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Interaction between BDNF Val66Met and *APOE*4 on biomarkers of Alzheimer's disease and cognitive decline

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Abstract

Background: Whether brain-derived neurotrophic factor (*BDNF*) Met carriage impacts the risk or progression of Alzheimer's disease (AD) is unknown.

Objective: To evaluate the interaction of *BDNF* Met and *APOE*4 carriage on cerebral metabolic rate for glucose (CMRgl), amyloid burden, hippocampus volume, and cognitive decline among cognitively unimpaired (CU) adults enrolled in the Arizona *APOE* cohort study.

Methods: 114 CU adults (mean age 56.85 years, 38% male) with longitudinal FDG PET, magnetic resonance imaging and cognitive measures were *BDNF and APOE* genotyped. A subgroup of 58 individuals also had Pittsburgh B (PiB) PET imaging. We examined baseline

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Conflict of Interest/Disclosure statement

The authors have no conflict of interest to report.

CMRgl, PiB PET amyloid burden, CMRgl and hippocampus volume change over time, and rate of change in cognition over an average of 15 years.

Results: Among *APOE*4 carriers, *BDNF* Met carriers had significantly increased amyloid deposition and accelerated CMRgl decline in regions typically affected by AD, but without accompanying acceleration of cognitive decline or hippocampal volume changes and with higher baseline frontal CMRgl and slower frontal decline relative to the Val/Val group. The *BDNF* effects were not found among *APOE*4 non-carriers.

Conclusion: Our preliminary studies suggest that there is a weak interaction between *BDNF* Met and *APOE*4 on amyloid-β plaque burden and longitudinal PET measurements of AD-related CMRgl decline in cognitively unimpaired late-middle-aged and older adults, but with no apparent effect upon rate of cognitive decline. We suggest that cognitive effects of *BDNF* variants may be mitigated by compensatory increases in frontal brain activity—findings that would need to be confirmed in larger studies.

Keywords

BDNF; APOE4; Positron-Emission Tomography; Fluorodeoxyglucose F18; Amyloid; cognition

Introduction

Apolipoprotein (*APOE*) ε4 allele is a major genetic risk factor for AD. *APOE*4 carriage (*APOE*4c) is associated with increased Aβ accumulation [1, 2]. Cognitively unimpaired (CU) *APOE*4 individuals exhibit cerebral Aβ accumulation as early as in their third or fourth decade [1, 3]. Age-related cognitive decline in *APOE*4 carriers also begins earlier relative to non-carriers [4], although the increased rate of memory decline in CU *APOE*4c was found to be associated with high Aβ levels compared with *APOE*4 non-carriers (*APOE*4nc) with low Aβ [5]. In addition, CU *APOE*4 carriers show lower cerebral metabolic rate of glucose (CMRgl) as measured by FDG-PET in regions known to be affected in AD [6] such as the posterior cingulate, precuneus, parietotemporal, and prefrontal cortex [7–11] cross-sectionally, had greater rate of CMRgl decline longitudinally in these same regions than *APOE*4nc CU [12], and nonsignificant trends for smaller hippocampal volumes as measured by MRI [13].

It is unknown whether brain derived neurotrophic factor (*BDNF*), a neurotrophin involved in synaptic modulation, is a genetic risk factor for AD. A common single nucleotide polymorphism (SNP) in the human *BDNF* gene (Val66Met; rs6265) substitutes a valine to methionine in the 5' pro-domain of the *BDNF* protein which attenuates the activity-dependent form of *BDNF* secretion without affecting its constitutive secretion [14, 15]. *In vitro* and *in vivo* model systems describing the molecular and cellular characteristics of the polymorphism have demonstrated that Val66Met differentially impacts *BDNF* protein availability, neuronal survival and morphology, and altered neuronal function [14, 16]. Literature regarding Val66Met describes diverse, conflicting patterns of effects. *BDNF* Met carriage has been linked with a positive effect in healthy adults for cognitive control function such as response inhibition [17], was associated with reduced cognitive decline in patients with multiple sclerosis and [18] systemic lupus erythematosus [19], and preservation of

general intelligence following traumatic brain injury [20]. With regard to AD, the literature on whether *BDNF* genetic variants are an AD susceptibility factor or mitigates the effects of the *APOE*4 risk for developing AD is divided, ranging from: 1) no evidence of increased risk for AD [21]; 2) modulation effects of aging on working memory but no interaction with *APOE*4 on hippocampal volumes or memory performance[22] to 3) increased rates of cognitive decline among those BDNF Met Carriers with *APOE*4c and higher Aβ load [23–26] and 4) significantly higher amyloid load in *BDNF* Met carriers than Val/Val homozygotes only among *APOE*4 carriers[27].

