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

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The brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism is implicated in synaptic excitation and neuronal integrity, and has previously been shown to moderate amyloid-β-related memory decline and hippocampal atrophy in preclinical sporadic Alzheimer's disease. However, the effect of BDNF in autosomal dominant Alzheimer's disease is unknown. We aimed to determine the effect of *BDNF* Val66Met on cognitive function, hippocampal function, tau and amyloid-β in preclinical autosomal dominant Alzheimer's disease. We explored effects of apolipoprotein E (*APOE*) ε4 on these relationships. The Dominantly Inherited Alzheimer Network conducted clinical, neuropsychological, genetic, biomarker and neuroimaging measures at baseline in 131 mutation non-carriers and 143 preclinical autosomal dominant Alzheimer's disease mutation carriers on average 12 years before clinical symptom onset. *BDNF* genotype data were obtained for mutation carriers (95 Val_{66} homozygotes, 48 Met_{66} carriers). Among preclinical mutation carriers, Met_{66} carriers had worse memory performance, lower hippocampal glucose metabolism and increased levels of cerebrospinal fluid tau and phosphorylated tau (p-tau) than Val_{66} homozygotes. Cortical amyloid-β and cerebrospinal fluid amyloid-β_{42} levels were significantly different from non-carriers but did not differ between preclinical mutation carrier Val_{66} homozygotes and Met_{66} carriers. There was an effect of *APOE* on amyloid-β levels, but not cognitive function, glucose metabolism or tau. As in sporadic Alzheimer's disease, the deleterious effects of amyloid-β on memory, hippocampal function, and tau in preclinical autosomal dominant Alzheimer's disease mutation carriers are greater in Met_{66} carriers. To date, this is the only genetic factor found to moderate downstream effects of amyloid-β in autosomal dominant Alzheimer's disease.

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Abbreviations: ADAD = autosomal dominant Alzheimer's disease; DIAN = Dominantly Inherited Alzheimer Network; FDG = fluorodeoxyglucose; PiB = Pittsburgh compound B; SUVR = standardized uptake value ratio

Introduction

Alzheimer's disease begins with the aggregation of amyloid-β, 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 Alzheimer's disease are still not understood completely; however, recent *in vitro* (Hariri *et al.*, 2003; Lee *et al.*, 2012), post-mortem (Peng *et al.*, 2005; Garzon and Fahnestock, 2007; Buchman *et al.*, 2016) 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 behavioural expression in Alzheimer's disease (Fahnestock, 2011; Lu *et al.*, 2013).

Clinical studies of the role of BDNF in Alzheimer's disease are limited by the absence of validated biomarkers for 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 (Egan *et al.*, 2003; Chen *et al.*, 2006). 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 Alzheimer's disease, 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.*, 2013, 2014*b*). These same studies observe rates of cortical amyloid-β accumulation to be unaffected by the Met_{66} allele (Lim *et al.*, 2013, 2014*b*), suggesting that *BDNF* Met_{66} may accelerate neuronal dysfunction and memory decline by moderating pathological processes downstream of cortical amyloid-β accumulation, such as tau aggregation.

While the processes that give rise to cortical amyloid-β accumulation are likely to differ between sporadic and autosomal dominant Alzheimer's disease, the effects of amyloid-β on neurodegeneration and cognition are similar, albeit occurring at markedly younger ages in autosomal dominant Alzheimer's disease (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, amyloid-β and tau in ADAD. The first hypothesis was that in preclinical ADAD mutation carriers, 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 amyloid-β 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 amyloid-$\beta_{1\text{-}42}$ 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 amyloid-β-related cognitive decline in preclinical sporadic Alzheimer's disease (Lim *et al.*, 2015*b*).

Therefore, we also explored the extent to which *APOE* acts independently, or with *BDNF*, to impact disease processes in ADAD.

