Genomics & Informatics Vol. 2(2) 81-85, June 2004

No Evidence of Association of Interleukin 1A (-889) Genetic Polymorphism with Alzheimer's Disease in Koreans

Jin Hyeong Jhoo[1], Woong Yang Park[2], Ki Woong Kim[3], Kwang Hyuk Lee[2], Dong Young Lee[7], Jong Chul Youn[5], Young Ju Suh[6], Jeong-Sun Seo[2] and Jong Inn Woo[4,7]*

[1]Department of Psychiatry, Pundang Jesaeng Hospital, Daejin Medical Center, Seongnam, Kyunggi, Korea
[2]Department of Biochemistry and Molecular Biology, College of Medicine, Seoul National University, Seoul, Korea
[3]Department of Neuropsychiatry, Seoul National University Bundang Hospital, Seongnam, Kyunggi, Korea
[4]Neuroscience Research Institute of the Medical Research Center, Seoul National University, and Clinical Research Institute of Seoul National University Hospital, Seoul, Korea
[5]Department of Neuropsychiatry, Kyunggi Provincial Hospital for Elderly, Yongin, Kyunggi, Korea
[6]Research division of Human Life Science, Seoul National University, Seoul, Korea
[7]Department of Neuropsychiatry, Seoul National University, College of Medicine and Seoul National University Hospital, Seoul, Korea

Abstract

To examine whether the IL-1A (-889) polymorphism associates with a risk for Alzheimer's disease (AD) and acts interactively with the apolipoprotein (APOE) ε4 in the development of AD, we performed genotype analyses of the IL-1A and the APOE of the 102 Korean AD patients and 200 Korean non-demented controls. We failed to detect a significant difference in genotypic and allelic frequencies of IL-1A between the AD group and control group. No overexpression of the IL-1A C/T genotype and IL-1A T allele was found when we analyzed the late-onset and early-onset patients, separately. There was no significant genetic interaction between IL-1A polymorphism and the APOE polymorphism. In conclusion, the IL-1A polymorphism did not contribute to the development of AD independently or interactively with the APOE ε4 allele in Koreans.

Key words: Alzheimer's disease (AD), Interleukin-1A (IL-1A), Koreans, association

*Corresponding author: E-mail jiwoomd@plaza.snu.ac.kr, Tel +82-2-760-2456, Fax +82-2-762-3176
Accepted 10 May 2004

Introduction

Interleukin-1 (IL-1), a potent acute phase proinflammatory cytokine, is known to play an important role in the development of Alzheimer's disease (AD). It was reported to regulate amyloid beta protein (Aβ) production and increase the phosphorylation of tau protein (Sheng *et al.*, 1996, 2000). Its level was elevated in cerebral cortex and cerebrospinal fluid of AD patients, and activated IL-1 immunoreactive microglia were also proliferated significantly in the cerebral cortex of AD patients (Griffin *et al.*, 1989; Cacabelos *et al.*, 1991).

In 2000, Grimaldi and his colleagues reported that the genetic polymorphism in the promoter region of IL-1A (-889) was significantly associated with the risk of AD. In their population, the IL-1A T allele conferred the risk of AD in dose-dependent manner. The odds ratios for AD in the IL-1A T-heterozygous subjects were 1.84 and those of IL-1A T-homozygous subjects were 6.33. The association of AD with IL-1A polymorphism was also replicated in several other populations (Du *et al.*, 2000; Nicoll *et al.*, 2000; Rebeck, 2000; Combarros *et al.*, 2002; Hedley *et al.*, 2002). Moreover, this polymorphism might be functional. The IL-1A TT genotype significantly increased the transcriptional activity of the IL-1 alpha gene with respect to the CC genotype (Dominici *et al.*, 2002).

However, there was substantial ethnic difference in the allelic frequency of the IL-1A (-889) T and IL-1A T-conferred AD risk. While the frequency of the IL-1A T was 17.8 % - 33% in Caucasians (Du *et al.*, 2000; Grimaldi *et al.*, 2000; Minster *et al.*, 2000; Nicoll *et al.*, 2000; Rebeck, 2000; Kolsch *et al.*, 2001; Combarros *et al.*, 2002; Fidani *et al.*, 2002; Green *et al.*, 2002; Hedley *et al.*, 2002; Pirskanen *et al.*, 2002), it was less than 11.3% in East Asians (Ki *et al.*, 2001; Kuo *et al.*, 2003; Tsai *et al.*, 2003) and the IL-1A T-AD association was not significant in Asians (Ki *et al.*, 2001; Kuo *et al.*, 2003; Tsai *et al.*, 2003).

