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BDNF VAL66MET POLYMORPHISM AND MEMORY DECLINE ACROSS THE SPECTRUM OF ALZHEIMER'S DISEASE

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ABSTRACT

The brain-derived neurotrophic factor (*BDNF*) Val66Met (rs6265) polymorphism has been shown to moderate the extent to which memory decline manifests in preclinical Alzheimer's disease (AD). To date, no study has examined the relationship between *BDNF* and memory in individuals across biologically confirmed AD clinical stages (i.e., Aβ+). We aimed to understand the effect of *BDNF* on episodic memory decline and clinical disease progression over 126-months in individuals with preclinical, prodromal and clinical AD. Participants enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) study who were Aβ+ (according to positron emission tomography), and cognitively normal (CN; n=238), classified as having mild cognitive impairment (MCI; n=80), or AD (n=66) were included in this study. Cognition was evaluated at 18-month intervals using an established episodic memory composite score over 126-months. We observed that in Aβ+ CNs, Met66 was associated with greater memory decline with increasing age and were 1.5 times more likely to progress to MCI/AD over 126-months. In Aβ+ MCIs, there was no effect of Met66 on memory decline or on disease progression to AD over 126-months. In Aβ+ AD, Val66 homozygotes showed greater memory decline, while Met66 carriers performed at a constant and very impaired level. Our current results illustrate the importance of time and disease severity to clinicopathological models of the role of *BDNF* Val66Met in memory decline and AD clinical progression. Specifically, the effect of *BDNF* on memory decline is greatest in preclinical AD and reduces as AD clinical disease severity increases.

INTRODUCTION

Understanding the role neurotrophins in central nervous system (CNS) disease could provide insight into the biological bases of how the brain benefits from experience and potentially for how brain functions could be restored after injury or disease.[1] However, there currently exist no well- understood and replicable models of the effects of BDNF on CNS functions in humans. We have identified and refined a model in early Alzheimer's disease (AD) where the influence of the brain derived neurotrophic factor (BDNF) on brain structure and function, and its expression in cognition, is large. Further challenge of this brain-behavior model has potential to increase understanding of how neurotrophic factors can influence neurodegeneration and its clinical expression in AD.

Allelic variation on the *BDNF* Val66Met (rs6265) polymorphism, is associated with changes in BDNF metabolism, and subsequent reductions in synaptic plasticity and long-term potentiation particularly in the hippocampus.[2] We have shown that this polymorphism may also moderate the magnitude of neurodegeneration and cognitive decline in preclinical sporadic AD (i.e., Aβ+ cognitively normal older adults).[3-6] In preclinical sporadic AD, loss of episodic memory and hippocampal volume increased substantially in Met66 carriers (d=0.70). However, Met66 did not influence memory or hippocampal volume in Aβ- adults, nor was it associated with rates of Aβ accumulation.[3, 5, 6] Together, these data suggest that carriage of *BDNF* Met66 may moderate the downstream effects of Aβ toxicity on neurodegeneration and cognition.

The observation that in preclinical sporadic AD, carriage of *BDNF* Met66 was associated with greater loss of episodic memory and hippocampal volume has been replicated and extended in studies of asymptomatic individuals who carry a causative mutation in presenilin-1, presenilin-2 or the amyloid precursor protein which confer nearly a 100% risk for AD, with clinical symptoms emerging around 45 years of age (i.e. preclinical autosomal dominant AD). Individuals with preclinical autosomal dominant AD who also carried the Met66 allele showed greater memory decline, hippocampal volume loss, and reduced functional connectivity in the hippocampus compared to age-matched Val66 homozygotes.[4, 7, 8] Consistent with the findings in preclinical sporadic AD, rates of Aβ accumulation in preclinical autosomal dominant AD were not influenced by Met66 carrier status. Interestingly though, in preclinical autosomal dominant AD, cerebrospinal fluid (CSF) levels of total tau (t-tau) and phosphorylated tau at threonine 181 ($ptau_{181}$) increased faster in Met66 carriers than in Val66 homozygotes (d = 0.90-1.5).[8]

These findings suggest that in both sporadic and autosomal dominant AD, allelic variation in *BDNF* Val66Met may influence the course of AD. However, a challenge to this hypothesis comes from the absence of *BDNF* single nucleotide polymorphisms (SNPs) in genome-wide association studies (GWAS) of sporadic AD.[9, 10] This is surprising as the substantially greater memory and hippocampal volume loss observed in Aβ+ Met66 carriers with early AD should result in these individuals progressing to clinically classified AD dementia faster and in increased numbers, or having more severe phenotypic characteristics, than Val66 homozygotes. One explanation for this discrepancy is that GWAS typically use a clinical classification of AD dementia as the target phenotype and therefore contributions of the *BDNF* Val66Met polymorphism may be overlooked because the polymorphism, and its effects on BDNF protein synthesis, occur most strongly in the earliest stages of the disease. For example, neurotrophic factors may have a greater effect when at-risk individuals are asymptomatic and before widespread neuronal damage has occurred. However, to date, no study of which we are aware has examined the relationship between *BDNF* Val66Met and memory decline across the clinical disease stages of biologically confirmed AD. To determine the extent to which the influence of the *BDNF* Val66Met polymorphism changes with disease severity, we studied rates of decline in episodic memory in Aβ+ individuals who were cognitively normal (CN) (i.e., preclinical AD), and who meet clinical criteria for mild cognitive impairment (MCI; prodromal AD) and AD dementia. We also evaluated the effect of *BDNF* Val66Met polymorphism on clinical disease progression over 126 months in Aβ+ CN and Aβ+ MCI individuals.

