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APOE and BDNF polymorphisms moderate amyloid β -related cognitive decline in preclinical Alzheimer's disease

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ORIGINAL ARTICLE

APOE and *BDNF* polymorphisms moderate amyloid β -related cognitive decline in preclinical Alzheimer's diseaseYY Lim^{1,2,3}, VL Villemagne^{1,4,5}, SM Laws^{6,7,8}, RH Pietrzak⁹, PJ Snyder^{2,3}, D Ames^{10,11}, KA Ellis^{1,11}, K Harrington¹, A Rembach¹, RN Martins⁶, CC Rowe^{4,5}, CL Masters¹ and P Maruff^{1,12}

Accumulation of β -amyloid ($A\beta$) in the brain is associated with memory decline in healthy individuals as a prelude to Alzheimer's disease (AD). Genetic factors may moderate this decline. We examined the role of apolipoprotein E ($\epsilon 4$ carrier [$\epsilon 4^+$], $\epsilon 4$ non-carrier [$\epsilon 4^-$]) and brain-derived neurotrophic factor (*BDNF*^{Val/Val}, *BDNF*^{Met}) in the extent to which they moderate $A\beta$ -related memory decline. Healthy adults ($n = 333$, $M_{age} = 70$ years) enrolled in the Australian Imaging, Biomarkers and Lifestyle study underwent $A\beta$ neuroimaging. Neuropsychological assessments were conducted at baseline, 18-, 36- and 54-month follow-ups. $A\beta$ positron emission tomography neuroimaging was used to classify participants as $A\beta^-$ or $A\beta^+$. Relative to $A\beta^- \epsilon 4^-$, $A\beta^+ \epsilon 4^+$ individuals showed significantly faster rates of cognitive decline over 54 months across all domains ($d = 0.40$ – 1.22), while $A\beta^+ \epsilon 4^-$ individuals showed significantly faster decline only on verbal episodic memory (EM). There were no differences in rates of cognitive change between $A\beta^- \epsilon 4^-$ and $A\beta^- \epsilon 4^+$ groups. Among $A\beta^+$ individuals, $\epsilon 4^+/BDNF^{Met}$ participants showed a significantly faster rate of decline on verbal and visual EM, and language over 54 months compared with $\epsilon 4^-/BDNF^{Val/Val}$ participants ($d = 0.90$ – 1.02). At least two genetic loci affect the rate of $A\beta$ -related cognitive decline. $A\beta^+ \epsilon 4^+/BDNF^{Met}$ individuals can expect to show clinically significant memory impairment after 3 years, whereas $A\beta^+ \epsilon 4^+/BDNF^{Val/Val}$ individuals can expect a similar degree of impairment after 10 years. Little decline over 54 months was observed in the $A\beta^-$ and $A\beta^+ \epsilon 4^-$ groups, irrespective of *BDNF* status. These data raise important prognostic issues in managing preclinical AD, and should be considered in designing secondary preventative clinical trials.

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INTRODUCTION

In healthy individuals, high β -amyloid ($A\beta$) levels suggest that preclinical Alzheimer's disease (AD) has begun.^{1,2} However, variability in the extent of cognitive and clinical impairment in $A\beta^+$ healthy individuals suggests other factors influence $A\beta$ -related cognitive decline.^{3,4} The major genetic risk factor for sporadic AD is the apolipoprotein E (*APOE*) $\epsilon 4$ allele.^{5,6} apoE may be involved in AD pathogenesis directly, through increasing $A\beta$ accumulation, reducing clearance of $A\beta$ or modifying $A\beta$ -synaptic toxicity,^{5–7} or indirectly, through reducing synaptic plasticity, increasing neuroinflammation or affecting concurrence of cerebrovascular events.^{3,8} In accord with this, a recent study of 490 healthy individuals aggregated from the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study, the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the Harvard Aging Brain Study showed that carriage of the $\epsilon 4$ allele increased substantially the rate of memory decline in healthy individuals with high $A\beta$ levels ($A\beta^+ \epsilon 4^+$) over a median follow-up period of 1.5 years.⁹ This analysis also showed that individuals who were $\epsilon 4$ carriers but with low $A\beta$ ($A\beta^- \epsilon 4^+$) showed no memory decline compared with $\epsilon 4$ non-carriers with low $A\beta$ ($A\beta^- \epsilon 4^-$), suggesting that, by itself, the *APOE* $\epsilon 4$ allele is not associated with memory decline. However, as the *APOE* $\epsilon 4$ allele is

associated with increased cognitive decline in healthy individuals,¹⁰ and earlier diagnosis of AD,¹¹ the effect of *APOE* $\epsilon 4$ on $A\beta$ -related cognitive decline warrants further investigation over time intervals greater than 18 months.