Therefore we investigated the association of AD with the IL-1A (-889) polymorphism and examined its interaction with APOE polymorphism in the development of AD in Koreans.

Materials and Methods

The AD patients were selected from the patients who had visited the dementia clinic at Seoul National University Hospital and from the community-dwelling

elderly individuals in three districts of Seoul (Kwanak-gu, Seocho-gu, Nowon-gu). All the non-demented normal control subjects were community-dwelling elderly individuals randomly selected from those three districts.

We administered the Korean version of the CERAD assessment packet (Lee *et al.,* 2002) and Modified Hachinski Ischemic Score (MHIS) (Rosen *et al.,* 1980) to all the subjects. Following this evaluation, a diagnosis for each subject was made at a consensus meeting; diagnoses for dementia were made according to DSM-IV criteria (APA, 1994) and diagnoses for probable AD according to NINCDS-ADRDA criteria (McKhann *et al.,* 1984). The subjects who were diagnosed as cognitively normal and got less than 3 of MHIS were included in the normal control group. Age-at-onset was defined as the age at which the patient or his family first noticed the symptoms required for the diagnosis. The cut-off between late-onset AD and early-onset AD was 65 years of age. The control subjects were also divided into the young controls aged 64 years or less and the old controls aged 65 years or over. The subjects with cerebrovascular disorders, neurologic disorders, and psychiatric disorders including depression were excluded from the patient group as well as the control group. The Institutional Review Board of Seoul National University Hospital, Korea, approved the study protocol and informed consent was obtained from all participants or their guardians.

Genomic DNA for genotyping was extracted from peripheral venous blood. IL-1A (-889) polymorphism was determined using a dynamic allele specific hybridization method (DASH) (Howell *et al.,* 1999). The polymerase chain reaction (PCR) primers (forward, 5'-TTTTTACATAT GAGCCTTCAAT-3'; biotinylated reverse, 5'- TTAATAATAG TAACCAGGCAAC-3') and the two fluorescent labeled probes (5'-AGGCAACATCAT TGAAG-3' and 5'-AGGC A ACACCATTGAAG-3'), complementary to the both allelic sequences of the IL-1A (-889) polymorphism, were added to a 96-well microtiter plate, and PCR was then performed. After PCR, the plate was placed in a DASH instrument (Hybaid ltd.) and heated from 35°C to 85°C at a rate of 0.3°C/sec. Probe-duplex denaturation was determined by the decrease in fluorescence. The IL-1A (-889) genotypes were determined from the fluorescence curves as previously described (Howell *et al.,* 1999). APOE genotyping was performed using a slight modification of the method reported by Wenham *et al.* (1991).

The differences in both allele and genotype frequency between the AD patients and the control subjects were compared using either a Fisher's exact test or Pearson Chi square test when appropriate. Logistic regression analyses controlling for age (age at onset for AD), gender and APOE genotype were performed to calculate the odds ratio (OR) associated with the IL-1A (-889) polymorphism on the risk for AD. And the age at onset between groups was compared using Student T test. SPSS version 10.0 for Windows was used for these statistical analyses.

Results

A total of 102 sporadic AD patients and 200 controls were enrolled in this study. Among the 102 AD patients, 34 subjects were early-onset AD patients (age at onset = 58.8±3.7 years, age = 62.8±3.6 years, range = 54-71 years, 58.3% female) and 68 subjects were late-onset AD patients (age at onset = 75.3±35.9 years, age = 78.3±5.8 years, range = 67-97 years, 67.6% female). The 200 control subjects were also stratified by age into 38 young controls (age = 60.8±3.0 years, range = 53-64 years, 89.5% female) and 162 old controls (age = 72.6±5.7 years, range = 65-88 years, 72.8% female).