Materials and methods

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 USA, one in the UK and three in Australia (Morris *et al.*, 2012). The process of recruitment and enrolment has been described in detail previously (Bateman *et al.*, 2012; Morris *et al.*, 2012). Baseline data from 274 participants (131 non-carriers, 143 preclinical mutation carriers) who were cognitively normal, as defined by a Clinical Dementia Rating (CDR) of 0, and who had completed assessments of cognitive function, neuroimaging and CSF sampling were included. *APOE* genotype was determined for all individuals as part of the DIAN study protocol. Additionally, for mutation carriers, 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 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 non-carriers 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 single nucleotide polymorphisms (SNPs) and individuals (98%). SNPs not in Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$) were excluded. No SNPs were removed due to low minor allele frequency. 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 were 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 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 determination. Volumetric (3 T) T_1-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 ~15 mCi of ^{11}C-PiB. Dynamic imaging acquisition started either at injection for 70 or 40 min post-injection for 30 min. For analysis, PiB-PET data between 40 to 70 min were used. For PiB-PET, total neocortical standardized uptake value ratio (SUVR) was used to determine levels of cortical amyloid-β 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 ^{18}F-FDG-PET was performed with a 3D dynamic acquisition begun 40 min after a bolus injection of ~5 mCi of FDG and lasted for 20 min. In accordance with previous reports (Bateman *et al.*, 2012), the regions of interest 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 amyloid-β_{42}, total tau, and tau phosphorylated at threonine 181 (p-tau$_{181}$) 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 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, mutation carrier *BDNF* Met$_{66}$ carriage would be associated with greater impairment in memory and hippocampal function, higher CSF tau but not cortical amyloid-β levels were tested by submitting the episodic memory composite, PiB-PET amyloid-β, CSF tau and glucose metabolism in the hippocampus (FDG-PET) to separate analyses of covariance (ANCOVA). In each ANCOVA, estimated year of onset was added as a covariate, and Group (non-carriers, Val$_{66}$/Val$_{66}$ mutation carrier, Met$_{66}$ mutation carrier) as a fixed factor. Within each ANCOVA, two planned comparisons were constructed with the first comparing Val$_{66}$ homozygotes and Met$_{66}$ mutation carriers and the second comparing Val$_{66}$ homozygote mutation carriers to the non-carriers group. Exploratory analyses were conducted only if a statistically significant difference between the Val$_{66}$ homozygote and Met$_{66}$ mutation 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 amyloid-β_{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, amyloid-β burden, tau and neuronal function was determined by repeating these analyses with ε4 status (carrier versus 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 estimated year of onset. For the primary outcomes, statistical significance was classified as $P < 0.05$. This was to balance the risk of false positive findings against the identification of important relationships because (i) this is an exploratory investigation in a relatively new area in which an important clinical issue has been identified; (ii) as all four primary outcome measures are recognized as part of the Alzheimer's disease pathological process, changes in these will be correlated; and (iii) 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

Mutation carriers were significantly younger than non-carriers, although the estimated year of onset between Val$_{66}$ homozygotes and Met$_{66}$ mutation carriers did not differ significantly. Non-carriers and mutation carrier 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 mutation carrier Met$_{66}$ carriers than in mutation carrier Val$_{66}$ homozygotes and non-carriers (Table 1). Groups did not differ in MMSE total scores or levels of depressive symptoms.

Effect of *BDNF* Val66Met on episodic memory, cortical amyloid-β, 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 Fig. 1 for episodic memory and Fig. 2 for the Alzheimer's disease biomarkers. Statistically significant group differences between Val$_{66}$ homozygot- and Met$_{66}$ mutation carriers were observed for episodic memory (Fig. 1), glucose metabolism in the hippocampus and CSF tau, but not cortical amyloid-β (Fig. 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 amyloid-β. No statistically significant differences between non-carriers and Val$_{66}$ homozygote mutation carriers were observed for any of the primary outcome measures, with all differences small in magnitude.

Effect of *BDNF* Val66Met on cognition, CSF amyloid-β_{42}, CSF p-tau$_{181}$ and glucose metabolism in the precuneus

For each exploratory cognitive and biomarker outcome measure, raw group means and standard deviations are summarized on Table 2. Figures 1 and 2 also summarize the outcomes of the exploratory analyses for cognitive measures and Alzheimer's disease biomarkers, respectively.

Table 1 Demographic and clinical characteristics

	Mutation non-carriers ($n = 131$)	Mutation carrier Val_{66}/Val_{66} ($n = 95$)	Mutation carrier Met_{66} ($n = 48$)	*P*-value
n (%) Female	58 (44.3%)	37 (38.9%)	25 (52.1%)	0.325
n (%) *APOE* ε4 carrier	39 (29.8%)	24 (25.3%)	12 (25.0%)	0.784
Age	**38.37 (10.13)**	**34.45 (8.54)**	**35.12 (9.51)**	**0.012**
Estimated year of onset	N/A	−12.44 (8.11)	−12.70 (7.60)	0.855
Years of education	14.79 (2.64)	14.72 (3.54)	14.24 (2.56)	0.328
GDS	1.24 (1.66)	1.45 (1.83)	1.47 (1.60)	0.566
CDR sum of boxes	**0.01 (0.06)**	**0.02 (0.10)**	**0.06 (0.17)**	**0.005**
MMSE	29.20 (1.17)	28.97 (1.37)	29.04 (0.99)	0.340

