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RESEARCH ARTICLE

Longitudinal analysis of *APOE-ε4* genotype with the logical memory delayed recall score in Alzheimer's disease

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Abstract. No study has focussed on the longitudinal effect of *APOE-ε4* genotype on the logical memory delayed recall total (LDELTOTAL) score in late-onset Alzheimer's disease (AD). The LDELTOTAL scores were collected at baseline, 12, 24, 36 and 48 months from 382 participants with AD, 503 with cognitive normal (CN), 1293 with mild cognitive impairment (MCI) in the Alzheimer's Disease Neuroimaging Initiative (ADNI). A linear mixed model (LMM) was used to investigate the effect of *APOE-ε4* on the longitudinal changes in the LDELTOTAL scores adjusted for age, gender, education and baseline Mini Mental State Examination score. There were significant differences in LDELTOTAL scores among AD, CN, and MCI ($P < 0.0001$) and among *APOE-ε4* alleles at baseline ($P < 0.0001$). In the multivariable LMM, elders with 75+ years ($P = 0.0051$), females ($P < 0.0001$), lower education ($P < 0.0001$), AD and MCI (both P values < 0.0001) were associated with decreased LDELTOTAL values, while the individuals with 1 or 2 *APOE-ε4* allele revealed significantly lower LDELTOTAL scores (both P values <0.0001) compared with individuals without *APOE-ε4* allele. Further, *APOE-ε4* alleles had significant interactions with four follow-up visits, where all follow-up visits showed significantly higher LDELTOTAL score. In addition, gender showed interaction with age, education and *APOE-ε4* with follow-up visits. Our findings provide the first evidence of the effect of *APOE-ε4* genotype on the logical memory declines related to AD. Further, *APOE-ε4* alleles showed interactions with gender and follow-up visits.

Keywords. Alzheimer's disease; longitudinal study; logical memory; *APOE-ε4*; mixed model; gender difference.

Introduction

Alzheimer's disease (AD) is a progressive, degenerative disorder resulting in loss of memory at first, and eventually affecting all cognition and behaviour (El Haj *et al.* 2016; Lipnicki *et al.* 2017). About 5.8 million Americans of all ages are estimated to live with Alzheimer's dementia in 2019 (Alzheimer's Association 2019). Worldwide, 36 million people were living with dementia in 2010, which was

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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predicted to rise to 66 million by 2030 and to 115 million by 2050 (Prince *et al*. 2015).

Almost 50% to 80% of the AD has been associated with genetics cause according to twin and family studies (Gatz *et al.* 2006; Wingo 2012). The Apolipoprotein (*APOE*) gene located at 19q13.32 (Olaisen *et al.* 1982; Das *et al.* 1985; Lusis *et al.* 1986) encodes an apolipoprotein E, a glycoprotein, which is highly expressed in the brain, transports cholesterol and lipoproteins in link to memory (Mattson and Arumugam 2018). *APOE-ε4* is the most significant genetic risk factor for accelerated cognitive decline and AD (Wisdom *et al.* 2011; Oliveira and Lourenco 2016; Sibbett *et al.* 2018; Najm *et al.* 2019; Yamazaki *et al.* 2019). Further, the *APOE-ε4* allele has been associated with decline in verbal memory in middle-aged and older adults without dementia (Lavretsky *et al.* 2003) and episodic and working memory in healthy and cognitively normal older adults (Liu *et al.* 2010; Lim *et al.* 2012). Moreover, the *APOE-ε4* allele was associated with decreased interhemispheric resting-state functional connectivity, which was attributed to memory performance in carriers (Luo *et al.* 2016) and contributed to poorer performance on a logical memory test in dementia cases (Sibbett *et al.* 2018). Other studies have found that *APOE-ε4* carriers perform worse on measures of memory than noncarriers while remaining clinically asymptomatic (Caselli *et al.* 2011; Wisdom *et al.* 2011). However, some studies found that *APOE-ε4* positivity does not correlate with memory decline (Kim *et al.* 2002; Bunce *et al.* 2004) and *APOE-ε4* does not influence memory abilities with verbal and spatial memory in a normal population of 70-year-old (Luciano *et al.* 2009).

