BDNF Val66Met moderates memory impairment, hippocampal function and tau in preclinical autosomal dominant Alzheimer's disease

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Abstract

Background: The brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism is implicated in synaptic excitation and neuronal integrity, and has previously been shown to moderate Aβ-related memory decline and hippocampal atrophy in preclinical sporadic Alzheimer's disease (AD). However, the effect of *BDNF* in autosomal dominant AD (ADAD) is unknown. We aimed to determine the effect of *BDNF* Val66Met on cognitive function, hippocampal function, tau and Aβ in preclinical ADAD. We explored effects of apolipoprotein E (*APOE*) ε4 on these relationships.

Methods: The Dominantly Inherited Alzheimer Network (DIAN) conducted clinical, neuropsychological, genetic, biomarker and neuroimaging measures at baseline in 131 mutation non-carriers (NC) and 143 preclinical ADAD mutation carriers (MC) on average 12 years prior to clinical symptom onset. *BDNF* genotype data were obtained for MCs (95 Val_{66} homozygotes, 48 Met_{66} carriers).

Findings: Among preclinical MCs, Met_{66} carriers had worse memory performance, lower hippocampal glucose metabolism and increased levels of CSF tau and phosphorylated tau (p-tau) than Val_{66} homozygotes. Cortical Aβ and CSF $Aβ_{42}$ levels were significantly different from NC's but did not differ between preclinical MC Val_{66} homozygotes and Met_{66} carriers. There was an effect of *APOE* on Aβ levels but not cognitive function, glucose metabolism or tau.

Interpretation: As in sporadic AD, the deleterious effects of Aβ on memory, hippocampal function, and tau in preclinical ADAD mutation carriers are greater in Met_{66} carriers. To date, this is the only genetic factor found to moderate downstream effects of Aβ in ADAD.

Introduction

Alzheimer's disease (AD) begins with the aggregation of beta-amyloid (Aβ), the development and spread of hyperphosphorylated tau (Ballatore *et al.* , 2007, Ittner and Götz, 2011), and ultimately neuronal and synaptic loss. This characteristic pathological process manifests initially as cognitive impairment which increases progressively so eventually classification of dementia is warranted (Hardy and Higgins, 1992, Ittner and Götz, 2011, Spires-Jones and Hyman, 2014). Clinical pathological relationships in AD are still not understood completely, however recent *in vitro* (Hariri *et al.* , 2003, Lee *et al.* , 2012), *post-mortem* (Buchman *et al.* , 2016, Garzon and Fahnestock, 2007, Peng *et al.* , 2005) and animal (Caccamo *et al.* , 2010, Lee *et al.* , 2012, Rosa and Fahnestock, 2015) studies suggest neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) moderate neuronal and synaptic dysfunction and their behavioral expression in AD (Fahnestock, 2011, Lu *et al.* , 2013).

Clinical studies of the role of BDNF in AD are limited by the absence of validated biomarkers for central nervous system (CNS) BDNF (Forlenza *et al.* , 2010, Kim *et al.* , 2015). However, the *BDNF* Val66Met (rs6265) polymorphism Met protein can result in reduced dendritic trafficking and synaptic localization of the protein and up to a 30% reduction in activity-dependent BDNF secretion (Chen *et al.* , 2006, Egan *et al.* , 2003). In healthy young adults, memory dependent hippocampal activity is reduced in Met_{66} carriers (Hariri *et al.* , 2003). In the preclinical and prodromal stages of sporadic AD, prospective studies show Met_{66} carriers to have increased rates of decline in episodic memory and hippocampal atrophy relative to Val_{66} homozygotes (Feng *et al.* , 2013, Lim *et al.* , 2014, Lim *et al.* , 2013, Lim *et al.* , 2014). These same studies observe rates of cortical Aβ accumulation to be unaffected by the Met_{66} allele (Lim *et al.* , 2014, Lim *et al.* , 2013), suggesting that *BDNF* Met_{66} may accelerate neuronal dysfunction and memory decline by moderating pathological processes downstream of cortical Aβ accumulation, such as tau aggregation.

While the processes that give rise to cortical Aβ accumulation are likely to differ between sporadic and autosomal dominant AD, the effects of Aβ on neurodegeneration and cognition are similar, albeit occurring at markedly younger ages in ADAD (mean age of onset is 45 years) (Bateman *et al.* , 2012, Jack and Holtzman, 2013, Ryman *et al.* , 2014). Therefore the aim of this study was to investigate the effects of the *BDNF* Met_{66}

allele on episodic memory, hippocampal function, Aβ and tau in ADAD. The first hypothesis was that in preclinical ADAD mutation carriers (MCs), impairment in episodic memory and hippocampal function would be greater in individuals who carry at least one copy of the *BDNF* Met_{66} allele compared to Val_{66} homozygotes. The second hypothesis was that cortical Aβ levels would be unrelated to variation in *BDNF* Val66Met. The third hypothesis was that CSF tau levels would be greater in *BDNF* Met_{66} carriers compared to Val_{66} homozygotes. We also explored the extent to which carriage of the *BDNF* Met_{66} allele was associated with domains of cognition beyond episodic memory, neuronal function in the precuneus and CSF biomarkers of $A\beta_{1-42}$ and phosphorylated tau (p-tau_{181}). Finally, while the apolipoprotein E (*APOE*) ε4 allele does not increase severity of clinical presentation in ADAD (Ryman *et al.* , 2014), we observed previously additive effects of the *BDNF* Met_{66} and *APOE* ε4 alleles on Aβ related cognitive decline in preclinical sporadic AD (Lim *et al.* , 2014). Therefore, we also explored the extent to which *APOE* acts independently, or with *BDNF,* to impact disease processes in ADAD.

Method

Participants

Individuals at risk for carrying a mutation for ADAD (i.e., *presenilin 1* [*PSEN1*], *presenilin 2* [*PSEN2*], or *amyloid precursor protein* [*APP*] mutations) were enrolled in the Dominantly Inherited Alzheimer Network (DIAN) study. Participants from families with known pathogenic ADAD mutations were recruited from 197 families at six sites in the United States, one in the United Kingdom and three in Australia (Morris *et al.* , 2012). The process of recruitment and enrollment has been described in detail previously (Bateman *et al.* , 2012, Morris *et al.* , 2012). Baseline data from 274 participants (131 NCs, 143 preclinical MCs) who were cognitively normal, as defined by a Clinical Dementia Rating of 0, and who had completed assessments of cognitive function, neuroimaging and cerebrospinal fluid (CSF) sampling were included. *APOE* genotype was determined for all individuals as part of the DIAN study protocol. Additionally, for MCs, only individuals whose *BDNF* Val66Met polymorphism was available were included. Table 1 shows the demographic characteristics of each participant group.

