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In Thai Nationals, the ApoE4 Allele Affects Multiple Domains of Neuropsychological, Biobehavioral, and Social Functioning Thereby Contributing to Alzheimer's Disorder, while the ApoE3 Allele Protects Against Neuropsychiatric Symptoms and Psychosocial Deficits

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Abstract

The apolipoprotein E epsilon 4 (ApoE4) allele is the strongest genetic risk factor for Alzheimer's disorder (AD) and is associated with semantic and episodic memory deficits. The aim of this study was to examine the associations between ApoE alleles (E2, E3, E4) and genotypes and neuropsychological tests, behavioral functions, and dementia symptoms as assessed using Consortium to Establish a Registry for Alzheimer's Disease (CERAD). This study included 60 patients with Alzheimer's disorder (AD), 60 with mild cognitive disorder (MCI), and 62 normal volunteers. ApoE4 carriers and individuals with E3/E4 and E4/E4 genotypes show an increased incidence of AD, but not MCI. ApoE4 carriers and especially E4/E4 homozygotes show a worse outcome on the CERAD total score, Blessed Dementia Scale, and Short Blessed Test and lower scores on the Verbal Fluency Test, Boston Naming Test, Constructional Praxis Recall, and Word List Memory, Recall, and Recognition. ApoE4 carriers and E4/E3 heterozygotes show higher scores on the Clock Drawing Test. ApoE4 carriers show a worse outcome on the CERAD clinical history scores of memory, language, personality, ADL, orientation, and social skills, while allele AopE3 carriers show better scores on activities of daily living (ADL) and social skills. ApoE3 carriers show lower total weighted, irritability/aggression, and behavioral dysregulation scores on the Behavior Rating Scale for Dementia. The results show that in Thai

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individuals, the presence of ApoE4 allele is accompanied by a multifarious decline in neurocognitive functions and behavioral features and that ApoE3 may convey protection against neuropsychiatric symptoms and a decline in social skills. ApoE4 and especially the E4/E4 genotype may affect multiple domains of cognitive, biobehavioral, and social functioning thereby contributing to AD phenomenology.

Keywords Dementia · Alzheimer · ApoE · ApoE4 · Cognition · CERAD · Behavior

Introduction

Alzheimer's disease (AD) is a neurodegenerative and neuroinflammatory brain disorder and the major cause of dementia [1, 2]. In the early phase, AD is characterized by a gradual decline in neurocognitive functioning including problems with learning and memory causing deficits in episodic and semantic memory, while some individuals also develop language difficulties, such as decreased word fluency [3–5]. When AD progresses, patients may experience more memory loss, apraxia, problems with language, communication, social skills, and executive functions, while it may become difficult to carry out activities of daily living (ADL), uphold social interactions, and cope with new situations [6]. At that stage, behavioral dysregulation and neuropsychiatric problems may be evident including vegetative, depressive, and psychotic symptoms, personality changes, aggression, and inertia [3–6]. In later stages of AD, patients are unable to communicate and recognize close family members.

The pathophysiological characteristics of AD comprise positive lesions, including neurofibrillary tangles, amyloid plaques, dystrophic neurites with tau protein, neuroinflammation and astrogliosis, and negative lesions, such as synaptic, neuropil, and neuronal loss causing more widespread brain damage [7–10]. These neuropathological processes, which appear first in the hippocampus, may start years before the onset of neurocognitive and neuropsychiatric symptoms.

The two most important unmodifiable risk factors for late-onset AD are aging and genetic factors. Genetically, the apolipoprotein E epsilon 4 (ApoE4) allele is the strongest genetic risk factor for AD. ApoE4 is overrepresented in AD with AopE4 carriers being at higher risk for both early-onset AD and late-onset AD (LOAD) as compared with AopE3 carriers, while the AopE2 allele may be protective against AD [11–14]. The ApoE gene consists as polymorphic E2, E3, and E4 alleles with worldwide frequencies of 8.4, 77.9, and 13.7%, respectively, with AD patients showing a 40% increase in ApoE4 allele frequency [15]. In Caucasians, two copies of the ApoE4 allele, namely E4/E4 (odds ratio = 14.9) and one copy of this allele, as in E2/E4 (odds ratio = 2.6) and E3/E4 (odd ratio = 3.2), increase risk for AD [15]. ApoE genotypes influence delivery of lipids to cells and deposition of amyloid-β (Aβ) thereby playing a role in brain lipid transport, amyloid-β aggregation, synaptic dysfunctions, neurodegeneration, and neuroinflammation [13].

During normal aging, the ApoE4 allele is associated with an age-related cognitive decline in multiple cognitive domains, including working, episodic, and semantic memory [13, 16–18]. In healthy middle-aged adults, the presence of ApoE4 coupled with increased systemic blood pressure is associated with lower cognitive performance before the onset of clinically significant memory impairments [19]. As such, the ApoE4 allele may be associated with an increased risk for mild cognitive impairment (MCI) [17]. MCI is defined as a decline in cognitive functions beyond that expected by age while not interfering with daily activities [20]. While individuals with MCI have an increased risk to develop AD, some regard MCI as a prodromal stage of AD although MCI may remain stable over time or even remit [20–22]. In African AD patients, ApoE4 was significantly associated with constructional praxis, but not with verbal fluency or immediate or delayed recall [23]. There is also some evidence that the ApoE4 allele may increase the risk for some behavioral deficits and neuropsychiatric symptoms in AD. For example, a review shows that ApoE4 is associated with an increased risk for psychosis and irritability, but not with other behavioral disturbances including depression, apathy, and agitation [24].

Nevertheless, the effects of the three polymorphic alleles ApoE2, ApoE2, and ApoE4 and their genotypes on neuropsychological and behavioral dysfunctions and clinical symptoms of dementia as measured with a standardized instrument such as the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [25] have been difficult to establish. Therefore, the aim of this study was to examine the associations between ApoE alleles and phenotypes and comprehensive tests of neuropsychological and behavioral functions and clinical dementia and biobehavioral symptoms as well.