The logical memory is a clinical measure of the episodic memory from the Wechsler Memory Scale-Revised (Wechsler 1987), which is widely used (Battista *et al.* 2017) and involves repeated verbal words learned, considered to be one of the best predictors for the conversion of mild cognitive impairment (MCI) to AD (Bondi *et al.* 2008; El Haj *et al.* 2016). Mixed-effects model including both fixed effects and random effects has been proposed to analyse longitudinal correlated data, for example in the effect of *APOE-ε4* on AD-related phenotypes (e.g., Manning *et al.* 2014; Mormino *et al.* 2014; Paranjpe *et al.* 2019). However, no study has been done on the longitudinal effect of the *APOE-ε4* genotype on the logical memory delayed recall total score (LDELTOTAL) in AD, MCI, and cognitive normal (CN) individuals. The aims of this study were to detect the longitudinal effect of *APOE-ε4* genotype on the LDELTOTAL among AD, CN and MCI individuals using linear mixed models (LMMs) and to test whether there is gender difference.

Materials and methods

Study subjects

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). The ADNI study began in 2004 as a multicentre that provides services to the United States and Canada. The ADNI is an ongoing, longitudinal, multicentre study designed to develop clinical, imaging, genetic and biochemical biomarkers for the early detection and tracking of AD. For this study, the merged data was used from several components of ADNI. The total sample size of the baseline data is 2178 including 382 with AD, 503 with CN, and 1293 with MCI.

Measures

Demographic variables included gender, age and educational levels. Gender was self-reported as either male or female. Age was classified into three groups: ≤ 65 years, 66–75 years and 76+ years. Race consisted of three subgroups: non-Hispanic White, non-Hispanic African American, and Hispanic. Years of education was classified into ≤ 12 years, 13–16 years, and 17+ years.

The Mini Mental State Examination (MMSE) was administered to provide a global measure of mental status, evaluating five cognitive domains: orientation, registration, attention and calculation, recall, and language (Cockrell and Folstein 1988). The logical memory (LM) test is a modified version of the episodic memory assessment from the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987)—one of the most widely used clinical measures of memory. Subjects were asked to recall a short story that consists of 25 pieces of information, both immediately after it was read to the subject, and after a 30-min delay (Hua *et al.* 2008). In our study, we used the LDELTOTAL reported in the ADNI database. The LDELTOTAL were measured at baseline, 12, 24, 36 and 48 months.

The data of *APOE-ε4* genotypes were extracted from the ADNI database. APOE genotyping was performed on DNA samples obtained from subjects' blood, using an APOE genotyping kit, the details are described in http://adni.loni.usc.edu/wp-content/uploads/2010/09/ADNI_GeneralProceduresManual.pdf. *APOE-ε4* carriers were defined as individuals with at least one ε4 allele (ε4/ε4 designated as *APOE-ε4*-2, ε4/ ε3 or ε4/ ε2 as *APOE-ε4*-1), while noncarriers were defined as individuals with no ε4 allele (*APOE-ε4*-0) (table 1).

Statistical methods

The categorical variables are presented in their raw counts along with the proportions for categorical variables and

Table 1. Descriptive statistics at baseline.

Variable	CN	MCI	AD	χ^2/F	*P*
Gender					
Male	236	707	219	11.71	0.0029
Female	267	586	163		
APOE-ε4					
0	357	644	116	159.01	< 0.0001
1	125	464	170		
2	13	110	72		
Age (years)					
≤ 65	19	203	47	79.49	< 0.0001
66–75	285	580	136		
76+	199	510	199		
Race					
White	449	1181	350	2.70	0.6092
AA	32	60	18		
Hispanic	22	52	14		
Education (years)					
≤ 12	50	55	89	46.52	< 0.0001
13–16	221	162	182		
17+	244	161	113		
MMSE					
Mean ± SD	29.08 ± 1.11	28.35 ± 1.54	23.17 ± 2.07	951.27	< 0.0001
LDELTOTAL					
Mean ± SD	13.34 ± 3.35	7.33 ± 4.52	1.35±1.84	999.35	< 0.0001

CN, cognitive normal; MCI, mild cognitive impairment; AD, Alzheimer disease; AA, non-Hispanic African American; MMSE, mini mental state examination; LDELTOTAL, logical memory delayed recall total score; SD, standard deviation. *P* value is based on chi-square test or *F* test in ANOVA.

Table 2. One-way ANOVA of LDELTOTAL scores by *APOE-ε4* status.