The objective of our study was to better understand the role of BDNF and its interaction with *APOE*4c on the endophenotypes of AD in CU adults by examining the interaction between *BDNF* Met and *APOE*4 carriage in baseline and longitudinal glucose metabolism, baseline and longitudinal hippocampal volume, baseline amyloid burden, and longitudinal cognitive decline in CU adults enrolled in the Arizona *APOE* cohort study. We tested the hypothesis that within the *APOE*4 carrier group, individuals with *BDNF* Met carriage would have higher Aβ burden at baseline, differing glucose metabolism, differing hippocampal volumes, and increased cognitive decline over time compared to individuals with *BDNF* Val/Val. Our results reveal an intriguing interaction between *BDNF* Met and *APOE*4 in AD pathogenesis and disease progression.

Methods

Study Participants:

The included participants (N=114) were drawn from the longitudinal Arizona *APOE* cohort study [4, 28–30], specifically those who were both *APOE* and *BDNF* genotyped and had undergone longitudinal FDG PET and magnetic resonance (MR) imaging and cognitive testing. The Arizona *APOE* cohort study began in 1994 and is composed of CU individuals residing in Maricopa County, Arizona, mostly 47–68 years old, recruited through local media advertisements for inclusion in a study of cognitive aging. At entry, participants must score at least a 27/30 on the Mini-Mental State Examination (with at least 1of 3 on the recall subject) and exhibit no evidence of depression quantified by 10 points or less on the Hamilton Depression Rating Scale. The participants must also have perfect scores on the Functional Activities Questionnaire and Instrumental Activities of Daily Living Questionnaire, absence of a current psychiatric or vascular disease, normal neurological examination, and no clinically significant imaging abnormalities. All participants had a family history of dementia, were *APOE* genotyped, completed a full battery of neuropsychological testing (including the Auditory Verbal Learning Test, Controlled Oral Word Association Test, Mini-Mental State Exam), and had both FDG PET and T1 MRI measurements at every two-year visit as initially designed. In year 2007, we also added PiB PET for the amyloid measurements. Study participants were followed and seen in the clinic also every 2 years. All individuals gave written informed consent to participate in the study and the study protocol was approved by the institutional review boards of Banner Good Samaritan Medical Center (now Banner-University Medical Center, Phoenix, AZ, USA) and the Mayo Clinic.

APOE/BDNF genotyping:

Blood for plasma analysis was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Samples were centrifuged at 2000x g at 4C for 10 minutes. Centrifuged samples were aliquoted and immediately frozen at −80C in polypropylene vials pending biochemical analysis. Single nucleotide polymorphisms (SNPs) for *APOE* (rs429358, rs7412) were genotyped as described elsewhere [31]. BDNF genotypes (rs6265, Val66Met variant, located on chromosome 11:27,658,369 in human genome build GRCh38 38.1/142) were generated using KASP chemistry (LGC Biosearch Technologies, Teddington, Middlesex, United Kingdom). KASP reactions were comprised of sample DNA, KASP Master Mix, and KASP Assay Mix which contains competitive, allele-specific forward primers with differing FRET tags and one common reverse primer. KASP-based polymerase chain reaction amplification results in fluorescent signals that indicate genotypes for the Val66Met variant.

Brain Imaging:

PiB-PET: 58 participants who were both *APOE* and *BDNF* genotyped also had one-time PiB PET imaging. Amyloid PET imaging was performed using a HR+ scanner (Siemens, Knoxville, TN) in a three-dimensional mode after intravenous injection of approximately 15 mCi of 11C-PiB for a 90-minute dynamic sequence of emission scans. For quantification of amyloid burden, PiB PET images between 50 to 70 minutes post-injection were summed and normalized to the cerebellum to generate cerebral-to-cerebellar standard uptake value ratio (SUVR). All the quantification was performed using SPM8 in the MNI template space. For the cross-sectional PiB-PET voxel-wise analysis, we used general linear model (GLM) procedure with uncorrected p-value of 0.005 for brain regions known to be associated with beta amyloid. To address possible inflated type I error in the examination of group differences in the voxel wise analysis, we used the same post hoc Monte Carlo simulation procedure with 1000 iterations as in our previous study [32]. Using this procedure, we tested the hypothesis that the number of PiB SUVR differences observed in the postulated direction (i.e., SUVR higher in the *APOE*4c-BDNF Met carriers than in the *APOE*4nc-BDNF Val/Val group) was significantly greater than the number of voxels with elevations in the opposite direction (i.e., SUVR higher in the *APOE*4nc-BDNF Val/Val group than in the *APOE*4c-BDNF Met carriers group). We note this Monte-Carlo simulation based test is global and had no localization power for group difference for any brain region.