Subjects and Methods

Participants

Participants with AD and MCI were recruited at the Dementia Clinic, Outpatient Department, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Normal controls were normal elderly caregivers of patients with dementia visiting the Dementia Clinic, senior Red Cross volunteers, community senior club members, and

individuals who visited the Health Check Up Clinic. AD and MCI patients and controls were recruited from the same catchment area, namely Bangkok province. All subjects underwent cognitive screening at baseline, including history taking, physical and neurological examination, mental state examination, neuropsychological tests, and laboratory tests. AD was diagnosed using the diagnostic criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [26], while other inclusion were (a) Thai Mental Status Examination (TMSE) score between 10 and 23 [27, 28] and (b) Thai version of Clinical Dementia Rating Scale (CDR) score between 1 and 2 [29]. MCI was diagnosed using the Peterson criteria [30], while other inclusion criteria were: (a) a TMSE score more than 23 and (b) CDR score equal to 0.5.

Exclusion criteria for AD patients were brain tumors, head trauma, epilepsia, neuro-inflammatory, and neurodegenerative disorders other than AD, including stroke, Parkinson's disease, and multiple sclerosis; immune-inflammatory disorders, including systemic lupus erythematosus, diabetes type 1, inflammatory bowel disease, rheumatoid arthritis, and chronic obstructive pulmonary disease, and other DSM-IV-TR axis-1 mental disorders other than AD and dementia, including schizophrenia, bipolar disorder, major depression, and substance use disorders. Magnetic resonance imaging of the brain was performed in all AD patients to rule out vascular dementia. The same clinical exclusion criteria were applied to MCI patients and controls who were also excluded if they had a diagnosis of dementia. A current depression was excluded using the Thai Geriatric Depressive Rating Scale (TGDS) > 12 [31]. Normal controls were additionally excluded for any disorder that may affect cognition or cognitive impairments. Individuals with abnormal blood test results including lower vitamin B12, abnormal thyroid tests, and BUN and a chest X-ray indicating infiltrations were also excluded. After considering inclusion and exclusion criteria, we recruited 62 healthy volunteers, 60 patients with MCI, and 60 AD patients.

All participants and 60 guardians of patients with MCI and 60 guardians of patients with AD gave written informed consent prior to participation in this study. The study was conducted according to Thai and international ethics and privacy laws. Approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (No. 359/56), which is in compliance with the International Guideline for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Methods

Clinical Assessments

A senior psychiatrist, neurologist, and neuro-psychologist, all specialized in dementia evaluated all participants using a semi-structured interview consisting of clinical history, diagnostic criteria, and interviews with informants (the participants' close relatives) and neurological and physical examinations. Subjects were interviewed and clinically assessed by one psychiatrist and one neurologist for diagnosis of dementia or MCI. All subjects were screened for cognitive impairment using the Mini-mental Examination—Thai version 2002 [27] and Montreal Cognitive Assessment Scale (MoCA)—Thai version [32]. The CDR was measured as a tool to estimate staging of dementia as to confirm the diagnosis of AD [29]. The psychiatrist and neurologist completed the semi-structured interview and clinical and physical examinations, while the CERAD Neuropsychological Assessment Battery (CERAD-NP) was completed by the neuro-psychologist, who was blinded to the clinical diagnosis.

In this study, we analyzed the clinical history (C1) items memory, language, personality ad behavior, orientation for time and place, ADL, social activities, judgment and problem solving, and other cognitive problems. We also completed data on C2, namely the Blessed Dementia Scale (BDS), C3: Behavior Rating Scale for Dementia (BRSD), C4: the Short Blessed Test (SBT), and C5: calculation, clock, and language. In this study, we used the total scores on the BDS and SBT and the BRSD subdomains, namely depressive features, psychotic features, defective self-regulation, irritability/agitation, vegetative features, and inertia/apathy. We also measured the CERAD-NP (CERAD-NB) Total Score [33] and analyzed all subdomains of the CERAD-NP. We used a new validated translation for use in a Thai population (submitted). The CERAD-NP subtests comprise the Verbal Fluency Test (VFT), assessing semantic memory, verbal productivity, and cognitive flexibility and Modified Boston Naming Test (BNT), measuring visual naming and confrontational word retrieval. The BNT includes three measures that vary according to frequency of use, and here, we use the total sum of those three measures. Mini-mental State Examination (MMSE), which tests concentration, orientation, language, memory, and ideatoric and constructional praxis. Here, we use the Thai-validated version 2002 (MMSE Thai) [27, 28]. Word List Memory, including three trials and measuring free recall memory, learning ability for new verbal information, and thus verbal episodic memory or immediate working memory. Word List Recall, Delayed, and True Recall, which measure the ability to recall and verbal episodic memory. Word List Recognition, which measures verbal episodic memory-discriminability or verbal learning recall recognition. Constructional Praxis Test and Recall, which measures visuoconstructive abilities and later recall task [34].

***APOE* Genotyping** Genomic DNA was extracted from peripheral blood leukocytes by standard procedures with a DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). DNA was amplified by using two primers, 5′-ACAG AATTCGCCCCGGCCTGGTACACAC-3′ and 5′-TAAG CTTGGCACGGCTGAAGGA-3′. Each amplification reaction contained 1 g of leukocyte DNA, 1 pmol/l of each primer, 10% dimethyl sulfoxide, and 0.025 units/pl of *Taq* polymerase in a final volume of 30 l. Each reaction mixture was heated at 95 °C for 5 min followed by 40 cycles of 95 °C for 60 s, 65 °C for 80 s, and 72 °C for 80 s with a final extension at 72 °C for 7 min. The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, USA) according to the protocols supplied by the manufacturer, and sent for direct sequencing to Macrogen Inc., Seoul, South Korea. We defined three APOE genotype groups: (a) the APOE2 group including subjects carrying E2/E2 or E3/E2 genotypes; (b) the APOE3 group including those carrying E3/E3 genotypes; and (c) the APOE4 group including those carrying the E4/E3 or E4/E4 genotypes.