MATERIALS AND METHODS

Participants.

Aβ+ participants enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) study who were cognitively normal (n=238), classified as MCI (n=80), and classified as AD (n=66) were included in this study. Recruitment and diagnostic classification procedures have been described in detail previously.[11, 12] Briefly, participants were excluded from the AIBL study at baseline if they had schizophrenia, elevated depressive symptoms (Geriatric Depression Score, GDS, of 6 or greater), Parkinson's disease, cancer (other than basal cell skin carcinoma) within the last two years, untreated sleep apnea, symptomatic stroke, uncontrolled diabetes, or alcohol use exceeding two standard drinks per day for women or four per day for men.

All participants with AD met NINCDS-ADRDA criteria for AD,[13] and in all cases, the clinical review panel comprised of geriatricians, neurologists and clinical neuropsychologists, reviewed available clinical and neuropsychological data to ensure that the diagnosis was consistent with agreed criteria. Similarly, available clinical and neuropsychological data for participants with MCI were reviewed to ensure that their classification was consistent with internationally agreed criteria.[14, 15] The clinical review panel also ensured the cognitive normality of all participants classified as cognitively normal. Participants were classified as cognitively normal if they performed greater than -1 standard deviation on all neuropsychological tests when compared to Australian age- and education-matched normative data, had an MMSE score of 26 or greater, and a CDR sum of boxes score of 0 or 0.5 (CDR sum of boxes score of 0.5 was allowable if all neuropsychological tests were within normative ranges). Clinical classification was blinded to all neuroimaging and genetic information. Approximately 98% of all participants were Caucasian.

The AIBL study was approved by the institutional research and ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital and Edith Cowan University. All participants provided written informed consent prior to participating in the study.

Assessments.

Episodic memory. The episodic memory composite was computed by averaging standardized outcome measures for tests of verbal and visual episodic memory administered as part of the AIBL neuropsychological battery, specifically Logical Memory delayed recall, California Verbal Learning Test, Second Edition (CVLT-II) and Rey Complex Figure Test delayed recall. Consistent with previous methods in AIBL, each test was standardized using the baseline mean and standard deviation of the entire CN sample.[16-19] This episodic memory composite has been used extensively in previous studies of memory across clinical disease stages in the AIBL cohort, with its development described in these studies.[16-19]

Neuroimaging. Aβ imaging with positron emission tomography (PET) was conducted using one of three radioligands, that is, Pittsburgh Compound B (PiB), florbetapir or flutemetamol. The acquisition protocol for each radioligand has been detailed previously.[20, 21] Briefly, a 30-minute acquisition was started 40 minutes after PiB-injection, and 20-minute acquisitions were performed 50 minutes after florbetapir injection and 90 minutes after flutemetamol injection. For PiB acquisition, standardized uptake value (SUV) data for key regions of interest were summed and normalized to the

cerebellar cortex SUV. This resulted in a region-to-cerebellar ratio which was termed SUV ratio (SUVR). For florbetapir, SUVR was generated using the whole cerebellum as the reference region and for flutemetamol, the pons was used as the reference region. Threshold values for elevated Aβ deposition vary by radiotracer, so all SUVR values were transformed into the Centiloid scale.[22, 23] Aβ+ was classified in participants for whom Centiloid scores were greater than 15 at their first PET scan.[22, 23]

Genetics. An 80ml blood sample was taken from each participant at baseline and 10ml forwarded for large-scale DNA extraction using either the QIAamp DNA blood Midi or Maxi kits (Qiagen), applying the protocol given by the manufacturer. As previously described,[24] TaqMan® genotyping assays were used to determine *APOE* (rs7412, assay ID: C____904973_10; rs429358, assay ID: C___3084793_20) and *BDNF* (rs6265, assay ID: C__11592758_10) genotypes (Life Technologies, Carlsbad, CA). TaqMan® genotyping assays were performed on a QuantStudio 12K Flex™ Real-Time-PCR systems (Applied Biosystems, Foster City, CA) using the TaqMan® GTXpress™ Master Mix (Life Technologies) as per manufacturer instructions. The Val66Met polymorphism had a call rate of greater than 99% and did not depart from Hardy-Weinberg equilibrium (p =.57).

Procedure.

Participants underwent clinical and neuropsychological assessments at 18-month intervals as part of the AIBL study. In this study, we report PET neuroimaging and genetic data obtained at baseline, and episodic memory performance assessed at baseline, and follow-up assessments conducted every 18-months up to the most recent 126-month visit.

Data Analysis.