FDG-PET: Cerebral glucose metabolism was measured with ^{18}F-fluorodeoxyglucose (FDG) PET imaging on the same HR+ scanner (Siemens, Knoxville, TN), with an intravenous injection of approximately 10 mCi of FDG and a 60-min dynamic sequence of emission scans as the subjects, who had fasted for at least 4 hours, lay quietly in a darkened room with their eyes closed and directed forward. For quantification of brain glucose metabolic rate (CMRgl) for this study, the last 30 minutes of the dynamic FDG PET images were summed and proportionally scaled (normalized) by whole brain average counts to allow assessment of relative CMRgl at regional and voxel level. For baseline FDG-PET voxel-wise analysis, we used general linear model (GLM) procedure with uncorrected p-value of 0.005 with the brain regions known to be affected by AD[8, 33]. For longitudinal FDG-PET voxel-wise analysis, assuming the CMRgl changes over time are linear, we estimated the voxel-wise rate of changes for each of those subjects who had multiple time

points FDG PET scans using linear regression with subject's visit ages as the independent variable. Thus, a slope image was created for each subject. Two sample independent t-test was then used to compare the difference of the longitudinal change rates between *APOE*4-carrier + BDNF-met and *APOE*4-Carrier + BDNF-val. For the FDG-PET analyses, the same Monte-Carlo simulation test described above was also performed.

T1-MRI: T1-weighted MRI scans were acquired on a 3T GE Discovery MR750 system. They were preprocessed with FreeSurfer 5.3. Cortical and subcortical regions were labeled and the automated segmentations were manually inspected and corrected as needed (surfer.nmr.mgh.harvard.edu/fswiki/).[34, 35] The relative hippocampal volume was calculated by taking the sum of the left and right hippocampal volume and dividing by the intracranial volume (ICV) for each subject.

Statistical analysis:

Descriptive data results are shown as mean +/− standard deviation. For baseline comparisons under the ANOVA framework, we examined the differences between the following groups: BDNF Met carriers versus non-carriers, *APOE*4 carriers versus non-carriers, and especially *APOE*4c-BDNF Met carriers/*APOE*4nc-BDNF Val/Val. Equivalent to the interaction test under the general two-factor ANOVA framework, the interaction examined in this study is with the SPM contrast settings under the general linear model to examine the difference between *BDNF* Met versus Val/Val groups in *APOE*4c minus the difference between *BDNF* Met versus Val/Val groups in *APOE*4nc (the difference of difference). Likewise, the same interaction can be examined by the *APOE*4 difference in *BDNF* Met vs the *APOE*4 difference in *BDNF* Val/Val group (the difference of difference). To illustrate, using subscript 1 for *APOE*4 carriers and 2 for non-carriers, the difference between *BDNF* Met and Val/Val in *APOE*4 carriers is (*BDNF*m1 - *BDNF*v1). The same difference in *APOE*4 non-carriers is (*BDNF*m2 – *BDNF*v2). The difference of difference is, in this case, (*BDNF*m1 - *BDNF*v1) - (*BDNF*m2 – *BDNF*v2), which is examining the *BDNF* differential effects between *APOE*4 carriers vs non-carriers. The same expression can be re-arranged equivalently as (*BDNF*m1-*BDNF*m2)- (*BDNF*v1-*BDNF*v2), which is examining the *APOE*4 differential effects between *BDNF* Met vs *BDNF* Val/Val. For longitudinal cognitive measures, we used linear mixed effect modeling approach taking the *APOE*4 by *BDNF* interactions into consideration. Linear mixed effect models were adjusted for baseline age, sex, and education. Only findings with values of $p \leq 0.05$ (2-tailed) were considered significant. Analyses were conducted with R software (www.r-project.org/).

To correct for the multiple comparisons associated with the voxel-wise analyses, we adopted an omnibus Monte Carlo Simulation (MCS) strategy previously developed in our laboratory.[32] This Monte Carlo simulation procedure assumes that a) the null hypothesis is that there is no difference between the two groups everywhere in the brain (using the PiB-PET as an example, the mean SUVR at a given voxel in one group is the same as in another group); b) the noise is of Gaussian at each voxel for each subject; c) the measurements are inter-voxel correlated with the smoothness resulted from preprocessing steps. The simulation procedure then generated the group difference t-score map based on the same GLM model used in the analysis of the real data, number of subjects in each of the two groups and the

assumptions above, repeated such t-score map generations N times (in our study, N=1,000). For each of such maps, we counted the number of voxels at one direction vs. the number of voxels in the opposite direction, all at uncorrected p=0.05 level (note this p-value is not for statistical inferences about regional changes). Over N iterations, we then counted the number of times, referred to n, the simulated t-score had the number of voxels in the hypothesized direction equal or exceed the observed in the real data. Finally, the ratio of n/N is the type-I error of interest. Our simulation procedure is conceptually very similar to the widely used Alpha-Sim approach https://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf), but we deliberately decided not to consider the individual cluster sizes, rather to compare over the whole spatial extent (number of voxels), noting the low resolution of the PET data.