CDR = Clinical Dementia Rating scale; GDS = Geriatric Depression Scale; HADS = Hospital Anxiety and Depression Scale; MACQ = Memory Complaints Questionnaire; 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

	Estimated year of onset		Group		Mutation non-carriers		Mutation carrier Val_{66}/Val_{66}		Mutation carrier Met_{66}	
	(df)*F*	*P*	(df)*F*	*P*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*
Primary outcomes										
Episodic memory	**(1,268) 20.87**	**0.00**	**(2,268) 5.35**	**0.00**	0.03 (0.82)	131	−0.12 (0.83)	95	−0.43 (0.83)	48
PiB-PET SUVR	**(1,223) 18.31**	**0.00**	**(2,223) 38.85**	**0.00**	1.04 (0.54)	106	1.62 (0.53)	82	1.74 (0.53)	39
CSF tau	**(1,216) 16.20**	**0.00**	**(2,216) 19.94**	**0.00**	57.18 (40.42)	101	82.83 (40.29)	80	102.15 (40.29)	39
FDG-PET hippocampus	**(1,225) 12.13**	**0.00**	**(2,225) 3.91**	**0.02**	1.25 (0.09)	109	1.26 (0.09)	80	1.21 (0.09)	40
Exploratory outcomes										
Executive function	(1,268) 2.23	0.14	(2,268) 2.10	0.12	0.02 (0.80)	131	−0.15 (0.81)	95	−0.22 (0.81)	48
Language	(1,267) 0.28	0.60	(2,267) 2.90	0.06	0.03 (0.86)	131	−0.15 (0.86)	95	−0.29 (0.86)	48
Attention	(1,268) 2.20	0.14	**(2,268) 4.00**	**0.02**	0.01 (0.81)	131	−0.15 (0.81)	95	−0.37 (0.81)	48
Global Cognition	**(1,267) 8.36**	**0.00**	**(2,267) 3.58**	**0.03**	0.02 (0.65)	131	−0.12 (0.65)	95	−0.26 (0.66)	48
CSF amyloid-β_{42}	**(1,213) 8.03**	**0.01**	**(2,213) 7.55**	**0.00**	430.72 (147.29)	99	355.78 (146.91)	78	346.57 (146.79)	40
CSF p-tau_{181}	**(1,217) 8.12**	**0.01**	**(2,217) 32.22**	**0.00**	29.27 (22.75)	101	48.48 (22.69)	80	60.62 (22.67)	40
FDG-PET precuneus	**(1,225) 4.62**	**0.03**	(2,225) 1.13	0.33	2.79 (0.29)	109	2.74 (0.29)	80	2.73 (0.29)	40

Group = effect of group membership as non-carriers, mutation carrier Val_{66} homozygote or mutation carrier Met_{66} carrier; all models have been adjusted for estimated year of symptom onset; bolded values are significant at the $P < 0.05$ or $P < 0.001$ level.

Statistically significant group differences of a moderate-to-large magnitude, were observed between Val_{66} homozygotes and Met_{66} mutation carriers for CSF p-tau_{181} levels (Fig. 2), but not for glucose metabolism in the precuneus or for the executive function, language, attention or global cognition composites (Fig. 1). There were also no statistically significant differences between mutation carrier Val_{66} homozygotes and mutation carrier Met_{66} carriers on CSF amyloid-β_{42} levels, with these differences small in magnitude (Fig. 2).

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

Effect of *APOE* ε4 on cognitive function, neuronal dysfunction, amyloid-β and tau

Reanalyses 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 amyloid-β_{42}, although there were no significant interactions between *APOE* and *BDNF* for either measure (Table 3). *Post hoc* analyses showed that when compared to mutation carrier ε4 non-carriers, mutation carrier ε4 carriers had significantly increased PiB-PET SUVR {*d* [95%CI = 0.45 (0.05,