Analyses were conducted in RStudio using R version 3.5.0, using the following packages: "lme4", "lmerTest", "ggplot2", "emmeans", "survminer", "effects", and "cowplot". Differences between groups on demographic, clinical and neuroimaging characteristics were determined using independent-samples t-tests for continuous variables and chi-square tests for categorical variables. To determine the effect of *BDNF* Val66Met on episodic memory performance at baseline in CN, MCI and AD groups, we conducted a series of analyses of covariances (ANCOVA), with age, premorbid intelligence, sex and *APOE* ε4 status as fixed factors/covariates. To determine the effect of *BDNF* Val66Met on episodic memory change, we conducted a series of linear mixed models (unstructured covariance matrix, maximum likelihood) with increasing age as the time-dependent variable,

participant and time as random factors, and premorbid intelligence, sex and *APOE* ε4 status as fixed factors/covariates. LMMs were employed because they are robust to missing data (see Supplementary Table 1), because they model both fixed and random effects (thus accounting for multiple sources of variability), and because they provide improved estimates of random effects (within-subject coefficients) in prospective studies.[25, 26] Mean slopes for Val66 homozygotes and Met66 carriers were derived from these models, and the magnitudes of between-group differences were expressed using Cohen's d and 95% confidence intervals. Cox proportional hazard ratios and log-rank tests were conducted to evaluate the effect of *BDNF* Val66Met on clinical disease progression in Aβ+ CN and Aβ+ MCI groups.

Data Availability.

Data from the AIBL study is publicly available through https://ida.loni.usc.edu, although data is systematically released after a delay (e.g., 2 years) to allow AIBL investigators the opportunity to publish main study findings. Earlier access to the data will be considered by the AIBL management committee for researchers interested in collaboration.

RESULTS

Demographic and clinical characteristics

Table 1 summarizes the demographic and clinical characteristics of all participant groups. Aβ+ CN Val66 homozygotes had higher levels of premorbid intelligence than Aβ+ CN Met66 carriers. Val66 homozygotes and Met66 carriers were equivalent across all other demographic and clinical characteristics in Aβ+ CN, Aβ+ MCI and Aβ+ AD groups.

Effect of *BDNF* Val66Met on episodic memory impairment at baseline

There was no effect of *BDNF* Val66Met on episodic memory performance at baseline in Aβ+ CN, Aβ+ MCI or Aβ+ AD groups, with the difference between Val66 homozygotes and Met66 carriers in all three clinical groups, by convention, small in magnitude (Fig 1, Table 2).

Effect of *BDNF* Val66Met on episodic memory decline and clinical disease progression

In the Aβ+ CN group, we observed a significant BDNF x age interaction, where Met66 carriers showed greater deterioration in episodic memory with increasing age than Val66 homozygotes, and the magnitude of this difference was moderate (Table 3, Fig 2). Over the 126-month period, Aβ+ CN Met66 carriers were also more likely to progress to a classification of MCI or AD than Aβ+ CN Val66

homozygotes, Cox proportional hazard ratio (95%CI) = 1.50 (1.2-1.9), χ^2 (1) = 13.3, $p < .001$ (Fig 3A). This effect remained even after covarying for age, sex, *APOE* ε4, and premorbid intelligence levels, Cox proportional hazard ratio (95%CI) = 1.36 (1.14-1.58), χ^2 (5) = 203.70, $p < .001$.

No significant BDNF x age interaction was observed in the Aβ+ MCI group, and the magnitude of difference in episodic memory decline between groups was very small. Similarly, no differences were observed between Aβ+ MCI Met66 carriers and Val66 homozygotes on the likelihood of progressing to a classification of AD over 126-months, Cox proportional hazard ratio (95%CI) = 0.95 (0.72, 1.20), χ^2 (1) = 0.1, $p = .709$ (Fig 3B).

For the Aβ+ AD group, we observed a significant BDNF x age interaction, where Val66 homozygotes showed greater rates of episodic memory with increasing age than Met66 carriers, and the magnitude of this difference was moderate (Table 3, Fig 2). No statistically significant change in episodic memory performance was observed in Aβ+ AD Met66 carriers, β(SE) = -0.184 (0.154), $p = .237$.

DISCUSSION

The results of this study demonstrate that the effect of *BDNF* Val66Met on episodic memory decline is greatest in preclinical AD and reduces in magnitude with clinical disease progression (Table 3, Fig 2A). Consistent with the faster memory decline in early disease, Met66 carriers were 1.5 times more likely than Val66 homozygotes to progress from preclinical to prodromal AD (Fig 3A). Together, the results of this study are consistent with previous reports of Aβ+ CNs assessed over shorter follow-up periods where Met66 carriers show greater episodic memory decline compared to matched Val66 homozygotes.[3, 5]. In Aβ+ MCI (i.e., prodromal AD), Met66 carriage was also associated with an increased episodic memory decline, although given the greater variability in this sample, the magnitude of difference from Val66 homozygotes (d=0.14) was too small to reach statistical significance (Fig 2B). Previously, in a small sample of Aβ+ MCI followed over 3 years, we observed that Met66 was associated with a moderately (*d*=0.4) faster decline in episodic memory.[27] The smaller effect of Met66 carriage on memory in the current study may have arisen because the longer study interval resulted in disease severity in the MCI group increasing from when they had been studied previously. For example, in the current Aβ+ MCI participants, 71% progressed to AD over the 126-month period, with carriage of Met66 adding no additional risk for disease progression (Fig 3B). Thus, in the current study, the smaller effect of Met66 on episodic memory decline in the Aβ+ MCI group was because the disease had become too severe over the study period. Consistent with this notion, in Aβ+ AD

dementia, episodic memory decline was not observed at all in Met66 carriers. Rather, performance remained at a constant and very impaired level across the study period (Fig 2C). However, Aβ+ AD Val66 homozygotes did continue to show memory decline, and this resulted in differences between the two groups being moderate in magnitude (Fig 2C). The results of this study suggest that the clinical consequences of Met66 carriage are greatest in the earliest disease stages and that this influence reduces as a function of clinical disease progression.

