Effect of BDNF Val66Met on memory decline and hippocampal atrophy in prodromal Alzheimer's disease: A preliminary study

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10.1371/journal.pone.0086498
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Abstract

**Objective:** Cross-sectional genetic association studies have reported equivocal results on the relationship between the brain-derived neurotrophic factor (BDNF) Val66Met and risk of Alzheimer’s disease (AD). As AD is a neurodegenerative disease, genetic influences may become clearer from prospective study. We aimed to determine whether BDNF Val66Met polymorphism influences changes in memory performance, hippocampal volume, and Aβ accumulation in adults with amnestic mild cognitive impairment (aMCI) and high Aβ.

**Methods:** Thirty-four adults with aMCI were recruited from the Australian, Imaging, Biomarkers and Lifestyle (AIBL) Study. Participants underwent PiB-PET and structural MRI neuroimaging, neuropsychological assessments and BDNF genotyping at baseline, 18 month, and 36 month assessments.

**Results:** In individuals with aMCI and high Aβ, Met carriers showed significant and large decline in episodic memory (d = 0.90, p = .020) and hippocampal volume (d = 0.98, p = .035). BDNF Val66Met was unrelated to the rate of Aβ accumulation (d = −0.35, p = .401).

**Conclusions:** Although preliminary due to the small sample size, results of this study suggest that high Aβ levels and Met carriage may be useful prognostic markers of accelerated decline in episodic memory, and reductions in hippocampal volume in individuals in the prodromal or MCI stage of AD.


Introduction

Current models of Alzheimer’s disease (AD) emphasize beta-amyloid (Aβ) as precipitating a cascade of events that result in synaptic loss and memory impairment [1]. Recent in vivo evidence suggests neurotrophic factors such as brain-derived neurotrophic factor (BDNF) may be an indirect moderator of Aβ neurotoxicity, as BDNF and its main receptor, tropomyosin-related kinase B (TrkB) are not involved in amyloidogenesis, but rather in synaptic excitation and neuronal plasticity, which may provide an ability for the central nervous system (CNS) to withstand Aβ-related neuronal death [2–4]. Further, in individuals with AD or mild cognitive impairment (MCI), BDNF messenger ribonucleic acid (mRNA) is reduced substantially in the hippocampus and temporal lobe, with the extent of BDNF loss associated with the magnitude of cognitive impairment [5,6]. Unfortunately, there are currently no validated peripheral markers of central nervous system (CNS) BDNF [3,4]. Therefore, in humans, conclusions about the role of BDNF in AD have been based on the measurement of the effect of BDNF polymorphisms (e.g., Val66Met [rs6265]) on clinical or pathological features of the disease, or on risk for AD [3,4]. Unfortunately, evidence from such studies has been mixed with some showing BDNFVal66Met carriers to have increased memory impairment, brain atrophy or risk of AD while others show that these same impairments are associated with BDNFVal66Met homozygosity [3,4,7].

We [8] and others [3] have argued that the inconsistency of relationships between the BDNFVal66Met polymorphism and AD in human studies suggest the involvement of a moderating factor. As a proportion of healthy individuals at risk for AD have high Aβ [9,10], and healthy individuals with high Aβ show substantial decline in episodic memory as well as increased hippocampal atrophy [10–12]_ENREF_5, the effects of BDNF polymorphisms may be moderated by Aβ levels. Recently, we observed an epistatic relationship between the BDNF Val66Met polymorphism and Aβ deposition in which BDNFVal66Met healthy individuals showed a faster rate of hippocampal atrophy and episodic memory decline than BDNFVal66Met homozygotes, but only if they had abnormally high Aβ [8]. Thus, this suggests that Aβ deposition may moderate the relationship between the BDNFVal66Met polymorphism and risk of AD.

Objective but subtle memory impairment with corroborating evidence of memory difficulties from a reliable informant is codified as the clinical classification mild cognitive impairment (MCI), and is associated with increased risk of progression to AD [12–14]. This risk is increased if the MCI classification is accompanied by the presence of abnormal Aβ levels [14]. Therefore, another test of the hypothesis that the BDNF Val66Met polymorphism increase risk of AD would be if individuals with MCI and high Aβ who are BDNFVal66Met carriers show greater decline in episodic memory and hippocampal volume than BDNFVal66Met homozygotes. However, as BDNF is an indirect mediator of Aβ toxicity, a second hypothesis is that the BDNF Val66Met polymorphism will not affect Aβ accumulation.

Methods

Participants

Thirty-four adults with aMCI and high Aβ levels enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study were included in this study [9,15]. Participants had undergone BDNF genotyping at baseline, and positron emission tomography (PET) neuroimaging using Pittsburgh Compound B (PiB), structural magnetic resonance imaging (MRI), and neuropsychological assessment at baseline, 18 and 36 month follow-up (Table 1).