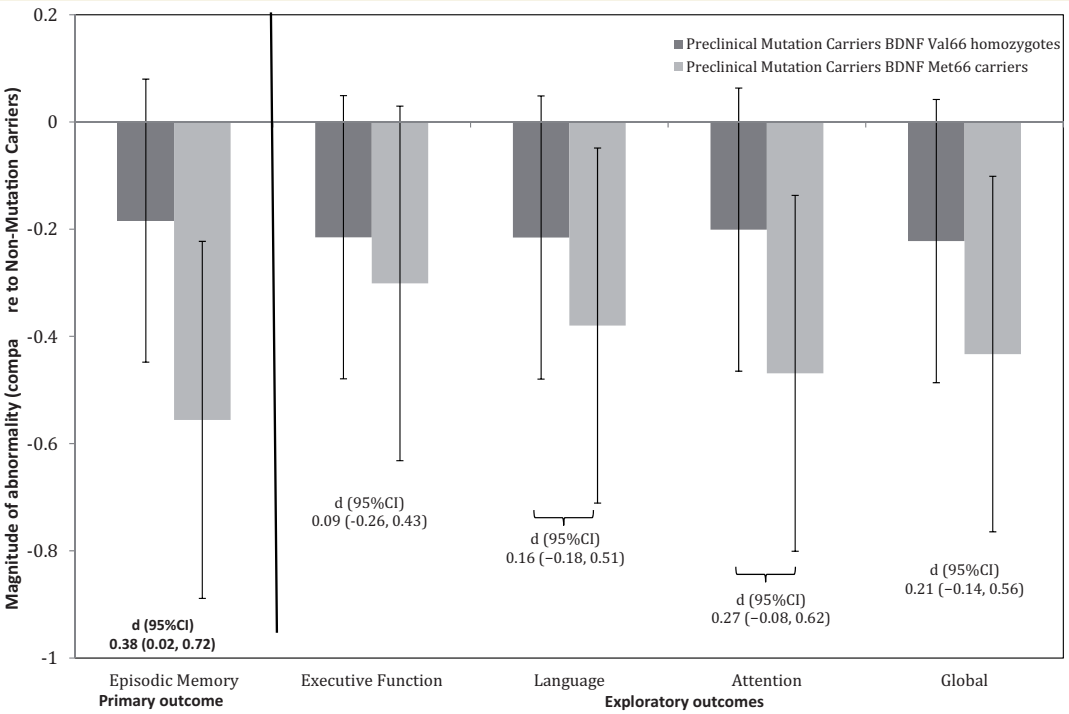

Figure 1 Magnitude of cognitive impairment in preclinical mutation carrier Val_{66} homozygotes and preclinical mutation carrier Met_{66} carriers when compared to mutation non-carriers. Error bars represent 95% confidence intervals. Statistical significance occurs when 95% confidence intervals do not cross '0' line.

0.85), $P = 0.03$]} and decreased CSF amyloid-β_{42} levels {d [95%CI = 0.76 (0.34, 1.17), $P < 0.001$]}.

Effect of *BDNF* Val66Met on the relationship between estimated year of onset and markers of cognitive and neuronal function, amyloid-β and tau

There were no statistically significant relationships between level of cognitive function and estimated year of onset in non-carriers or in Val_{66} homozygote mutation carriers. However, the relationship between estimated year of onset and episodic memory was statistically significant and moderate in magnitude for Met_{66} mutation carriers (Fig. 3A). Similarly, there were no statistically significant relationships between glucose metabolism in the hippocampus and estimated year of onset in non-carriers or in mutation carrier Val_{66} homozygotes. However, the relationship between glucose metabolism in the hippocampus and estimated year of onset was moderate in magnitude and statistically significant for mutation carrier Met_{66} carriers (Fig. 3B).

There was no relationship between levels of cortical amyloid-β and estimated year of onset in non-carriers, but there was a significant moderate association between cortical amyloid-β levels and estimated year of onset in mutation carriers irrespective of *BDNF* Val66Met polymorphism (Fig. 3C). Similarly, while there was no association between CSF tau and estimated year of onset in non-carriers, there was a significant moderate association between CSF tau and estimated year of onset in mutation carriers, irrespective of *BDNF* Val66Met genotype (Fig. 3D), with Met_{66} mutation carriers showing systematically higher levels of CSF tau relative to their estimated year of onset than Val_{66} homozygote mutation carriers (Fig. 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 Met_{66} mutation carriers was approximately double that observed in Val_{66} homozygote mutation carriers. 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 Alzheimer's disease from the AIBL and ADNI studies (Feng *et al.*, 2013; Lim *et al.*, 2013, 2014*b*). The results confirm therefore, in an independent sample, that *BDNF* is important to the preclinical presentation of Alzheimer's disease.