The observation that in AD dementia, Val66 homozygotes had greater memory decline than Met66 carriers is unlikely to reflect that in dementia, *BDNF* risk switches from carriage of Met66 to Val66. Instead, the declining memory function in Aβ+ AD Val66 homozygotes may reflect that any protective effects of Val66 are now exhausted. It may also suggest that episodic memory performance in Aβ+ AD Met66 carriers had reached the lowest possible values (i.e., floor) at approximately three standard deviations below that of CNs (Fig 2C). However, the extent to which the low and stable memory performance in the Aβ+ AD Met66 group reflects a floor effect should be investigated in future studies that focus on the AD dementia stage and use tests that can accommodate the variation in performance that occurs from the increase in dementia severity over time. Taken together, these data indicate that when cognitive abilities are at a level that can be assessed using conventional neuropsychological tests, the effects of Met66 are reduced as AD clinical disease severity increases. Unfortunately, there are currently no validated *in vivo* markers of BDNF protein levels in the central nervous system (CNS), and most models seeking to understand the effect of BDNF on neurodegeneration have occurred through the study of *BDNF* polymorphisms, particularly rs6265.[28, 29] On this polymorphism there is a substitution of valine (Val) to methionine (Met) at codon 66 (Val66Met, c.196G>A, dbSNP: rs6265) and with this, the pro-domain structure of the gene is altered. This substitution leads to improper protein folding and a reduced binding of the mature BDNF to its receptor TrkB, which in turn can alter BDNF protein-protein interactions, binding affinities in the CNS.[1, 30] Lower levels of neurotrophins can disrupt normal neuronal and synaptic function and thereby result in reduced cellular function and even increased cell death.[31, 32] Considered in the context of the current data, reduced neurotrophic activity resulting from Met66 carriage may result in substantially faster memory decline and clinical disease progression in preclinical AD. In AD dementia, the clinical consequences of Met66 are blunted. One potential explanation for this change is that neurotrophins that act to restore AD-damaged neurons may lack efficacy as the extent of neuronal damage increases. For example, with increasing disease severity, biological processes such as

tau toxicity, neuroinflammation and microvascular damage all contribute to cell death.[33] Consequently, the potential for neurotrophins to influence these processes is diminished. Clinicopathological studies are necessary to test such hypotheses, and the current data provide a strong foundation for the design of such studies.

Despite the significant associations between Met66 and memory decline observed from prospective analyses (Fig 2), when memory performance was considered cross-sectionally, no differences were observed between Met66 carriers and Val66 homozygotes at any stage of AD (Fig 1). The necessity for prospective designs to detect the effects of *BDNF* Met66, has been observed previously in AIBL and other cohorts,[3, 4, 8] and likely reflects that with sample sizes such as those studied here (i.e. <200), the greater precision afforded by control of within-individual variance in prospective study designs is necessary to detect subtle gene-cognition relationship. The differential effect of the *BDNF* Val66Met polymorphism across the different AD stages, and the dependence of such relationships on analyses of prospective data, may provide an explanation for why *BDNF* has not been identified in GWAS of AD. First, while very large samples are considered in GWAS, the target phenotypes for these are typically classification of AD dementia based on clinical assessment or performance on a cognitive screening tool (e.g., MMSE or MOCA) rather than from more sensitive neuropsychological markers of AD, such as tests of episodic memory. For example, in the current study, clinical classifications of symptomatic disease (i.e., MCI or AD) were sufficiently sensitive only to *BDNF* Val66Met variation in the preclinical AD stage. Second, all relevant GWAS have been performed on data collected in a single assessment which, as shown here for both memory performance and risk of clinical disease progression, has no relation with Met66 carriage beyond the preclinical stage of AD.[9, 10] Additionally, the observation that in preclinical AD, Met66 carriage is associated with greater rates of progression to MCI or AD dementia over 12 years, and that in AD dementia, memory performance is stable at the lowest possible level in Met66 carriers, raises the possibility that dementia onset may occur earlier in Met66 carriers.