Visit	*n*	*APOE-ε4*-0 mean ± SD	*APOE-ε4*-1 mean ± SD	*APOE-ε4*-2 mean ± SD	*F*-value, *P*
Baseline (months)	2190	9.08 ± 5.21	6.62 ± 5.42	4.36 ± 4.64	94.28, < 0.0001
12	1768	9.41 ± 5.91	6.06 ± 5.93	4.13 ± 5.13	88.84, < 0.0001
24	1370	10.37 ± 5.98	6.80 ± 6.55	4.15 ± 5.22	80.90, < 0.0001
36	827	10.21 ± 5.96	6.97 ± 6.40	3.60 ± 5.23	49.41, < 0.0001
48	695	11.43 ± 5.50	8.07 ± 6.68	4.87 ± 5.61	39.78, < 0.0001

SD, standard deviation. *P* value is based on *F* test in one-way ANOVA.

continuous variables were presented in the form of mean ± SD. Chi-square test was used to examine the associations of categorical variables with AD diagnostics, while one-way ANOVA was performed to determine the differences in continuous variables among AD diagnostics.

The multivariable LMMs including *APOE-ε4* as fixed effect and subject as random effect was used to examine the longitudinal changes in LDELTOTAL as a continuous trait adjusting for age, sex, race, education and MMSE. To investigate whether the association between *APOE-ε4* status and LDELTOTAL differs at different years, the *APOE-ε4* × follow-up visit interaction was created and tested. To test the effects of gender, interaction terms between gender and each of other factors were added in the LMMs; especially a three-way interaction (gender × *APOE-ε4* × visit interaction) was included. To examine gender differences of the associations, multivariable LMM stratified by gender was applied to adjust for potential risk factors. The repeated measures longitudinal analyses were performed using PROC MIXED in SAS 9.4. All statistical analyses were performed using SAS (v.9.4).

This multicentered research project was approved by institutional review boards at each site and has obtained authorized written informed consent from participants (http://adni.loni.usc.edu/). There was an Institutional Review Board exemption for current study due to secondary data analysis.

Data availability statement

The data were downloaded from the ADNI database (http://adni.loni.usc.edu/). Application for access to the ADNI data can be submitted by anyone at http://adni.loni.usc.edu/data-

samples/access-data/. The process includes completion of an online application form and acceptance of Data Use Agreement. All data used in the study were downloaded from ADNI in May 2020.

Results

Baseline descriptive statistics

There are significant associations of diagnostic status with gender, *APOE-ε4*, age group, and education levels ($P = 0.0029$, < 0.0001, < 0.0001 and < 0.0001, respectively) (table 1). There was no difference in diagnosis among the three racial groups. Further, one-way ANOVA revealed significant differences in MMSE and LDELTOTAL among AD, CN and MCI (all P values < 0.0001); while AD group has lower mean values in the MMSE and LDELTOTAL than those in CN and MCI (table 1).

Cross-sectional differences in LDELTOTAL by APOE-ε4

One-way ANOVA revealed significant differences in LDELTOTAL among *APOE-ε4* alleles at baseline and four years follow-up (all P values <0.0001) (table 2). Further, the individuals with 1 or 2 *APOE-ε4* allele revealed lower LDELTOTAL scores compared with individuals without *APOE-ε4* allele (figure 1).

Three-way interaction of gender × APOE-ε4 × visit

Gender had significant interactions with age group ($P = 0.0482$), education levels ($P = 0.0192$), and APOE-ε4 and visits ($P = 0.001$) (data not shown). The results of three-way interaction (gender × *APOE-ε4* × visit interaction) are presented in table 3. A graphical display of three-way interactions was further shown by two-way interactions between *APOE-ε4* and visit separately for male and female (figure 2, a&b).

Table 3. Gender × *APOE-ε4* × visit interaction with the LDELTOTAL scores.

Variable	β ± SE	t-value, *P*
Visit * *APOE-ε4* * gender		
12 months*1*male	−0.83 ± 0.24	−3.48, 0.0005
12 months*1*female	−0.48 ± 0.26	−1.84, 0.0663
12 months*2*male	−0.33 ± 0.36	−0.90, 0.3693
12 months*2*female	−1.00 ± 0.44	−2.27, 0.0232
Visit * *APOE-ε4* * gender		
24 months*1*male	−0.61 ± 0.27	−2.27, 0.0236
24 months*1*female	−0.61 ± 0.29	−2.08, 0.0380
24 months*2*male	−1.12 ± 0.43	−2.60, 0.0095
24 months*2*female	−0.60 ± 0.51	−2.17, 0.2425
Visit * *APOE-ε4* * gender		
36 months*1*male	−1.06 ± 0.35	−3.03, 0.0025
36 months*1*female	−0.84 ± 0.40	−2.12, 0.0345
36 months*2*male	−1.31 ± 0.57	−2.29, 0.0221
36 months*2*female	−1.89 ± 0.68	−2.77, 0.0057
Visit * *APOE-ε4* * gender		
48 months*1*male	−0.82 ± 0.37	−2.21, 0.0270
48 months*1*female	−1.04 ± 0.40	−2.56, 0.0104
48 months*2*male	−0.18 ± 0.63	−0.29, 0.7711
48 months*2*female	−1.40 ± 0.78	−1.79, 0.0738