Another strong genetic candidate for moderating $A\beta$ -related memory decline is the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism. *BDNF* is important in the biological basis of learning and memory in animals and humans.^{4,12–14} Prospective studies show that in healthy and mild cognitive impairment groups from both the AIBL and ADNI cohorts, *BDNF*^{Met} carriage is associated with faster $A\beta$ -related memory decline and hippocampal atrophy over 3 years but is unrelated to $A\beta$ accumulation,^{15–17} suggesting that *BDNF*Val66Met moderates the effects of $A\beta$ on synaptic integrity in preclinical AD.¹⁷ To our knowledge, no study has examined the interaction between *BDNF*, *APOE* and $A\beta$ -related memory decline.

The overarching aim of this study was to explore potential interactions between $A\beta$, *APOE* and *BDNF* on cognitive decline in 333 healthy individuals who had undergone $A\beta$ neuroimaging, genetic testing and 54-month clinical follow-up as part of the AIBL study. First, we examined whether episodic memory (EM) and other aspects of cognition would remain stable over 54 months in

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healthy Aβ[−]ε4⁺ participants, where any cognitive decline would provide an estimate of Aβ-independent effects of ε4. We then examined whether the Aβ⁺ε4⁺ group, compared with Aβ[−]ε4[−] healthy individuals, would show faster rates of decline in EM. Finally, we explored whether BDNF^{Met} moderated any relationship between Aβ, ε4 and cognition.

MATERIALS AND METHODS

Participants

Participants were recruited from the AIBL healthy adult group, the recruitment of which has been described previously.^{18,19} Briefly, exclusion criteria included the following: schizophrenia, depression (15-item Geriatric Depression Score ≥ 6), Parkinson's disease, symptomatic stroke, uncontrolled diabetes and alcohol use exceeding two standard drinks per day for women or four per day for men. Participants underwent medical, psychiatric and neuropsychological assessments at baseline, 18-, 36- and 54-month follow-up.¹⁹ At each assessment, a clinical review panel considered all available medical, psychiatric and neuropsychological information to classify clinical status.¹⁹ Clinical classification was blinded to neuroimaging results. Group demographic and clinical characteristics are provided in Table 1, with the number of participants whose diagnostic classification changed or who withdrew from the study shown in Figure 1. The study was approved by and complied with the regulations of three institutional research and ethics committees.¹⁹ All participants provided written informed consent.

Measures

Neuroimaging. Aβ imaging with positron emission tomography (PET) was conducted using either ¹¹C-Pittsburgh Compound B (PiB), ¹⁸F-florbetapir or ¹⁸F-flutemetamol. PET methodology has been described in detail previously.^{18,20,21} A 30-min acquisition was started 40 min post injection of PiB and a 20-min acquisition was performed 50 min post injection of florbetapir and 90 min post injection of flutemetamol. For PiB-PET, standardized uptake value (SUV) data were summed and normalized to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVr). The whole cerebellum was the reference region for florbetapir,²¹ while for flutemetamol the reference region was the pons. Consistent with these studies, SUVr was classified dichotomously as either negative (Aβ[−]) or positive (Aβ⁺). PiB studies were classified Aβ⁺ when SUVr ≥ 1.5,¹⁸ florbetapir, when SUVr ≥ 1.11^{ref. 21} and for flutemetamol when SUVr ≥ 0.62.²⁰ Aβ⁺ levels were further classified as being 'high' Aβ⁺ (SUVr PiB > 1.9; flutemetamol > 0.82; florbetapir > 1.29) or 'low' Aβ⁺ (SUVr PiB = 1.5–1.9; flutemetamol = 0.62–0.82; florbetapir = 1.11–1.29).^{22,23}

Genotyping. A blood sample was taken from each participant for genotyping. The BDNFVal66Met polymorphism (rs6265) was included in a custom Illumina GoldenGate assay, which included 1536 single-nucleotide polymorphisms, and was performed by the Beijing Genomics

Institute. Of the 333 healthy individuals who had undergone PET neuroimaging for Aβ, BDNFVal66Met data was available for 314 healthy individuals, of which 191 were BDNF^{Val/Val} homozygotes and 123 were BDNF^{Met} carriers (111 BDNF^{Met/Val} heterozygotes and 12 BDNF^{Met/Met} homozygotes).