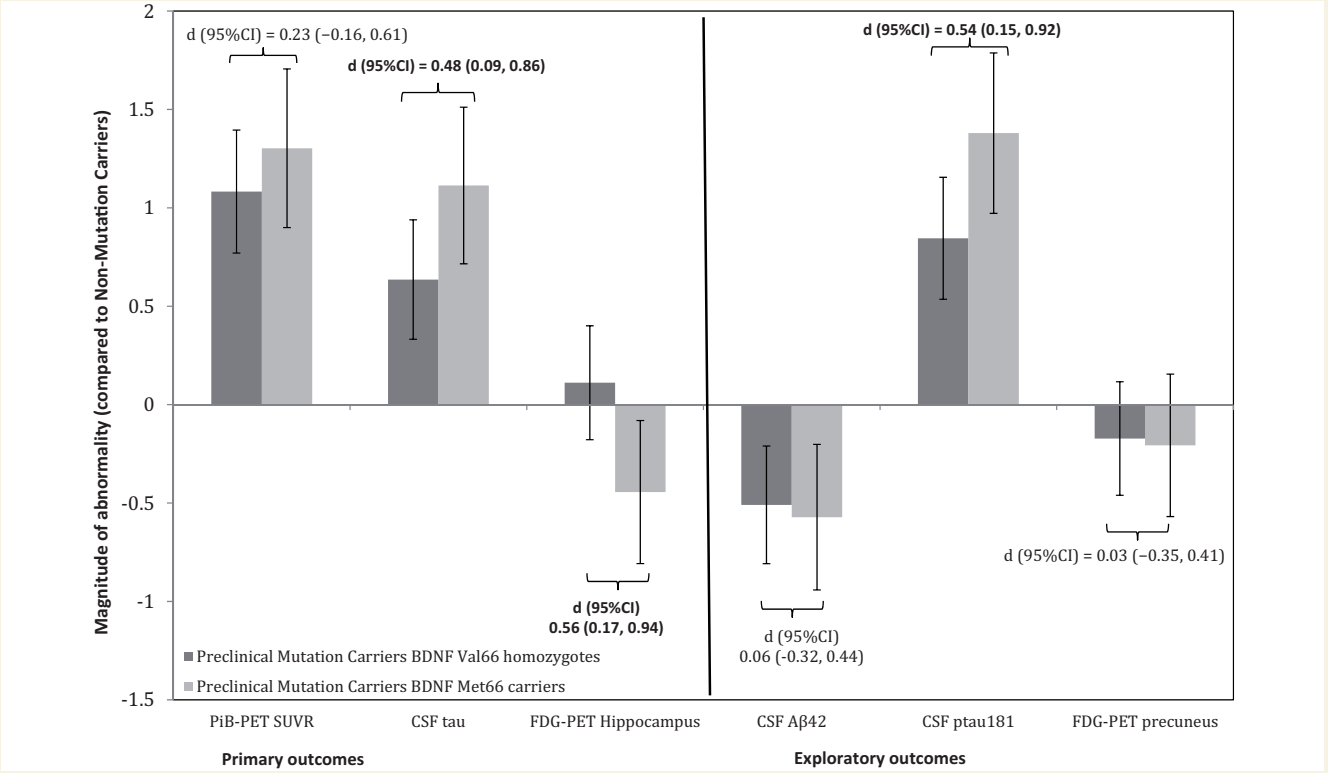

Figure 2 Magnitude of abnormality on markers of amyloid-β, tau and glucose metabolism in preclinical mutation carrier Val_{66} homozygotes and preclinical mutation carrier Met_{66} carriers when compared to mutation non-carriers. Error bars represent 95% confidence intervals. Statistical significance occurs when 95% confidence intervals do not cross '0' line.

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

	Estimated year of onset		*APOE* Group		*BDNF* Group		*APOE* × *BDNF* Group	
	(df) *F*	*P*	(df) F	*P*	(df) *F*	*P*	(df) *F*	*P*
Primary outcomes								
Episodic memory	**(1,266) 19.32**	**0.00**	(1,266) 1.79	0.18	**(1,266) 3.84**	**0.05**	(1,266) 0.08	0.77
PiB-PET SUVR	**(1,221) 16.73**	**0.00**	**(1,221) 7.21**	**0.01**	(1,221) 2.65	0.11	(1,221) 1.18	0.28
CSF tau	**(1,214) 15.50**	**0.00**	(1,214) 0.24	0.63	**(1,214) 4.06**	**0.04**	(1,214) 0.05	0.82
FDG-PET hippocampus	**(1,223) 11.88**	**0.00**	(1,223) 0.05	0.83	**(1,223) 6.03**	**0.02**	(1,223) 0.09	0.77
Exploratory outcomes								
Executive function	(1,266) 2.50	0.12	(1,266) 0.93	0.34	(1,266) 0.08	0.78	(1,266) 0.08	0.78
Language	(1,266) 0.20	0.65	(1,266) 0.39	0.54	(1,266) 0.99	0.32	(1,266) 0.15	0.70
Attention	(1,266) 2.40	0.12	(1,266) 1.46	0.23	(1,266) 2.78	0.10	(1,266) 0.49	0.49
DIAN Composite	**(1,266) 8.14**	**0.01**	(1,266) 0.04	0.84	(1,266) 0.84	0.36	(1,266) 0.06	**0.81**
CSF amyloid-β_{42}	**(1,211) 6.94**	**0.01**	**(1,211) 9.28**	**0.00**	(1,211) 0.02	0.90	(1,211) 1.39	0.24
CSF p-tau_{181}	**(1,215) 7.39**	**0.01**	(1,215) 2.00	0.16	**(1,215) 4.60**	**0.03**	(1,215) 0.34	0.56
FDG-PET precuneus	**(1,223) 4.82**	**0.03**	(1,223) 0.65	0.42	(1,223) 0.001	0.98	(1,223) 0.02	0.91