Clinical Assessment

Without reference to participants' performance on the neuropsychological test battery, a clinician assessed each participant for the presence and severity of clinical symptoms of dementia at baseline. This was operationalized using the Clinical Dementia Rating (CDR) scale, for which a CDR total score of 0 indicates cognitive normality (Morris, 1983). Participants also completed the Mini Mental State Examination (MMSE) and the Geriatric Depression Scale (GDS) at baseline.

Neuropsychological Assessment

All participants were assessed using the DIAN neuropsychological test battery, which includes the Wechsler Memory Scale–Revised Logical Memory (Story A only, immediate and delayed recall) and Digit Span; Category Fluency (animals, vegetables); Trail Making Test A and B; Digit Symbol from the Wechsler Adult Intelligence Scale–Revised (WAIS–R); the Boston Naming Test (30 odd items), letter fluency for F, A, and S, and immediate and delayed recall of a single presentation of a 16-item word list (Storandt *et al.* , 2014). These tasks have been described previously, and were administered according to standard protocols by trained research assistants (Storandt *et al.* , 2014). The process of standardization and quality control of neuropsychological assessments across all DIAN sites have also been described previously (Storandt *et al.* , 2014).

Outcome measures for each neuropsychological test were standardized against the baseline mean and standard deviation for the NC group. Standardized scores were then averaged to form four cognitive domain-specific composite scores for *episodic memory* (Logical Memory delayed recall, word list learning delayed recall); *executive function* (Letter Fluency, Trail Making Test B); *language* (Category Fluency animals + vegetables, Boston Naming Test); *attention* (Digit Span Forwards, Digit Symbol); and *global cognition* (Logical Memory delayed recall, word list learning delayed recall, Digit Symbol, MMSE) (Donohue *et al.* , 2014).

Genotyping

Genotyping for pathogenic mutations in the *APP*, *PSEN1*, and *PSEN2* genes were performed on DNA extracted from peripheral blood samples using methods described previously (Talbot *et al.* , 1994). Samples were also genotyped with the Infinium HumanExomeCore V1.0 Beadchip (Illumina, Inc). Genotyping was performed at The Genome Technology Access Center (GTAC; https://gtac.wustl.edu/) at Washington University. All samples and genotypes underwent stringent quality control (QC). Genotype data were cleaned by applying a minimum call rate for SNPs and individuals (98%). SNPs not in Hardy-Weinberg equilibrium ($P< 1\times10^{-6}$) were excluded. No SNPs were removed because low mAF. Gender identification was verified by analysis of X-chromosome SNPs. We tested for unanticipated duplicates using pairwise genome-wide estimates of proportion identity-by-descent using PLINK v1.9. Genotype data for the *BDNF* Val66Met (rs6265) polymorphism was extracted from using PLINK. Clinicians were blinded to all genetic information and genetic polymorphisms were not used diagnostically. *BDNF* Val66Met genotyping was performed only in samples from individuals with a known ADAD mutation.

Neuroimaging

Images obtained through positron emission tomography (PET) with the use of fluorodeoxyglucose (FDG) and Pittsburgh compound B (PiB) (FDG-PET and PiB-PET, respectively) were co-registered with individual MRI images for region-of-interest (ROI) determination. 3 Tesla volumetric T1-weighted MRI scans from DIAN participants were acquired and processed through FreeSurfer (Martinos Center, Boston, MA) as previously described (Benzinger *et al.* , 2013). Amyloid imaging was performed with a bolus injection of approximately 15 mCi of [11C] PiB. Dynamic imaging acquisition started either at injection for 70 minutes or 40 minutes post-injection for 30 minutes. For analysis, PiB-PET data between 40 to 70 minutes were used. For PiB-PET, total neocortical SUVR was used to determine levels of cortical Aβ deposition, using cerebellar grey matter as the reference region and applying partial volume correction using a regional point spread function as previously described (Su *et al.* , 2015).

Metabolic imaging with [18F] FDG-PET was performed with a 3D dynamic acquisition began 40 minutes after a bolus injection of approximately 5 mCi of FDG and lasted for 20 minutes. In accordance with previous reports (Bateman *et al.* , 2012), the

ROIs selected for this study were the hippocampus and the precuneus, with decreased FDG SUVR indicating decreased glucose metabolism and therefore reduced neuronal function in that area. The reference region used was the cerebellar cortex.

Biochemical Analysis

Fasted CSF was collected in the morning via lumbar puncture. Samples were shipped on dry ice to the DIAN biomarker core laboratory. CSF concentrations of $A\beta_{42}$, total tau, and tau phosphorylated at threonine 181 ($ptau_{181}$) were measured by immunoassay (INNOTEST β-Amyloid1-42, Innogenetics). All values had to meet quality-control standards, including a coefficient of variation of 25% or less, kit "controls" within the expected range as defined by the manufacturer, and measurement consistency between plates of a common sample that was included in each run.

Estimated year of onset

The estimated year from expected symptom onset (EYO) was calculated as the age of the participant at the time of the baseline assessment minus the mean age at onset of all other individuals with the same mutation type (Ryman *et al.* , 2014).

Data Analysis

The study hypotheses that in ADAD, MC *BDNF* Met_{66} carriage would be associated with greater impairment in memory~~, greater~~ and hippocampal function, higher CSF tau but not cortical AB levels were tested by submitting the episodic memory composite, PiB-PET Aβ, CSF tau and glucose metabolism in the hippocampus (FDG-PET) to separate analyses of covariance (ANCOVA). In each ANCOVA, EYO was added as a covariate, and Group (NC, MC Val_{66}/Val_{66}, MC Met_{66}) as a fixed factor. Within each ANCOVA, two planned comparisons were constructed with the first comparing MC Val_{66} homozygotes and MC Met_{66} carriers and the second comparing MC Val_{66} homozygotes to the NC group. Exploratory analyses were conducted only if a statistically significant difference between the MC Val_{66} homozygote and MC Met_{66} carrier groups was observed for at least one of the primary outcome measures. With this criterion met, the ANCOVAs were repeated for the remaining cognitive composite scores, CSF $A\beta_{42}$, CSF p-tau_{181}, and FDG-PET in the precuneus. The extent to which the

presence of the *APOE* ε4 allele influenced the effect of *BDNF* on cognitive function, Aβ burden, tau and neuronal function was determined by repeating these analyses with ε4 status (carrier vs. non-carriers) entered into all statistical models. Finally, to further understand the effect of *BDNF* Val66Met on cognitive and biomarker outcomes in ADAD, we expressed each cognitive and biomarker outcome variable as a function of EYO. For the primary outcomes, statistical significance was classified as $p<.05$. This was to balance the risk of false positive findings against the identification of important relationships because (a) this is an exploratory investigation in a relatively new area in which an important clinical issue has been identified, (b) as all four primary outcome measures are recognized as part of the AD pathological process, changes in these will be correlated and (c) effect sizes (Cohen's d) were used to guide interpretation about the meaningfulness of statistical tests and comparisons with effect sizes <0.2 were classified as trivial and not interpreted regardless of statistical significance (Cohen, 1988).