Cognitive assessments. Composite cognitive scores were computed by standardizing outcome measures for each neuropsychological test against the baseline mean and s.d. for the Aβ[−] group. Standardized scores were averaged to form composite scores for verbal EM (Logical Memory delayed recall, California Verbal Learning Test, Second Edition [CVLT-II] long delay, CVLT-II d'); visual EM (Rey Complex Figure Test [RCFT] 3-min delayed recall, RCFT 30-min delayed recall, CogState One-Card Learning); executive function (CogState One-Back, Letter Fluency, Category Fluency Switching [Fruit/Furniture]); language (Category Fluency [Animals/Boys' Names], Boston Naming Test); and attention (Digit Symbol, CogState Detection, CogState Identification). The development and validation of each cognitive composite score has been described previously.^{23,24}

Data analysis

For each composite cognitive score, three planned comparisons were constructed using repeated-measures linear mixed-effects model with maximum likelihood estimation and an unstructured covariance matrix. Linear mixed modeling was employed because of its ability to model both fixed and random effects, which accounts for multiple sources of variability in longitudinal studies. In addition, both empirical and theoretical models of AD show that once the threshold for Aβ positivity is reached, there is a linear trend in cognitive decline, neurodegeneration and amyloid accumulation until a clinical diagnosis of AD is reached.^{1,2,25} In these analyses, Aβ status (Aβ[−], Aβ⁺), APOE status (ε4⁺, ε4[−]), time, APOE × Aβ interaction, APOE × time interaction, Aβ × time interaction and APOE × Aβ × time interaction were entered as fixed factors; participant as a random factor; age, premorbid intelligence and anxiety levels as covariates; and cognitive composite score as the dependent variable. Within the model, the magnitude of difference from the Aβ[−]ε4[−] group was expressed using Cohen's *d*.²⁶ Where the planned comparisons indicated differences between group trajectories, group means (95% confidence intervals (95% CIs)) for each assessment were estimated from the model and differences in these between Aβ⁺ε4[−] and Aβ⁺ε4⁺ groups at each assessment were determined by the extent of overlap between the 95% CIs associated with those means.

To examine the effect of BDNFVal66Met, separate linear mixed-effects models were conducted for each composite score in Aβ⁺ individuals. In these analyses, APOE status, BDNF status (BDNF^{Val/Val}, BDNF^{Met}), time, APOE × BDNF interaction, APOE × time interaction, BDNF × time interaction and APOE × BDNF × time interaction were entered as fixed factors; participant as a random factor; age, premorbid intelligence and anxiety levels as covariates and composite cognitive test score as the dependent variable. Within this model, rate of change over 18-month intervals in each group (ε4[−] BDNF^{Met}; ε4⁺ BDNF^{Val/Val}; ε4⁺ BDNF^{Met}) was compared with that

Table 1. Demographic and clinical characteristics of the full sample and of study groups

	Full sample (n = 333)	Aβ [−] ε4 [−] (n = 188)	Aβ [−] ε4 ⁺ (n = 61)	Aβ ⁺ ε4 [−] (n = 36)	Aβ ⁺ ε4 ⁺ (n = 48)	P-value
	N (%) or mean (s.d.)	N (%) or mean (s.d.)	N (%) or mean (s.d.)	N (%) or mean (s.d.)	N (%) or mean (s.d.)	
N (%) Female	173 (52.0%)	95 (50.5%)	33 (54.1%)	19 (52.8%)	26 (54.2%)	0.947
Age (years)	69.95 (6.80)	69.22 (6.28)	66.98 (5.20)	76.06 (7.27)	72.04 (7.03)	0.000
Premorbid IQ	108.59 (7.07)	108.51 (6.84)	106.98 (7.75)	111.47 (6.55)	108.75 (6.93)	0.000
HADS depression subscale	2.58 (2.24)	2.58 (2.26)	2.73 (2.11)	1.83 (1.42)	2.92 (2.70)	0.151
HADS anxiety subscale	4.20 (2.78)	4.18 (2.72)	4.12 (2.82)	3.26 (1.99)	5.10 (3.21)	0.026
CDR sum of boxes	0.04 (0.16)	0.04 (0.18)	0.04 (0.14)	0.06 (0.16)	0.02 (0.10)	0.777
MMSE	28.87 (1.19)	28.94 (1.18)	28.84 (1.23)	28.69 (1.26)	28.75 (1.14)	0.578
N (%) high Aβ ⁺	44 (13.2%)	n.a.	n.a.	18 (50.0%)	26 (54.2%)	0.705
N (%) progressed at 54 months	23/296 (7.8%)	11/178 (6.2%)	2/57 (3.5%)	3/26 (11.5%)	7/35 (20.0%)	0.020

Abbreviations: CDR, Clinical Dementia Rating scale; HADS, Hospital Anxiety and Depression Scale; MMSE, Mini Mental State Examination; PET, positron emission tomography; SUVr, standardized uptake value ratio. Bolded values are significant at the *P* < 0.05 or the *P* < 0.001 level; of the 333 healthy older adults who underwent PET neuroimaging, 183 were scanned using ¹¹C Pittsburgh Compound B, 76 using ¹⁸F florbetapir and 74 using ¹⁸F flutemetamol; high Aβ⁺ was classified when SUVr PiB > 1.9, flutemetamol > 0.82 and florbetapir > 1.29.