In this study, we did not measure AD biomarkers other than Aβ levels. However, our results showing greater cognitive decline in Met66 carriers early in the disease course may help inform brain-behavior models of the role of the *BDNF* Val66Met polymorphism in the development of AD. While we have previously shown that Met66 is not associated with change in Aβ (measured via PET or CSF),[5, 8] in preclinical autosomal dominant AD (i.e., CDR=0), Met66 was associated with greater increases in CSF t-tau and $ptau_{181}$.[8] Therefore, it is likely that the earlier onset of memory decline in Aβ+ Met66 carriers

may be due to higher levels of t-tau and ptau$_{181}$. For example, *in vitro* studies have shown that reduced BDNF slows the dephosphorylation of tau by slowing TrkB activation,[34] and that in AD postmortem samples, BDNF loss is specific to tangle-bearing neurons.[35,36] As such, in addition to the known effects of BDNF on synaptic excitation, long-term potentiation and neuronal plasticity, another potential pathway by which BDNF may moderate AD dementia onset may be through its effects on the hyperphosphorylation of tau. Although, it remains possible that the hyperphosphorylation of tau is itself a consequence of BDNF effects on neurons.[37]

Several methodological caveats need to be considered before the results of this study can be applied to existing models of AD. First, while this is the largest prospective investigation of *BDNF* Val66Met on episodic memory performance in biologically confirmed preclinical, prodromal and clinical AD to date, the sample sizes are relatively small for an investigation of the relationships between genes and cognition. We and others have previously observed the effects of *BDNF* Val66Met on memory in modest samples sizes (e.g., n<100). This suggests that the effects of this polymorphism on cognitive outcomes can be substantial; however, our current results also illustrate the importance of time and disease severity to clinicopathological models of the role of *BDNF* in AD. As such, to understand these effects and their interactions with AD clinical and biological processes, it will be necessary to increase sample sizes further. Secondly, in this study, we focused on episodic memory as the main cognitive expression of Met66 carriage. This is because dysfunction in episodic memory is the cornerstone of cognitive dysfunction across all stages of AD, and because animal and post-mortem human studies have emphasized the importance of BDNF on brain regions that subserve memory. The effects of *BDNF* Val66Met on episodic memory observed here provides a solid theoretical and quantitative reference point for future investigations into other cognitive domains. Furthermore, clarification of the conditions under which the effects of *BDNF* Val66Met manifest clinically in older adults with AD provide a strong foundation for the development of brain-behavior challenges with greater sophistication to increase understanding of the biological actions of neurotrophins.

FINANCIAL DISCLOSURES

YY Lim, SM Laws, S Perin, RH Pietrzak and C Fowler have no financial disclosures to report. CL Masters holds shares in Prana Biotechnology Ltd. P Maruff is a full-time employee of Cogstate Ltd.

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Table 1. Demographic and clinical characteristics of the Cognitively normal, MCI, and AD groups by *BDNF* Val66Met genotype

	Cognitively normal			MCI			AD		
	Val66 (n=151)	Met66 (n=87)	*p*	Val66 (n=51)	Met66 (n=29)	*p*	Val66 (n=44)	Met66 (n=22)	*p*
N (%) Female	80 (53.0%)	43 (49.4%)	.597	23 (45.1%)	13 (44.8%)	.981	25 (56.8%)	14 (63.6%)	.595
N (%) *APOE* ε4	65 (43.0%)	35 (40.2%)	.610	33 (64.7%)	20 (69.0%)	.710	29 (65.9%)	17 (77.3%)	.466
Age	72.28 (6.61)	72.38 (6.94)	.910	75.02 (5.61)	76.52 (7.14)	.301	73.47 (7.56)	74.92 (7.23)	.458
Premorbid IQ	**109.12 (6.82)**	**105.98 (8.24)**	**.002**	105.80 (7.69)	107.76 (8.52)	.297	103.56 (8.39)	100.67 (12.85)	.284
HADS depression	2.66 (2.22)	3.13 (2.79)	.220	3.30 (2.44)	3.76 (2.62)	.500	4.10 (3.39)	2.94 (2.65)	.222
HADS anxiety	4.21 (3.34)	4.78 (3.00)	.256	4.87 (2.43)	4.81 (2.66)	.927	5.60 (3.44)	4.65 (3.74)	.381
MMSE	28.86 (1.16)	28.35 (1.41)	.191	27.33 (1.75)	27.00 (1.00)	.773	22.00 (6.06)	22.50 (2.12)	.919
CDR sum of boxes	0.05 (0.26)	0.07 (0.19)	.616	1.12 (0.83)	1.09 (0.63)	.860	5.11 (2.16)	4.68 (1.94)	.432
Centiloid	54.39 (31.83)	57.47 (32.26)	.474	87.40 (35.92)	88. 72 (32.64)	.871	93.53 (31.58)	99.68 (25.68)	.432
PET Time Difference	2.36 (2.38)	2.96 (2.73)	.080	0.62 (1.15)	0.96 (1.60)	.273	0.51 (1.08)	0.53 (1.38)	.930
N Assessments	5 (2-7)	5 (2-7)	.700	4 (2-7)	3 (2-7)	.559	3 (2-7)	3 (2-7)	.318

Note: Val66 = *BDNF* Val66 homozygotes; Met66 = *BDNF* Met66 carriers; MCI = Mild Cognitive Impairment; AD = Alzheimer's Disease; *APOE* = apolipoprotein E; HADS = Hospital Anxiety and Depression Scale; MMSE = Mini Mental State Examination; CDR = Clinical Dementia Rating; PET = Positron Emission Tomography imaging

Table 2. Mean episodic memory performance (SD) and Cohen's *d* representing group mean differences at baseline in cognitively normal, MCI and AD groups.