Multivariable LMM analysis of LDELTOTAL scores

The longitudinal changes in LDELTOTAL were examined in the multivariable LMM adjusted for baseline

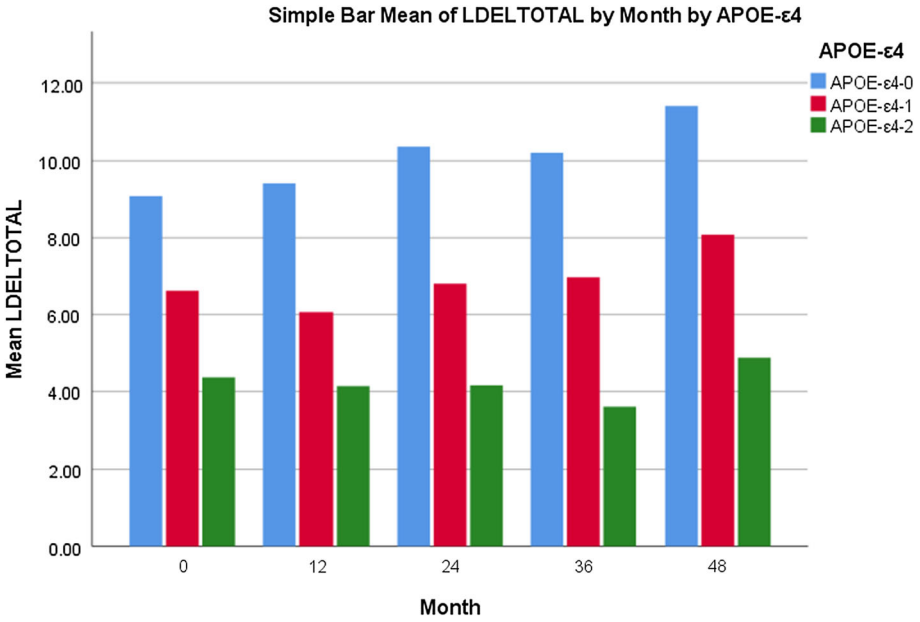

Figure 1. Logic memory delayed recall total score by follow-up visits and *APOE-ε4* alleles.

