Lack of association of cathepsin D genetic polymorphism with Alzheimer’s disease in Koreans

Jin Hyeong Jhoo a, Woong Yang Park b, Ki Woong Kim c,f, Kwang Hyuk Lee b, Dong Young Lee d,f, Jong Chul Youn e, Il Han Choo d,f, Jeong Sun Seo b, Jong Inn Woo d,f,g,*

a Department of Psychiatry, Pundang Jesaeng Hospital, Daejin Medical Center, 255-2 Seohyun, Seongnam, Kyunggi 463-774, Republic of Korea
b Department of Biochemistry and Molecular Biology, College of Medicine, Seoul National University, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Republic of Korea
c Department of Neuropsychiatry, Seoul National University Bundang Hospital, 300 Kumi, Seongnam, Kyunggi 463-707, Republic of Korea
d Department of Neuropsychiatry, Seoul National University Hospital, 28 Yongon-dong, Chongno-gu, Seoul 110-774, Republic of Korea
e Department of Neuropsychiatry, Kyunggi Provincial Hospital for Elderly, 4 Sangha, Kuseong, Yongin, Kyunggi 449-910, Republic of Korea
f Department of Psychiatry, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-774, Republic of Korea
g Neuroscience Research Institute of the Medical Research Center, Seoul National University, and Clinical Research Institute of Seoul National University Hospital, 28 Yongon-dong, Chongno-gu, Seoul 110-774, Republic of Korea

Received 4 November 2004; accepted 15 December 2004
Available online 25 February 2005

Abstract

Cathepsin D (CatD) is a good candidate susceptibility marker for Alzheimer’s disease (AD), since it was found to be involved in the processing of the amyloid precursor protein and the formation of the hyperphosphorylated tau. And recently, a CatD genetic polymorphism was found to be associated with the risk of Alzheimer’s disease (AD) in a German population. However, the CatD T–AD association has not been replicated in a series of the successive independent studies in other races. Therefore, we determined CatD genotypes to examine the possible association of the CatD polymorphism with AD in Koreans. We failed to find significant association between the CatD T
allele and AD. In addition, the CatD T–AD association was not significant regardless of the age at onset or the occurrence of the apolipoprotein ε4 allele. However, we cannot exclude the possible contribution of the CatD in the development of AD, since the power of the present study was not high enough because of low allelic frequency of the CatD T in Koreans and small sample size. In conclusion, the association between the CatD genetic polymorphism and AD was not found in Koreans, although it waits for further replication in an extended sample.

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Keywords: Alzheimer’s disease (AD); Cathepsin D (CatD); Koreans; Association

1. Introduction

Recently, Papassotiropoulos et al. (1999, 2000) reported that the CatD 224C/T polymorphism strongly conferred the risk of AD in the German populations. And it was also found to be associated with general intelligence in a healthy older population (Payton et al., 2003). Cathepsin D (CatD) is a major lysosomal/endosomal aspartic protease, which is known to be involved in the amyloid precursor protein processing (Sadik et al., 1999; Wolfe et al., 1999) and the hyperphosphorylated tau formation (Bi et al., 2000). The CatD gene has a single biallelic nucleotide polymorphism at position 224. A C to T transition at this position converts Ala to Val in its pro-fragment. This missense polymorphism was found to be associated with the increased proCatD secretion and altered intracellular maturation (Touitou et al., 1994).

However, the CatD T–AD association has not been replicated in a series of the successive independent studies in other races (Crawford et al., 2000; Bertram et al., 2001; Matsui et al., 2001; Bagnoli et al., 2002; Mateo et al., 2002; Ingegni et al., 2003; Styczynska et al., 2003), and thus, it is a matter of controversy and the influence of racial difference on the CatD–AD association needs to be investigated further.

Therefore, in Koreans, we determined CatD and APOE genotypes of the AD patients and the cognitively normal elderly controls and examined whether the CatD T confers the risk of AD independently or interactively with the APOE ε4.