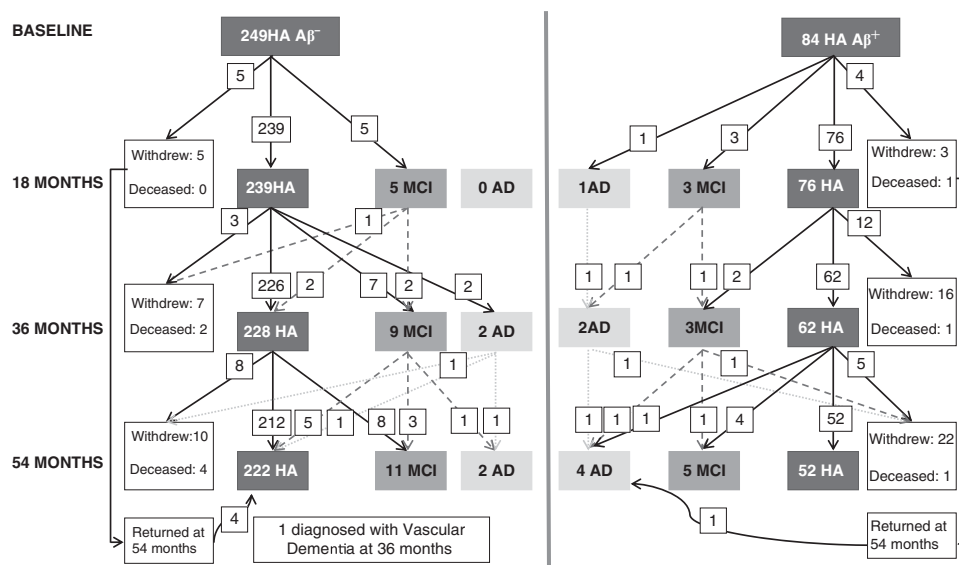


Figure 1. Clinical classification and disease progression of β -amyloid negative ($A\beta^-$) and β -amyloid positive ($A\beta^+$) participants over 54 months.

for the $\epsilon 4^-$ $BDNF^{Val/Val}$ group. For each comparison, the magnitude of difference from the $\epsilon 4^-$ $BDNF^{Val/Val}$ group was expressed using Cohen's d .²⁶ Group means (95% CIs) at each assessment were estimated from the linear mixed-effects model, and differences in performance between $\epsilon 4^+$ $BDNF^{Val/Val}$ and $\epsilon 4^+$ $BDNF^{Met}$ groups at each assessment was determined by the extent of overlap of 95% CIs associated with those means. Bonferroni correction was applied to all pairwise comparisons.

To estimate the clinical meaning for the effect of each genetic risk factor on decline in cognition, a group mean of 1.5 s.d. below the $A\beta^-$ $\epsilon 4^-$ group was defined as clinically important cognitive impairment.²⁷ The time to reach this criterion was estimated for each group based on linear mixed-effects model-derived linear functions.

RESULTS

Demographic and clinical characteristics

At baseline, statistically significant differences between groups were observed for age, premorbid intelligence and anxiety symptoms (Table 1). No other demographic or clinical characteristics differed between groups. There was no difference in the proportion of individuals in the $\epsilon 4^+$ or $\epsilon 4^-$ groups who were classified as high $A\beta^+$ (Table 1).

Effect of $A\beta$ levels and $\epsilon 4$ on cognitive change

Group mean slopes for each $A\beta$ - $\epsilon 4$ group for each composite cognitive score are summarized in Table 2. Relative to $A\beta^-$ $\epsilon 4^-$, the $A\beta^+$ $\epsilon 4^+$ group showed a significantly faster decline on all cognitive composites, with these differences moderate to large in magnitude (Table 2). Extrapolation of the rate of decline in verbal EM in the $A\beta^+$ $\epsilon 4^+$ group indicated it would meet criterion for clinically significant impairment (<1.5 s.d. from controls) in ~9 years (Supplementary Table 1). Compared with $A\beta^-$ $\epsilon 4^-$, the $A\beta^+$ $\epsilon 4^-$ group showed a faster rate of decline only for verbal EM composite (Table 2). Inspection of performance on each assessment indicated no overlap between 95% CIs for group mean verbal and visual EM composites between $A\beta^+$ $\epsilon 4^+$ and $A\beta^+$ $\epsilon 4^-$ groups at 18-, 36- and 54-month assessments (Supplementary Table 1). Extrapolation of the rate of verbal EM decline in the $A\beta^+$ $\epsilon 4^-$ group indicated it would meet criterion for clinically significant impairment in 27 years (Supplementary Table 1). Group mean slopes did not differ significantly for any cognitive composite between the $A\beta^-$ $\epsilon 4^-$ and $A\beta^-$ $\epsilon 4^+$ groups.

