Angiotensin-converting enzyme gene and plasma protein level in Alzheimer’s disease in Taiwanese

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Abstract

Background: angiotensin-converting enzyme (ACE) gene insertion/deletion (indel) polymorphism is considered a biomarker for Alzheimer’s disease (AD). However, the associations of ACE gene and protein level to AD are undetermined among Taiwanese.

Methods: this study investigated 257 Taiwanese cases with AD and 137 ethnically matched controls using ACE gene indel genotype association methods with logistic regression adjusted for other variables. Besides, 65 out of 257 AD patients, 11 with D/D genotype, 28 with I/I genotype and 26 with I/D genotype were recruited. Their plasma ACE protein levels were measured by enzyme-linked immuno-sorbent assay and compared for their corresponding ACE gene indel polymorphism.

Results: patients with ACE-I/I homozygote were less likely to be associated with AD, compared with both I/D and D/D (OR: 0.601; 95% CI: 0.372–0.969; P = 0.037), or only I/D genotype (OR: 0.584; 95% CI: 0.349–0.976; P = 0.040). There were significantly different plasma ACE protein levels among these three different genotype groups (P = 0.023). The I/I genotype group had significantly lower ACE plasma levels [114.79 ± 31.32 ng/ml (mean ± SD)], compared with D/D (164.07 ± 86.36 ng/ml; P = 0.010), but not I/D (141.45 ± 51.50 ng/ml; P = 0.064).

Conclusion: ACE-I/I homozygote corresponds to lower plasma ACE protein level and it is independently but less likely to be associated with AD. These findings signal the importance of ACE indel polymorphisms to their corresponding protein levels and to AD.

Keywords: angiotensin-converting enzyme, Alzheimer’s disease, Taiwanese, elderly
Introduction

The angiotensin-converting enzyme (ACE) gene located on chromosome 17q23 [1, 2] is regarded as a plausible biological candidate susceptibility gene for AD because ACE protein can degrade amyloid β [3–5], a hallmark of AD [6]. Individuals diagnosed with AD have higher ACE activity in the hippocampal, para-hippocampal and temporal cortex [7–9], but have decreased plasma ACE level [10] than non-demented individuals. Meanwhile, ACE gene insertion/deletion polymorphism (indel) is associated with ACE levels and activity [11] and has been previously associated with risk for AD [12–15].

Two meta-analyses addressing the relationship between ACE indel polymorphism and AD have shown that the D/D genotype is associated with reduced risk for AD [12, 13]. However, such results vary with different races or ethnicities. In Taiwan, a study has reported that D/D is a risk for AD [15], whereas the results are heterogeneous in the Japanese [16]. The associations of ACE indel polymorphism and corresponding ACE protein levels to AD are not well-determined yet, especially among Taiwanese.

To further investigate these issues, this study examined the distribution of ACE indel polymorphism in a well-characterised and genetically and diagnostically distinct cohort of AD cases and non-demented controls in Taiwan. Plasma ACE levels in AD patients were also examined with respect to their ACE indel genotype in order to have a comprehensive exploration of the ACE gene and AD.

Material and methods

Subjects

Based on a self-reported process, 257 cases and 137 controls were recruited by the Department of Neurology Kaohsiung Medical University Hospital (KMUH), a medical centre at southern Taiwan. The diagnosis of AD was based on the NINCDS-ADRDA criteria [17] referring to a series of comprehensive neuro-psychological tests, including the Mini-Mental Status Examination (MMSE) [18], Cognitive Assessment Screening Instrument (CASI) [19], Neuropsychiatric Inventory (NPI) [20] and Clinical Dementia Rating (CDR) scale [21]. Patients with other conditions possibly contributing to the diagnosis of AD were excluded.

All AD patients were being treated with acetylcholinesterase inhibitors before their study entry diagnosis. The mean treatment duration was 4.13 ± 0.44 years for very mild, 3.43 ± 0.17 for mild and 2.76 ± 0.20 for moderate stage AD. For ACE protein plasma measurement, only AD patients who gave consent for measurements, apart from previous genetic association studies, were recruited into the protein level analysis.

The Kaohsiung Medical University Hospital Institutional Review Board (IRB) approved of all the procedures, and all participants or their legal representatives provided written informed consent.