Results

Study Participant Characteristics

Table 1 summarizes the demographics of the entire study cohort (N=114) with *BDNF* status (*BDNF* Met carrier, BDNF Val/Val) and *APOE*4 subgroups. Note that the overall *BDNF* Met carrier group included both Met/Met homozygotes (n=4) and Val/Met heterozygotes (n=36). The average age in the entire cohort was 56.85 years old and 38% of participants were men. No significant differences ($p \leq 0.05$) were identified in baseline characteristics of age, sex, education, and the cardiovascular risk factors of diabetes, hypertension, hyperlipidemia, and smoking for the *BDNF* and the *APOE*4 main effects. We however observed *APOE*4 effects on AVLT-LTM, AVLT-STM and AVLT-TL ($p<0.05$) and no significant *BDNF/APOE*4 interaction effects for baseline characteristics. Similarly, Table 2 summarizes the demographics and baseline characteristics of the PiB-PET cohort (N=58) subgrouped by *BDNF* and *APOE*4 status. However, we found no significant main or interactive effects in this cohort, most likely due to the much-reduced sample size.

Cognitive Change over Time

Table 3 summarizes the rate of cognitive change over time from baseline for the entire cohort (N=114, subgrouped both by *APOE*4 and *BDNF* status) as determined by linear mixed effects model. The mean rate of change for a given group is the fixed slope together with its standard deviation for that group. The only statistically significant difference was identified between the *APOE*4c and *APOE*4nc groups, where the *APOE*4c group had a greater decline in the rate of cognitive change over time as compared to *APOE*4nc in the AVLT TL (−0.45 vs −0.22 (p=0.02)), AVLT STM (−0.14 vs −0.04 (p=0.01)), AVLT LTM (−0.17 vs −0.06 (p=0.01)) and number of subjects who progressed to MCI (p=0.0004). However, the *APOE*4 by *BDNF* interaction for the cognitive longitudinal change was not statistically significant.

Hippocampal Volume (MRI)

We found no hippocampus volume differences at baseline for any of the two main effects and their interaction. We also did not see significant longitudinal changes.

Amyloid Deposition (PiB-PET)

Within the PiB-PET imaging subgroup (N=58), an interaction between *APOE*4c and *BDNF* Met carriage was identified but only at uncorrected p=0.05 level. Figure 1 shows regions of higher amyloid deposition in *APOE*4c than in *APOE*4nc in *BDNF* Met group as compared to that same *APOE*4c/*APOE*4nc contrast in the BDNF Val/Val group. Equivalent to the interaction in the general two-factor ANOVA framework, the interaction examined in this study is the *APOE*4 difference in *BDNF* Met vs the *APOE*4 difference in *BDNF* Val/Val group (the difference of difference). Using our Monte-Carlo computer simulation to assess overall global significance, we found that there were 2827 voxels in the *APOE*4c>*APOE*4nc direction in the *BDNF* Met group (in contrast to that in the *BDNF* Val/Val group) and 377 voxels in the opposite direction. The overall global significance was estimated to be $p<0.001$ over 1000 simulations. The locations where we observed the significances are provided in the Table in Figure 1. Between the *APOE*4c and *APOE*4nc, only the *APOE*4c showed higher amyloid deposition in frontal regions (image not shown), consistent with our previous findings.[2]

Glucose Metabolism (FDG-PET)

Figure 2 shows the *APOE*4 and *BDNF* interactive effects (the difference of difference) at baseline. We found higher CMRgl in *BDNF* Met carriers than in *BDNF* Val/Val groups among *APOE*4 carriers, as compared to the same difference among *APOE4* non-carriers, with the interaction appearing primarily in the frontal regions. For assessing the overall global significance for the *APOE*4 by *BDNF* interaction, we found that there were 7216 voxels in the direction of *BDNF* Met> BDNF Val/Val among *APOE*4 carriers and 267 voxels in the opposite direction. The global significance is also $p<0.001$ with 1000 simulations. Consistent with our prior studies, Figure 3 shows lower CMRgl uptake in *APOE*4 carriers than non-carriers in various brain regions known to be affected by AD. For post-hoc assessing the overall global significance using the Monte Carlo simulation, we found that there were 2097 voxels in this hypothesized direction and 223 voxels in opposite direction. The global significance is again $p<0.001$ with 1000 simulations.

For longitudinal change, Figure 4 shows faster CMRgl decline in *BDNF* Met than *BDNF* Val/Val among APOE4c, as compared to *BDNF* Met versus *BDNF* Val/Val groups among *APOE*4nc (difference of difference) over an average of 8 years' time. We observed significantly faster CMRgl decline in parahippocampus (p=0.002), precuneus (p=0.003), temporal (p=0.0001), and thalamus (p=0.0007) regions. In addition to areas of faster CMRgl decline, we looked for areas of slower CMRgl decline over time. We observed *BDNF* Met carriers had slower CMRgl decline in frontal regions than the *BDNF* Val/Val group among *APOE*4 carriers, as compared to *BDNF* Met/BDNF Val/Val groups among *APOE*4 non-carriers (difference of difference, image not shown).