Effect of $BDNF^{Val66Met}$ on the relationship between $A\beta$, $\epsilon 4$ and cognitive change

In $A\beta^-$ participants, mean slopes between $\epsilon 4^-$ $BDNF^{Val/Val}$ and the three subgroups ($\epsilon 4^-$ $BDNF^{Met}$, $\epsilon 4^+$ $BDNF^{Val/Val}$ and $\epsilon 4^+$ $BDNF^{Met}$) did not differ for any other composite (data not shown).

In $A\beta^+$ participants, relative to the $\epsilon 4^-$ $BDNF^{Val/Val}$ group, the $\epsilon 4^+$ $BDNF^{Met}$ group showed a faster decline on the verbal and visual EM and language composites, and differences between slopes were moderate to large in magnitude (Table 3). Inspection of group means for individual assessments indicated no overlap between 95% CIs for the mean verbal and visual EM and language composite between $\epsilon 4^+$ $BDNF^{Met}$ and $\epsilon 4^+$ $BDNF^{Val/Val}$ groups at the 36- and 54-month assessments (Supplementary Table 1). The rate of verbal EM decline in the $\epsilon 4^+$ $BDNF^{Met}$ group indicated it met criterion for clinically significant impairment within 3 years from enrolment (Figure 2b). In contrast, extrapolation of the rate of verbal EM decline suggested that $\epsilon 4^+$ $BDNF^{Val/Val}$ group would meet criterion for clinically significant impairment within 10 years. Groups did not differ in the rate of decline on the executive function and attention composites (Table 3). Finally, relative to the $\epsilon 4^-$ $BDNF^{Val/Val}$ group, the $\epsilon 4^-$ $BDNF^{Met}$ group did not show a significantly faster decline on any cognitive composite.

DISCUSSION

EM and all other aspects of cognition remained stable over 54 months in $A\beta^-$ individuals, irrespective of $\epsilon 4$ status, which replicates and extends previous observations from AIBL^{23,28} and other cohorts.^{29–31} The absence of any $\epsilon 4$ -related cognitive decline in the current $A\beta^-$ individuals is also consistent with findings of a recent study⁹ and supports the hypothesis that there are no $A\beta$ -independent effects of APOE on cognitive decline in healthy individuals, even when studied over more than 4 years. Compared with the $A\beta^-$ $\epsilon 4^-$ group, $A\beta^+$ individuals showed faster decline in EM, and this decline was increased by $\epsilon 4^+$ (Figure 2a). Recently, the exacerbation of $A\beta$ -related memory decline by $\epsilon 4$ carriage in healthy individuals was shown over 1.5 years.⁹ The current findings support and extend this report by demonstrating that APOE $\epsilon 4$ carriage does exacerbate $A\beta$ -related cognitive decline in healthy individuals, and persists over more than 4 years. Neurologically, APOE can affect both intrinsic (for example,

Table 2. Mean slopes (s.d.) per 18-month interval for each cognitive composite score and magnitudes of difference (Cohen's *d*) in slopes

	Mean slope (s.d.)				Cohen's <i>d</i> (95% CIs) (vs Aβ [−] ε4 [−])		
	Aβ [−] ε4 [−] (n = 188)	Aβ [−] ε4 ⁺ (n = 61)	Aβ ⁺ ε4 [−] (n = 36)	Aβ ⁺ ε4 ⁺ (n = 48)	Aβ [−] ε4 ⁺	Aβ ⁺ ε4 [−]	Aβ ⁺ ε4 ⁺
Verbal EM	0.021 (0.239)	0.034 (0.206)	−0.075 (0.197)	−0.263 (0.206)	−0.06 (−0.34, 0.23)	0.41 (0.05, 0.77)	1.22 (0.88, 1.55)
Visual EM	0.026 (0.276)	0.030 (0.238)	−0.001 (0.229)	−0.198 (0.237)	−0.01 (−0.30, 0.27)	0.10 (−0.26, 0.46)	0.83 (0.51, 1.16)
Executive Function	−0.011 (0.220)	−0.003 (0.190)	−0.051 (0.180)	−0.103 (0.188)	−0.04 (−0.33, 0.25)	0.19 (−0.17, 0.54)	0.43 (0.11, 0.75)
Language	−0.033 (0.252)	−0.035 (0.217)	−0.086 (0.206)	−0.176 (0.216)	0.01 (−0.28, 0.30)	0.22 (−0.14, 0.57)	0.58 (0.26, 0.90)
Attention	−0.101 (0.201)	−0.125 (0.174)	−0.100 (0.164)	−0.180 (0.177)	0.12 (−0.17, 0.41)	−0.01 (−0.36, 0.35)	0.40 (0.08, 0.72)

Abbreviations: CI, confidence interval; EM, episodic memory. Bolded values are significant at the $P < 0.05$ or $P < 0.001$ level; values are adjusted for age, premorbid intelligence and anxiety.