Results

Demographic and clinical characteristics

MCs were significantly younger than NCs, although the EYO between MC Val_{66} homozygotes and MC Met_{66} carriers did not differ significantly. NC and MC groups did not differ on any other demographic characteristic. While the inclusion criteria required all individuals to have a CDR score of 0, the CDR sum of boxes score was significantly higher in MC Met_{66} carriers than in MC Val_{66} homozygotes and NCs (Table 1). Groups did not differ in MMSE total scores or levels of depressive symptoms.

Effect of *BDNF* Val66Met on episodic memory, cortical Aβ, CSF tau and glucose metabolism in the hippocampus

Group means and standard deviations for raw scores on each of the primary outcome cognitive and biomarker measures for each group are summarized on Table 2. The outcomes of the primary analyses are summarized on Figure 1 for episodic memory and Figure 2 for the AD biomarkers. Statistically significant group differences between MC Val_{66} homozygotes and MC Met_{66} carriers were observed for episodic memory (Figure 1), glucose metabolism in the hippocampus and CSF tau, but not cortical Aβ (Figure 2). Effect sizes for these comparisons were, by convention, moderate-to-large in

magnitude for episodic memory, glucose metabolism in the hippocampus and CSF tau levels, but were trivial for levels of cortical Aβ. No statistically significant differences between NC and MC Val_{66} homozygotes were observed for any of the primary outcome measures, with all differences small in magnitude.

Effect of *BDNF* Val66Met on cognition, CSF $Aβ_{42}$, CSF p-tau_{181} and glucose metabolism in the precuneus

For each exploratory cognitive and biomarker outcome measure, ~~Group~~ raw group means and standard deviations ~~for raw scores on each of the primary outcome cognitive and biomarker measures for each group~~ are ~~also~~ summarized on Table 2. Figure 1 and 2 also summarises the ~~and~~ outcomes of the exploratory analyses ~~are summarized on Figure 1~~ for cognitive measures and ~~Figure 2 for the~~ AD biomarkers respectively. Statistically significant group differences, of a moderate-to-large magnitude, were observed between MC Val_{66} homozygotes and MC Met_{66} carriers for CSF p-tau_{181} levels (Figure 2, exploratory outcomes) but not for glucose metabolism in the precuneus or for the executive function, language, attention or global cognition composites (Figure 1, exploratory outcomes). There were also no statistically significant differences between MC Val_{66} homozygotes and MC Met_{66} carriers on CSF $Aβ_{42}$ levels, with these differences small in magnitude (Figure 2, exploratory outcomes).

When compared to NCs, MC Val_{66} homozygotes showed no statistically significant impairment in any domain of cognitive function (Figure 1) and did not differ significantly in the extent of glucose metabolism in the hippocampus or the precuneus (Figure 2). Compared to NCs, both MC Val_{66} homozygotes and MC Met_{66} carriers showed elevated levels of CSF tau and p-tau_{181}, and increased PiB-PET SUVR and decreased CSF $Aβ_{42}$ levels (Table 2).

Effect of *APOE* ε4 on cognitive function, neuronal dysfunction, Aβ and tau

Re-analyses of the primary hypotheses with the addition of *APOE* status indicated no significant main effect of *APOE* status and no significant interaction between *APOE* and *BDNF* status on any measure of cognitive function (Table 3). Similarly, there was no significant main effect of *APOE* or interaction between *APOE* and *BDNF* for any outcome measure of glucose metabolism or tau (Table 3). However, there

was a significant main effect of *APOE* for both PiB-PET SUVR and CSF $A\beta_{42}$, although there were no significant interactions between *APOE* and *BDNF* for either measure (Table 3). Post-hoc analyses showed that when compared to MC ε4 non-carriers, MC ε4 carriers had significantly increased PiB-PET SUVR (*d* [95%CI = 0.45 [0.05, 0.85], *p* = .03) and decreased CSF $A\beta_{42}$ levels (*d* [95%CI = 0.76 [0.34, 1.17], *p* <.001).

Effect of *BDNF* Val66Met on the relationship between EYO and markers of cognitive and neuronal function, Aβ and tau

There were no statistically significant relationships between level of cognitive function and EYO in NCs or in MC Val_{66} homozygotes. However, the relationship between EYO and episodic memory was statistically significant and moderate in magnitude for MC Met_{66} carriers (Figure 3A). Similarly, there were no statistically significant relationships between glucose metabolism in the hippocampus and EYO in NCs or in MC Val_{66} homozygotes. However, the relationship between glucose metabolism in the hippocampus and EYO was moderate in magnitude and statistically significant for MC Met_{66} carriers (Figure 3B).

There was no relationship between levels of cortical Aβ and EYO in NCs, but there was a significant moderate association between cortical Aβ levels and EYO in MCs irrespective of *BDNF* Val66Met polymorphism (Figure 3C). Similarly, while there was no association between CSF tau and EYO in NCs, there was a significant moderate association between CSF tau and EYO in MCs, irrespective of *BDNF* Val66Met genotype (Figure 3D), with MC Met_{66} carriers showing systematically higher levels of CSF tau relative to their EYO than MC Val_{66} homozygotes (Figure 3D).

Discussion

The results show that the presence of one copy of the *BDNF* Met_{66} allele increased the severity of impairment in episodic memory and hippocampal function in preclinical ADAD. This effect is clinically important as the magnitude of memory impairment related to MC Met_{66} carriers was approximately double that observed in MC Val_{66} homozygotes. These findings in the DIAN cohort are consistent with the greater memory decline and hippocampal volume loss observed in older adults with preclinical or prodromal sporadic AD from the AIBL and ADNI studies (Feng *et al.* , 2013, Lim *et al.* ,

2014, Lim *et al.* , 2013). The results confirm therefore, in an independent sample, that *BDNF* is important to the preclinical presentation of AD.