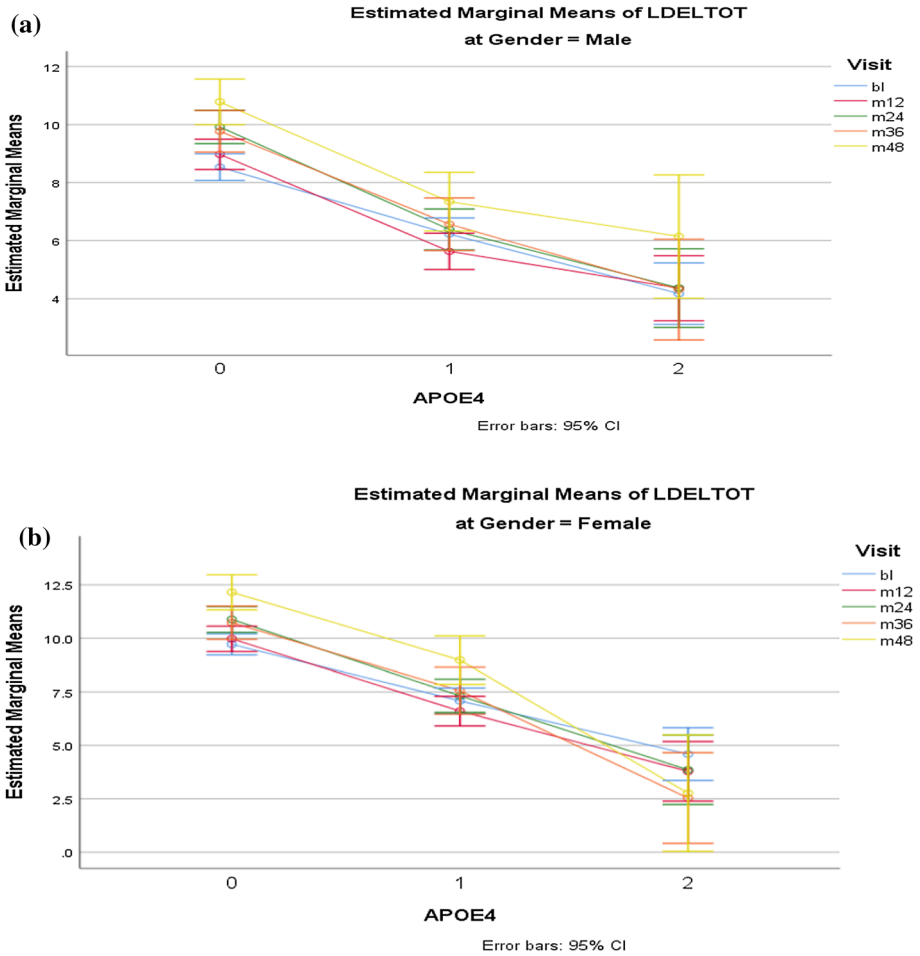

Figure 2. Gender × A*POE-ε4* × visit interaction for logic memory delayed recall total score. The *y* axis is the estimated marginal means of the logic memory delayed recall total score. The *x* axis is A*POE-ε4* alleles. Three-way interaction is expressed by two-way interaction between A*POE-ε4* and visit separately for male (a) and female (b).

characteristics such as age, gender, race, education, diagnostic status and MMSE (table 4). In the whole sample, all the independent variables except race were significantly associated with longitudinal changes in LDELTOTAL ($P < 0.05$). Particularly elders with 75+ years ($P = 0.0051$), gender ($P < 0.0001$), AD and MCI (both P values < 0.0001) were associated with decreased LDELTOTAL values. The individuals with one or two *APOE-ε4* alleles revealed significantly lower LDELTOTAL scores (both P values < 0.0001) compared with individuals without *APOE-ε4* allele, whereas individuals with higher education were associated with increased LDELTOTAL values ($P < 0.0001$). Further, there was significant interaction between *APOE-ε4* alleles and years of follow-up at years 1, 2, 3 and 4 (except for, at year 4 comparing *APOE-ε4*-2 with *APOE-ε4*-0) when one or two *APOE-ε4* alleles decreased LDELTOTAL scores.

Stratified by gender, 75+ years was associated with LDELTOTAL just in females ($P = 0.0024$), while there were gender differences in the interaction between *APOE-ε4* alleles and years of follow-up at years 1, 2, 3 and 4.

Discussion

In the present study, we conducted a longitudinal study of the association between *APOE-ε4* gene and LDELTOTAL scores. The results revealed that there were significant differences in LDELTOTAL scores among AD, CN, and MCI at baseline and among *APOE-ε4* alleles at baseline and a four-year follow-up. Further, the individuals with one or two *APOE-ε4* alleles revealed significant lower LDELTOTAL scores compared with individuals without *APOE-ε4* allele. The *APOE-ε4* gene was significantly associated with LDELTOTAL scores adjusting for age, gender, education, and MMSE; meanwhile significant interactions between *APOE-ε4* and time of follow-up were found. Additionally, gender showed interaction with age, education and *APOE-ε4* with follow-up visits.

Previous study found that the effect of *COMT* genotype on verbal declarative memory in a cohort of healthy 79 years old was independent of the effect of *APOE* genotype, and similar in effect size (Harris *et al*. 2005). One study reported significant interactions between Aβ and *APOE-ε4* status in

Table 4. Linear mixed model analysis of *APOE-ε4* gene with the LDELTOTAL scores.