2. Subjects and methods

This study was performed on 107 sporadic AD patients (age at onset, 69.6 ± 9.4 years; age, 72.9 ± 9.0 years; range, 54–97 years; 65.4% female) and 216 cognitively intact control subjects (age, 70.1 ± 7.1 years; range, 52–88 years; 78.2% female). All the AD patients and control subjects were unrelated Koreans. Among the 107 AD patients, 36 AD patients were classified as having early-onset AD (age at onset, 58.7 ± 3.9 years; age, 62.7 ± 3.7 years; range, 54–71 years; 61.1% female) and 71 patients were classified as having late-onset AD (age at onset, 75.1 ± 6.0 years; age, 78.1 ± 5.9 years; range, 67–97 years; 67.6% female) based on the age at onset of 65 years. The control subjects were also divided into the controls under 65 years (age, 72.5 ± 5.7 years; range, 65–88 years; 75.0% female). The AD patients were enrolled at the Dementia Special Clinic of
Seoul National University Hospital in Seoul, Korea. The controls were either the patients’ spouses or people selected from three districts in Seoul (Nowon-Gu, Seocho-Gu and Gwanak-Gu). The AD patients and controls were evaluated using the Korean version of the CERAD Clinical Assessment Battery, which were performed by a neuropsychiatrist (Lee et al., 2002). The age at onset for the AD patients was determined based on the information from patients’ spouses or their families obtained by a neuropsychiatrist. The diagnosis of AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria. All the AD patients met the criteria for probable AD. 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. Even when the consent was obtained from guardians, we explained this study to our patients and confirmed they had no objections to the study.

Genomic DNA for genotyping was extracted from peripheral venous blood. CatD polymorphism was determined using a dynamic allele specific hybridization method (DASH), which was found to be reliable for detecting all single nucleotide polymorphism types, including the C/T polymorphism (Howell et al., 1999). The polymerase chain reaction (PCR) primers (forward, 5′-CCCCGTCTCAAAGTACTCCCAG-3′; biotinylated reverse, 5′-GCACCTCAGTCACGACTGGCAC-3′) and the two fluorescent-labeled probes (5′-CTCCCCAGGCGGTGCCAG-3′ and 5′-CTCCCCAGGTGGTGCCAG-3′), complementary to both allelic sequences of CatD 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/s. Probe–duplex denaturation was determined by the decrease in fluorescence. The CatD genotypes were determined from the fluorescence curves as previously described (Howell et al., 1999).

Apolipoprotein (APOE) genotyping was performed using a slight modification of the method reported by Wenham et al. (1991).

There was no significant difference in the T allele and C/T genotype frequencies between the AD patients and control subjects (Table 1). In the late-onset AD patients, the frequency of the CatD T was nearly double the frequency of the CatD T in the age-matched controls. However, the difference did not reach a statistical significance (Table 1). The distributions of both the CatD and APOE genotypes in the AD patients and controls were in Hardy-Weinberg equilibrium.

There was no significant difference in the frequencies of the CatD T and CatD C/T between the AD patients and the control subjects after stratifying the subjects by age at
onset, either. The APOE ε4 carrier was significantly higher in the AD patients than in the controls \((p < 0.00001)\). In the APOE ε4-negative subjects, although the CatD T and the CatD C/T were more prevalent in the AD patients compared to the controls, the difference was not statistically significant (Table 2).

Logistic regression analyses adjusted for gender and age (age at onset for AD) showed that the odds ratio for being affected due to carrying a CatD T was 1.77 (95% C.I. = 0.70–4.46). The odds in the late-onset AD alone were 1.97 (95% C.I. = 0.67–5.78), and those in the APOE ε4-negative AD were 2.24 (95% C.I. = 0.76–6.58). The odds in the APOE ε4-negative late-onset AD were 3.01 (95% C.I. = 0.78–12.03) (Table 3).

