Endothelin-converting enzyme-1 promoter polymorphisms and susceptibility to sporadic late-onset Alzheimer's disease in a Chinese population

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Abstract. Endothelin converting enzyme (ECE-1) is a candidate Alzheimer disease susceptibility gene. It was previously reported that western individuals homozygous for the C-338A polymorphism (AA) within the *ECE1* gene promoter region are at reduced risk of developing late onset Alzheimer disease (LOAD). A further polymorphism, T-839G, is present within the *ECE1* promoter region but a potential association with LOAD has not been studied. We therefore studied possible associations between these *ECE1* polymorphisms and LOAD in a Chinese population. Subjects comprised 376 Chinese LOAD patients and 376 age- and sex-matched controls; all subjects were typed for the *ECE1* C-338A and the T-839G polymorphisms. We report that the frequency of the 338A allele was decreased in LOAD patients compared to controls (adjusted OR = 0.73; 95% CI = 0.54–0.98; *P* = 0.03). There was no significant association between T-839G genotype and LOAD, however the combined 839T/338A haplotype was significantly associated with decreased risk of LOAD (OR = 0.73; 95% CI = 0.57–0.93; *P* = 0.01). This study argues that the *ECE1* 338A allele is protective against LOAD in a Chinese population.

Keywords: Alzheimer's disease, polymorphism, ECE-1

1. Introduction

Cerebral accumulation of β -amyloid peptide ($A\beta$) is thought to be central to the pathogenesis of Alzheimer's disease (AD) [1]. Endothelin-converting enzyme-1 (ECE-1) is a candidate $A\beta$ -degrading enzyme in brain, and polymorphisms affecting expression levels of the *ECE1* gene may therefore affect the risk of developing AD.

The promoter region of the *ECE1* gene contains a polymorphic site at position 338 (C-338A); alleles containing A at this position are associated with increased transcriptional activity in promoter-reporter assays *in vitro* as well as with increased ECE-1 mRNA expression in human neocortex [2]. It has also been reported in large case-control studies that individuals homozygous for C-338A (ie. AA) are at reduced risk of developing AD [3,4]. A further functional polymorphism, T-839G, has also been identified within the *ECE1* promoter region and gene alleles containing the 338A polymorphism (338A/839T and 338A/839G) were reported to have increased transcriptional activity [2].

A potential association between *ECE1* T-839G polymorphisms and the risk of late onset AD (LOAD) has

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Table 1
Baseline characteristics of LOAD patients and controls

Characteristics	LOAD (<i>n</i> = 376)	Controls (<i>n</i> = 376)	<i>P</i>
Age ^a (y)	68.2 (62–94)	68.4 (62–94)	NS
Sex (male), <i>n</i> (%)	123 (32.7%)	123 (32.7%)	NS
Education(≤ 6y)	157 (41.8%)	107 (28.5%)	< 0.001
Smoking, <i>n</i> (%)	162 (43.1%)	90 (23.9%)	< 0.001
Drinking, <i>n</i> (%)	243 (64.6%)	164 (43.6%)	< 0.001
Hypertension, <i>n</i> (%)	211 (56.1%)	131 (34.8%)	< 0.001
Diabetes, <i>n</i> (%)	141 (37.5%)	68 (18.1%)	< 0.001

^aMedian (25th–75th percentiles).

so far not been investigated. In present study we genotyped LOAD patients and control subjects in a Chinese population for their genotypes and haplotypes at the C-338A and T-839G polymorphic sites in order to study a possible association with LOAD.

2. Material and methods

2.1. Patients and controls

A cohort of 376 Chinese Han subjects with sporadic late-onset (> 60 years) Alzheimer's disease was recruited at the Division of Neurology of Daping Hospital (Chongqing, China). Dementia was diagnosed according to DSM-IV-R criteria [5]. Cognitive performance was assessed using the mini mental state evaluation and the global deterioration scale. All LOAD cases were defined as sporadic because reported family histories contained no first-degree relatives with dementia. Probable sporadic LOAD was diagnosed according to the NINCDS-ADRDA Work Group guidelines (revised) [6]. The control group comprised 376 unrelated and age- and sex-matched individuals randomly selected from the same area. These subjects had complete neurological, laboratory and medical examinations and were shown to be free of neurodegenerative disorders. The following risk factors for LOAD development were evaluated: low education, smoking, alcohol consumption, hypertension and diabetes. All patients and controls were free of anti-hypertensive treatment. Participants who had ever smoked within the 10 years prior to study entry were defined as smokers. All participants gave informed consent for the study and the outline of the study was approved by the Institutional Review Board.

