating sample proportions. Hardy-Weinberg equilibrium was tested by the likelihood-ratio test [Lynch and Walsh, 1998]. Comparisons of the allele and genotype frequencies were made using chi-square analysis and Fisher's exact test. Logistic regression analysis was done to calculate the odds ratios (ORs) for AD. Comparison of the age-at-onset among groups with different genotypes were made using analysis of variance (ANOVA).

RESULTS

A total of 110 patients with probable AD and 209 controls were enrolled in the present study. Although the AD group was slightly older than the control group (70.0±7.8 years versus 68.2±4.6 years, $P<0.05$), the two groups did not differ by gender. The AD group was comprised of 62 patients with late-onset AD (LOAD) and 48 patients with early-onset AD (EOAD). The mean age-at-onset of the EOAD patients was 58.7±4.3 years and that of the LOAD patients 72.7±5.0 years, while the mean age of the EOAD patients was 62.8±4.4 years and that of the LOAD patients 75.7±4.4 years. The EOAD and LOAD groups did not differ by gender nor degree of impairments in cognitive functions and activities in daily life (data not shown).

The distributions of the *ACT* genotypes and alleles of the AD, EOAD, LOAD, and control groups are presented in Table I. No significant difference in *ACT* genotype frequency ($\chi^2 = 1.98$, df = 2, $P>0.1$) and its allele frequency ($\chi^2 = 1.61$, df = 1, $P>0.1$) was obtained between the AD control groups. No overexpression of the *ACT* A/A genotype or *ACT* A allele was found when we analyzed the LOAD and EOAD patients separately. Then we stratified the *ACT* genotypes based on the presence or absence of the APOE ε4 allele to evaluate the possible interaction between them (Table II). In the APOE ε4-negative subjects, although the *ACT* A allele tended to be overexpressed in the AD group, the differences in the frequencies of the *ACT* A allele ($\chi^2 = 2.79$, df = 1, $P>0.1$) and *ACT* A/A genotype ($\chi^2 = 0.16$, df = 1, $P>0.1$) were not statistically significant. No significant overrepresentation of the *ACT* A allele ($\chi^2 = 0.02$, df = 1, $P>0.1$) and *ACT* A/A genotype ($\chi^2 = 0.17$, df = 1, $P>0.1$) was found in the APOE ε4-positive subjects, either. We also performed logistic regression analysis to examine the possible interaction between the APOE ε4 allele and *ACT* genotype. The occurrence of the APOE ε4 allele and the *ACT* genotype was entered in the first step to examine their independent effects and then the interaction between the two was entered in the second step to examine specifically for interactive effects (Table III). In the first step, the effect of the APOE ε4 allele was confirmed, whereas no effect of the *ACT* genotype was seen. Also, no significant additional effect was seen in the second step ($\chi^2 = 2.80$, df = 2, $P>0.1$), i.e., the interaction between the occurrence of the APOE ε4 allele and the *ACT* genotype did not met the

TABLE I. Distribution of *ACT* Genotypes and Alleles Among Alzheimer's Disease (AD), Early-Onset AD Patients (EOAD), Late-Onset AD Patients (LOAD), and Control Groups*

	Number (%) of individuals			
	AD with			
ACT	All	EOAD	LOAD	Control
Genotype				
A/A	17 (15.5)	6 (12.5)	11 (17.7)	27 (12.9)
A/T	60 (54.5)	25 (52.1)	35 (56.5)	103 (49.3)
T/T	33 (30.0)	17 (35.4)	16 (25.8)	79 (37.8)
Total	110	48	62	209
Allele				
A	94 (42.7)	37 (38.5)	57 (46.0)	157 (37.6)
T	126 (57.3)	59 (61.5)	67 (54.0)	261 (62.4)
Total	220	96	124	418

*EOAD, AD developed before age 65; LOAD, AD developed at age 65 or later. Distribution of *ACT* genotypes in each group is in Hardy-Weinberg equilibrium.

TABLE II. Distribution of the *ACT* Genotypes and Alleles Stratified by the Occurrence of the APOE ε4 Allele

	Number (%) of individuals with			
	APOE ε4 (–)		APOE ε4 (+)	
ACT[a]	AD[b]	Control	AD[b]	Control
Genotype				
A/A	10 (14.9)	24 (13.0)	7 (16.3)	3 (12.5)
A/T	41 (61.2)	90 (48.6)	19 (44.2)	13 (54.2)
T/T	16 (23.9)	71 (38.4)	17 (39.5)	8 (33.3)
Total	67	185	43	24
Allele				
A	61 (45.5)	138 (37.3)	33 (38.4)	19 (39.6)
T	73 (54.5)	232 (62.7)	53 (61.6)	29 (60.4)
Total	134	370	86	48

[a]*ACT* genotypes in each group were in Hardy-Weinberg equilibrium.
[b]AD = Alzheimer's disease.