Although not statistically significant, there were a higher proportion of *APOE*4 homozygotes in the *BDNF* Met carriers than in the *BDNF* Val/Val group. We therefore did a post-hoc analysis to examine the *BDNF* effects by covarying out *APOE*4 gene allele, and interactive effects remained even when covarying for APOE4 allele gene dose.

Discussion

Our initial hypothesis that in contrast to *BDNF* Val/Val and *APOE*4 non-carriers, *BDNF* Met and *APOE*4 carriage will be associated with higher Aβ burden, differing glucose metabolism, and greater cognitive decline was only partially supported. Among *APOE*4 carriers, *BDNF* met carriage was associated with increased amyloid deposition and accelerated CMRgl decline in regions typically affected by AD, but without accompanying acceleration of cognitive decline or hippocampal volume change over a nearly 15 year follow up period in our study. In contrast, these Met carriers had increased baseline frontal CMRgl and reduced frontal decline. Thus, while the *BDNF* Met and *APOE*4 carriage interaction of increased amyloid deposition and greater decline of glucose metabolism in regions typically affected by AD does suggest increased risk for AD, the preserved frontal metabolism may have been compensatory so that cognitive decline was not observed at this earlier, presymptomatic stage.

With regard to the PiB-PET results, when comparing *APOE*4 carriers and *APOE*4 non-carriers, the *APOE*4 carriers had significantly higher frontal amyloid deposition, a result that is consistent with previous literature [1, 2]. Consistent with our hypothesis and in line with a previous study by Adamczuk et al [27], we found an interaction between *BDNF* Met and *APOE*4 carriage associated with increased amyloid deposition and that the presence of *BDNF* Met in the context of *APOE*4 non-carriers does not result in increased amyloid deposition. With FDG-PET, we also found an interaction between *BDNF* Met and *APOE*4 carriage. Although, as expected [36, 37], the *APOE*4 carriers had significantly decreased CMRgl in a pattern similar to AD hypometabolism compared to *APOE*4nc, among *APOE*4 carriers, *BDNF* Met carriers had significantly higher frontal CMRgl and faster decline of parahippocampus, precuneus, temporal, and thalamus CMRgl over an 8-year period than the *BDNF* Val/Val group. We also showed, consistent with the amyloid imaging results, the baseline *BDNF* effects of higher CMRgl with slower longitudinal decline (average 8 years) in the frontal regions for *BDNF* Met carriage as compared to BDNF Val/Val individuals were not found among *APOE*4 non-carriers. This reflects a different, although not initially unfavorable, glucose metabolism mechanism that *APOE*4 *BDNF* Met carriers employ, as we did not observe statistically significant cognitive deficits related to *APOE*4c *BDNF* Met carriers relative to the *APOE*4c Val/Val group, nor did we observe hippocampal volume decline.

In line with other studies, our results showed a decline in the rate of cognitive change over 15 years' time in the *APOE*4 carriers as compared with the *APOE*4 non-carriers. However, we found no differences in the rate of cognitive decline between the *BDNF* Met and Val/Val groups either as a whole or within the *APOE*4 group, which did not support our initial hypothesis that the combination of *APOE*4 and *BDNF* Met carriage would result in greater cognitive decline. The only statistically significant difference was found on the baseline MMSE mean score ($p=0.04$), where among *BDNF* Met carriers the mean (SD) was 29.6 (0.68) and *BDNF* Val/Val was 29.9 (0.38). While this achieved statistical significance, it is not clinically relevant as both groups scored well within the normal limits for normal cognition for the MMSE test (30 possible points). A recent study by Xia et al 2019 [38] investigated the influence of *BDNF* Val66Met on cognition, CSF and neuroimaging