Statistics

Analyses of variance (ANOVAs) were used to assess differences in continuous variables between the three study groups. The protected least significant difference (LSD) was used to examine the differences among group means. Analyses of contingence tables (X^2 test) were used to assess the associations between categorical variables. Multivariate general linear model (GLM) analyses were employed to check the effects of explanatory variables (e.g., diagnosis, ApoE alleles and genotypes, gender, age, etc.) on dependent variables, including the MMSE, CERAD total score and subscores, BDS and SBT total and subscores, and the BRSD total score and 6 subscores). We subsequently used tests for between-subject effects to delineate the univariate effects of the significant explanatory variables (e.g., ApoE alleles and genotypes) on the dependent variables. Binary regression analyses were used to delineate the significant explanatory variables (ApoE alleles and genotypes, etc.) that predict Alzheimer disorder as dependent variable (and no Alzheimer disorder as reference group). Consequently, we computed odds ratios with upper-lower 95% confidence intervals (CI) of the ApoE alleles and genotypes predicting Alzheimer disorder. Stepwise automatic multinomial logistic regression analysis was used to delineate the associations between the diagnostic groups (Alzheimer versus MCI; MCI versus controls; and Alzheimer versus controls) and the ApoE distributions and thus to examine possible differences between Alzheimer's disease and MCI and MCI and controls. All statistical analyses were performed using IBM SPSS windows version 22. Tests were two-tailed and a p value of 0.05 was used for statistical significance.

Results

Descriptive Statistics

Table 1 shows the demographic, clinical, and ApoE allelic and genotypic data in the three study groups. Patients with AD were somewhat older than those with MCI and normal controls and MCI subjects as compared with controls. There were no significant differences in the sex ratio between the three study groups. There were significant differences in education among the three study groups. Consequently, we have adjusted all statistical analyses concerning ApoE alleles and genotypes and neuropsychological testing for age, sex, and education. Table 1 also shows that there were highly significant differences in the MMSE and CERAD scores among the three study groups, both decreasing from normal to MCI and from MCI to AD. There were also highly significant differences in BDS and total weighted score (TWS) scales with higher values in AD as compared with controls and MCI patients. Table 1 also shows the ApoE allele and genotype distributions in the three study groups. There was a significant association between Apo4 allele and ApoE3/E4 genotype and the diagnostic categories with higher frequencies of the Apo4 allele and ApoE3/E4 genotype in those with AD but not MCI. We did not use a p-correction to assess the significance of the multiple univariate analyses in Table 1 because we used these results (and the correlation matrix between the variables) to define the relevant explanatory variables that were subsequently used as determinants of independent association with the diagnostic groups and neuropsychological testing/behaviors in the ultimate multivariate GLM analyses and logistic regression analyses.

Differences in ApoE among AD, MCI, and Controls

In order to delineate the differences in alleles and genotypes between AD, MCI, and controls, we have carried out a multinomial logistic regression analysis with the three study groups as dependent variables and the alleles and genotypes (analyzed in an automatic stepwise regression) and age, sex, and education (forced entry) as explanatory variables. Table 2 shows that there was a significant separation ($X^2 = 101.68$, $df = 8$, $p < 0.001$, Nagelkerke = 0.482) of the three study groups and that AopE4 (but not the other alleles or genotypes) was a significant explanatory variable ($X^2 = 11.90$, $df = 2$, $p = 0.003$). The apoE4 allele was significantly associated with AD (versus MCI and versus controls), while there were no significant differences between MCI and controls.

Table 1 Demographic, clinical, and ApoE data of 62 healthy controls (HC), 60 patients with mild cognitive impairment (MCI), and 60 patients with Alzheimer's disorder (AD)

Variables	HC (A)	MCI (B)	AD (C)	F/X^2	*df*	*p* value
Age (years)	68.0 (5.7) B, C	74.8 (6.3) A, C	78.8 (7.7) B, C	44.40	2/179	< 0.001
Sex (M/F)	11/51	16/44	19/41	3.22	2	0.200
Education (years)	12.4 (5.0) B, C	10.0 (5.5) A, C	6.9 (5.7) B, C	15.65	2/179	< 0.001
MMSE	27.5 (1.8) B, C	26.2 (2.3) A, C	16.6 (3.7) A, B	293.37	2/179	< 0.001
CERAD	84.3 (5.8) B, C	72.8 (11.3) A, C	36.8 (10.8) A, B	402.60	2/179	< 0.001
Blessed Dementia Scale	0.04 (0.14) C	0.42 (0.99) C	3.33 (2.42) A, B	86.64	2/179	< 0.001
Short Blessed Test	2.19 (2.89) B, C	5.72 (3.68) A, B	19.60 (4.97) A, C	356.96	2/179	< 0.001
Behavioral Rating Scale (TWS)	0.10 (0.39) C	1.30 (5.25) C	10.27 (10.94) A, B	37.44	2/179	< 0.001
Allele E2	0.104	0.083	0.050	A/B: 0.33	2	0.848
Allele E3	0.823	0.842	0.692	A/C: 16.57	2	<0.001
Allele E4	0.073	0.075	0.258	B/C: 14.86	2	< 0.001
E2/E2 (no/yes)	61/1	60/0	60/0	–	–	–
E2/E3 (no/yes)	52/10	51/9	57/3	4.27	2	0.118
E2/E4 (no/yes)	61/1	59/1	57/3	–	–	–
E3/E3 (no/yes)	20/42	17/43	29/31	5.85	2	0.054
E3/E4 (no/yes)	54/8	54/6	42/18	9.70	2	0.008
E4/E4 (no/yes)	62/0	59/1	55/5	–	–	–

Results are shown as mean (± SD)

F, results of analyses of variance; X^2 , results of analyses of contingency; MMSE, Mini-mental State Examination; CERAD, Consortium to Establish a Registry for Alzheimer's Disease

Differences in ApoE Between AD Patients and Other Subjects

Consequently, we have examined which genetic markers differentiated AD from subjects without AD (controls and MCI). Table 3 shows the results of binary logistic regression analyses with AD as dependent variable (and no AD as reference group) and with computation of the odds ratios after adjusting for age, sex, and education. Regression 1 shows that there was a significant association between ApoE4 allele (positively) and ApoE3 allele (inversely), but not ApoE2 allele, and AD. However, after forced entry of both allele types in one logistic regression analysis, we found that the ApoE4 allele (Wald = 6.10, $df = 1$, $p = 0.01$), but not the ApoE3 allele (Wald = 2.23, $df = 1$, $p = 0.132$), was significantly associated with AD. In Table 3, regression 2 shows the associations among four ApoE phenotypes and AD. We found significant associations between E2/E3 (protective) and E3/E4 and E4/E4 (both risk factors) and AD. Table 3 shows also the results of a regression analysis with ApoE alleles and genotypes and age, sex, and education as explanatory variables. We found that AD was strongly and positively associated with ApoE4 allele and ApoE4/E4 genotype ($X^2 = 72.43$, $df = 5$, $p < 0.001$, Nagelkerke = 0.457).