TABLE III. Odds Ratio (OR) With 95% Confidence Interval (CI) for Developing Alzheimer's Disease for the Occurrence of the APOE ε4 Allele and *ACT* Genotype*

	Independent model		Interactive model	
	OR	95% CI	OR	95% CI
APOE ε4 allele	1.0		1.0	
Absence				
Presence	5.08	2.85–9.06	9.43	3.47–25.63
ACT genotype	1.0		1.0	
T/T				
A/T	1.538	0.88–2.65	2.02	1.05–3.90
A/A	1.58	0.73–3.42	1.85	0.74–4.62
APOE+/ACT interaction				
Absence	T/T		1.0	
Presence	A/T		0.34	0.09–1.22
Presence	A/A		0.59	0.09–3.73
Likelihood	376.65, df = 3		373.85, df = 5	

*No significant difference ($\chi^2 = 2.80$, df = 2, $P > 0.1$).

criteria for the entry into the regression equation (W = 2.76 , df = 2, P>0.1).

The *ACT* genotype did not influence on the age-at-onset of AD (F = 0.03, df = 2, P>0.1 by ANOVA), The mean age-at-onset of the AD patients with A/A, A/T, and T/T genotypes were 65.9±9.3, 65.6±15.3, and 65.0±7.8 years, respectively. Also, the influence of the *ACT* genotype on age-at-onset did not show a significant interaction with the occurrence of the APOE ε4 allele (F = 0.43, df = 2, P>0.1) or APOE ε2 allele (F = 0.93, df = 2, P>0,1).

DISCUSSION

Kamboh et al. [1995] reported that the *ACT* polymorphism conferred a significant risk for AD as well as modified the APOE ε4-conferred risk for AD. However, in the present study, we could not find any independent association between AD and the *ACT* polymorphism, or a significant interaction between the *ACT* polymorphism and the APOE ε4 allele in the development of AD. There are several possible reasons, including the ethnicity, type of AD, or other unknown differences in the populations, for the conflicting results. A difference in the allele frequency regarding the *ACT* polymorphism was evident between Koreans and Caucasians. The frequency of the *ACT* A allele in normal Koreans was significantly lower than that in Caucasian controls (37.6% versus 48.4%, P<0.01) [Kamboh et al., 1997]. However, it is less likely that the difference of the frequency among ethnic groups is a main source of the conflicting results, since the effect of the *ACT* polymorphism was not consistent even within a single ethnic group, e.g., in the Japanese [Itabashi et al., 1998; Muramatsu et al., 1996; Yamanaka et al., 1998; Yoshiiwa et al., 1997; Yoshizawa et al., 1997]. Considering that the significant association between the *ACT* A/A genotype and AD was observed only in late-onset familial AD cases by a few studies [Nacmias et al., 1998; Yoshizawa et al., 1997], the difference in the distribution of age-at-onset may also have produced the conflicting results. In the present study, the *ACT* A allele and A/A genotype tended to be more prevalent in the LOAD group than in the EOAD group, but the difference did not reach statistical significance (46.0% versus 38.5%, P>0.1).

Talbot et al. [1996] reported that the AD patients with the *ACT* A/A genotype showed onset of AD six years earlier than those with the *ACT* A/T or T/T genotype in the APOE ε4 positive group. However, we could not find any association between the number of the *ACT* A alleles and age-at-onset of AD either independently or interactively with the APOE ε4 allele or ε2 allele. Our results are in agreement with those by Nacmias et al. [1996] and Yoshizawa et al. [1997]. This discrepancy may be attributable to the difference in the population studied, e.g., the proportion of familial cases or other unknown factors contributing to the progression of AD other than the status of the APOE polymorphism. Actually, in Talbot et al. [1996], the *ACT* A/A genotype was not overexpressed in AD patients, and an interaction between the *ACT* A/A genotype and APOE ε4 allele for the development of AD was not significant, either.

In conclusion, the *ACT* polymorphism does not significantly contribute to the development of AD independently or interactively with the APOE ε4 allele in the Korean.

REFERENCES

Abraham CR, Selkoe DJ, Potter H. 1988. Immunochemical identification of the serine protease inhibitor alpha 1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell 52:487–501.

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders, 4th ed. Washington DC: American Psychiatric Association.