The current data support the first hypothesis that in preclinical MCs, impairment in memory and hippocampal function would be greater in Met_{66} carriers compared to Val_{66} homozygotes. Compared to MC Val_{66} homozygotes, MC Met_{66} carriers had worse episodic memory function (Figure 1). In contrast, no memory impairment was observed in MC Val_{66} homozygotes compared to NCs. Similarly, hippocampal function, determined by cerebral glucose metabolism, was also reduced in MC Met_{66} carriers compared to MC Val_{66} homozygotes. However, MC Val_{66} homozygotes did not show lower glucose metabolism compared to NCs. As increased oxidative stress has been previously observed in females (Keaney *et al.* , 2003), it is possible that the sex of participants may better account for the memory impairment in MC Met_{66} carriers. However, re-analysis of all primary outcome measures suggest that even when the sex of participants was considered, the effect of *BDNF* Val66Met on memory impairment, hippocampal function and tau remains (Table 4). Finally, MC Met_{66} carriers who were estimated to be nearer to their expected year of clinical symptom onset (EYO) showed increased memory impairment and lower glucose metabolism in the hippocampus (Figure 3). In contrast, EYO was not associated with memory impairment or glucose metabolism in NCs or MC Val_{66} homozygotes.

The second hypothesis that cortical Aβ and CSF $A\beta_{42}$ levels would be unrelated to allelic variation in *BDNF* Val66Met was also supported. Preclinical MC Met_{66} carriers and Val_{66} homozygotes had equivalent levels of higher cortical Aβ and CSF $A\beta_{42}$. Furthermore, these group differences were, by convention, small (i.e., $d<0.2$; Figure 2) in magnitude indicating that absence of statistically significant differences was not due to insufficient statistical power. Compared to NCs, both Met_{66} carriers and Val_{66} homozygotes showed increased levels of cortical Aβ deposition and decreased levels of CSF $A\beta_{42}$. Similarly, cortical Aβ burden was higher in preclinical MCs who were nearer to their EYO; although this relationship was not moderated by the *BDNF* Val66Met polymorphism (Figure 3C). Increased cortical Aβ and lower CSF $A\beta_{42}$ levels have been observed previously in preclinical ADAD (Bateman *et al.* , 2012, Ryman *et al.* , 2014). The absence of any effect of Met_{66} carriage on Aβ burden in preclinical ADAD is also consistent with observations that *Met* carriage was unrelated to rates of cortical Aβ

accumulation over 3 years in preclinical and prodromal sporadic AD (Feng *et al.* , 2013, Lim *et al.* , 2014, Lim *et al.* , 2013). Together, these findings suggest that the effect of the *BDNF* Met_{66} allele is independent of the effect of Aβ on risk for, and progression of, AD.

The results also support the third hypothesis that CSF levels of tau would be greater in MC Met_{66} carriers compared to MC Val_{66} homozygotes. Levels of both CSF tau and p-tau_{181} were increased substantially in preclinical MC Met_{66} carriers compared to preclinical MC Val_{66} homozygotes (Figure 2). Compared to NCs, preclinical MC Val_{66} homozygotes also showed increased levels of CSF tau and p-tau_{181}, although not to the same extent as MC Met_{66} carriers. Despite the overall increase in these biochemical markers, strong relationships between EYO and CSF tau were observed in both MC Val_{66} homozygotes and MC Met_{66} carriers, and the magnitude of these relationships were equivalent (Figure 3D). Thus, while the Met_{66} allele hastens memory dysfunction in preclinical ADAD, it does not necessarily affect the rate at which p-tau_{181} accumulates in CSF. Instead, substantial differences in CSF p-tau_{181} levels between MC Met_{66} carriers and MC Val_{66} homozygotes (Figure 2) suggest that MC Val_{66} homozygotes may have an increased level of resilience to the neurotoxic effects of tau and Aβ.

Finally, we explored the extent to which *APOE* acts independently or with *BDNF* to impact disease processes in ADAD. There were no independent effects of *APOE* ε4, or combined effects of *APOE* and *BDNF*, on cognition, neuronal function or CSF tau (Table 3). However, compared to MC ε4 non-carriers, MC ε4 carriers showed increased cortical Aβ and decreased CSF $Aβ_{42}$. This indicates that in prelinical ADAD, the abnormal accumulation of cortical Aβ resulting from pathogenic mutations is increased further by the *APOE* ε4 allele, although this increased Aβ was not associated with any greater impairment in cognition or neuronal function. Importantly, the increase in cortical Aβ in MC ε4 carriers was not affected by the *BDNF Met* allele. Thus, allelic variation in *BDNF* and *APOE* may affect different AD processes with ε4 increasing cortical Aβ accumulation and *BDNF* Met_{66} moderating Aβ related impairment in cognition and neuronal function through its effects on tau.

Neuronal and synaptic loss characteristic of both sporadic and autosomal dominant AD is due to the combined accumulation of Aβ plaques and tau aggregation (Ballatore *et al.* , 2007, Ittner and Götz, 2011, Spires-Jones and Hyman, 2014).

Neuropathological and CSF biomarker studies show that in AD, cognitive impairment and synaptic loss are associated more strongly with the presence and number of neurofibrillary tangles than Aβ plaques (Bennett *et al.* , 2004, Giannakopoulos *et al.* , 2003, Ingelsson *et al.* , 2004). However, neuroimaging studies in preclinical AD report that higher cortical Aβ load is associated with greater rates of cognitive decline and progression to MCI (Lim *et al.* , 2014, Rowe *et al.* , 2013), with these effects mediated by the effect of Aβ on neurodegeneration (Jack and Holtzman, 2013, Lim *et al.* , 2015). In this context, dissociation of the effects of *BDNF* on Aβ and tau associated cognitive impairment observed here are important because they provide evidence that *BDNF* Met_{66} influences disease progression through effects on neuronal dysfunction and cognitive impairment associated with tau.

The current observation that *BDNF* Met_{66} in preclinical ADAD was associated with increased tau, hippocampal dysfunction and memory impairment is consistent with the role that CNS BDNF plays in synaptic excitation, long-term potentiation and neuronal plasticity (Fahnestock, 2011, Forlenza *et al.* , 2010, Garzon and Fahnestock, 2007, Hariri *et al.* , 2003, Lee *et al.* , 2012, Lu *et al.* , 2013, Peng *et al.* , 2005). Evidence of a mechanistic relationship between BDNF and tau has been shown in cellular studies which demonstrate that BDNF can induce rapid dephosphorylation of tau through TrkB activation (Elliott *et al.* , 2005) and that BDNF loss in AD is specific to tangle-bearing neurons (Ferrer *et al.* , 1999). This has prompted the hypothesis that there may be a direct relationship between CNS BDNF levels and tau (Belrose *et al.* , 2013), although this remains under investigation. Even in the absence of a direct mechanistic link, the large and clinically important effects of *BDNF* Met_{66} on memory, hippocampal function and tau, observed in the current ADAD sample, indicate that studying allelic variation in *BDNF* Val66Met may help clarify pathological models of AD and may even provide a reference for the investigation of the effects and clinical consequences of other neurotrophic factors in AD.