We have also examined the associations among the different CERAD tests (neuropsychological battery, behavioral scores, and clinical history) and AD diagnosis. Toward this end, we have carried out four different stepwise automatic binary regression analyses with AD as dependent variable and the CERAD Neuropsychological tests, SBT tests, BRSD scores, and clinical history total subscores as explanatory variables. Regression 4 shows that of the different neuropsychological tests of the CERAD, Word List Recall and Recognition best

Table 2 Results of automatic stepwise multinomial logistic regression analysis with Alzheimer disorder (AD) and mild cognitive impairments (MCI) as dependent variables and normal controls (NC) or MCI as reference group

Contrasts	Explanatory variables	Wald	*df*	*p* value	OR	CI 95%
AD → HC	Allele E4	5.42	1	0.020	4.03	1.25–13.00
MCI → HC	Allele E4	0.07	1	0.788	1.17	0.37–3.74
AD → MCI	Allele E4	9.96	1	0.002	4.72	1.80–12.37

Entered were E2, E3 and E4, all genotypes, age, sex, and education

OR, odds ratio; CI 95%, 95% confidence intervals with lower and upper limits

Table 3 Results of binary logistic regression analyses with Alzheimer's disorder (AD) as dependent variable

Regressions	Explanatory variables	Wald	*df*	*p* value	OR	CI 95%
1. ApoE alleles*	Allele E2	2.73	1	0.099	0.39	0.13–1.19
	Allele E3	7.05	1	0.008	0.11	0.02–0.57
	Allele E4	11.01	1	0.001	4.38	1.83–10.99
2. ApoEgenotypes*	E2/E3	4.55	1	0.044	0.23	0.06–0.96
	E3/E3	2.57	1	0.109	0.54	0.25–1.15
	E3/E4	3.89	1	0.049	2.56	1.01–6.50
	E4/E4	9.41	1	0.002	47.89	4.04–566
3. Best prediction*	Age	15.06	1	< 0.001	1.13	1.06–1.20
	Sex	5.69	1	0.017	3.21	1.23–8.34
	Education	13.29	1	< 0.001	0.86	0.80–0.93
	Allele E4	5.73	1	0.017	3.08	1.23–7.72
	E4/E4	5.20	1	0.023	19.62	1.52–253
4. CERAD Neuropsychological Tests	Word List Recall	14.27	1	< 0.001	0.28	0.15–0.54
	Word List Recognition	5.40	1	0.020	0.61	0.40–0.92
5. Short Blessed Test	Clock drawing	50.91	1	<0.001	6.04	3.69–9.90
6. Behavioral Rating Scale for Dementia	Irritability/aggression	9.98	1	0.002	1.81	1.25–2.61
	Vegetative symptoms	10.55	1	0.001	3.14	1.58–6.27
	Psychotic symptoms	5.83	1	0.016	2.05	1.15–3.68
7. Clinical history	Behavior dysregulation, total	6.97	1	0.008	34.68	2.49–482
	Orientation, total	8.51	1	0.004	15.45	2.45–97
	ADL, total	7.39	1	0.007	17.77	2.23–141
	Other cognitive dysfunctions, total	16.45	1	< 0.001	54.21	7.87–373

OR, odds ratio; CI 95%, 95% confidence intervals with lower and upper limits, CERAD, Consortium to Establish a Registry for Alzheimer's Disease; ADL, activities of daily living

*All result of binary logistic regression analysis with age, sex, and education as covariates

predicted AD ($X^2 = 200.84$, $df = 2$, $p < 0.001$, Nagelkerke = 0.931; 97.3% of all subjects were classified correctly with a sensitivity of 96.7% and a specificity of 97.5%). Regression 5 shows that of the three SBT tests, Clock Drawing is the single best predictor of AD ($X^2 = 84.53$, $df = 1$, $p < 0.001$, Nagelkerke = 0.531; 85.4% of all subjects were classified correctly with a sensitivity of 64.3% and a specificity of 95.1%). Regression 6 shows that AD was significantly and positively associated with irritability/aggression and vegetative and psychotic symptoms ($X^2 = 67.29$, $df = 3$, $p < 0.001$, Nagelkerke = 0.430; 82.4% of all subjects were classified correctly with a sensitivity of 48.3% and a specificity of 99.2%). Regression 7 shows that AD was significantly and positively associated with behavioral dysregulation, orientation, ADL, and other cognitive symptoms ($X^2 = 186.51$, $df = 4$, $p < 0.001$, Nagelkerke = 0.907; 96.6% of all subjects were classified correctly with a sensitivity of 94.8% and a specificity of 97.5%). Finally, we found that 100% of all AD patients were correctly classified using ADL total score, Clock Drawing, Word List Recall, and irritability/aggression as explanatory variables ($X^2 = 151.43$, $df = 4$, $p < 0.001$, Nagelkerke = 1.000; results not shown as separate Wald tests and odds ratios cannot be computed).