Variable	Whole sample β ± SE	t-value, *P*	Male β ± SE	t-value, *P*	Female β ± SE	t-value, *P*
Gender (ref=male)						
Female	−0.74 ± 0.16	−4.73, < 0.0001	–	–	–	–
APOE-ε4 (ref=0)						
1	−0.79 ± 0.17	−4.62, < 0.0001	−0.65 ± 0.22	−2.98, 0.0030	−1.00 ± 0.27	−3.76, 0.0002
2	−1.81 ± 0.29	−6.35, < 0.0001	−1.54 ± 0.35	−4.35, < 0.0001	−2.26 ± 0.46	−4.88, < 0.0001
Age (ref= ≤ 65 year)						
66–75	0.46 ± 0.25	1.82, 0.0695	0.47 ± 0.35	1.34, 0.1820	0.52 ± 0.36	1.45, 0.1473
76+	−0.72 ± 0.25	−2.83, 0.0047	−0.40 ± 0.35	−1.15, 0.2514	−1.13 ± 0.37	−3.04, 0.0024
Race (ref = white)						
AA	−0.38 ± 0.38	−1.00, 0.3168	0.15 ± 0.58	0.26, 0.7958	−0.72 ± 0.50	−1.43, 0.1540
Hispanic	0.17 ± 0.42	0.40, 0.6871	−0.30 ± 0.60	−0.50, 0.6166	0.47 ± 0.59	0.80, 0.4255
Education (ref = ≤ 12 year)						
13–16	1.42 ± 0.23	6.21, < 0.0001	1.57 ± 0.31	4.98, < 0.0001	1.22 ± 0.33	3.67, 0.0003
17+	2.62 ± 0.23	11.21, < 0.0001	2.35 ± 0.31	7.55, < 0.0001	2.95 ± 0.35	8.30, < 0.0001
MMSE	0.33 ± 0.02	19.12, < 0.0001	0.33 ± 0.02	14.50, < 0.0001	0.34 ± 0.03	12.86, < 0.0001
Diagnostics (ref= CN)						
MCI (3)	−5.13 ± 0.19	−26.84, < 0.0001	−4.99 ± 0.25	−19.82, < 0.0001	−5.24 ± 0.29	−18.17, < 0.0001
AD (2)	−8.83 ± 0.28	−31.90, < 0.0001	−8.44 ± 0.36	−23.69, < 0.0001	−9.17 ± 0.43	−21.24, < 0.0001
Visit (ref= baseline)						
12 months	0.89 ± 0.11	7.84, < 0.0001	0.71 ± 0.15	4.71, < 0.0001	1.13 ± 0.17	6.60, < 0.0001
24 months	1.14 ± 0.13	9.10, < 0.0001	0.98 ± 0.17	5.70, < 0.0001	1.34 ± 0.18	7.35, < 0.0001
36 months	1.20 ± 0.16	7.43, < 0.0001	1.12 ± 0.21	5.21, < 0.0001	1.31 ± 0.25	5.35, < 0.0001
48 months	1.22 ± 0.17	7.31, < 0.0001	1.01 ± 0.22	4.54, < 0.0001	1.49 ± 0.25	5.92, < 0.0001
Visit * *APOE-ε4* (ref= m12*0)						
12 months*1	−0.67 ± 0.18	−3.77, 0.0002	−0.83 ± 0.24	−3.51, 0.0005	−0.47 ± 0.26	−1.80, 0.0719
12 months*2	−0.61 ± 0.28	−2.16, 0.0312	−0.32 ± 0.36	−0.89, 0.3711	−1.00 ± 0.45	−2.24, 0.0252
Visit * *APOE-ε4* (ref= m24*0)						
24 months*1	−0.60 ± 0.20	−3.04, 0.0024	−0.62 ± 0.27	−2.29, 0.0223	−0.60 ± 0.29	−2.04, 0.0417
24 months*2	−0.93 ± 0.33	−2.80, 0.0052	−1.11 ± 0.43	−2.57, 0.0102	−0.57 ± 0.51	−1.12, 0.2628
Visit * *APOE-ε4* (ref= m36*0)						
36 months*1	−0.96 ± 0.26	−3.63, 0.0003	−1.08 ± 0.34	−3.14, 0.0017	−0.81 ± 0.41	−1.97, 0.04491
36 months*2	−1.54 ± 0.44	−3.52, 0.0004	−1.30 ± 0.56	−2.35, 0.0190	−1.84 ± 0.71	−2.60, 0.0095
Visit * *APOE-ε4* (ref= m48*0)						
48 months*1	−0.91 ± 0.27	−3.33, 0.0009	−0.81 ± 0.36	−2.26, 0.0243	−1.02 ± 0.42	−2.45, 0.0145
48 months*2	−0.68 ± 0.49	−1.38, 0.1685	−0.19 ± 0.62	−0.31, 0.7559	−1.41 ± 0.81	−1.74, 0.0826