As we have noted (Lim *et al.* , 2013), genome-wide association studies (GWAS) of AD do not identify the *BDNF* Val66Met polymorphism as increasing the risk for AD (Lambert *et al.* , 2013). One possible explanation for this is that GWAS typically use a clinical classification of dementia as the target phenotype. Consequently, they may overlook the contribution of *BDNF* because the effects of this gene manifest only in the

earliest stages of the disease (Feng *et al.* , 2013, Lim *et al.* , 2014, Lim *et al.* , 2013). This hypothesis is supported by GWAS of cognitive aging in non-demented older adults, where *BDNF* Val66Met has been associated with memory impairment and decline (Harris and Deary, 2011, Papenberg *et al.* , 2015). Thus, the hypothesis arising from the current and previous studies (Lim *et al.* , 2014, Lim *et al.* , 2013, Lim *et al.* , 2014) is that in studies of cognitive aging, memory decline associated with *BDNF* Met_{66} may reflect occult AD as opposed to the effects of normal aging. In contrast to *BDNF*, GWAS of AD identify carriage of *APOE* ε4 as increasing risk for AD (Lambert *et al.* , 2013). We have also reported that in preclinical sporadic AD, the *APOE* ε4 allele increases the rate of memory decline and brain volume loss associated with high Aβ (Dore *et al.* , 2013, Lim *et al.* , 2014, Lim *et al.* , 2015). We have also observed that Aβ+ older adults who carry both the *APOE* ε4 and *BDNF* Met_{66} allele show greater memory decline than those who carry either one by itself (Lim *et al.* , 2014). Re-analysis of the current data taking into account *APOE* ε4 did not indicate any effect of *APOE* or any interaction between *APOE* and *BDNF* on cognition (Table 3). The absence of any effect of *APOE* on cognition in this study is consistent with the results of a detailed meta-analysis of three ADAD cohorts which showed that *APOE* did not moderate age of clinical symptom onset (Ryman *et al.* , 2014). However, despite having no effect on cognitive function or clinical symptom onset, *APOE* ε4 was associated with increasing cortical Aβ levels in preclinical MCs. Consequently, one hypothesis for the absence of any *APOE* effect on cognitive and clinical outcomes in ADAD is that these outcomes are related more strongly to neuronal dysfunction and tau than to Aβ accumulation.

This study demonstrates that the deleterious effects of Aβ in ADAD were increased in preclinical individuals who carried the *BDNF* Met_{66} allele. Therefore, the results of this study also confirm the similarity between the development of dementia in ADAD and sporadic AD. However, as the current findings are based on cross-sectional data, it will be necessary to replicate these results prospectively. Nonetheless, the strength and consistency of our results with that in sporadic AD is important because they suggest that strategies designed to increase CNS BDNF levels may be a viable therapeutic alternative or addition to those which seek to reduce the neurotoxic effects of Aβ. Our results also suggest strongly that the *BDNF* Val66Met polymorphism should be considered as a potential moderator of clinical trial outcomes in current treatment

and prevention trials in ADAD and sporadic AD (Donohue *et al.* , 2014, Mills *et al.* , 2013).

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Conflicts of Interests

YY Lim, J Hassenstab, C Cruchaga, P Maruff, PJ Snyder, R Allegri, MR Farlow, NR Graff-Radford, C Laske, E McDade, and J Ringman have no disclosures.

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Table 1. Demographic and clinical characteristics

	NC (n=131)	MC Val_{66}/Val_{66} (n=95)	MC Met_{66} (n=48)	*p*
N (%) Female	58 (44.3%)	37 (38.9%)	25 (52.1%)	.325
N (%) *APOE* ε4 carrier	39 (29.8%)	24 (25.3%)	12 (25.0%)	.784
Age	**38.37 (10.13)**	**34.45 (8.54)**	**35.12 (9.51)**	**.012**
Est. Year of Onset	N/A	-12.44 (8.11)	-12.70 (7.60)	.855
Years of Education	14.79 (2.64)	14.72 (3.54)	14.24 (2.56)	.328
GDS	1.24 (1.66)	1.45 (1.83)	1.47 (1.60)	.566
CDR sum of boxes	**0.01 (0.06)**	**0.02 (0.10)**	**0.06 (0.17)**	**.005**
MMSE	29.20 (1.17)	28.97 (1.37)	29.04 (0.99)	.340

Note: NC = Mutation non-carrier; MC = mutation carrier; GDS = Geriatric Depression Scale; HADS = Hospital Anxiety and Depression Scale; MACQ = Memory Complaints Questionnaire; CDR = Clinical Dementia Rating scale; MMSE = Mini-Mental State Examination

Table 2. Differences in each cognitive marker and biomarker between mutation non-carriers, mutation carriers who are *BDNF* Val_{66} homozygotes, and mutation carriers who are *BDNF* Met_{66} carriers.

	EYO		Group		NC		MC Val_{66}/Val_{66}		MC Met_{66}	
Primary Outcomes	(df) F	*p*	(df) F	*p*	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Episodic Memory	**(1,268) 20.87**	**.00**	**(2,268) 5.35**	**.00**	0.03 (0.82)	131	-0.12 (0.83)	95	-0.43 (0.83)	48
PiB-PET SUVR	**(1,223) 18.31**	**.00**	**(2,223) 38.85**	**.00**	1.04 (0.54)	106	1.62 (0.53)	82	1.74 (0.53)	39
CSF tau	**(1,216) 16.20**	**.00**	**(2,216) 19.94**	**.00**	57.18 (40.42)	101	82.83 (40.29)	80	102.15 (40.29)	39
FDG-PET hippocampus	**(1,225) 12.13**	**.00**	**(2,225) 3.91**	**.02**	1.25 (0.09)	109	1.26 (0.09)	80	1.21 (0.09)	40
Exploratory Outcomes										
Executive Function	(1,268) 2.23	.14	(2,268) 2.10	.12	0.02 (0.80)	131	-0.15 (0.81)	95	-0.22 (0.81)	48
Language	(1,267) 0.28	.60	(2,267) 2.90	.06	0.03 (0.86)	131	-0.15 (0.86)	95	-0.29 (0.86)	48
Attention	(1,268) 2.20	.14	**(2,268) 4.00**	**.02**	0.01 (0.81)	131	-0.15 (0.81)	95	-0.37 (0.81)	48
Global Cognition	**(1,267) 8.36**	**.00**	**(2,267) 3.58**	**.03**	0.02 (0.65)	131	-0.12 (0.65)	95	-0.26 (0.66)	48
CSF $A\beta_{42}$	**(1,213) 8.03**	**.01**	**(2,213) 7.55**	**.00**	430.72 (147.29)	99	355.78 (146.91)	78	346.57 (146.79)	40
CSF p-tau_{181}	**(1,217) 8.12**	**.01**	**(2,217) 32.22**	**.00**	29.27 (22.75)	101	48.48 (22.69)	80	60.62 (22.67)	40
FDG-PET precuneus	**(1,225) 4.62**	**.03**	(2,225) 1.13	.33	2.79 (0.29)	109	2.74 (0.29)	80	2.73 (0.29)	40