Table 3. Mean slopes (s.d.) per 18-month interval for each cognitive composite score and magnitudes of difference (Cohen's *d*) in slopes in Aβ⁺ healthy individuals

	Mean slope (s.d.)				Cohen's <i>d</i> (95% CIs) (vs ε4 [−] BDNF ^{Val/Val})		
	ε4 [−] BDNF ^{Val/Val} (n = 19)	ε4 [−] BDNF ^{Met} (n = 11)	ε4 ⁺ BDNF ^{Val/Val} (n = 27)	ε4 ⁺ BDNF ^{Met} (n = 14)	ε4 [−] BDNF ^{Met}	ε4 ⁺ BDNF ^{Val/Val}	ε4 ⁺ BDNF ^{Met}
Verbal EM	−0.058 (0.341)	−0.046 (0.326)	−0.223 (0.451)	−0.400 (0.423)	−0.04 (−0.76, 0.69)	0.40 (−0.16, 0.96)	0.91 (0.19, 1.59)
Visual EM	0.039 (0.325)	−0.091 (0.315)	−0.146 (0.428)	−0.328 (0.407)	0.40 (−0.32, 1.12)	0.48 (−0.09, 1.03)	1.02 (0.29, 1.71)
Executive Function	−0.017 (0.262)	−0.087 (0.249)	−0.018 (0.345)	−0.181 (0.323)	0.27 (−0.46, 0.99)	0.00 (−0.55, 0.56)	0.57 (−0.12, 1.24)
Language	−0.063 (0.282)	−0.141 (0.269)	−0.130 (0.372)	−0.341 (0.349)	0.28 (−0.45, 1.00)	0.20 (−0.36, 0.75)	0.90 (0.18, 1.58)
Attention	−0.028 (0.218)	−0.143 (0.200)	−0.207 (0.279)	−0.134 (0.263)	0.54 (−0.21, 1.26)	0.70 (0.12, 1.26)	0.45 (−0.24, 1.12)

Abbreviations: CI, confidence interval; EM, episodic memory. Bolded values are significant at the $P < 0.05$ or $P < 0.001$ level; values are adjusted for age, premorbid intelligence and anxiety.

synaptic plasticity and neuroinflammation) and extrinsic (for example, cerebrovascular disease) factors.^{3,8,32} As all participants in the AIBL study have well-controlled risk factors for cardiovascular disease,¹⁹ the risk of concomitant cerebrovascular events over the period of observation was reduced. Further, as there is increasing experimental evidence that APOE isoforms have a direct effect on Aβ deposition, clearance and Aβ-mediated synaptotoxicity,^{5–7} a likely explanation for the cognitive decline seen in the Aβ⁺ε4⁺ group is that APOE moderates the direct effects of Aβ accumulation. These results differ from previous reports, where we and others found no effect of ε4 carriage on Aβ-related cognitive decline.^{23,28,30,33} The most likely reason for this lack of effect was that the smaller sample sizes used in these studies resulted in reduced power to detect any effects of ε4 on Aβ-related cognitive decline.^{23,28,30,33}

We reported previously that BDNFVal66Met moderated Aβ-related changes in cognition and neurodegeneration in both healthy older and mild cognitive impairment groups in AIBL and ADNI, although it did not moderate the rate of Aβ deposition.^{15–17} Consistent with evidence indicating that BDNF is necessary for synaptic plasticity in the hippocampus,⁴ we proposed that BDNFVal66Met affects the clinical manifestation of early AD by influencing the ability of the brain to tolerate Aβ toxicity.^{16,17} In the current study, we explored whether BDNFVal66Met moderated any relationship between Aβ, ε4 and cognition. The Aβ⁺ε4⁺BDNF^{Met} group showed faster decline in EM and language compared with both the ε4[−]BDNF^{Val/Val} and ε4[−]BDNF^{Met} groups (Figure 2b). The ε4⁺BDNF^{Val/Val} group showed a moderate rate of cognitive decline. Consistent with our previous observations,^{15–17} BDNFVal66Met, such as APOE, did not moderate any cognitive variable in Aβ[−] individuals.

