

Association between butyrylcholinesterase K variant and mild cognitive impairment in the Thai community-dwelling patients

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Objective: To study the association of the butyrylcholinesterase K variant (BChE-K) and the plasma BChE activity with mild cognitive impairment (MCI) in Thai community-dwelling patients.

Methods: One hundred patients diagnosed with MCI and 100 control subjects were recruited from the community-dwelling setting in Bangkok, Thailand. The genotype and allele distributions of the BChE-K were determined by polymerase chain reaction and subsequent DNA sequencing. The BChE activity was measured in plasma according to the Ellman's method.

Results: The BChE-K allele frequencies in the Thai community-dwelling patients were in accordance with other ethnics. The BChE-K allele frequency in the control subjects (12%) was higher than that of MCI patients (5.5%), suggesting a protective role of BChE-K for MCI in the Thai community-dwelling patients. The BChE-K homozygotes were significantly associated with lower BChE activity.

Conclusion: Our results suggested that the BChE-K may be implicated as a protective factor for MCI in the Thai community-dwelling patients, although a further study with a large sample size is warranted to confirm this.

Keywords: butyrylcholinesterase K variant, butyrylcholinesterase activity, mild cognitive impairment, Thai community-dwelling patients

Introduction

Mild cognitive impairment (MCI) is a common problem in the elderly and a part of a cognitive continuum between normal aging and Alzheimer's disease (AD).¹ As the number of elderly people, as well as the prevalence of AD, is increasing rapidly due to the demographic changes in the country, identifying those who are likely to have clinical manifestation of AD in the earliest stage would benefit the patients, family and society as a whole.^{2,3} AD is the most common age-dependent neurodegenerative disorder noticeable by cognitive and memory deterioration, a variety of neuropsychiatric symptoms, behavioral disturbances and impairment of activities of daily living (ADL).⁴ In hippocampus and temporal cortex of AD patients, an increase in butyrylcholinesterase (BChE) and a reduction in acetylcholinesterase activity are observed.⁵ It is suggested that the increased level of BChE is associated with cognitive decline in dementias. Furthermore, BChE is associated with amyloid plaques and neurofibrillary tangles in AD.^{6,7} One of the most important challenges in research on cognitive impairment in the elderly is to identify genes that predispose to MCI and could be used to predict which subjects will progress to dementia. Recently, genetic variation is used as biomarkers for MCI and progression of MCI leading to dementia.⁸

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One of the candidates that has been identified to date is the K variant of BChE (BChE-K). The K variant, a mutation at codon 539, leads to the replacement of an alanine by a threonine residue (A539T) and is associated with 30% reduction in enzyme activity.⁹ The BChE-K has an essential role in AD progression.¹⁰ The BChE-K is observed in various ethnic populations with homozygote frequencies between 2% and 32%.^{11–23} AD has a multifactorial pathogenesis and its challenge is the early diagnosis. Thus, genotyping BChE-K and measuring BChE activity in subjects with MCI will help identify potential biomarkers for MCI in easily accessible peripheral blood samples. The purpose of this study is to investigate the association of the BChE-K and plasma BChE activity with MCI in Thai community-dwelling patients.

Methods

Participants were volunteers from the community-dwelling visit in the “Health Promotion and Common Disease Prevention in the Thai Elderly Project” being run in a community-dwelling setting in Bangkok, Thailand, as described by Muangpaisan et al.²⁴ The study protocol was approved by the Mahidol University Ethics Committee 145/2004, and a written informed consent was obtained from each subject prior to their participation. Inclusion criteria were patients aged >50 years who had a subjective memory complaint and intact instrumental ADL (IADL) and were willing to participate. They had a Thai Mental State Examination (TMSE) score of 24 or more and showed no abnormality of judgment and insight during the structured interview.²⁵ Exclusion criteria were patients who had dementia and depression diagnosed by the 4th edition of the Diagnostic and Statistical Manual criteria.²⁶ They had a TMSE score of <24, illiteracy, communication problems, impairment in insight and judgment. The diagnosis of MCI was made according to the Petersen criteria: 1) impairment in one or more measures of memory, 2) clinician’s judgment based on a structured interview by using the Clinical Dementia Rating Scale score of 0.5, 3) the Global Deterioration Scale score of 3, but with normal performances in other cognitive domains and 4) no impairment in IADL.^{27–29} The control subjects had TMSE score of at least 24, no evidence of dementia or memory impairment on assessment, no diagnosis of major medical, neurological or psychiatric illness, no history of head injury and substance abuse and no impairment in ADL.

Genomic DNA was extracted from peripheral blood cells using a QIAamp DNA Blood Mini Kit according to the

manufacturer’s instructions. BChE-K genotyping was performed as previously described.³⁰ The BChE activity of plasma samples were measured according to the Ellman’s colorimetric method,³¹ with 5,5′-dithio-bis 2 nitrobenzoic acid (DTNB) and butyrylthiocholine iodide as substrate.