markers in the non-demented elderly using the ADNI cohort. While this study discusses non-demented elderly, it is important to note that this group included both CU and MCI (CDR 0.5, MMSE 23–30) together and did not analyze the groups separately. They found in this combined group of average age of 74 years that there was an interaction between Aβ load and *BDNF* Val66Met in cognition. The *BDNF* Val66Met polymorphism had significant association with atrophy of the entorhinal cortex and MMSE scores in the non-demented elderly and the A+ (abnormal Aβ) subgroup, while no association was found in the A-subgroup. Another recent study of very mild amnestic MCI patients (average age 72) found that the combination of *BDNF* Met and *APOE*4 carriage is associated with memory dysfunction but not with structural brain changes [39]. In contrast, Gomar and colleagues did see a trend for thinner posterior cingulate and precuneus cortices but, consistent with our findings, no significant differences in cognitive measures in CU *APOE*4c Met carriers compared to Val homozygotes (average age 76) [24]. Lim and colleagues [26] observed accelerated memory decline in CU amyloid positive/*APOE*4c/*BDNF* Met carriers (average age 72). They and Boots et al, who also observed accelerated cognitive decline in a similar but younger aged cohort [23], did not report on FDG PET data. Our study included CU participants who, with the exception of the study by Boots and colleagues, were younger than the participants in the aforementioned studies and had no evidence of MCI. These demographic differences may account for the lack of association with memory decline for the *BDNF* Met/*APOE*4c group in our study. Our baseline FDG PET findings further support the conclusion that there may be some initial compensatory brain activity in this younger, CU cohort to offset dysfunction and accelerated decline associated with Aβ deposition.

Our FDG PET findings compliment findings from Xu et al [40], where the *BDNF* Met allele affects glucose metabolism in some specific regions with both hyper- and hypometabolism in cognitively unimpaired adults. While Xu and colleagues did not combine *BDNF* Met and *APOE*4 carriage nor report on differences in cognitive measures over time as our study did, they did investigate *BDNF* Met carriage and used age, sex, and *APOE*4 status as covariates. Hypermetabolism in *BDNF* Met carriers compared to Val/Val group in CU was found in the superior and middle frontal gyrus cortex. Another study reported hyperactivity in frontal and posterior parietal cortexes with fMRI in healthy *BDNF* Met carriers in comparison to the Val/Val group during a spatial working memory task [41].

The strength of our study is the longitudinal nature and addition of FDG PET and MRI data to help explain varying results reported in the literature. One limitation of our study is that study participants all had a family history of dementia, so it is possible that the findings may not generalize to those without a family history. Additionally, our small sample size may have affected the variability of the results. Indeed, with association studies carried out on a relatively small sample size, Type 1 error may exist and thus, further replication of this study is required before we have a more defined answer regarding the interaction between *BDNF* Met carriage and *APOE*4 on amyloid burden, glucose metabolism, hippocampal volume, and change in cognitive scores over time. In addition to an overall small sample size, we did not separate Met carriers into heterozygotes and homozygotes. In a population of European ancestry, 64% of individuals are Val homozygotes (Val/Val), another 3% are Met homozygotes (Met/Met), and the 34% that remain are the heterozygotes (Val/Met) [42]. It is possible that previous studies may have suffered from small effect sizes with few Met

homozygotes. Owing to the low frequency of the Met allele, studies, including ours, have combined Met/Met and Val/Met subjects, or only compared Val/Met and Val/Val subjects and excluded Met/Met due to the low sample size. Further studies are required to determine the consequences of *BDNF* Met homozygosity. Also, as with any study such as this, we cannot exclude the possibility of unexamined confounders such as medications for unrelated conditions. Our cohort was largely healthy and cognitively unimpaired and most likely confounders such as cardiovascular risk factors, which may differentially influence age-related memory decline in *APOE*4 homozygotes,[43] were evenly balanced among groups. We are unaware of cardiovascular risk factor influences with *BDNF* Val66Met. Although not statistically significant, there were more *APOE*4 homozygotes in the *BDNF* Met group than the *BDNF* Val/Val group, which could confound our conclusions, particularly with the small sample size. However the effects remained even when covarying for *APOE*4 allele gene dose, and, unlike the statistically greater proportion of *APOE*4 carriers than non-carriers who developed incident MCI, there were less in the *BDNF* Met group than the *BDNF* Val/Val who developed incident MCI (also not statistically significant). It is interesting to note the absence of significant hippocampal volume baseline and longitudinal differences in the presence of some significant PET and cognitive findings especially for *APOE* and for *APOE4/BDNF* interaction. We did not observe any significant differences in the hippocampal volume, even between *APOE*4c and *APOE*4nc groups, let alone the *BDNF* interactions. We previously reported the same insignificant findings for hippocampus volume.[13] The insignificant MRI based structural changes also contributed to the results of our one early report that CMRgl correlations with *APOE*4 gene dose remained the same with or without the correction for partial-volume averaging.[9]

Conclusion

We observed a weak interaction between *BDNF* Met and *APOE*4 carriage with the baseline PIB PET, baseline FDG PET, and longitudinal FDG PET findings. The increased baseline CMRgl in *APOE4*/*BDNF* Met carriers may reflect a compensatory response to offset the dysfunction resulting from higher frontal amyloid deposition. This reflects perhaps a different, although not initially unfavorable, glucose metabolism mechanism that *APOE*4c *BDNF* Met carriers employ, as we noticed preservation in cognition over time with no significant differences in decline of cognitive scores identified in either *APOE4*/*BDNF* Met carriers or the *BDNF* Met carriers alone. However, due to the small sample size, we cannot reach any definite conclusions regarding whether *BDNF* Met carriage is an AD genetic risk or protective factor. Further studies should examine *BDNF* Met carriage in both homozygotes and heterozygotes.