2.2. Genotyping of ECE1 polymorphisms

After overnight fasting approximately 5 ml samples of venous blood were drawn from each subject into tubes containing EDTA. Genomic DNA was extracted according to the salting-out procedure described by Miller et al. [7]. The ECE1 C-338A polymorphism was investigated according to the technique described by Funalot et al. and Scacchi et al. [4,8]. T-839G polymorphisms were detected by the same method. The PCR primers and restriction enzymes used in this work are summarized in Table 2.

2.3. Statistical analysis

Statistical analyses were conducted using the SPSS 13.0 program (SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium of the analyzed polymorphisms was tested in the study population by a χ^2 goodness-of-fit test. Odds ratios (ORs) are reported with 95% confidence interval (CI) and P-values were calculated using the χ^2 -test. Logistic regression analysis was performed with respect to the C-338A or T-839G genotypes and the following covariates: low education, diabetes, hypertension, smoking and alcohol consumption. Linkage disequilibrium was tested using the LINKDOS program of Garnier-Gere and Dillmann [9]. Haplotype analysis was conducted using modules implemented in the Haplo.stats program [10].

3. Results

The clinical characteristics of all study participants are shown in Table 1. As expected, known risk factors for LOAD including low education, smoking, alcohol consumption, hypertension and diabetes were more common among cases than controls ($P < 0.001$).

The ECE1 C-338A and T-839G variants were in Hardy-Weinberg equilibrium ($P = 0.293$ and $P = 0.697$ respectively for C-338A LOAD patients and controls; $P = 0.391$ and $P = 0.582$ respectively for G-839T LOAD patients and controls) and were in strong linkage disequilibrium (LD: $r^2 = 0.713$).

The distribution of C-338A genotypes (CC, CA and AA) was significantly different between LOAD patients (81.9%, 16.8%, and 1.3%) and controls (59.8%, 34.6%, and 5.6%; $\chi^2 = 7.168$, $P = 0.028$), indicating a strong association between 338A alleles and LOAD (adjusted OR = 0.73; 95% CI = 0.54–0.98; $P = 0.03$).

Table 2
Primers sequences

Polymorphism	refSNP ID	Primer pairs Sense/Antisense	RE
C-338A	rs213045	5/- TAG GGT TATAGG AGA GGG CTC AGG-3/ 5/-AAG TAT CAG GAA GGT GCC CTC AAT-3/	Tsp509I
T-839G	rs213046	5/- CCCCTCACCCCTCTAAACCT -3/ 5/- CACGGGATAAAGGAGCTGT-3/	AvaI

There was no significant different in the distribution of *ECE1* T-839G genotypes (CC, CA and AA) between LOAD patients (51.6%, 38.8%, and 9.6%) and controls (78.7%, 19.7%, and 1.6%; $\chi^2 = 1.213$, $P = 5.45$). There was also no significant difference between genotype at position 839 and LOAD even after adjustment for known risk factors including education, diabetes, hypertension, smoking and alcohol consumption (adjusted OR = 1.24; 95% CI = 0.87–1.79; $P = 0.28$) (Table 3).

The most frequent haplotype in the healthy Chinese control subjects was 839T/338C, followed by 839T/338A and 839G/338C. Similar haplotype frequencies were found in patients with LOAD. However, the combined 839T/338A haplotype was significantly associated with LOAD (OR = 0.73; 95% CI = 0.57–0.93; $P = 0.01$) (Table 4).

4. Discussion

In the present case-control study we compared the distribution of C-338A and T-839G polymorphisms within the *ECE1* gene between patients with LOAD and a control group of age- and sex-matched subjects without any neurodegenerative disorder. Both cohorts were extensively characterized with respect to known risk factors for LOAD in order to permit statistical adjustment. We report that *ECE1* 338A alleles and the 839T/338A haplotype were significantly under-represented in LOAD patients compared to control subjects.

Several genetic polymorphisms have been identified within the *ECE1* gene promoter. Previous studies addressed potential associations with AD, hypertension and coronary artery disease and further nonsynonymous *ECE1* gene variants have also been studied [3, 4,8,11]. Three different explanations have been put forward to explain the association between *ECE1* gene polymorphisms and disease status. First, the polymorphisms may be biologically relevant to the disease; second, they are in linkage disequilibrium with biologically relevant variants elsewhere within the *ECE1* gene; or third, these polymorphisms are in disequilibrium with

genetic variants in other adjacent genes [12]. *In vitro* or *in vivo* expression studies of the mutant gene and protein will be required to address the functional relationship between the different polymorphisms and disease risk.