The observed genotype and allele frequencies of BChE-K between MCI patients and controls were compared with the predicted values using chi-square (χ^2) test. Odds ratio (OR) and confidence interval (CI) calculations were performed based on a crosstabs module included in the SPSS statistical software package version 17.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean $\pm$ standard deviation. Analysis of variance was used to compare the differences among the groups and data were expressed as percentage. Two-tailed Student’s *t*-test was used to compare the differences between the two groups. $P < 0.05$ was considered statistically significant.

Results

The demographic characteristics of the Thai community-dwelling patients are shown in Table 1. In this study, females are predominant; however, MCI patients and controls showed no statistical significant differences in gender, age, TMSE scores. Furthermore, there is no statistically significant difference of plasma BChE activity between the MCI patients and controls ($P = 0.4738$).

The BChE-K genotype and allele frequencies for the Thai community-dwelling patients are summarized in Table 2. Distribution of the BChE-K genotype in MCI patients ($\chi^2 = 1.8$, degrees of freedom [*df*] = 1, $P = 0.1796$) and the controls ($\chi^2 = 2.8$, *df* = 1, $P = 0.5959$) were in Hardy–Weinberg equilibrium. It is noteworthy that 1% MCI patients and 2% controls were homozygous for the K allele (K/K). The BChE-K allelic frequency was found to be significantly different in MCI patients when compared with controls; 5.5% and 12%,

Table 1 Demographic data and clinical characteristics

Subjects	Controls	MCI patients
Total, n	100	100
Female, n	67	74
Male, n	33	26
Age (years)	63.85 $\pm$ 8.25	64.35 $\pm$ 7.91
Female	62.51 $\pm$ 7.99	62.96 $\pm$ 6.98
Male	66.58 $\pm$ 8.21	68.31 $\pm$ 9.16
TMSE score	27.55 $\pm$ 2.48	25.70 $\pm$ 2.95
Plasma BChE activity (U/L)	4,988 $\pm$ 1,229	4,999 $\pm$ 1,200

Notes: Data are presented as the mean $\pm$ SD. U/L, units/litre (reference range 4,300–12,900 U/L).

Abbreviations: BChE, butyrylcholinesterase; MCI, mild cognitive impairment; n, number; SD, standard deviation; TMSE, Thai Mental State Examination.

Table 2 Genotype and allele frequencies of the BChE-K in MCI patients and controls

Subjects	n	Genotypes (%)			χ^2	P-value	Alleles (%)	
		WT/WT	WT/K	K/K			WT	K
Control	100	78 (78)	20 (20)	2 (2)	0.28	0.5959	176 (88)	24 (12) ^a
Female	67	55 (82.1)	12 (17.9)	–			122 (91)	12 (9)
Male	33	23 (69.7)	8 (24.2)	2 (6.1)			54 (81.8)	12 (18.2)
MCI	100	90 (90)	9 (9)	1 (1)	1.8	0.1796	189 (94.5)	11 (5.5) ^a
Female	74	70 (94.6)	4 (5.4)	–			144 (97.3)	4 (2.7)
Male	26	20 (76.9)	5 (19.2)	1 (3.9)			45 (86.5)	7 (13.5)

Notes: The number (%) of patients in each group. ^aComparison of allele frequency of K variant in MCI patients and controls: $\chi^2=5.357$, $df=1$, $P=0.02$; OR =0.427, 95% CI =0.203–0.897, $P<0.05$. WT/WT, wild type of BChE gene; WT/K, BChE-K heterozygotes; K/K, BChE-K homozygotes.

Abbreviations: BChE, butyrylcholinesterase; BChE-K, butyrylcholinesterase K variant; MCI, mild cognitive impairment; df , degrees of freedom; OR, odds ratio; CI, confidence interval; n, number.

respectively ($\chi^2=5.357$, $df=1$, $P=0.02$). The OR as an estimate of relative risk for MCI was 0.427 (95% CI=0.203–0.897, $P<0.05$), which indicated that BChE-K is a protective factor for MCI in the Thai community-dwelling patients. Among 200 subjects, included in this study were 141 females (controls =67, MCI patients =74) and 59 males (controls =33, MCI patients =26). Although females are predominant in the Thai community-dwelling female, both MCI and controls did not exhibit homozygous for the BChE-K. Homozygous for the BChE-K was found in MCI male patients (3.9%) and male controls (6.1%).

The values of plasma BChE activity did not exhibit significant difference between MCI patients and controls (Table 1). To determine whether there were genotype and gender differences in the influence of BChE activity, we stratified BChE activity according to genotype and gender. The plasma BChE activities in each genotype and gender are given in Table 3. The significant reduction in BChE activities was observed in the BChE-K homozygotes (K/K) of both MCI patients (55%) and controls (39% and 65%). However, comparison of BChE-K heterozygotes (wild type [WT]/K) with homozygotes for WT of BCh allele (WT/WT) with

respect to their BChE activity and gender yielded no statistically significant difference. A reduction of BChE activity was observed only in males who are carrier homozygous for BChE-K.