	Cognitively normal		MCI		AD	
	(df) F	*p*	(df) F	*p*	(df) F	*p*
BDNF Val66Met	(1,216) 1.863	.174	(1,68) 0.230	.633	(1,45) 2.235	.142
Age	**(1,216) 26.931**	**<.001**	(1,68) 2.237	.139	(1,45) 1.333	.254
Premorbid IQ	**(1,216) 10.854**	**.001**	(1,68) 0.457	.501	(1,45) 0.204	.654
Sex	(1,216) 1.773	.184	(1,68) 0.000	.995	**(1,45) 5.155**	**.028**
APOE ε4	(1,216) 0.609	.436	(1,68) 1.252	.267	(1,45) 3.448	.070

	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Val66 homozygote	-0.100 (0.762)	151	-1.912 (0.743)	51	-2.534 (0.657)	44
Met66 carrier	-0.151 (0.765)	87	-1.947 (0.716)	29	-2.654 (0.643)	22
Cohen's d (95%CI)	0.07 (-0.20, 0.33)		0.05 (-0.41, 0.50)		0.18 (-0.33, 0.69)	

Note: Val66 = *BDNF* Val66 homozygotes; Met66 = *BDNF* Met66 carriers; MCI = Mild Cognitive Impairment; AD = Alzheimer's Disease; *APOE* = apolipoprotein

Table 3. Mean slopes for episodic memory (SD) and Cohen's *d* representing group mean slope differences over 126-months in cognitively normal, MCI and AD groups.

	Episodic Memory					
	Cognitively normal		MCI		AD	
	β(SE)	*p*	β(SE)	*p*	β(SE)	*p*
BDNF Val66Met	0.124 (0.106)	.243	-0.051 (0.196)	.795	0.079 (0.214)	.712
Age	**-0.442 (0.061)**	**<.001**	**-0.506 (0.131)**	**<.001**	-0.184 (0.155)	.237
Premorbid IQ	**0.174 (0.047)**	**<.001**	0.137 (0.098)	.166	0.082 (0.107)	.448
Sex	-0.120 (0.094)	.205	0.320 (0.195)	.106	**0.471 (0.205)**	**.026**
APOE ε4	0.131 (0.094)	.164	0.417 (0.210)	.052	0.389 (0.253)	.130
BDNF Val66Met x Age	**0.198 (0.078)**	**.011**	0.115 (0.175)	.514	**-0.492 (0.209)**	**.021**

	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Val66 homozygote	-0.244 (0.590)	151	-0.391 (0.843)	51	-0.677 (0.727)	44
Met66 carrier	-0.442 (0.569)	87	-0.505 (0.705)	29	-0.184 (0.955)	22
Cohen's d (95%CI)	**0.34 (0.07, 0.60)**		0.14 (-0.31, 0.60)		**0.56 (0.03, 1.07)**	

Note: Val66 = *BDNF* Val66 homozygotes; Met66 = *BDNF* Met66 carriers; MCI = Mild Cognitive Impairment; AD = Alzheimer's Disease; *APOE* = apolipoprotein

Supplementary Table 1. Number of participants who completed assessments at each timepoint.

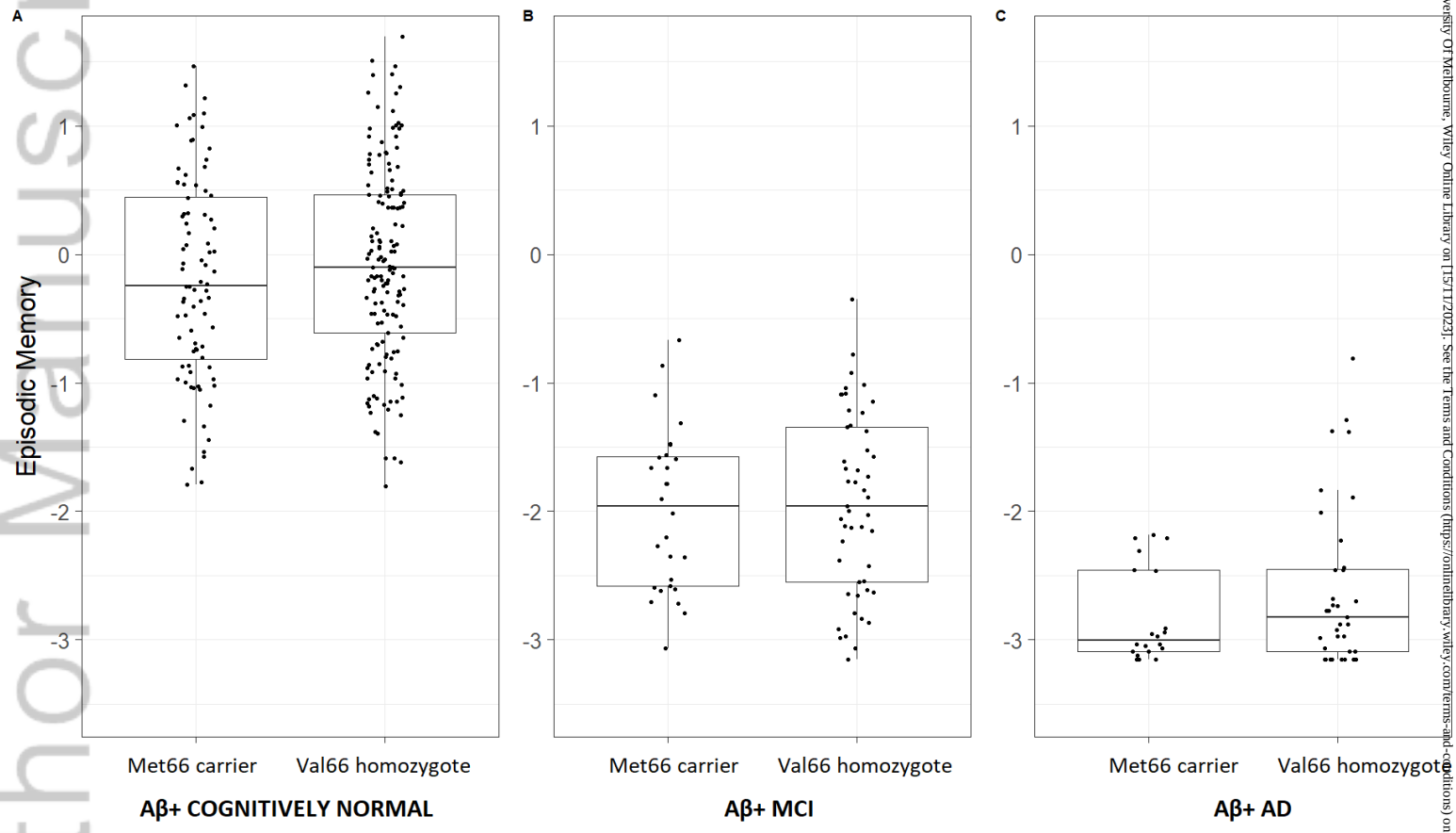
	Baseline	18M	36M	54M	72M	90M	108M	126M
Aβ+ CN	238	238	216	193	156	112	104	91
Aβ+ MCI	80	72	62	46	29	12	10	8
Aβ+ AD	66	66	50	30	12	7	3	2