ApoE4 and CERAD, MMSE, BDS, and SBT

In order to examine the associations between the MMSE, CERAD, BDS, and SBT and the genetic markers, we have carried out multivariate GLM analyses with the four rating scores as dependent variables and the alleles or genotypes as explanatory variables while adjusting for age, sex, and education. Table 4 shows that ApoE4 has a significant effect on the four rating scales, while age ($F = 8.87$, $df = 1/174$, $p < 0.001$) and education ($F = 11.03$, $df = 4/174$, $p < 0.001$), but not sex ($F = 2.07$, $df = 4/174$, $p = 0.086$), had significant effects. Tests for between-subject effects showed that ApoE4 had a significant effect on MMSE, CERAD, SBT, and BDS scale scores with a particularly strong effect on the first three. Parameter estimates showed that age was significantly related to the MMSE ($t = -4.02$, $p < 0.001$), CERAD ($t = -5.63$, $p < 0.001$), SBT ($t = +4.44$, $p < 0.001$), and BDS ($t = +4.36$, $p < 0.001$). Education was significantly associated with the MMSE ($t = +6.23$, $p < 0.001$), CERAD ($t = +6.14$, $p < 0.001$), SBT ($t = -4.74$, $p < 0.001$), and BDS ($t = -3.22$, $p = 0.002$).

Table 4 Results of multivariate GLM analysis with Mini-mental State Examination (MMSE), Consortium to Establish a Registry for Alzheimer's Disease (CERAD) total score, Blessed Dementia Scale (BDS), and the Short Blessed Test (SBT) as dependent variables

Type of test	Dependent variables	Explanatory variables	*F* value	*df*	*p* value
Multivariate 1	All 4 scores	Allele E4	4.73	4/174	< 0.001
Between-subject effects	MMSE total	Allele E4	18.34	1/177	< 0.001
	CERAD total	Allele E4	16.00	1/177	< 0.001
	BDS	Allele E4	8.49	1/177	0.004
	SBT	Allele E4	12.94	1/177	< 0.001
Multivariate 2	All 4 scores	E4/E4	5.17	4/173	0.001
		E3/E4	2.74	4/173	0.030
Between-subject effects	MMSE total	E4/E4	11.09	1/176	0.001
	CERAD total	E4/E4	18.93	1/176	< 0.001
	BDS	E4/E4	1.83	2/176	0.178
	SBT	E4/E4	12.61	1/176	< 0.001
Between-subject effects	MMSE total	E3/E4	11.03	1/176	0.001
	CERAD total	E3/E4	6.24	1/176	0.013
	BDS	E3/E4	4.14	1/176	0.043
	SBT	E3/E4	6.55	1/176	0.011

Model-predicted estimated marginal means (SE)

Tests	Allele E4		E4/E4		E3/E4	
	No	Yes	No	Yes	No	Yes
MMSE total	23.8 (0.4)	20.5 (0.7)	22.3 (0.5)	16.2 (1.9)	20.7 (0.9)	17.8 (1.3)
CERAD total	65.9 (1.6)	53.8 (2.8)	61.2 (1.8)	30.8 (7.2)	50.2 (3.6)	41.9 (4.8)
BDS	1.18 (0.17)	2.13 (0.31)	1.61 (0.20)	2.65 (0.79)	1.76 (0.40)	2.51 (0.53)
SBT	8.92 (0.65)	13.37 (1.15)	10.83 (0.75)	20.99 (2.94)	14.16 (1.49)	17.66 (1.96)

Table 4 shows also that ApoE3 ($F = 1.42$, $df = 1/173$, $p = 0.230$) and ApoE2 ($F = 0.70$, $df = 4/173$, $p = 0.591$) were not significant. ApoE4 coupled with age, sex, and education explained 40.3% of the variance in MMSE, 45.0% of the CERAD variance, 35.6% of the SBT, and 27.2% of the BDS variances. There were no significant interactions between ApoE4 × age ($F = 1.04$, $df = 4/173$, $p = 0.390$), ApoE4 × sex ($F = 0.61$, $df = 4/173$, $p = 0.655$), and sex × age ($F = 2.19$, $df = 4/173$, $p = 0.072$).

Table 4 shows that both the E4/E4 and E3/E4 genotypes had significant effects on MMSE, CERAD, BDS, and SBT, while E2/E2 ($F = 0.10$, $df = 4/172$, $p = 0.983$), E2/E3 ($F = 0.83$, $df = 4/172$, $p = 0.509$), E2E4 ($F = 1.17$, $df = 4/172$, $p = 0.326$), and E3/E3 ($F = 0.38$, $df = 4/172$, $p = 0.824$) had no significant effects. The ApoE4/E4 genotype explained 10.4% of the variance in the four rating scales, while ApoE3/E4 explained 4.9% of the variance. Tests for between-subject effects showed that ApoE4/E4 was significantly associated with MMSE, CERAD, and SBT, but not BDS, while ApoE3/E4 was significantly associated with the four scores. Table 4 shows also the model-predicted estimated mean values (SE) of the four rating scales obtained by the multivariate GLM analysis.

APOE and J (CERAD-NP) and C5 Subscale Scores

In order to examine the associations between the CERAD-NP subscales and the genetic markers, we have carried out multivariate GLM analyses with the CERAD-NP subscores (except MMSE) as dependent variables and ApoE alleles or genotypes as explanatory variables while adjusting for age, sex, and education. Table 5 shows that ApoE4 had a significant effect on the CERAD-NP subdomain scores. Tests for between-subject effects showed significant effects on all subscores, especially on Word List Memory, Word List Recall, Word List Recognition, and Recall Constructional Praxis. We have also examined the genotypes and found that only the APOE4/E4 genotype had significant effects on the CERAD-NP subscales. Test of between-subject effects showed that this genotype was associated with all CERAD-NP subscales, except constructional praxis.

Table 5 shows the effects of ApoE4 on the three C5 items. Multivariate GLM analysis showed a significant effect of ApoE4 on the three C5 items, while tests for between-subject effects showed that ApoE4 had a significant effect on clock drawing but not on calculation and expressive language. The ApoE3/E4 phenotype was the

Table 5 Results of two multivariate GLM analysis: (1) with the CERAD neuropsychological assessment measurements as dependent variables and (2) C5 CERAD items, i.e., calculation, clock drawing, and expressive language, as dependent variables