Discussion

In the present study, genotype and allele distributions of the BChE-K and the plasma BChE activity were investigated in Thai community-dwelling patients. The distributions of the genotype and allele frequencies of the BChE-K gene in both MCI patients and controls were in Hardy–Weinberg equilibrium. These results regarding the K allele and genotype distribution are in accordance with previous reports.^{11,12} In relation to the BChE polymorphism, our controls (12%) showed a frequency of the K allele and genotype distribution that is similar to British,¹³ Chinese,¹⁴ Finnish,¹⁵ Japanese,¹⁶ Korean,¹⁷ Northern Irish,¹⁸ Northern Spanish¹¹ and the US¹⁹ populations (9%–18%). On the contrary, patients from Australia,²⁰ the Canary Islands,¹² Turkey²¹ and the USA²² have shown a higher K allele frequency (23%–32%), while patients from Brazil²³ have a lower reported K allele frequency (2%). The K allele frequency shows a considerable ethnic variation.

The distribution of the BChE-K allelic frequency in the Thai community-dwelling patients was found to differ significantly in MCI patients (5.5%) compared with control (12%), suggesting that the BChE -K might be associated with a protective role for cognitive decline in MCI patients. So far, there has been no report on the association between the BChE-K and MCI in Thai population. However, the association of the BChE-K with AD is controversial. Some researchers^{18,32–35} found association between the BChE -K and AD, whereas others^{17,36} found no association. Alvarez-Arcaya et al¹¹ and Bizzarro et al³⁷ suggested a protective role of BChE -K. The correlation of BChE-K with MCI as well as AD is different between different ethnic groups. Thus, BChE-K may be either

Table 3 BChE-K genotype, gender and BChE activity in MCI patients and controls

Subjects	n	BChE activity (U/L)		
		WT/WT	WT/K	K/K ^a
Control	100	5,065±1,227	4,845±1,085	1,776, ^b 3,047 ^b
Female	67	5,100±1,023	4,898±1,260	–
Male	33	4,979±1,640	4,767±830	1,776, ^b 3,047 ^b
MCI	100	5,098±1,170	4,321±998	2,184 ^b
Female	74	5,193±1,011	4,598±1,023	–
Male	26	4,766±1,596	4,099±1,034	2,184 ^b

Notes: Data are presented as the mean ± SD. ^aNumber of K/K homozygotes in controls =2 and MCI patient =1. ^b $P<0.05$ compared with WT/WT. WT/WT, wild type of BChE gene; WT/K, BChE-K heterozygotes; K/K, BChE-K homozygotes.

Abbreviations: BChE, butyrylcholinesterase; BChE-K, butyrylcholinesterase K variant; MCI, mild cognitive impairment; n, number; SD, standard deviation.

a risk or a protective factor in AD.³⁸ Furthermore, the presence of the $\epsilon 4$ allele of apolipoprotein E (ApoE- $\epsilon 4$) is associated with an increased A β deposition and neurofibrillary tangle formation in the brain before the onset of dementia. The ApoE- $\epsilon 4$ allele is implicated as a risk factor associated with rate of cognitive decline in AD.³⁵ Several studies have demonstrated a positive association between ApoE- $\epsilon 4$, BChE-K allele and AD,^{32–35} whereas others have shown no relationship between the presence of these genes and the disease.^{15,17,18} This discrepancy may also reflect different distribution of BChE-K variant in different ethnic populations. The association of BChE-K and ApoE- $\epsilon 4$ allele in Thai community-dwelling patients warrants further investigation.

In addition, the progression from MCI to AD is influenced by the BChE genotype and gender.^{39,40} In 2013, Lane and He⁴¹ reviewed the influence of BChE genotype and gender in AD. Male and female gender may differentially affect the progression of cognitive impairment, neurodegenerative processes and response to treatment. In Thai community-dwelling, females do not have homozygous for the BChE-K (Table 2), suggesting that females have higher possibility to develop MCI or AD than males. In agreement with AD epidemiology, females have higher risk than males of developing MCI.² In our population, the protective effect of BChE-K was restricted to males. In MCI patients and controls, the BChE activity of the heterozygous for the BChE-K and the homozygous WT are not statistically different between males and females (Table 3). The homozygous for the BChE-K was associated with low BChE activity in males.

Genotyping for BChE-K is probably useful to predict which AD patients would favorably respond to BChE inhibitor. Thus, BChE inhibitor should not be prescribed to male AD patients who are homozygous for the BChE-K since they already have low BChE activity. However, Thai female AD patients are foreseeable to respond to BChE inhibitor since they have normal BChE activity.

Conclusion

The prevalence of MCI in the Thai community-dwelling patients was higher in females than males. The BChE-K may be implicated as a protective factor for MCI, although further study with a large sample size is warranted to confirm this.

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Author contributions

DP, NP, PA, PS, SU and SY participated in design experiments and recruitment of subjects and interpreted data. NP conducted genotyping, enzyme activity experiments and statistical analysis. AT, NP and PS were involved in the preparation of the manuscript. All authors read and approved the final manuscript, contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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