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Abbreviations:

BDNF	brain-derived neurotropic factor
APOE	apolipoprotein E
Val	valine
Met	methionine
MRI	magnetic resonance imaging
PiB	Pittsburgh Compound B
PET	positron emission tomography
FDG	Fluorodeoxyglucose
CMRgl	cerebral metabolic rate for glucose
AD	Alzheimer's disease
CU	cognitively unimpaired

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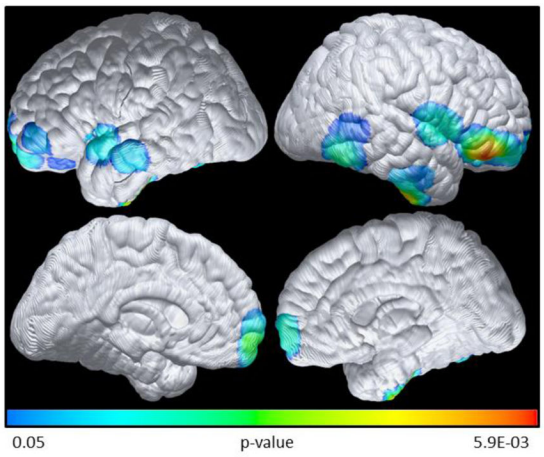


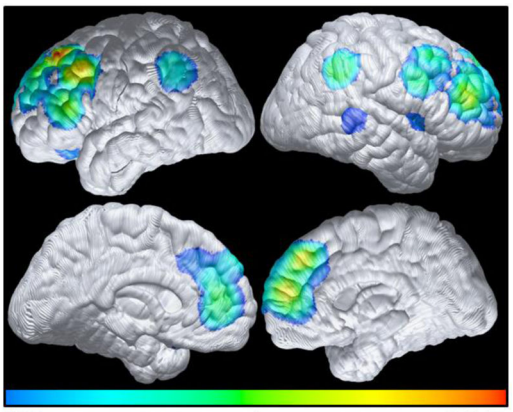
Colored areas indicate regions of higher amyloid deposition in *APOE4* carriers than in *APOE4* non-carriers in *BDNF* Met group as compared to that same *APOE4*carrier/*APOE4*non-carrier contrast in the BDNF Val/Val group (uncorrected threshold of p=0.05). See text for the global significance (p<0.001) based on the Monte Carlo simulation.

	X	Y	Z	Z-score	P value
Right Inferior Orbitofrontal	53	38	-15	2.5	8E-03
Right Superior Orbitofrontal	16	65	-13	1.9	3E-02
Left Superior Orbitofrontal	-10	61	-17	2.2	2E-02
Right Middle Orbitofrontal	40	46	-16	2.3	1E-02
Left Medial Orbitofrontal	-6	63	-13	2.5	8E-03
Left Inferior Temporal	-65	-17	-23	1.9	3E-02
Right Inferior Temporal	44	-15	-36	2.7	4E-03
Left Superior Temporal	-61	4	-7	2.0	3E-02
Right Superior Temporal Pole	63	10	0	2.4	10E-03
Left Superior Temporal Pole	-59	5	-10	2.0	2E-02
Right Fusiform	40	-14	-40	2.7	4E-03

Figure 1:

Higher amyloid deposition in *APOE4*c than in *APOE4*nc in *BDNF* Met group, as compared to the same directional difference in the *BDANF* Val/Val group

Note: The data were extracted from voxels associated with maximally significant in association with *BDNF* Met carriage and Met Val/Val ANOVA based analysis. At each location listed above, we observed higher amyloid deposition in *APOE*4c than in *APOE*4nc among *BDNF* Met individuals compared to the *APOE*4c/*APOE*4nc differences in the *BDNF* Val/Val individuals. Listed locations correspond to the brain maps shown in Figure 1, corresponding to p<=0.05, uncorrected. Coordinates were obtained from Talairach, X is the distance to the right or left of the midline, Y is the distance anterior or posterior to the anterior commissure, and Z is the distance superior or inferior to a horizontal plane through the anterior and posterior commissures.



Colored areas indicate regions of higher CMRgl in *BDNF* Met carriers than in *BDNF* Val/Val groups among *APOE4* carriers, as compared to the same difference among APOE4 non-carriers, with the interaction appearing primarily in the frontal regions (uncorrected threshold of p=0.005). See text for the global significance (p<0.001) based on the Monte Carlo simulation.