CN, cognitive normal; MCI, mild cognitive impairment; AD, Alzheimer disease; MMSE, mini mental state examination; LDELTOTAL, memory delayed recall total score; m12, m24, m36, and m48 refer to 12 months, 24 months, 36 months, and 48 months, respectively.

predicting change on both immediate and delayed logical memory scores in healthy individuals using LMM (Mormino *et al.* 2014). The joint presence of elevated SBP and *APOE-ε4* is associated with compromised memory function in midlife, when the risk for cognitive decline is otherwise minimal (Oberlin *et al*. 2015). However, *APOE-ε4* does not influence memory abilities with verbal and spatial memory in a normal population of 70-year-old (Luciano *et al.* 2009). A pilot study on heart failure patients also suggested no significant association of *APOE-ε4* alleles with delayed (recall) memory (Pressler *et al.* 2017). One study supported the role for *CR1* and *CLU* loci variants in influencing episodic memory in African Americans and Caucasians, respectively (Pedraza *et al.* 2014).

The mechanisms of *APOE-ε4* in AD pathogenesis and cognitive decline have been studied at molecular, cellular and brain circuitry levels, although it is probably not fully understood (Mattson and Arumugam 2018). *APOE-ε4* alleles are linked to aggregation of proteinpathies including tau, α-synuclein, and amyloid-β, the process of oxidative stress response and inflammation, dysregulation of synaptogenesis and lipid metabolism, leading to the loss of memory through the loss of neurons, for an example, loss of GABAergic interneurons in hippocampus (Oliveira and Lourenco 2016; Najm *et al.* 2019; Yamazaki *et al.* 2019). At anatomic connection-circuitry level, recent functional imaging studies suggest that right medial prefrontal cortex is associated with episodic memory and left prefrontal cortex is associated with semantic memory, along with medial temporal lobe. Functional connectivity appears to be compromised in healthy ageing individuals, but this decrease of connectivity is especially pronounced in AD with the specific network affected the most, such as default mode network (DMN), including prefrontal cortex, posterior parietal lobe, and

medial temporal lobe (MTL) (Dennis and Thompson 2014; El Haj *et al.* 2016). *APOE-ε4* gene variant/isoforms may affect this brain functional network connectivity in healthy older people and episodic memory performance in AD, through significant epistatic interaction with another memory gene KIBRA at the right medial prefrontal cortex, the posterior cingulate cortex, and the left superior frontal gyrus and angular gyrus in DMN (Wang *et al.* 2019). *APOE-ε4* carriers showed limited decreased functional connectivity between the two MTLs and orbital frontal cortex, which is correlated with the Wechsler memory scale-logical memory (WMS-LM), immediate and delayed recall tests scores (Luo *et al.* 2016). Our present study showed WMS-LM significantly declined in AD patients, providing the supporting evidence of episodic memory being impaired, less of semantic memory which is relatively preserved especially in mild AD (El Haj *et al.* 2016). There are several articles which concluded that *APOE-ε4* carriers are at higher risk of Alzheimer's diseases (Lim *et al.* 2012; Samieri *et al.* 2014). The present study is different from previous studies in several ways. First of all the Lim *et al.* (2012) and Samieri *et al.* (2014) used composite scores to prove the decline in episodic memory of *APOE-ε4* carriers in healthy adults.