Didierjean O, Martinez M, Campion D, Hannequin D, Dubois B, Martin C, Puel M, Thomas Anterion C, Pasquier F, et al. 1997. No effect of the alpha 1-antichymotrypsin A allele in Alzheimer's disease. J Neurol Neurosurg Psychiat 63:103–105.

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. J Am Med Assoc 278:1349–1356.

Hachinski VC, Lassen NA, Marshall J. 1974. Multi-infarct dementia. A cause of mental deterioration in the elderly. Lancet 2:207–210.

Haines JL, Pritchard ML, Saunders AM, Schildkraut JM, Growdon JH, Gaskell PC, Farrer LA, Auerbach SA, Gusella JF, Locke PA, et al. 1996. No genetic effect of alpha-1-antichymotrypsin in Alzheimer disease. Genomics 33:53–56.

Helisalmi S, Mannermaa A, Lehtovirta M, Ryynanen M, Riekkinen P Sr, Soininen H. 1997. No association between alpha1-1 antichymotrypsin polymorphism, apolipoprotein E and patients with late-onset Alzheimer's disease. Neurosci Lett 231:56–58.

Itabashi S, Arai H, Matsui T, Matsushita S, Muramatsu T, Higuchi S, Trojanowski JQ, Sasaki H. 1998. Absence of association of alpha1-antichymotrypsin polymorphisms with Alzheimer's disease: a report on autopsy-confirmed cases. Exp Neurol 151:237–240.

Kamboh MI, Sanghera DK, Ferrell RE, DeKosky ST. 1995. APOE*4- associated Alzheimer's disease risk is modified by alpha 1-antichymotrypsin polymorphism. Nature Genet 10:486–488.

Kamboh MI, Sanghera DK, Aston CE, Bunker CH, Hamman RF, Ferrell RE, DeKosky ST. 1997. Gender-specific nonrandom association between the alpha 1-antichymotrypsin and apolipoprotein E polymorphisms in the general population and its implication for the risk of Alzheimer's disease. Genet Epidemiol 14:169–180.

Lynch M, Walsh B. 1998. Properties of single loci. In: Lynch M, Walsh B, editors. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associates. p 51–60.

Ma J, Yee A, Brewer HB, Das S, Potter H. 1994. Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. Nature 372:92–94.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 34:939–944.

Morris JC, Hyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, Mellits ED, Clark C. 1989. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39:1159–1165.

Muramatsu T, Matsushita S, Arai H, Sasaki H, Higuchi S. 1996. Alpha 1-antichymotrypsin gene polymorphism and risk for Alzheimer's disease. J Neural Transm 103:1205–1210.

Muller U, Boeker RH, Gerundt I, Kurz A. 1996. Lack of association between alpha 1 antichymotrypsin polymorphism, Alzheimer's disease, and allele 4 of apolipoprotein E. Neurology 47:1575–1577.

Nacmias B, Tedde A, Latorraca S, Piacentini S, Bracco L, Amaducci L, Guarnieri BM, Petruzzi C, Ortenzi L, Sorbi S. 1996. Apolipoprotein E and alpha1-antichymotrypsin polymorphism in Alzheimer's disease. Ann Neurol 40:678–680.

Nacmias B, Marcon G, Tedde A, Forleo P, Latorraca S, Piacentini S, Amaducci L, Sorbi S. 1998. Implication of alpha1-antichymotrypsin polymorphism in familial Alzheimer's disease. Neurosci Lett 244:85–88.

Talbot C, Houlden H, Craddock N, Crook R, Hutton M, Lendon C, Prihar G, Morris JC, Hardy J, Goate A. 1996. Polymorphism in AACT gene may lower age of onset of Alzheimer's disease. Neuroreport 7:534–536.

Wenham PR, Price WH, Blandell G. 1991. Apolipoprotein E genotyping by one-stage PCR. Lancet 337:1158–1159.

Yamanaka H, Kamimura K, Tanahashi H, Takahashi K, Asada T, Tabira T. 1998. Genetic risk factors in Japanese Alzheimer's disease patients: alpha1-ACT, VLDLR, and ApoE. Neurobiol Aging 191(suppl):S43–S46.

Yoshiiwa A, Kamino K, Yamamoto H, Kobayashi T, Imagawa M, Nonomura Y, Yoneda H, Sakai T, Nishiwaki Y, Sato N, et al. 1997. Alpha 1-antichymotrypsin as a risk modifier for late-onset Alzheimer's disease in Japanese apolipoprotein E epsilon 4 allele carriers. Ann Neurol 42:115–117.

Yoshizawa T, Yamakawa-Kobayashi K, Hamaguchi H, Shoji S. 1997. α1-Antichymotrypsin polymorphism in Japanese cases of Alzheimer's disease. J Neurol Sci 152:136–139.