There were no significant differences in the allelic and genotypic frequencies of the IL-1A polymorphism between the AD patients and control subjects (Table 1). The distributions of the IL-1A (-889) and APOE genotypes were in Hardy-Weinberg equilibrium in both the AD patients and controls. The frequency of the T

Table 1. Interleukin-1A genotype and allele frequencies in the AD patients and control subjects

Group	N	Allele frequency		Genotype frequency		
		C (%)	T (%)	CC (%)	CT (%)	TT (%)
Total AD	102	193 (94.6)	11 (5.4)	91 (89.2)	11 (10.8)	0
Controls	200	370 (92.5)	30 (7.5)	170 (85)	30 (15)	0
EOAD	34	64 (94.1)	4 (5.9)	30 (88.2)	4 (11.8)	0
Controls	38	70 (92.1)	6 (7.9)	32 (84.2)	6 (15.8)	0
LOAD	68	129 (94.9)	7 (5.1)	61 (89.7)	7 (10.3)	0
Controls	162	300 (92.6)	24 (7.4)	138 (85.2)	24 (14.8)	0

Table 2. The frequencies of the interleukin-1A T allele and C/T genotype in the AD patients and control subjects stratified by the apolipoprotein (APOE) ε4 allele

Group	APOE ε4 negative			APOE ε4 positive		
	No	T (%)	C/T (%)	No	T (%)	C/T (%)
Total AD	50	5 (5.0)	5 (10.0)	52	6 (5.8)	6 (11.5)
Controls	172	25 (7.3)	25 (14.5)	28	5 (8.9)	5 (17.9)
EOAD	17	2 (5.9)	2 (11.8)	17	2 (5.9)	2 (11.8)
Controls	28	5 (8.9)	5 (17.9)	10	1 (5.0)	1 (10.0)
LOAD	33	3 (4.5)	3 (9.1)	35	4 (5.7)	4 (11.4)
Controls	144	20 (6.9)	20 (13.9)	18	4(11.1)	4 (22.2)

EOAD : Early-onset AD, LOAD: Late-onset AD

allele was 7.5% in our normal elderly controls, and the IL-1A T-homozygous subject was not observed in both the patient group and control group, which was quite consistent with the previously reported results in Koreans (Ki *et al.*, 2001).

As expected, the APOE ε4 was more prevalent in the AD patients than in the controls ($p<0.00001$). However, the IL-1A T allele was not overrepresented in the AD group compared with the control group ($P>0.1$). The IL-1A T-AD association was not significant either after stratifying the subjects by the type of AD (Table 1) or the presence of APOE ε4 allele (Table 2). The age- and gender-adjusted odds ratio for AD conferred to the IL-1A T was 0.61 (95% C.I. = 0.27- 1.37), and the interaction term between the IL-1A T and the APOE ε4 was not significant in logistic regression analysis.

The age-at-onset was not influenced by the IL-1A polymorphism. The mean age-at-onset of the AD patients with the IL-1A C/C genotype (69.9±9.4 years) was not different from that of the AD patients with IL-1A C/T genotype (69.0±9.4 years).

Discussion

In this study, we did not find a significant association between the IL-1A (-889) polymorphism and AD risk in Koreans, which is consistent with the three previous studies on Asians (Ki *et al.*, 2001; Kuo *et al.*, 2003; Tsai *et al.*, 2003). However, the absence of association between the IL-1A (-889) polymorphism and AD in the present study is not merely attributed to the ethnic difference. Five out of the 11 previous studies on Caucasians could not find any significant association either (Minster *et al.*, 2000; Kolsch *et al.*, 2001; Fidani *et al.*, 2002; Green *et al.*, 2002; Pirskanen *et al.*, 2002). Therefore, another factors including lack of power and linkage disequilibrium of IL-1A (-889) polymorphism with other nearby responsible gene on chromosome 2 could be the sources of the conflicting results.

The frequency of the IL-1A T allele in our controls, 7.5%, was much lower than those of Caucasians which were reported to be 17.8 % - 33% (Du *et al.*, 2000; Grimaldi *et al.*, 2000; Minster *et al.*, 2000; Nicoll *et al.*, 2000; Rebeck, 2000; Kolsch *et al.*, 2001; Combarros *et al.*, 2002; Fidani *et al.*, 2002; Green *et al.*, 2002; Hedley *et al.*, 2002; Pirskanen *et al.*, 2002), but it was similar to those of East Asians (7.1%-11.3%; Ki *et al.*, 2001; Kuo *et al.*, 2003; Tsai *et al.*, 2003). Therefore, in East Asians, much larger sample size may be required for verifying the same odds ratio for AD conferred to the IL-1A T compared with Caucasians. Because of this low T allele frequency as well as the insufficient sample size, the power of the present study was 65% if we considered that the odds of the IL-1A (-889) T allele for AD were 2.