FIGURE LEGENDS

Fig 1. Baseline episodic memory performance of Met66 carriers and Val66 homozygote in (A) Aβ+ CN, (B) Aβ+ MCI, and (C) Aβ+ AD groups.

Fig 2. Rate of decline between Val66 homozygotes and Met66 carriers on the AIBL episodic memory composite in (A) Aβ+ CN, (B) Aβ+ MCI, and (C) Aβ+ AD groups (shading indicates 95% confidence intervals).

Fig 3. Cumulative hazard of progressing to MCI or AD in (A) Aβ+ CN Val66 homozygotes and Met66 carriers, and (B) Aβ+ MCI Val66 homozygotes Met66 carriers (shading represents 95% confidence intervals).



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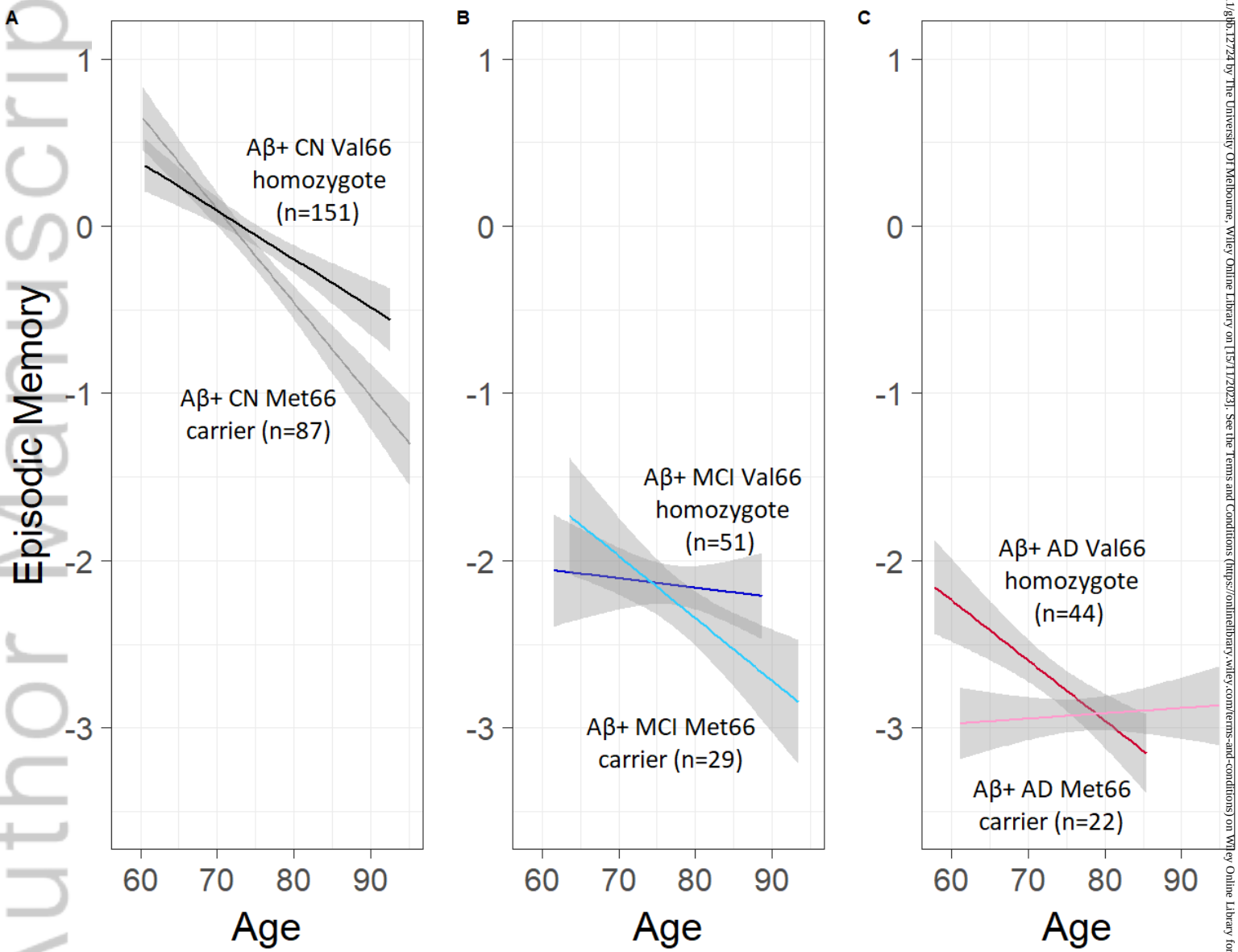