The process of recruitment and diagnostic classification of adults with aMCI enrolled in the AIBL cohort has been described in detail previously [15]. Participants who volunteered were excluded from the AIBL study if they had any of the following: schizophrenia; depression (Geriatric Depression Score (GDS) of 6 or greater); Parkinson’s disease; cancer (other than basal cell skin carcinoma) within the last two years; symptomatic stroke; uncontrolled diabetes; or current regular alcohol use exceeding two standard drinks per day for women or four per day for men. A clinical review panel chaired by DA reviewed all available medical, psychiatric and neuropsychological information to ensure that their clinical classification was consistent with international criteria [16,17]. Clinical classification was blinded to Aβ imaging data. The AIBL study was approved by the institutional ethics committees of Austin Health, St Vincent’s Health, Hollywood Private Hospital and Edith Cowan University [15]. All participants with MCI and their caregivers provided written informed consent prior to being tested.

Measures

Neuroimaging. PiB-PET imaging was conducted as described previously [9,10]. PET standardized uptake value (SUV) data acquired 40–70 minutes post-PiB injection were summed and normalized to the cerebellar cortex SUV, resulting in a region-to-cerebellar ratio termed SUV ratio (SUVR).

Magnetic resonance (MR) images were spatially normalized to the Montreal Neurological Institute (MNI) single-subject MRI brain template, [18] using MiXView®, software developed by the Australian e-Health Research Centre – BioMedIA (Brisbane, Australia). As described elsewhere, T1W MR images for each subject were classified into grey matter (GM), white matter (WM) and CSF using an implementation of the expectation maximization segmentation algorithm[19]. The algorithm computed probability maps for each tissue type and was used to assign each voxel to its most likely tissue type and subsequent segmentation. To improve the accuracy of analysis of the hippocampus, a separate, manually-delineated template was drawn on the MNI single-subject every 1 mm on coronal slices, and was subsequently used for hippocampal volume. The average hippocampal volumes were normalized for head size using the total intracranial volume, defined as the sum of GM, WM and CSF volumes.

Genotyping. An 80 ml blood sample was taken from each participant and 10 ml was used for large scale DNA extraction for AIBL bio-banking. The BDNF Val66Met polymorphism (rs6265) was included in a custom Illumina GoldenGate assay, which included 1536 SNPs, and was performed by the Beijing Genomics Institute. Val66Met polymorphism had a call rate of greater than 99% and did not depart from Hardy-Weinberg equilibrium in the AIBL aMCI group.

Clinical and cognitive assessments. The AIBL clinical and cognitive battery has been described in detail elsewhere and were administered according to standard protocols by trained research assistants [15], although the current study focused only on data for episodic memory. Clinical status was determined using information which included the Mini-Mental Status Examination (MMSE) and Clinical Dementia Rating (CDR) Scale. Premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR), and depressive and anxiety symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS).

Data Analysis

The episodic memory composite was computed by standardizing outcome measures on individual tests (Logical Memory
delayed recall, California Verbal Learning Test, Second Edition [CVLT-II] delayed recall) against the baseline mean and SD for the entire group and then averaging them. As in previous studies [9,10], adults with aMCI were classified as having high Aβ if they had an SUVR≥1.5. Linear mixed model (LMM) analyses of covariance were conducted to compare linear slopes of change in episodic memory, hippocampal volume and SUVR between BDNF groups across baseline, 18 and 36 month assessments. Linear mixed modelling was employed because of its ability to model both fixed and random effects, which accounts for multiple sources of variability, and because it provides improved estimates of within-subject coefficients (i.e., random effects) in longitudinal studies. For each LMM, BDNF, time, and BDNF-time interaction were entered as fixed factors; participant as a random factor; age and APOE status as covariates; and episodic memory, hippocampal volume or Aβ accumulation as dependent variables. For each outcome, the magnitude of the difference in slopes between the BDNF Val66Met polymorphism groups was expressed using Cohen’s $d$.

Results

Demographic and clinical characteristics of the total sample and BDNF Val66Met polymorphism groups are shown in Table 1. Study groups were matched on all demographic and clinical characteristics.

Relative to BDNF<sup>Val</sup> homoyzogtes, BDNF<sup>Met</sup> carriers showed a greater rate of decline in episodic memory and reduction in hippocampal volume over 36 months (Table 2, Figure 1a, 1b), with the effect size of difference between slopes for both measures, large in magnitude. Groups did not differ in the rate of Aβ accumulation over 36 months (Table 2, Figure 1c).

Discussion

The first hypothesis that the BDNF Val66Met polymorphism would moderate memory decline and hippocampal atrophy in aMCI with high Aβ was supported. In adults with aMCI for whom PiB-PET neuroimaging indicated high baseline Aβ, BDNF<sup>Met</sup> carriers showed greater episodic memory decline and hippocampal atrophy over 36 months compared to BDNF<sup>Val</sup> homozygotes, and the rate of decline between groups was, by convention, large in magnitude. While increased memory decline and hippocampal atrophy have been reported previously in adults with aMCI and high Aβ [10–12], results of the current study suggests a direct link between Aβ and the BDNF Val66Met polymorphism on progressive memory decline and hippocampal atrophy in prodromal AD.