Moreover, there was no IL-1A T homozygous subject in our sample. Among the six previous positive studies (Du *et al.*, 2000; Grimaldi *et al.*, 2000; Nicoll *et al.*, 2000; Rebeck, 2000; Combarros *et al.*, 2002; Hedley *et al.*, 2002), three studies found that only T/T genotype was significantly associated with AD risk (Nicoll *et al.*, 2000; Rebeck, 2000; Hedley *et al.*, 2002). Therefore the association between AD and IL-1A T/T in Korean was not examined in the present study, and waits for further investigation in an extended sample. In other three studies that the dose-dependent AD risk conferred to IL-1A T allele was confirmed (Du *et al.*, 2000; Grimaldi *et al.*, 2000; Combarros *et al.*, 2002), the AD risks conferred to IL-1A (-889) C/T genotype were less than 2 (1.39 – 1.84). This modest contribution of IL-1A T allele may further complicate the power of present study.

Age-at-onset was also suggested to be a confounding factor to influence on the association of IL-1A (-889) polymorphism with AD. While the association of AD with IL-1A (-889) polymorphism was significant only in early-onset cases in Glimaldi *et al.* (2000) and Rebeck (2000), it was significant only in

late-onset cases in Hedley *et al.* (2002). Therefore, the limited association of IL-1A (-889) polymorphism with AD according to age at onset is still controversial. In the present study, the association of IL-1A (-889) was not found either in the early onset AD patients or in the late-onset AD patients.

Combarros *et al.* (2002) reported that in the presence of the APOE ε4 allele the relative risk of IL-1A (-889) T allele carriers was approximately double the risk of IL-1A (-889) T allele non-carriers. However, in the present study, the association of AD with the IL-1A (-889) T was not significant regardless of the presence or absence of the APOE ε4 allele. Thus the absence of the AD- IL-1A (-889) T association in our sample was not a biased result confounded by the APOE polymorphism.

In conclusion, we failed to find any evidence supporting an association between the IL-1A (-889) genetic polymorphism and AD in Koreans. We also did not find any significant association after the stratification according to age at onset or APOE genotype.

Acknowledgments

This work was supported by Brain Science and Engineering Research Program (Grant No. 99-N1-02-03-A-12) sponsored by Korean Ministry of Science and Technology and was also supported by the Center for Functional Analysis of Human Genome, FG-2-3.

References

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition. (Washington DC: American Psychiatric Association).

Cacabelos, R., Barquero, M., Garcia, P., Alvarez, X.A., and Varela de Seijas, E. (1991). Cerebrospinal fluid interleukin-1 beta (IL-1 beta) in Alzheimer's disease and neurological disorders. *Methods Find. Exp. Clin. Pharmacol.* 13, 455-458.

Combarros, O., Sanchez-Guerra, M., Infante, J., Llorca, J., and Berciano, J. (2002). Gene dose-dependent association of interleukin-1A [-889] allele 2 polymorphism with Alzheimer's disease. *J. Neurol.* 249,1242-1245.

Dominici, R., Cattaneo, M., Malferrari, G., Archi, D., Mariani, C., Grimaldi, L.M., and Biunno, I. (2002). Cloning and functional analysis of the allelic polymorphism in the transcription regulatory region of interleukin-1 alpha. *Immunogenetics* 54, 82-86.

Du, Y., Dodel, R.C., Eastwood, B.J., Bales, K.R., Gao, F., Lohmuller, F., Muller, U., Kurz, A., Zimmer, R., Evans, R.M., Hake, A., Gasser, T., Oertel, W.H., Griffin, W.S., Paul, S.M., and Farlow, M.R. (2000). Association of an interleukin 1 alpha polymorphism with Alzheimer's disease. *Neurology* 55, 480-483.

Fidani, L., Goulas, A., Mirtsou, V., Petersen, R.C., Tangalos, E., Crook, R., and Hardy, J. (2002). Interleukin-1A polymorphism is not associated with late onset Alzheimer's disease. *Neurosci. Lett.* 323, 81-83.

Green, E.K., Harris, J.M., Lemmon, H., Lambert, J.C., Chartier-Harlin, M.C., St Clair, D., Mann, D.M., Iwatsubo, T., and Lendon, C.L. (2002). Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? *Neurology* 58,1566-1568.

Griffin, W.S., Stanley, L.C., Ling, C., White, L., MacLeod, V., Perrot, L.J., White, C.L. 3rd, and Araoz, C. (1989). Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 86, 7611-7615.

Grimaldi, L.M., Casadei, V.M., Ferri, C., Veglia, F., Licastro, F., Annoni, G., Biunno, I., De Bellis, G., Sorbi, S., Mariani, C., Canal, N., Griffin, W.S., and Franceschi, M. (2000). Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Ann. Neurol.* 47, 361-365.