Controls were either non-demented individuals who enrolled in longitudinal research projects for dementia of the Department of Neurology, KMUH, or non-demented spouses of demented individuals. All controls received a comprehensive medical evaluation, including clinical history, physical examination and blood chemistry examinations. For spouse controls, their CASI score should be greater than the cut-off values after controlling the effects of age and education. For other controls from the longitudinal studies, all of their clinical diagnoses were ‘no dementia with CDR0’ and their CASI score should be greater than the cut-off value adjusted by age and education on their last clinical assessment.

Methods for genotyping

Apolipoprotein E (APOE) genotyping was performed following a modification of the protocol developed by Pyrosequencer TM (http://www.pyrosequencing.com). Briefly, 10 ng of DNA was amplified in 20-μl reaction volume in which dGTP was replaced by a mixture of 25% dGTP and 75% dITP to facilitate the analysis of the GC-rich fragment. A 276-bp fragment was generated using forward primer AGA CGC GGG CAC GGC TGT and reverse, Biotin-labelled primer CTC GCG GAT GGC GCT GAG. Single-stranded DNA prepared using streptavidin-coated beads and the APOE gene variants at codons 112 and 158 were pyro-sequenced using the following primers and dispensation order: SNP112 GAC ATG GAG GAC GTG and SNP158 CCG ATG ACC TGC AGA and dispensation order GCTGAGCTAGCGT.

Detection of the insertion/deletion polymorphism of the human ACE gene was performed as described previously [2]. To confirm the genotype assignments, in particular D/I vs. D/D genotype, there was a second analysis as described by Lindpainter et al. [22]

ACE plasma concentration

Commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kits (Quantikine Human ACE Immunoassay, DACE00, R&D systems, Minneapolis, MN, USA) were used according to the manufacturer’s guidelines to measure the plasma ACE concentration. Assays were performed in duplicate and the optical density at 450 nm with background correction at 540 nm, and determined using a microplate ELISA reader (Multiskan EX, Thermo scientific, Vantaa, Finland).

Statistical analysis

Data analysis was performed using SPSS (version 12.0.1 for Windows, SPSS, Inc, Chicago, IL, USA). All statistical tests were two-tailed and an alpha of 0.05 was taken to indicate significance.

Chi-square test was used to evaluate the Hardy–Weinberg equilibrium and to examine whether the unadjusted likelihood of case status (vs. control status) was
associated with the frequency of the three ACE genotypes, at least one APOE 4 allele, gender and educational level. The t-test was conducted to determine differences in the mean age among groups, while logistic regression was used to examine whether ACE genotypes (I/D as the reference) were significantly associated with case status after adjusting for the effects of sex, age, educational level and possession of at least one APOE 4 allele [APOE 4 (+)].

Another logistic regression model was conducted to examine whether any of the ACE genotypes, compared with the other two genotypes, was significantly associated with case status after adjusting for co-variables. One-way analysis of variance (ANOVA) with Fisher’s least significant difference (LSD) was used to compare group means of ACE plasma level with respect to the ACE genotypes in AD patients.

The age of AD diagnosis among cases when they entered this study and age at the last assessment among controls were assigned to the age variable, treated as continuous variable by 1 ng/ml increment. Three educational levels were categorized according to educational years: <6, 6–9 and >9 years. The gender, APOE 4 (+) and ACE genotypes were treated as categorical variables.

**Results**

Both the case and control groups were in Hardy–Weinberg (H–W) equilibrium (P > 0.05). In unadjusted analyses, the likelihood of case status did differ significantly from controls among the three ACE genotypes (P < 0.001). Significant differences were also found in age (P < 0.001), educational level (P < 0.001), gender (P < 0.001) and APOE 4 (+) (P < 0.001) (Table 1).

In logistic regression, the I/I genotype was significantly associated with control status when compared with either I/D (OR: 0.584, 95% CI: 0.349–0.976, P = 0.040) or other non-I/I, I/D and D/D genotypes (OR: 0.601, 95% CI: 0.372–0.969, P = 0.037) (Table 2). Similarly, age (OR: 1.083, 95% CI: 1.047–1.120, P < 0.001), low educational level (OR: 5.273, 95% CI: 3.056–9.907, P < 0.001) and APOE 4 (+) (OR: 2.642, 95% CI: 1.423–4.903, P = 0.002) were significantly associated with case status.