The 338A allele was previously reported to display increased transcriptional activity in transient transfection assays compared to promoter regions containing 338C. Variants containing 839G did not appear to affect transcriptional activity, although a synergistic effect on promoter efficacy was reported when 839G was combined with 338C [2]. *In vivo*, *ECE1* mRNA expression levels were 2-fold higher in human prefrontal cortex of carriers of the 338A allele compared to non-carriers [3].

Expression of ECE-1 enzyme may be protective against A β deposition in Alzheimer disease. Over-expression of ECE-1 in Chinese hamster ovary cells lacking endogenous ECE activity was found to reduce extracellular A β concentrations by up to 90%; this effect was completely abolished by treatment with the ECE-1 inhibitor phosphoramidon [13]. Moreover, recombinant soluble ECE-1 was shown to hydrolyze synthetic A β -(1-40) and A β -(1-42) [14]. Because both the C-338A and T-839G polymorphisms affect expression levels of ECE-1 enzyme it would appear likely that different allelic variants at these sites might modulate the risk of AD development.

Although both 338A and 839G allele have been reported to increase *ECE1* promoter activity, we found that the 839G allele was not significantly associated with protection against LOAD. This inconsistency was also found in relation to coronary artery disease. Wang et al. [15] reported that *ECE1* 338A alleles were associated with increased risk of coronary artery disease (adjusted OR = 1.58, 95% CI = 1.07–2.32, $P = 0.020$) while, in contrast, Scacchia et al. [4] showed that 338A alleles were protective against coronary artery disease in subjects carrying the apolipoprotein E (*APOE*) ϵ^4 allele (adjusted OR = 0.51; 95% CI = 0.29–0.89; $P = 0.018$). Buhler et al. [11] found that subjects harboring the 839G allelic variant were at decreased risk of coronary artery disease (odds ratio 0.41; 95% CI = 0.18–0.90, $P = 0.024$). No satisfactory explanation has yet been put forward to explain why increased *ECE1* pro-

Table 3
Distributions and the risk estimates for the variants of *ECE1* C-338A and T-839G

genotype	Controls(%)	LOAD(%)	Crude OR	95% CI	P	Adjusted OR	95% CI	P
T-839G								
TT	308(81.9%)	296(78.7%)	1					
TG	63(16.8%)	74(19.7%)	1.22	(0.84–1.77)	0.29	1.27	(0.86–1.83)	0.31
GG	5(1.3%)	6(1.6%)	1.25	(0.38–4.14)	0.72	1.13	(0.25–3.98)	0.73
TG + GG	68(18.1%)	80(21.3%)	1.22	(0.85–1.76)	0.27	1.24	(0.87–1.79)	0.28
C-338A								
CC	194(51.6%)	225(59.8%)	1					
AC	146(38.8%)	130(34.6%)	0.77	(0.57–1.04)	0.09	0.78	(0.58–1.07)	0.09
AA	36(9.6%)	21(5.6%)	0.50	(0.28–0.89)	0.02	0.48	(0.25–0.87)	0.02
AA + AC	182(48.4%)	151(40.2%)	0.72	(0.54–0.96)	0.02	0.73	(0.54–0.98)	0.03

Patients and controls were matched by age and sex and data adjusted for low education, smoking, drinking, hypertension and diabetes.

Table 4
Distributions and the risk Estimates for *ECE1*/haplotypes

haplotype ^a	Controls(%)	LOAD(%)	Crude OR	95% CI	P
839T/338C	469(62.4%)	502(66.7%)	1		
839T/338A	210(27.9%)	164(21.8%)	0.73	0.57–0.93	0.01
839G/338C	65(8.6%)	78(10.4%)	1.12	0.79–1.60	0.53
839G/338A	8(1.1%)	8(1.1%)	0.93	0.35–2.51	0.89

moter activity is associated with increased risk of coronary artery disease in some studies, and with decreased risk in others.

In the present study 338A alleles were associated with reduced risk of developing LOAD, whereas alleles containing 839G were not protective, even though both variants are thought to increase *ECE1* promoter activity. These contrasting findings may possibly be explained by variations in genetic and environmental factors that modulate disease development. Only a few studies have addressed the functional effects of these allelic variants on *ECE1* gene transcription and enzyme activity. In addition, most investigations into potential associations between disease risk and *ECE1* allelic variants have been cross-sectional studies and/or limited to small series of patients; these may therefore be prone to selection bias. Furthermore, because the ECE-1 conversion target endothelin (ET-1) displays autocrine/paracrine activity, assessment of ET-1 levels in plasma may not afford an appropriate measure of ECE-1 activity [16]. More investigations will be required to clarify the association between ECE-1 enzyme expression levels and disease development including LOAD.

In conclusion, we report that the *ECE1* 338A alleles are associated with reduced risk of LOAD development in a Chinese population.

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