Type of test	Dependent variables	Explanatory variables	*F* value	*df*	*p* value
Multivariate 1	All 7 scores	Allele E4	2.59	7/171	0.015
Between-subject effects	Verbal Fluency Test	Allele E4	8.58	1/177	0.004
	Boston Naming Test	Allele E4	7.21	1/177	0.008
	Word List Memory	Allele E4	13.89	1/177	< 0.001
	Constructional Praxis	Allele E4	4.52	1/177	0.035
	Word List Recall	Allele E4	14.21	1/177	< 0.001
	Word List Recognition	Allele E4	14.35	1/177	< 0.001
	Constructional Praxis Recall	Allele E4	14.44	1/177	< 0.001
Between-subject effects	Verbal Fluency Test	E4/E4	10.65	1/173	0.001
	Boston Naming Test	E4/E4	9.09	1/173	0.003
	Word list Memory	E4/E4	15.36	1/173	< 0.001
	Constructional Praxis	E4/E4	0.86	1/173	0.350
	Word List Recall	E4/E4	19.73	1/173	< 0.001
	Word List Recognition	E4/E4	14.95	1/173	< 0.001
	Constructional Praxis Recall	E4/E4	10.69	1/173	0.001
Multivariate 2	C5 items	Allele E4	2.53	3/171	0.043
Between-subject effects	Calculation	Allele E4	0.29	1/173	0.589
	Clock Drawing	Allele E4	7.61	1/173	0.004
	Expressive Language	Allele E4	0.03	1/173	0.481
Between-subject effects	Calculation	E3/E4	1.63	1/173	0.209
	Clock Drawing	E3/E4	6.40	1/173	0.012
	Expressive Language	E3/E4	0.00	1/173	0.779
Model-predicted estimated marginal means (SE) after GLM analyses 1 and 2					
		No allele E4		Allele E4	
Verbal Fluency Test		15.8 (1.1)		13.1 (1.1)	
Boston Naming Test		9.7 (0.5)		8.9 (0.5)	
Word List Memory		15.0 (1.1)		12.3 (1.0)	
Constructional Praxis		9.6 (0.3)		8.8 (0.3)	
Word List Recall		4.7 (0.5)		3.3 (0.5)	
Word List Recognition		6.7 (0.5)		5.2 (0.5)	
Constructional Praxis Recall		5.2 (0.7)		3.2 (0.7)	
Calculation		0.578 (0.075)		0.658 (0.136)	
Clock Drawing		0.645 (0.079)		1.071 (0.143)	
Expressive Language		0.079 (0.022)		0.104 (0.040)	

only phenotype with significant effects on the three C5 items, while univariate tests showed a significant impact on clock drawing only. Table 5 shows the estimated marginal mean (SE) values in subjects with and without AopE4.

ApoE and BRSD

Table 6 shows that ApoE3 had a significant effect on the TWS and its six BRSD subscales (while adjusting for the effects of age, sex, and education) and explained 12.4% of the variance in the data. When entered together in the analysis, ApoE3 ($F = 2.75$, $df = 7/170$, $p = 0.010$) but not ApoE4 ($F = 1.51$, $df = 7/170$, $p = 0.168$) had a significant effect. Tests for between-subject effects showed that ApoE3 had a significant effect on TWS and the behavioral dysregulation and irritability/aggression subscale scores. Table 6 (see model-predicted estimated marginal means) shows that subjects with allele ApoE3 had significantly lower estimated marginal mean (SE) values of behavioral dysregulation and irritability and aggression subscale scores than those without ApoE3.

Table 6 Results of multivariate GLM analysis with the total weighted score (TWS) on the Behavior Rating Scale for Dementia (BRSD) and six behavioral subscales (depression, inertia, vegetative, irritability, behavioral dysregulation, and psychotic symptoms) as dependent variables

Type of tests	Dependent variables	Explanatory variables	*F* value	*df*	*p* value
Multivariate	TWS + all 6 subscales	Allele E3	3.45	7/171	0.002
Between subject effects	TWS	Allele E3	4.44	1/177	0.037
	Depressive symptoms	Allele E3	0.00	1/177	0.997
	Inertia subscale	Allele E3	2.46	1/177	0.118
	Vegetative symptoms	Allele E3	1.17	1/177	0.280
	Irritability aggression	Allele E3	7.76	1/177	0.006
	Behavioral dysregulation	Allele E3	10.54	1/177	0.001
	Psychotic symptoms	Allele E3	0.35	1/177	0.555
Model-predicted estimated marginal means (SE)					
		No allele E3		Allele E3	
Inertia subscale		2.693 (6.72)		0.801 (0.198)	
Behavioral dysregulation		1.145 (0.291)		0.191 (0.086)	

ApoE and Clinical History Obtained from Informants

Table 7 shows that there was a significant effect of ApoE3 and ApoE4 on the items of the clinical history scale. In this multivariate GLM analysis, ApoE3 explained 33.6% of the variance in the data and ApoE4 27.2%. Tests for between-subject effects showed significant protective effects of ApoE3 on three items, namely ADL-b (does the subject have difficulty remembering short list of shopping), social-b (has the subject lost special skills, interests or hobbies), and social-c (does the subject engage in socially inappropriate behavior or conversation). There was a significant negative impact of ApoE4 on memory-c (does the subject ask the same questions repeatedly), language-a (has the subject problems finding words in carrying a normal conversation), language-b (it is sometimes difficult to understand what he/she is talking about), personality-b (does the person ever see or hear things that are not there), orientation-b (does the subject forget approaching holidays, income tax days, etc.), ADL-c (does the subject has difficulties operating simple household appliances), social-a (does the subject participate in social or community functions less well), social-b (has the subject lost special skills), social-d (had the subject had impairment in job performance), problem solving-b (does the subject have difficulties relating to or understanding TV shows or newspaper articles), and others.