All models have been adjusted for estimated year of symptom onset; *APOE* Group indicates effect of group membership as non-carriers, *APOE* ε4 carrier or *APOE* ε4 non-carrier; *BDNF* Group indicates effect of group membership as non-carriers, mutation carrier Val_{66} homozygote or mutation carrier Met_{66} carrier; bold values are significant at the $P < 0.05$ level.

The current data support the first hypothesis that in preclinical mutation carriers, impairment in memory and hippocampal function would be greater in Met_{66} carriers compared to Val_{66} homozygotes. Compared to Val_{66} homozygote mutation carriers, Met_{66} mutation carriers had worse episodic memory function (Fig. 1). In contrast, no memory impairment was observed in Val_{66} homozygote mutation carriers compared to non-carriers. Similarly, hippocampal function, determined by cerebral glucose metabolism, was also reduced in Met_{66} mutation carriers compared to Val_{66} homozygote mutation carriers. However, Val_{66} homozygote mutation carriers did not

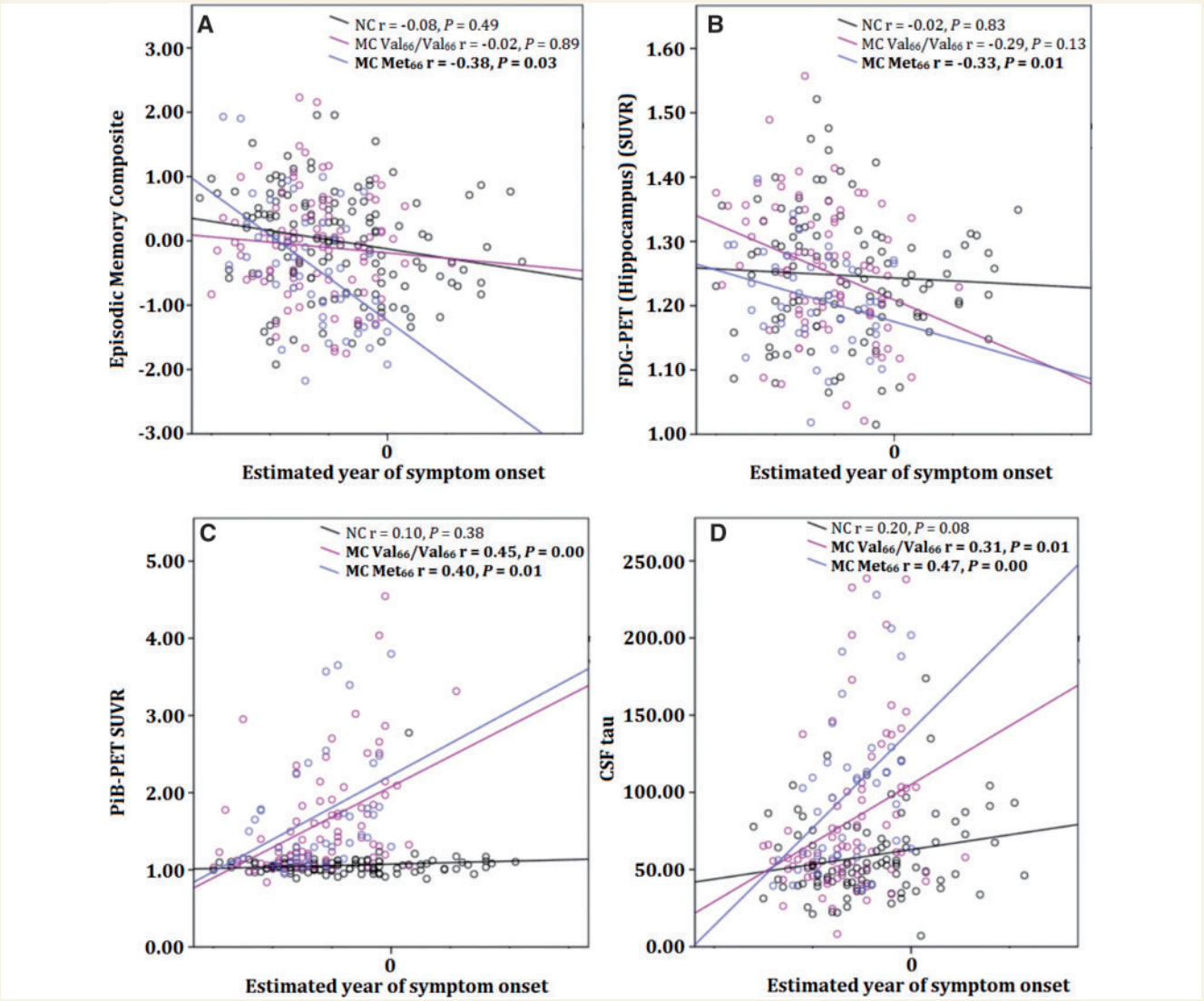

Figure 3 Relationship between estimated year of clinical symptom onset and episodic memory performance (A), glucose metabolism in the hippocampus (B), cortical amyloid-β levels (C), and CSF p-tau$_{181}$ levels (D), in mutation non-carriers, preclinical mutation carrier Val$_{66}$ homozygotes, and preclinical mutation carrier Met$_{66}$ carriers.

show lower glucose metabolism compared to non-carriers. 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 Met$_{66}$ mutation carriers. However, reanalysis 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, mutation carrier Met$_{66}$ carriers who were estimated to be nearer to their expected year of clinical symptom onset showed increased memory impairment and lower glucose metabolism in the hippocampus (Fig. 3). In contrast, estimated year of onset was not associated with memory impairment or glucose metabolism in non-carriers or Val$_{66}$ homozygote mutation carriers.