The second hypothesis that the BDNF Val66Met polymorphism would not moderate the rate of Aβ accumulation in adults with aMCI and high Aβ was also supported. While Aβ levels increased for both BDNF<sup>Met</sup> carriers and BDNF<sup>Val</sup> homozygotes over 36 months, the rate of Aβ accumulation across 36 months was not different between groups (Figure 1c). Thus, while high baseline Aβ was associated with memory decline and hippocampal atrophy, the deleterious effects were reduced in individuals who carried the BDNF Val66Met polymorphism that has been associated with greater secretion of the BDNF protein (i.e., BDNF<sup>Val</sup> homozygotes).

The results of this study are consistent with our previous finding in healthy individuals, where BDNF<sup>Met</sup> carriers with high Aβ showed significantly higher rates of episodic memory decline and hippocampal atrophy than BDNF<sup>Val</sup> homozygotes, despite no differences in the rates of Aβ accumulation [8]. Further, these results are consistent with animal studies, which have shown that the secretion of mature BDNF is crucial in the neuronal integrity of the hippocampus [2,20], and that Aβ decreases BDNF levels by reducing phosphorylated cAMP response element binding protein, which in turn regulates BDNF transcript expression [5]. Human and animal neuropathological studies have also found that interactions between BDNF Val66Met and Aβ-related synaptic changes occur in the hippocampus, and that these changes are related directly to memory [2,20,21]. Finally, genetic databases do not identify BDNF Val66Met polymorphism as increasing risk for AD [22]. Taken together, these data suggest that while BDNF Val66Met is unrelated to the presence of Aβ or to its accumulation, it may moderate the extent to which Aβ affects brain structure and memory function, at least in the prodromal stages of AD.

An important caveat of the current study is that the AIBL study is not an epidemiological sample. The selection of MCI groups was biased towards the inclusion of individuals with aMCI, and participants were predominantly highly educated, and had few existing or untreated medical or psychiatric illnesses. As such, it
would be important for these findings to be replicated in adults with MCI and high A\(\beta\) in population-based studies, such as the Mayo Clinic Study of Aging, where it is possible that the effect of the \textit{BDNF} Val66Met polymorphism on A\(\beta\)-related decline may be greater than that observed here. A second caveat is that we only investigated indirect interactions between \textit{APOE}, \textit{BDNF} Val66Met, A\(\beta\) and cognitive decline in adults with aMCI. This was primarily due to the small sample size, equivalence in the proportion of \textit{APOE} \(\varepsilon4\) carriers in \textit{BDNF} Met carriers and \textit{BDNF} Val homozygotes, and the previous observation that \textit{APOE} \(\varepsilon4\) does not interact with BDNF levels to affect cognitive function [23]. However, further investigation of the relationship between \textit{APOE}, \textit{BDNF} Val66Met, and A\(\beta\) on change in cognition needs to be conducted in larger, prospective studies of \textit{APOE} allele groups [24,25]. Finally, due to small sample sizes, we were unable to investigate whether \textit{BDNF} Val66Met polymorphism has an effect on cognitive decline in individuals with MCI and low A\(\beta\). However, as individuals with MCI and low A\(\beta\) do not show memory decline over 18 or 36 months,
months [12,14], and as we have previously shown that in healthy older adults, BDNFVal66Met polymorphism only exerts its effects on cognitive function and brain structure in individuals with high Aβ [9], we hypothesize that the BDNFVal66Met polymorphism would not have an effect on cognitive function and hippocampal volume in individuals with MCI and low Aβ.

Notwithstanding this limitation, results of the current study provide preliminary support for high Aβ and BDNF Val66Met polymorphism as important prognostic markers of increased memory decline and hippocampal atrophy in individuals with prodromal AD [13]. Further, as pharmacologically increasing BDNF levels in AD mouse models can ameliorate synaptic dysfunction and improve memory [26], and increasing BDNF secretion through aerobic exercise have been shown to improve memory performance in humans at risk for AD [27], interventions geared toward increasing BDNF levels may be a potential therapeutic strategy for the early stages of AD.

Acknowledgments
Alzheimer’s Australia (Victoria and Western Australia) assisted with promotion of the study and the screening of telephone calls from volunteers. The AIBL team wishes to thank the clinicians who referred patients with AD to the study: Associate Professor Brian Chambers, Professor Edmond Chin, Dr Roger Clarinette, Dr Mary Davison, Dr John Drago, Dr Peter Drysdale, Dr Jacqueline Gilbert, Dr Kwang Lim, Professor Nicola Lautenschlager, Dr Dina LoGiudice, Dr Peter McCardle, Dr Steve McFarlane, Dr Alastair Mander, Dr John Merony, Professor Daniel O’Connor, Dr Ron Schodes, Dr Mathew Samuel, Dr Darshan Trivedi, and Associate Professor Michael Woodward. We thank all those who participated in the study for their commitment and dedication to helping advance research into the early detection and causation of AD.

Author Contributions
Conceived and designed the experiments: YYL PM SML. Performed the experiments: YYL KE VLV CR PB SML. Analyzed the data: YYL VLV SML PM. Contributed reagents/materials/analysis tools: DA RNM CLM PM KE VLV CR PB. Wrote the paper: YYL PM. Provided substantial revisions of manuscript for scientific content: SML DA KE CR VLV KH RNM RHP PB AIB CLM.

References