Table 7 Results of multivariate GLM analysis with the clinical history items as dependent variables

Type of tests	Dependent variables	Explanatory variables	*F* value	*df*	*p* value
Multivariate 1	All clinical items	Allele E3	2.49	26/128	< 0.001
		Allele E4	1.84	26/128	0.014
Between subject effects	ADL memorized items (2)	Allele E3	7.00	1/153	0.009
	Social hobbies (2)	Allele E3	4.31	1/153	0.040
	Social inappropriate (3)	Allele E3	13.81	1/153	< 0.001
	Frequently asking (3)	Allele E4	4.24	1/153	0.041
	Language (1)	Allele E4	4.35	1/153	0.039
	Communication (2)	Allele E4	7.85	1/153	0.086
	Personality (2)	Allele E4	11.37	1/153	0.001
	Forget the date (2)	Allele E4	5.92	1/153	0.010
	Judgment (3)	Allele E4	7.29	1/153	0.008
	Less interaction (1)	Allele E4	15.03	1/153	< 0.001
	Lost hobbies (2)	Allele E4	10.33	1/153	0.002
	Ability to work (4)	Allele E4	13.33	1/153	< 0.001
	Problem solving (2)	Allele E4	7.47	1/153	0.007
	Other cognitive	Allele E4	6.38	1/153	0.013

Discussion

The first major finding of this study is that the ApoE4 allele had strong effects on many domains of neuropsychological functioning. Figure 1 summarizes the key findings of our study. Thus, ApoE4 is associated with defects in working memory, episodic memory, semantic memory, recall, executive functions, naming, and praxis (marginally), while ApoE4 carriers show significantly more clinical symptoms of neurocognitive dysfunction with language and communication problems. Moreover, the results show that 100% of all AD patients versus MCI/control subjects were correctly classified using a combination of episodic memory decline (Word List Recall Test), executive cognitive dysfunction (Clock Drawing Test), irritability and aggression (BDRS), and lowered ADL (clinical symptom on C1). As such, ApoE4 plays a role in many domains of cognitive and biobehavioral functions and thus also in AD. This explains that the AopE4 allele and the E4/E4 and E4/E3 genotypes are significantly and positively associated with AD. Notwithstanding the significant inverse association between the E2/E3 genotype and AD, the ApoE2 or ApoE3 alleles or the E2/E4 and E3/E3 genotypes were not associated with cognitive dysfunctions.

These findings extend those of previous reports showing that ApoE4 is associated with an age-associated cognitive decline in working, episodic, and semantic memory [13, 16–18]. In clinically healthy persons, those with E3/E4 and E4/E4 genotypes performed worse on semantic memory and delayed free recall tasks as compared with ApoE3 and ApoE2 carriers [35, 36]. Matura et al. [36] concluded that the AopE4 allele promotes memory deficits and altered intrinsic functional brain network connectivity in normal people, while being the strongest genetic risk factor for Alzheimer's disease. Based on these results, some authors describe the association between the ApoE4 allele and cognitive decline as an "ApoE4 cognitive phenotype" [37].

Nevertheless, negative results were reported by Mount et al. [23] who were unable to find an association between the ApoE4 allele and immediate or delayed recall and verbal fluency. Such negative results may be explained by problems with sample selection techniques. For example, the latter authors examined associations between ApoE4 and cognitive functions in AD patients only and not in a combined group of non-demented patients, MCI, and AD patients. Thus, these authors computed association in a restricted range of cognitive functioning (only patients suffering from AD are included), which may artificially weaken existing correlation coefficients. On the other hand, we included a more complete range of cognitive dysfunctions from normal to disturbed. As such, our results extend those of a previous paper showing that in a cross-sectional study performed in elderly AD patients and control subjects, ApoE4 was associated with episodic memory, semantic memory, and working memory. Interestingly, in the latter study, the associations between cognitive decline and ApoE4 allele disappeared or were highly significantly reduced after adjusting for the pathological correlates of AD, including measures of neurofibrillary tangles and plaques [38]. These results show that the links between ApoE4 and cognitive decline may at least in part be explained by pathological hallmarks of AD. The association between ApoE alleles and neurocognitive dysfunctions is also observed in prospective studies. For example, in population-based prospective studies (3–5 years), ApoE4 carriers deteriorated cognitively, while those with E2/2 or E2/3 genotypes maintained their verbal learning performance [12, 39]. Interestingly, in healthy volunteers who developed subtle

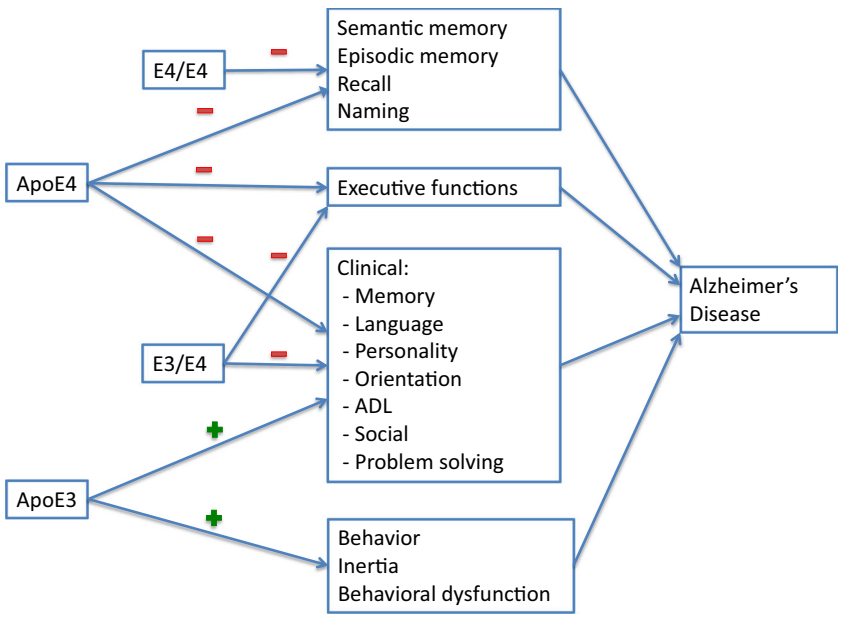

Fig. 1 Summary of the key findings of this study

cognitive decline, but not in cognitively stable subjects, ApoE4-associated gray matter loss was detected in brain areas vulnerable for neurodegeneration [40].

In our study, we did not find that the ApoE2 or E3 alleles had any significant effects on neurocognitive functions. Salo et al. were also unable to find an association between the ApoE2 allele and better cognitive performance [41]. Nevertheless, Martins et al. found that the ApoE2 allele is protective against cognitive decline [42], while the possession of one or more ApoE2 alleles may be associated with reduced decline in episodic memory [18]. In this respect, we detected that the E2/E3 phenotype may convey some protection against AD.