These results raise some important prognostic issues in managing preclinical AD and the implications for communicating these group data to individuals. The data demonstrates clearly that the rate of cognitive decline in preclinical AD is moderated by the combination of at least two genetic loci: ε4⁺ and BDNF^{Met}. Aβ⁺ε4⁺ individuals showed significantly greater EM decline than Aβ⁺ε4[−] individuals and this difference became evident 18 months after enrolment. BDNF^{Met} carriage increased the rate of memory decline related to Aβ and ε4, with differences in memory between Aβ⁺ε4⁺BDNF^{Val/Val} and Aβ⁺ε4⁺BDNF^{Met} groups becoming evident 36 months after enrolment (Supplementary Table 1). Another way of expressing these observations is to consider the length of time between establishing an individual's Aβ, ε4 and BDNF status and a criterion for clinically significant cognitive impairment (performance < 1.5 s.d. below controls,²⁷ dashed horizontal line in Figure 2). In the Aβ[−] group, irrespective of ε4 status, there was no evidence of decline over 4.5 years (Figure 2a). In contrast, extrapolation of the EM decline observed in the Aβ⁺ε4⁺ group showed that clinically significant memory impairment would be met ~ 9 years after enrolment, while the Aβ⁺ε4[−] group would take ~ 27 years. Taking into consideration the additional effect of BDNF in Aβ⁺ individuals, the effect of BDNF is seen clearly in the ε4⁺ subgroup where BDNF^{Val/Val} homozygotes would meet criteria for clinically significant impairment after ~ 10 years (similar rate based on ε4⁺ status alone) but the accelerated rate of memory decline in the BDNF^{Met} group meant that they met this criteria after only 3 years (Figure 2b). Although there is some evidence in the model that even at baseline the Aβ⁺ε4⁺BDNF^{Met} group performs worse than the other three groups, this difference was not statistically significant. One limitation of natural history cohorts is that the baseline performance of each individual is