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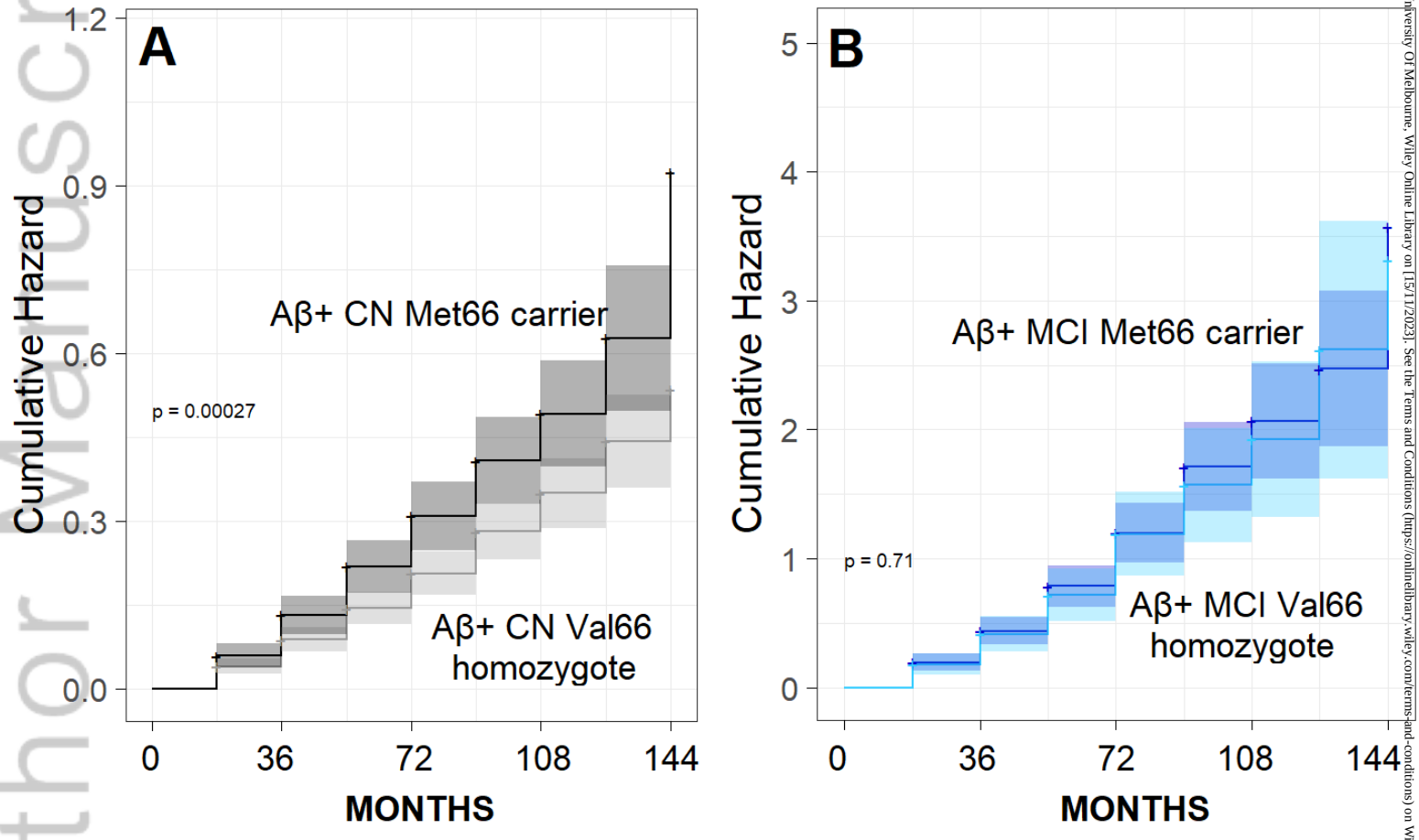

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