Moreover, we found that ApoE4 is not only associated with a decline in episodic and semantic memory but also in visual naming and confrontational word retrieval (Boston Naming Test). These findings are in agreement with a previous report showing that object and animal naming were significantly lowered in ApoE4 carriers [43]. Other results indicate that semantic fluency may serve as a prodromal marker for the onset of AD [43]. Another study showed an interaction between ApoE4 and age at onset on object naming in AD patients [44].

In our study, we were unable to observe interactions effects among ApoE allele × age.

Nichols et al. [45], on the other hand, concluded that aging and the ApoE allele may have interacting effects on the neural substrate of episodic memory. Salmon et al. [46] observed that age and an age × ApoE4 interaction were associated with a faster decline in memory, language, and executive functions over a 4-year period in ApoE4 carriers but not in ApoE4 individuals. Another study reported that age-associated decreases in regional glucose metabolism in the frontal and anterior cingulate areas are more pronounced in ApoE4+ AD patients [47]. While we could not detect a significant interaction between ApoE4 × education, education had a highly significant protective effect on AD. Shadlen et al. [48] found that lower education in ApoE4 homozygotes, but not heterozygotes, was accompanied by a significant 4-year cognitive decline. Also, Arenaza-Urquijo et al. [49] reported that education may counteract the effects of ApoEe4 on metabolism and that education is a protective factor, which may postpone cognitive decline in ApoEe4 carriers. A number of other papers showed that education may lower the risk of AD due to the ApoE allele by impacting gray matter volume and brain function [50–52]. Lower education is associated with a significant 4-year cognitive decline in ApoE4 homozygotes but not in ApoE4 heterozygotes [48].

While we did not observe significant effects of sex or an interaction between sex × ApoE4 on MMSE, CERAD, BDS, and SBT scores, some previous studies reported sex differences. For example, Lehmann et al. [53] reported that in ApoE4 homozygote men, there was a strong decline in episodic memory scores, while in women only a modest effect of ApoE4 was observed. Swan et al. [54] observed a significant ApoE4 × sex interaction for changes in short delay recall and a greater decline in executive functions in men and Trail making test performance in women. Moreover, the ApoE-ε4 risk for AD may be greater in females than males, especially in ApoE4 heterozygote women, while ApoE4 homozygote men may show an increased risk for AD [55]. These sex effects are in part explained by changes related to the transition from perimenopause to menopause leading to an increased risk profile in females [55].

A second finding of our study is that E4/E4 homozygotes showed a more profound cognitive decline as compared with E3/E4 heterozygotes. These results extend those of previous studies showing that ApoE4 homozygotes show a significantly accelerated decline in neurocognitive functions [56]. E4/E4 homozygosity may confer increased risk for cognitive decline (odds ratio = 3.1) as compared with E4 heterozygosity [57]. Individuals carrying two copies of the ApoE4 variant are at a significantly increased risk to develop AD [58], namely 10- to 15-fold compared with 2–3-fold in E4 heterozygotes [59]. In a 3.2-year follow-up study, memory ratings of immediate and delayed tests decreased 8.9 to 13.8% in E4/E4 homozygotes, but only 4.3% in E4 heterozygotes and 2.5% in ApoE4 non-carriers [60]. Caselli et al. [61] showed that E4/E4 homozygotes decline more quickly than ApoE4 non-carriers on the Wechsler adult intelligence scale-revised digit span and mental arithmetic tasks.

The third major finding of this study is that allele E3 conveyed some protection against irritability, aggression, and behavioral dysregulation and protected against a decline in ADL and social interactions. To the best of our knowledge, there are no other papers showing a protective activity of ApoE3 on biobehavioral measurements. These findings further substantiate recent reports that ApoE3 has neuroprotective activities [62]. For example, the ApoE3 allele plays a role in remodeling after neuronal injury, supports neuronal repair, displays antioxidant effects, and protects from neurodegeneration [62]. Finally, we found that allele E4 carriers show more personality changes, language, communication and judgment problems, and less social interactions and problem solving. Previously, no significant association was found between any of the Apo alleles and the Instrumental Activities of Daily Living score [63, 64] on the other hand, reported that the ApoE4 allele does not play a major role in personality changes. ApoE4 mice show a genotype-dependent lack of inhibitory control [65]. Although we found an inverse correlation between ApoE4 status and behavioral dysregulation, some previous studies, but not all, reported a significant positive correlation between the ApoE allele and delirium [66–69]. Moreover, our study was unable to find any significant association between ApoE alleles and depression and psychotic and vegetative symptoms. While previous research did not find an association between ApoE4 and depression [70], some reported significant associations with psychotic [24, 71, 72] and vegetative [73, 74] symptoms.

In our study, we were unable to detect any significant association between the ApoE4 allele and MCI. In fact, the frequency of the ApoE4 allele was the same in MCI and control individuals, this notwithstanding the presence of mild cognitive dysfunctions in MCI patients as compared with controls, including lowered MMSE and higher SBT and BRSD scores. This suggests that ApoE4 does not play a major role in the cognitive symptoms present in MCI. Caselli et al. [75], on the other hand, observed that, in a cohort with mean age of 60 years, memory declined in ApoE4 carriers before the symptomatic presentation of MCI. Boyle et al. [17] found that the ApoE4 allele is associated with MCI in community-dwelling older persons. Our negative results could maybe be attributed to differences in MCI diagnostic criteria among studies or the heterogeneity of MCI. For example, a further subdivision of our MCI sample in subgroups may be relevant, including single and multiple domain amnestic MCI, which is accompanied by cognitive decline and is probably an earlier stage of AD, and non-amnestic MCI [76]. Nevertheless, the frequency of ApoE4 in Italian subjects with MCI is 11% which is only slightly higher than that in controls (4%), while in AD patients it was 40.5% [77].

In summary, the results show that in Thai individuals, the presence of ApoE4 allele is accompanied by a multifarious decline in neurocognitive functions, including semantic and episodic memory, recall and naming, and constructional praxis, while ApoE4 may additionally affect multiple domains of social functioning, including language, communication, problem solving, and social skills, while causing changes in personality. The ApoE3 allele may perhaps convey protection against neuropsychiatric symptoms and the decline in social skills, but not neurocognitive functions.

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