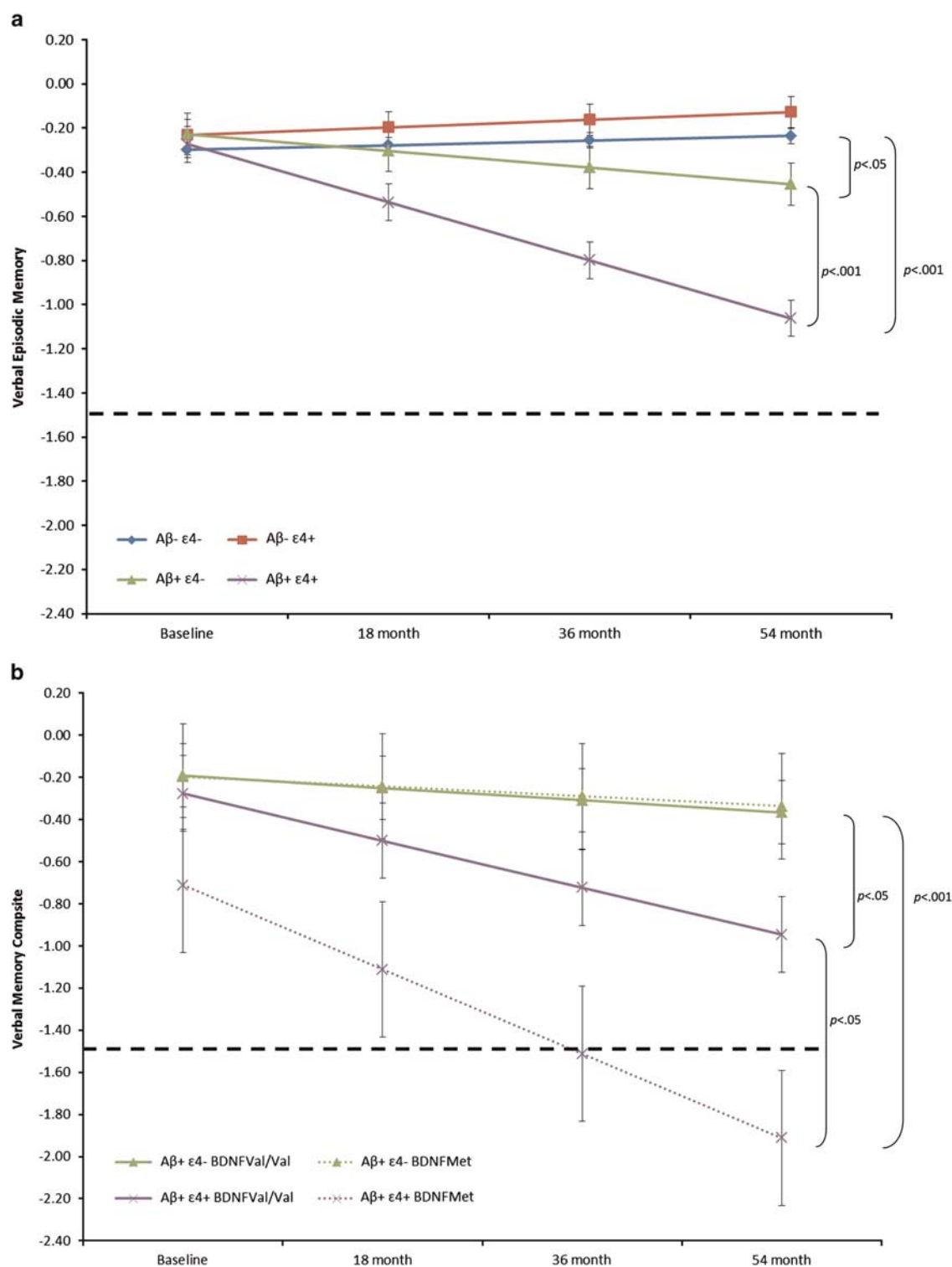


Figure 2. (a) Trajectories of change over 54 months on the Verbal Episodic Memory composite for A β ⁻ε4⁻, A β ⁻ε4⁺, A β ⁺ε4⁻ and A β ⁺ε4⁺ groups, with age and premorbid IQ as covariates (error bars represent 95% confidence intervals). Dotted line indicates 1.5 s.d. decline for clinically significant memory impairment. (b) Trajectories of change over 54 months in A β ⁺ healthy individuals on the Verbal Episodic Memory composite for ε4⁻/BDNF^{Val/Val}, ε4⁻/BDNF^{Met}, ε4⁺/BDNF^{Val/Val} and ε4⁺/BDNF^{Met} groups, with age and premorbid IQ as covariates (error bars represent 95% confidence intervals). Dotted line indicates 1.5 s.d. decline for clinically significant memory impairment.

defined by their first visit, rather than symptom onset. As such, the data presented here can be interpreted as suggesting that the combination of A β ⁺, ε4⁺ and BDNF^{Met} does accelerate cognitive decline significantly such that even at the first assessment this

decline is already evident. A diagnosis of mild cognitive impairment is typically made when objective evidence of clinically significant memory impairment is accompanied by individuals' acknowledgement of that impairment, usually corroborated by an

informant.²⁷ General cognitive function and functional activities are also typically preserved.²⁷ Over the course of this study, relatively few healthy individuals were classified as meeting clinical criteria for mild cognitive impairment/AD (Figure 1). This indicates that although some individuals met criteria for clinically significant impairment, these individuals, or their caregivers, had not acknowledged any problems with cognition. We have reported previously that subjective memory impairment in the AIBL healthy cohort does not predict objectively defined cognitive impairment or Aβ levels.³⁴ Presumably, individuals with clinically significant memory decline observed here will begin to report subjective memory complaints in the future.

An important caveat is that as three radioligands were used to measure Aβ, SUVr data could not be integrated to form a single continuous measure of Aβ burden. However, we found no relationship between the proportions of individuals who were high and low Aβ⁺ in the Aβ⁺ε4⁺ and Aβ⁺ε4⁻ groups, suggesting that the faster decline observed in Aβ⁺ε4⁺ was not due to more advanced disease at enrolment. Second, although the large number of healthy individuals who have undergone Aβ PET neuroimaging in the AIBL cohort has allowed for this report to investigate the effects of APOE and BDNF on the clinical manifestation of high Aβ, the resultant sample sizes remain relatively small. Further, the AIBL study is also not a representative population sample. Healthy participants in the AIBL study were highly educated, were of Caucasian backgrounds and had few existing or untreated medical, neurological or psychiatric illnesses. Participants selected for neuroimaging were also enriched for APOE ε4 carriers. As such, these results need to be replicated in other more representative and ethnically diverse prospective cohorts of healthy individuals. Finally, APOE and BDNF are unlikely to be the only factors that moderate the clinical manifestation of Aβ; rather, other co-morbidities (for example, cerebrovascular disease) and lifestyle and genetic factors will need to be considered in future work.

CONFLICT OF INTEREST

CLM is an advisor to Prana Biotechnology Ltd and a consultant to Eli Lilly. RHP and PJS are scientific consultants to Cogstate Ltd. PM is a full-time employee of Cogstate Ltd. DA has served on scientific advisory boards for Novartis, Eli Lilly, Janssen and Pfizer Inc. RNM is a consultant to Alzhyme. CCR has served on scientific advisory boards for Bayer Pharma, Elan Corporation, GE Healthcare and AstraZeneca, has received speaker honoraria from Bayer Pharma and GE Healthcare and has received research support from Bayer Pharma, GE Healthcare, Piramal Lifesciences and Avid Radiopharmaceuticals. VLV served as a consultant for Bayer Pharma and has received research support from a NEDO grant from Japan. All other authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

YYL, PM and CLM developed the concept and hypothesis for this study. DA, RNM, CLM, PM, KAE, VLV, AR and CCR are senior investigators of the AIBL study and are responsible for the design of the AIBL study and selection of study endpoints. VLV and CCR conducted and oversaw neuroimaging for all participants. YYL, KH and KAE conducted neuropsychological assessments. SML undertook all genetic analyses for all participants. YYL, PM and VLV conducted all statistical analyses. YYL, PM, VLV and CLM interpreted the data. YYL and PM reviewed all literature and prepared the manuscript. CLM, DA, SML, KAE, KH, AR, RNM, VLV, PJS, RHP and CCR drafted and revised the manuscript.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)