The present research uses the raw scores and detects differences in LDELTOTAL scores among AD, CN, and MCI using a LMM model. In addition to LMM, the generalized estimating equation (GEE) model can be used to account for repeated measures in longitudinal studies (Liang and Zeger 1986). GEE models are generalized linear marginal models, which combine the generalized linear model for a non-normal residual with the repeated measures of a marginal model; while LMMs account for the fact that clustered observations are similar by estimating the variance among cluster means. Several publications had given valuable discussions about GEE models and mixed models (Gardiner *et al.* 2009; Hubbard *et al.* 2010; Wang 2014; McNeish and Harring 2017). Generally, there were no major differences in the parameters estimated between mixed models and GEEs (Alencar *et al.* 2012; Ma *et al.* 2012; Coelho *et al.* 2020). Specifically, there are some differences. First, GEE is a population-level approach based on a quasi-likelihood function and is useful for exploring overall average effects, whereas LMM is an individual-level approach by adopting random effects to capture the correlation between the observations of the same subject. Hence, LMM model should be used when subject-specific effects (in addition to overall average effects) are of primary interest (Gardiner *et al.* 2009; Hubbard *et al.* 2010; Ma *et al.* 2012; Wang 2014; Naseri *et al.* 2016; Coelho *et al.* 2020). Second, GEE is still valid even though a full likelihood solution is not available or the correlation structure is misspecified; however, GEE is an asymptotic method and requires larger sample sizes to provide correct standard error for fixed effect. Third, GEE is not robust to nonrandomly missing longitudinal data and not extendible to random slopes and three level models; whereas LMMs assumes only missing at random data and can obtain straightforwardly with the mixed, multilevel random-effects approach (Liang and Zeger 1986; DeSouza *et al.* 2009; Gardiner *et al.* 2009; Hubbard *et al.* 2010; Ma *et al.* 2012; Wang 2014; McNeish and Harring 2017). LMMs have been used to analyse longitudinal correlated data in the effect of *APOE-ε4* on AD-related phenotypes (e.g., Manning *et al.* 2014; Mormino *et al.* 2014; Sutphen *et al.* 2015; Dhilla Albers *et al*. 2016; Paranjpe *et al.* 2019; Baek *et al.* 2020). However, no study has been done on the longitudinal effect of the *APOE-ε4* genotype on the LDELTOTAL in AD. Because of the advantage that LMM has over GEE model, such as observing individual specific effect over population average, LMM was used to take individual features into account and treat subjects as random effects in this analysis of the longitudinal correlated data in the effect of APOE4 on AD-related phenotypes. To the best of our knowledge, this is the first study to investigate the association of *APOE-ε4* gene with LDELTOTAL scores among individuals with AD, MCI or CN using a LMM model.

There are several strengths in this study. First, the sample size in ADNI was relatively large for this type of study, while the ADNI is a longitudinal study. Second, our study uses the logic memory to study the association of *APOE-ε4* alleles with cognitive decline in AD. This memory test represents the core comprehensive episodic feature type of memory, which is lacking in other studies (El Haj *et al.* 2016). Third, we used the LMM model to detect the longitudinal effects of *APOE-ε4* on LDELTOTAL scores. Last, we detected interactions among gender, *APOE-ε4* alleles and follow-up visits.

There are some limitations in this study. First, the study group participants are heterogenous in age, for example from under 75 and over 75 years old. Studies have shown that factors for interpreting the WMS-R results can be different for elderly patients aged over 75 years old in a memory clinic setting such as paired associate memory, visual-and-working memory, and attention can be crucial factors for interpreting the WMS-R results of patients over 75 years old (Kinno *et al*. 2017). Additionally, age may affect this association of *APOE-ε4* allele association with verbal memory performance differently for persons below versus over 57 years old (Jochemsen *et al*. 2012). Our findings of *APOE-ε4* allele's association with logical memory may best fit to patients within certain age groups. Second, the study group included participants of both early onset and late onset AD patients. Further study can investigate whether there are differences how genetic risk factor *APOE-ε4* alleles affects early onset AD patients more significantly than the late onset AD patients in link to logical memory test, and whether other types of core episodic features of memory are associated with *APOE-ε4* alleles. Last, other neuropsychological measures for AD could be further studied, including CDRSB measuring cognitive impairment, modified preclinical Alzheimer cognitive composite with trails test (mPACCtrailsB), which determines performance of processing speed, and modified preclinical Alzheimer cognitive composite with

digit test (mPACCdigit) measuring working memory and MMSE, which is used to screen for dementia, its staging and involves registration, recall, and attention (Donohue *et al.* 2014, 2017; Insel *et al.* 2019; Nagaraj and Duong 2021). Considering a knowledge gap of APOE4 with LDELTOTAL score in AD, current study focussed on the LDELTOTAL score and included MMSE as a covariate using LMM model.

In conclusion, the results from present study revealed significant differences in LDELTOTAL scores among AD, CN, and MCI at baseline and among *APOE-ε4* alleles at baseline and a four-year follow-up. Specially, the individuals with one or two *APOE-ε4* alleles showed significant lower LDELTOTAL scores compared with individuals without *APOE-ε4* allele, meanwhile there were significant interactions between *APOE-ε4* and time of follow-up. Additionally, gender showed interaction with age, education, and *APOE-ε4* with follow-up visits. Using linear mixed model analysis, our findings provide the first evidence of the longitudinal effect of *APOE-ε4* genotype on the logical memory related to AD and the logical memory examination scores could be a good predictor for AD.

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