### Table 2
The frequencies of the cathepsin D T allele and C/T genotype in the AD patients and control subjects stratified by the APOE ε4 allele

<table>
<thead>
<tr>
<th>Group</th>
<th>APOE ε4 negative</th>
<th>APOE ε4 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>T (%)</td>
</tr>
<tr>
<td>Total AD</td>
<td>107</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>Controls</td>
<td>216</td>
<td>10 (2.6)</td>
</tr>
<tr>
<td>EOAD</td>
<td>36</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Controls</td>
<td>44</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>LOAD</td>
<td>71</td>
<td>4 (5.6)</td>
</tr>
<tr>
<td>Controls</td>
<td>172</td>
<td>8 (2.6)</td>
</tr>
</tbody>
</table>

EOAD, early-onset AD; LOAD, late-onset AD.
4. Discussion

As in a series of previous studies (Crawford et al., 2000; Bertram et al., 2001; Matsui et al., 2001; Menzer et al., 2001; Bagnoli et al., 2002; Mateo et al., 2002; Ingegni et al., 2003; Styczynska et al., 2003), we could not found a significant association between the CatD T and AD in Koreans which had been reported by Papassotiropoulos et al. (1999, 2000).

Several sources might have contributed to the inconsistent finding in the CatD T–AD association. Although further investigations are warranted, the ethnicity of the subjects might be one of the possible sources. The allelic frequency of the CatD T varied significantly according to the ethnicity. It was 0.9% (Matsui et al., 2001) and 14.3% (Crawford et al., 2000) in Japanese and Hispanics, respectively. Even in Caucasians, it ranged from 4.5% to 11.1% (Papassotiropoulos et al., 1999, 2000; Crawford et al., 2000; Bertram et al., 2001; Menzer et al., 2001; Bagnoli et al., 2002; Mateo et al., 2002; Ingegni et al., 2003; Styczynska et al., 2003). As shown in the present study, the allelic frequency of the CatD T in Koreans was 2.5%, which was only about one-sixths of that in Hispanics. This clear ethnic difference in the allelic frequency of the CatD T might have influenced on the power detecting the association.

Linkage disequilibrium of the CatD with other risk genes for AD might also have contributed to the discrepancy. Crawford et al. (2000) suggested that the association between the CatD and AD was more easily detected in a relatively homogeneous Caucasian group. However, considering that Koreans and Japanese are ethnically homogeneous, ethnic homogeneity, if any, may not be the only source for the conflicting results.

The weaker association than previously reported by Papassotiropoulos et al. (1999, 2000) may be another source for the inconsistent results. Even though a significant association has not been observed other than Papassotiropoulos et al. (1999, 2000), a pooled data from several association studies of CatD polymorphisms indicate a statistically significant association between AD and CatD polymorphism (Menzer et al., 2001). Even in those studies, which did not find a significant association between the CatD and AD, the odd for AD were bigger than 1.0, and those from the pooled data were 1.39, indicating that the CatD T–AD association is weak.

In the present study, we could not find any interaction of the CatD with APOE e4 allele, age or gender in the development of AD. However, according to Menzer et al. (2001), CatD T-conferred AD risk was higher in males and its interaction with APOE genotypes was shown only in males, suggesting that the interaction of the CatD polymorphism with the APOE e4 status or gender may influence its association with AD. According to the meta-analysis by Ntais et al. (2004), the CatD T allele was found to enhance the APOE e4-conferred AD risk. Since the power of the present study was not high enough, we cannot exclude the possible contribution of the CatD T allele in the development of AD. The power of the present study was only 32% if we considered the odds of the CatD T allele for AD in Koreans were 2. This lack of power is attributed to the low allelic frequency of the CatD T in Koreans as well as small sample size. As presented in Table 1, the frequency of the CatD T in the Korean controls was only 0.025, which was less than a half of that in most Caucasian populations (about 0.045–0.11).
In conclusion, the association between the CatD genetic polymorphism and AD was not found in Koreans, although it waits for further replication in an extended sample.

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.

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