The second hypothesis that cortical amyloid-β and CSF amyloid-β$_{42}$ levels would be unrelated to allelic variation in *BDNF* Val66Met was also supported. Preclinical Met$_{66}$ and Val$_{66}$ homozygote mutation carriers had equivalent levels of higher cortical amyloid-β and CSF amyloid-β$_{42}$. Furthermore, these group differences were, by convention, small (i.e. $d < 0.2$; Fig. 2) in magnitude indicating that absence of statistically significant differences was not due to insufficient statistical power. Compared to non-carriers, both Met$_{66}$ carriers and Val$_{66}$ homozygotes showed increased levels of cortical amyloid-β deposition and decreased levels of CSF amyloid-β$_{42}$. Similarly, cortical amyloid-β burden was higher in preclinical mutation carriers who were nearer to their estimated year of onset; although this relationship was not moderated by the *BDNF* Val66Met polymorphism (Fig. 3C). Increased cortical

Table 4 Reanalysis of the effect of *BDNF* Val66Met on each primary outcome variable, covarying for the potential confounding effect of sex

	Estimated year of onset		Sex		*BDNF* group	
	(df) *F*	*P*	(df) *F*	*P*	(df) *F*	*P*
Episodic memory	**(1,267) 13.96**	**0.00**	**(1,267) 8.24**	**0.00**	**(2,267) 5.17**	**0.00**
PiB-PET SUVR	**(1,222) 18.43**	**0.00**	(1,222) 0.01	0.92	**(2,222) 40.59**	**0.00**
CSF tau	**(1,215) 17.02**	**0.00**	(1,215) 0.56	0.46	**(2,215) 20.98**	**0.00**
FDG-PET hippocampus	**(1,224) 5.60**	**0.02**	(1,224) 1.35	0.25	**(2,224) 3.95**	**0.02**

amyloid-β and lower CSF amyloid-β_{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 amyloid-β burden in preclinical ADAD is also consistent with observations that Met carriage was unrelated to rates of cortical amyloid-β accumulation over 3 years in preclinical and prodromal sporadic Alzheimer's disease (Feng *et al.*, 2013; Lim *et al.*, 2013, 2014*b*). Together, these findings suggest that the effect of the *BDNF* Met_{66} allele is independent of the effect of amyloid-β on risk for, and progression of, Alzheimer's disease.