In plasma ACE protein level analyses of AD patients, plasma samples recruited from 65 AD subjects (26 with I/I genotype, 28 I/D and 11 D/D were collected and analysed by ELISA). ANOVA with LSD post hoc analyses revealed that ACE plasma level significantly varied with ACE genotypes (P = 0.023). ACE plasma level in individuals with the I/I genotype (114.79 ± 31.32 ng/dl) was significantly lower than that in patients with the ACE D/D homozygote (164.07 ± 86.36 ng/ml, P = 0.010), but not more significant than that in I/D (141.45 ± 51.50 ng/ml, P = 0.064) (Table 3).

**Discussion**

This study shows that the I/I genotype of ACE indel polymorphism is significantly associated with decreased risk of AD among the Taiwanese. The plasma ACE level is lower in AD patients with ACE I/I genotype, compared with that in other genotypes.

In genetic association with AD, the results are partly similar to a previous study among Taiwanese [15], which report a significantly increased risk of AD in subjects with the D/D genotype and D allele. However, the analysis of that study is not adjusted for factors like age, gender and education in the diagnosis of AD. That study also does not conduct other objectively cognitive evaluation to ensure the status of their control groups. However, the pooled samples’ analyses for these two studies are encouraging in addressing these issues. The results here are also different from the results of a meta-analysis report in East Asia.
China and Japan, wherein the I/I genotype, compared with the I/D and D/D genotypes, is a risk factor of AD [12]. Such differences may be due to the possibility of different races, which is also reported in another study [23].

In ACE plasma level, the findings here are partly similar to previously reported results. A study examining the association of ACE plasma level with respect to the corresponding ACE indel polymorphisms in healthy individuals has found that subjects with the ACE I/I genotype have lower ACE plasma levels compared with other genotypes [11]. Another study examining the ACE plasma level has also found that AD patients, compared with control subjects, have a greater reduction in plasma ACE level after a 2-year evaluation [10].

The findings here reveal that individuals with ACE I/I homozygote tend to have lower ACE plasma level, thereby making them less likely to be associated with cerebral infarction [24–26] or other cardiovascular disorder like hypertension [27, 28] thus increasing risk for AD [29, 30]. These susceptibilities imply that the possible effects of ACE inhibitors in the treatment of AD may be varied, depending on the ACE genotypes of AD patient. Although recent reports indicate that in vitro, ACE may modulate susceptibility to progression of AD by degrading beta-amyloid [3–5], it is not known whether it will have a significant effect in vivo. Furthermore, it is not well known whether the association of ACE plasma level and ACE indel polymorphism is sufficient to reflect the influence of ACE level and activity on the brain (10). More advanced studies using a single cohort or pooled sample analysis to examine the mechanisms from ACE gene to AD are warranted.

This study has several limitations. First, there is a significant difference in the mean age between the cases and controls. Nonetheless, the cases in this study have been under treatment for years before enrolment. Thus, such difference, if any, may be minor. The mean age of the controls is 71.7 ± 4.9 years, which may be less likely to convert into AD later, compared with other controls in a study carried out in Taiwan (15), in which the mean age is 62.5 ± 8.7 years. Second, this study does not measure the plasma ACE level in the controls because the specific aims are mainly on the association of ACE indel polymorphism and its corresponding protein level. The difference of ACE protein level between AD and normal subjects has been examined in another study (10).

Nonetheless, this study has several strengths. First, the more stringent criteria for cases and controls groups are used and other covariates that may contribute to AD are controlled, compared with a previous study in Taiwan (15). Second, the ACE genotype and its corresponding plasma level to AD is examined in one cohort to avoid differences among various study designs, criteria of patient recruitment and controlled co-variables in statistical analyses. The results, compared with other previously published genetic association studies, have more objective evidences to highlight the significant association of ACE gene indel polymorphism and AD because significant differences in ACE protein level with regard to their ACE genotypes are shown. The findings also highlight the importance of ACE gene and its subsequent effects in the further research, prevention and treatment of AD.

### Key points

- ACE gene insertion/deletion (indel) polymorphism is significantly associated with the AD in Taiwanese.
- The different ACE protein levels encoded by ACE indel polymorphisms are significantly associated with AD in Taiwanese.
- A comprehensive study adjusted other co-variables to examine the associations.

### Conflicts of interest

None declared.

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### References


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