Note: EYO = estimated year of symptom onset; Group = effect of group membership as NC, MC Val_{66} homozygote or MC Met_{66} carrier; all models have been adjusted for estimated year of symptom onset; bolded values are significant at the $p < .05$ or $p < .001$ level

Table 3. Effect of estimated year of symptom onset (EYO), *APOE* ε4 status, *BDNF* Val66Met status, and the interaction between *APOE* and *BDNF* on each cognitive and biomarker outcome measure

	EYO		*APOE* Group		*BDNF* Group		*APOE x BDNF Group*	
Primary Outcomes	(df) F	*p*	(df) F	*p*	(df) F	*p*	(df) F	*p*
Episodic Memory	**(1,266) 19.32**	**.00**	(1,266) 1.79	.18	**(1,266) 3.84**	**.05**	(1,266) 0.08	.77
PiB-PET SUVR	**(1,221) 16.73**	**.00**	**(1,221) 7.21**	**.01**	(1,221) 2.65	.11	(1,221) 1.18	.28
CSF tau	**(1,214) 15.50**	**.00**	(1,214) 0.24	.63	**(1,214) 4.06**	**.04**	(1,214) 0.05	.82
FDG-PET hippocampus	**(1,223) 11.88**	**.00**	(1,223) 0.05	.83	**(1,223) 6.03**	**.02**	(1,223) 0.09	.77
Exploratory Outcomes								
Executive Function	(1,266) 2.50	.12	(1,266) 0.93	.34	(1,266) 0.08	.78	(1,266) 0.08	.78
Language	(1,266) 0.20	.65	(1,266) 0.39	.54	(1,266) 0.99	.32	(1,266) 0.15	.70
Attention	(1,266) 2.40	.12	(1,266) 1.46	.23	(1,266) 2.78	.10	(1,266) 0.49	.49
DIAN Composite	**(1,266) 8.14**	**.01**	(1,266) 0.04	.84	(1,266) 0.84	.36	(1,266) 0.06	**.**81
CSF $A\beta_{42}$	**(1,211) 6.94**	**.01**	**(1,211) 9.28**	**.00**	(1,211) 0.02	.90	(1,211) 1.39	.24
CSF p-tau_{181}	**(1,215) 7.39**	**.01**	(1,215) 2.00	.16	**(1,215) 4.60**	**.03**	(1,215) 0.34	.56
FDG-PET precuneus	**(1,223) 4.82**	**.03**	(1,223) 0.65	.42	(1,223) 0.001	.98	(1,223) 0.02	.91

Note: all models have been adjusted for estimated year of symptom onset (EYO); *APOE* Group indicates effect of group membership as NC, *APOE* ε4 carrier or *APOE* ε4 non-carrier; *BDNF* Group indicates effect of group membership as NC, MC Val_{66} homozygote or MC Met_{66} carrier; bolded values are significant at the $p < .05$ level

Table 4. Re-analysis of the effect of *BDNF* Val66Met on each primary outcome variable, covarying for the potential confounding effect of sex

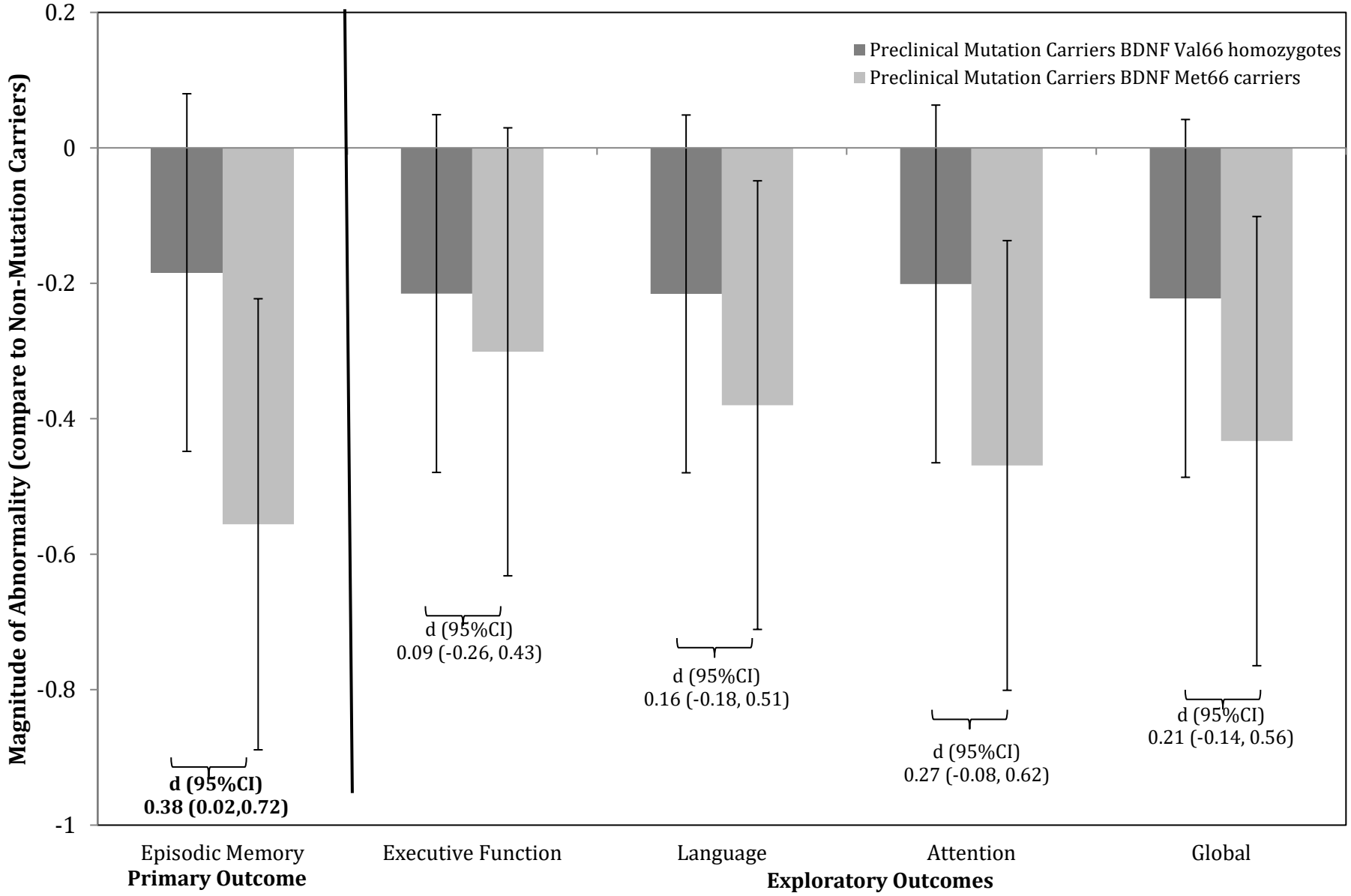
	EYO		Sex		*BDNF* Group	
	(df) F	*p*	(df) F	*p*	(df) F	*p*
Episodic Memory	**(1,267) 13.96**	**.00**	**(1,267) 8.24**	**.00**	**(2,267) 5.17**	**.00**
PiB-PET SUVR	**(1,222) 18.43**	**.00**	(1,222) 0.01	.92	**(2,222) 40.59**	**.00**
CSF tau	**(1,215) 17.02**	**.00**	(1,215) 0.56	.46	**(2,215) 20.98**	**.00**
FDG-PET hippocampus	**(1,224) 5.60**	**.02**	(1,224) 1.35	.25	**(2,224) 3.95**	**.02**