Hedley, R., Hallmayer, J., Groth, D.M., Brooks, W.S., Gandy, S.E., and Martins, R.N. (2002). Association of interleukin-1 polymorphisms with Alzheimer's disease in Australia. *Ann. Neurol.* 51, 795-797.

Howell, W.M., Jobs, M., Gyllensten, U., and Brookes, A.J. (1999). Dynamic allele-specific hybridization. A new method for scoring single nucleotide polymorphisms. *Nat. Biotechnol.* 17, 87–88.

Ki, C.S., Na, D.L., Kim, D.K., Kim, H.J., and Kim, J.W. (2001). Lack of association of the interleukin-1alpha gene polymorphism with Alzheimer's disease in a Korean population. *Ann. Neurol.* 49, 817-818.

Kolsch, H., Ptok, U., Bagli, M., Papassotiropoulos, A., Schmitz, S., Barkow, K., Kockler, M., Rao, M.L., Maier, W., and Heun, R. (2001). Gene polymorphisms of interleukin-1alpha influence the course of Alzheimer's disease. *Ann. Neurol.* 49, 818-819.

Kuo, Y.M., Liao, P.C., Lin, C., Wu, C.W., Huang, H.M., Lin, C.C., and Chuo, L.J. (2003). Lack of association between interleukin-1alpha polymorphism and Alzheimer disease or vascular dementia. *Alzheimer Dis. Assoc. Disord.* 17, 94-97.

Lee, J.H., Lee, K.U., Lee, D.Y., Kim, K.W., Jhoo, J.H., Kim, J.H., Lee, K.H., Kim, S.Y., Han, S.H., and Woo, J.I. (2002). Development of the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet (CERAD-K): clinical and neuropsychological assessment batteries. *J. Gerontol. B. Psychol. Sci. Soc. Sci.* 57, 47-53.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E.M. (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.

Minster, R.L., DeKosky, S.T., Ganguli, M., Belle, S., and Kamboh, M.I. (2000). Genetic association studies of interleukin-1 (IL-1A and IL-1B) and interleukin-1 receptor antagonist genes and the risk of Alzheimer's disease. *Ann. Neurol.* 48, 817-819.

Nicoll, J.A., Mrak, R.E., Graham, D.I., Stewart, J., Wilcock, G., MacGowan, S., Esiri, M.M., Murray, L.S., Dewar, D., Love, S., Moss, T., and Griffin, W.S. (2000). Association of interleukin-1 gene polymorphisms with Alzheimer's disease. *Ann. Neurol.* 47, 365-268.

Pirskanen, M., Hiltunen, M., Mannermaa, A., Iivonen, S., Helisalmi, S., Lehtovirta, M., Koivisto, A.M., Laakso, M., Soininen, H., and Alafuzoff, I. (2002). Interleukin 1 alpha gene polymorphism as a susceptibility factor in Alzheimer's disease and its influence on the extent of histopathological hallmark lesions of Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 14,123-127.

Rebeck, G.W. (2000). Confirmation of the genetic association of interleukin-1A with early onset sporadic Alzheimer's disease. *Neurosci. Lett.* 293, 75-77.

Rosen, W.G., Terry, R.D., Fuld, P.A., Katzman, R., and Peck, A. (1980). Pathological verification of ischemic score in differentiation of dementias. *Ann. Neurol.* 7, 486-488.

Sheng, J.G., Ito, K., Skinner, R.D., Mrak, R.E., Rovnaghi, C.R., Van Eldik, L.J., and Griffin, W.S. (1996). In vivo and in vitro evidence supporting a role for the inflammatory cytokine interleukin-1 as a driving force in Alzheimer pathogenesis. *Neurobiol. Aging* 7, 761-766.

Sheng, J.G., Zhu, S.G., Jones, R.A., Griffin, W.S., and Mrak, R.E. (2000). Interleukin-1 promotes expression and phosphorylation of neurofilament and tau proteins *in vivo. Exp. Neurol.* 163, 388-391.

Tsai, S.J., Liu, H.C., Liu, T.Y., Wang, K.Y., and Hong, C.J. (2003). Lack of association between the interleukin- 1alpha gene C(-889)T polymorphism and Alzheimer's disease in a Chinese population. *Neurosci. Lett.* 343, 93-96.

Wenham, P.R., Price, W.H., and Blandell, G. (1991). Apolipoprotein E genotyping by one-stage PCR. *Lancet* 337, 1158-1159.