The results also support the third hypothesis that CSF levels of tau would be greater in Met_{66} mutation carriers compared to Val_{66} homozygote mutation carriers. Levels of both CSF tau and p-tau_{181} were increased substantially in preclinical Met_{66} mutation carriers compared to preclinical Val_{66} homozygote mutation carriers (Fig. 2). Compared to non-carriers, preclinical mutation carrier Val_{66} homozygotes also showed increased levels of CSF tau and p-tau_{181}, although not to the same extent as Met_{66} mutation carriers. Despite the overall increase in these biochemical markers, strong relationships between estimated year of onset and CSF tau were observed in both Val_{66} homozygotes and Met_{66} mutation carriers, and the magnitude of these relationships were equivalent (Fig. 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 Met_{66} mutation carriers and Val_{66} homozygote mutation carriers (Fig. 2) suggest that Val_{66} homozygotes may have an increased level of resilience to the neurotoxic effects of tau and amyloid-β.

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 mutation carrier ε4 non-carriers, mutation carrier ε4 carriers showed increased cortical amyloid-β and decreased CSF amyloid-β_{42}. This indicates that in preclinical ADAD, the abnormal accumulation of cortical amyloid-β resulting from pathogenic mutations is increased further by the *APOE* ε4 allele, although this increased amyloid-β was not associated with any greater impairment in cognition or neuronal function. Importantly, the increase in cortical amyloid-β in mutation carrier ε4 carriers was not affected by the *BDNF* Met allele. Thus, allelic variation in *BDNF* and *APOE* may affect different Alzheimer's disease processes with ε4 increasing cortical amyloid-β accumulation and *BDNF* Met_{66} moderating amyloid-β-related impairment in cognition and neuronal function through its effects on tau.

Neuronal and synaptic loss characteristic of both sporadic and autosomal dominant Alzheimer's disease is due to the combined accumulation of amyloid-β 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 Alzheimer's disease, cognitive impairment and synaptic loss are associated more strongly with the presence and number of neurofibrillary tangles than amyloid-β plaques (Giannakopoulos *et al.*, 2003; Bennett *et al.*, 2004; Ingelsson *et al.*, 2004). However, neuroimaging studies in preclinical Alzheimer's disease report that higher cortical amyloid-β load is associated with greater rates of cognitive decline and progression to MCI (Rowe *et al.*, 2013; Lim *et al.*, 2014*a*), with these effects mediated by the effect of amyloid-β on neurodegeneration (Jack and Holtzman, 2013; Lim *et al.*, 2015*a*). In this context, dissociation of the effects of *BDNF* on amyloid-β 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 (Hariri *et al.*, 2003; Peng *et al.*, 2005; Garzon and Fahnestock, 2007; Forlenza *et al.*, 2010; Fahnestock, 2011; Lee *et al.*, 2012; Lu *et al.*, 2013). Evidence of a mechanistic relationship between BDNF and tau has been shown in cellular studies that demonstrate that BDNF can induce rapid dephosphorylation of tau through TrkB activation (Elliott *et al.*, 2005) and that BDNF loss in Alzheimer's disease 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 Alzheimer's disease and may even provide a reference for the investigation of the effects and clinical consequences of other neurotrophic factors in Alzheimer's disease.

As we have noted (Lim *et al.*, 2013), genome-wide association studies (GWAS) of Alzheimer's disease do not identify the *BDNF* Val66Met polymorphism as increasing the risk for Alzheimer's disease (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.*, 2013, 2014*b*). This hypothesis is supported by GWAS of cognitive ageing 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.*, 2013, 2014*b*) is that in studies of cognitive ageing, memory decline associated with *BDNF* Met_{66} may reflect occult Alzheimer's disease as opposed to the effects of normal ageing. In contrast to *BDNF*, GWAS of Alzheimer's disease identify carriage of *APOE* ε4 as increasing risk for Alzheimer's disease (Lambert *et al.*, 2013). We have also reported that in preclinical sporadic Alzheimer's disease, the *APOE* ε4 allele increases the rate of memory decline and brain volume loss associated with high amyloid-β (Dore *et al.*, 2013; Lim *et al.*, 2014*a*, 2015*c*). We have also observed that amyloid-β+ 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.*, 2015*b*). Reanalysis 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 amyloid-β levels in preclinical mutation carriers. 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 amyloid-β accumulation.

This study demonstrates that the deleterious effects of amyloid-β 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 Alzheimer's disease. 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 Alzheimer's disease 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 amyloid-β. 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 Alzheimer's disease (Mills *et al.*, 2013; Donohue *et al.*, 2014).

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