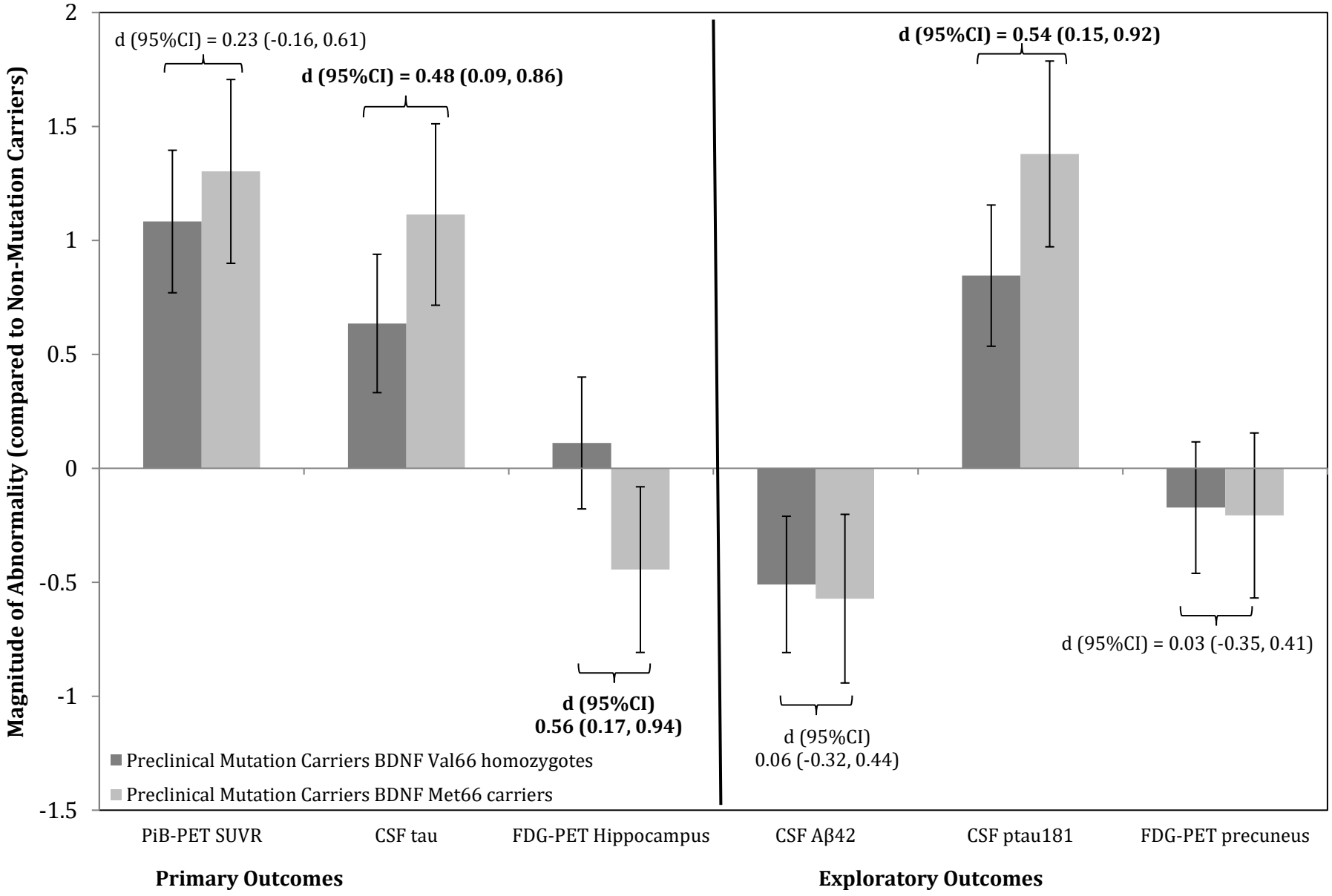
Figure Captions

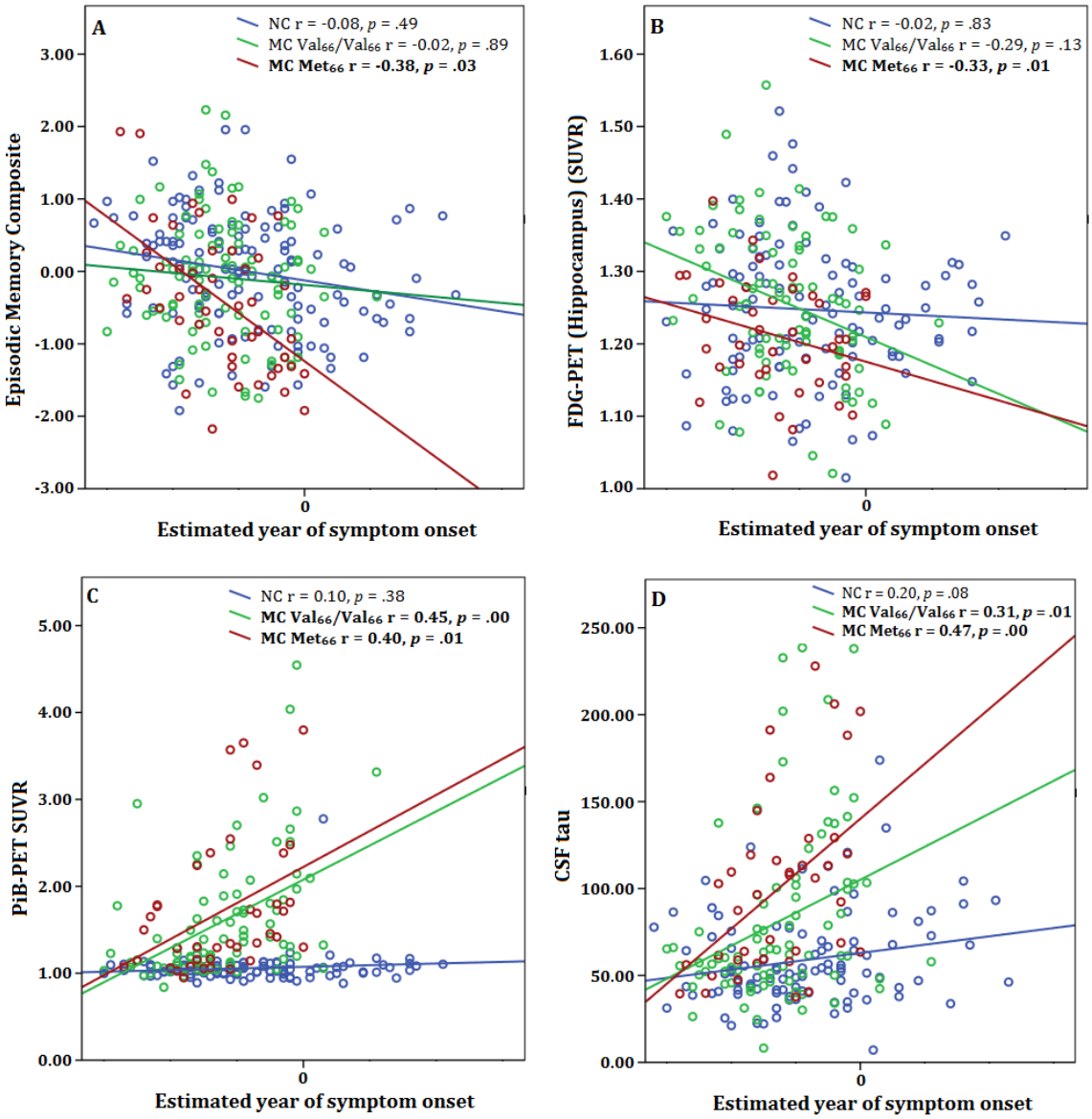
Figure 1. Magnitude of cognitive impairment in preclinical MC Val_{66} homozygotes and preclinical MC Met_{66} carriers when compared to mutation non-carriers (error bars represent 95% confidence intervals) (statistical significance occurs when 95% confidence intervals do not cross "0" line).

Figure 2. Magnitude of abnormality on markers of Aβ, tau and glucose metabolism in preclinical MC Val_{66} homozygotes and preclinical MC Met_{66} carriers when compared to mutation non-carriers (error bars represent 95% confidence intervals) (statistical significance occurs when 95% confidence intervals do not cross "0" line).

Figure 3. Relationship between estimated year of clinical symptom onset and episodic memory performance (A), glucose metabolism in the hippocampus (B), cortical Aβ levels (C), and CSF p-tau_{181} levels (D), in mutation non-carriers, preclinical MC Val_{66} homozygotes, and preclinical MC Met_{66} carriers.


0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Magnitude of Abnormality (compare to Non-Mutation Carriers)
Preclinical Mutation Carriers BDNF Val66 homozygotes
Preclinical Mutation Carriers BDNF Met66 carriers
d (95%CI)
0.38 (0.02,0.72)
d (95%CI)
0.09 (-0.26, 0.43)
d (95%CI)
0.16 (-0.18, 0.51)
d (95%CI)
0.27 (-0.08, 0.62)
d (95%CI)
0.21 (-0.14, 0.56)
Episodic Memory
Primary Outcome
Executive Function
Language
Attention
Global
Exploratory Outcomes


Magnitude of Abnormality (compared to Non-Mutation Carriers)
2
1.5
1
0.5
0
-0.5
-1
-1.5
d (95%CI) = 0.23 (-0.16, 0.61)
d (95%CI) = 0.48 (0.09, 0.86)
d (95%CI)
0.56 (0.17, 0.94)
d (95%CI)
0.06 (-0.32, 0.44)
d (95%CI) = 0.54 (0.15, 0.92)
d (95%CI) = 0.03 (-0.35, 0.41)
Preclinical Mutation Carriers BDNF Val66 homozygotes
Preclinical Mutation Carriers BDNF Met66 carriers
PiB-PET SUVR
CSF tau
FDG-PET Hippocampus
CSF Aβ42
CSF ptau181
FDG-PET precuneus
Primary Outcomes
Exploratory Outcomes


A
NC r = -0.08, p = .49
MC Val66/Val66 r = -0.02, p = .89
MC Met66 r = -0.38, p = .03
Episodic Memory Composite
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
0
Estimated year of symptom onset
B
NC r = -0.02, p = .83
MC Val66/Val66 r = -0.29, p = .13
MC Met66 r = -0.33, p = .01
FDG-PET (Hippocampus) (SUVR)
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0
Estimated year of symptom onset
C
NC r = 0.10, p = .38
MC Val66/Val66 r = 0.45, p = .00
MC Met66 r = 0.40, p = .01
PiB-PET SUVR
5.00
4.00
3.00
2.00
1.00
0.00
0
Estimated year of symptom onset
D
NC r = 0.20, p = .08
MC Val66/Val66 r = 0.31, p = .01
MC Met66 r = 0.47, p = .00
CSF tau
250.00
200.00
150.00
100.00
50.00
0.00
0
Estimated year of symptom onset

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