	X	Y	Z	Z-score	P value
Left Middle Frontal	-50	21	32	4.1	4E-05
Right Middle Frontal	48	47	9	3.6	3E-04
Left Inferior Frontal Operculum	-51	21	28	4.0	5E-05
Right Inferior Frontal Operculum	55	23	28	3.5	4E-04
Left Superior Frontal	-20	54	23	2.9	2E-03
Right Superior Frontal	24	65	12	3.0	2E-03
Right Superior Medial Frontal	12	40	31	3.4	4E-04
Right Inferior Frontal Triangulus	40	27	2	4.2	3E-05
Left Inferior Frontal Triangulus	-36	29	2	3.6	2E-04
Right Insula	40	23	-1	4.1	4E-05
Left Anterior Cingulum	-6	45	12	2.8	3E-03
Right Supramarginal	63	-43	39	3.5	4E-04
Left Supramarginal	-63	-37	39	3.0	2E-03
Left Inferior Parietal	-63	-37	42	2.8	3E-03
Left Precentral	-48	3	29	3.41	5E-04

Figure 2:

Higher CMRgl in *BDNF* Met carriers than in *BDNF* Val/Val group among *APOE*4c, as compared to the same directional difference among *APOE*4nc

Note: The data were extracted from voxels where maximally significantly higher CMRgl in *BDNF* Met than in *BDNF* Val/Val groups among *APOE*4 carriers, as compared to the same difference among *APOE*4 non-carriers were observed. Listed locations correspond to the brain maps shown in Figure 2, corresponding to p<=0.005, uncorrected. Coordinates were obtained from Talairach, X is the distance to the right or left of the midline, Y is the distance anterior or posterior to the anterior commissure, and Z is the distance superior or inferior to a horizontal plane through the anterior and posterior commissures.

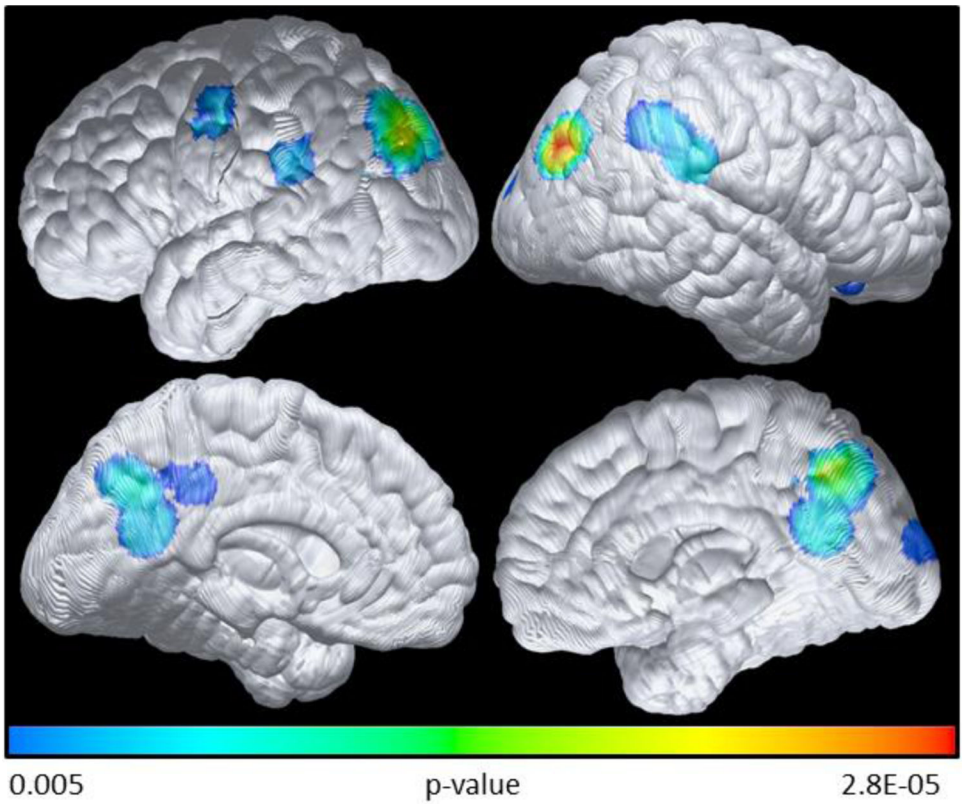


Figure 3:

Lower baseline *CMRgl* in *APOE4* carriers compared with *APOE4* non-carriers

Statistical difference brain maps (uncorrected threshold of p=0.005) of the metabolic reduction between *APOE4* carriers and non-carriers based on voxel-wise FDG analysis. As expected, compared with *APOE4* non-carriers there is a reduction in CMRgl uptake in the *APOE4* carriers. Colored areas indicate regions of lower CMRgl in*APOE4* carriers compared to non-carriers. See text for the global significance (p<0.001) based on the Monte Carlo simulation.

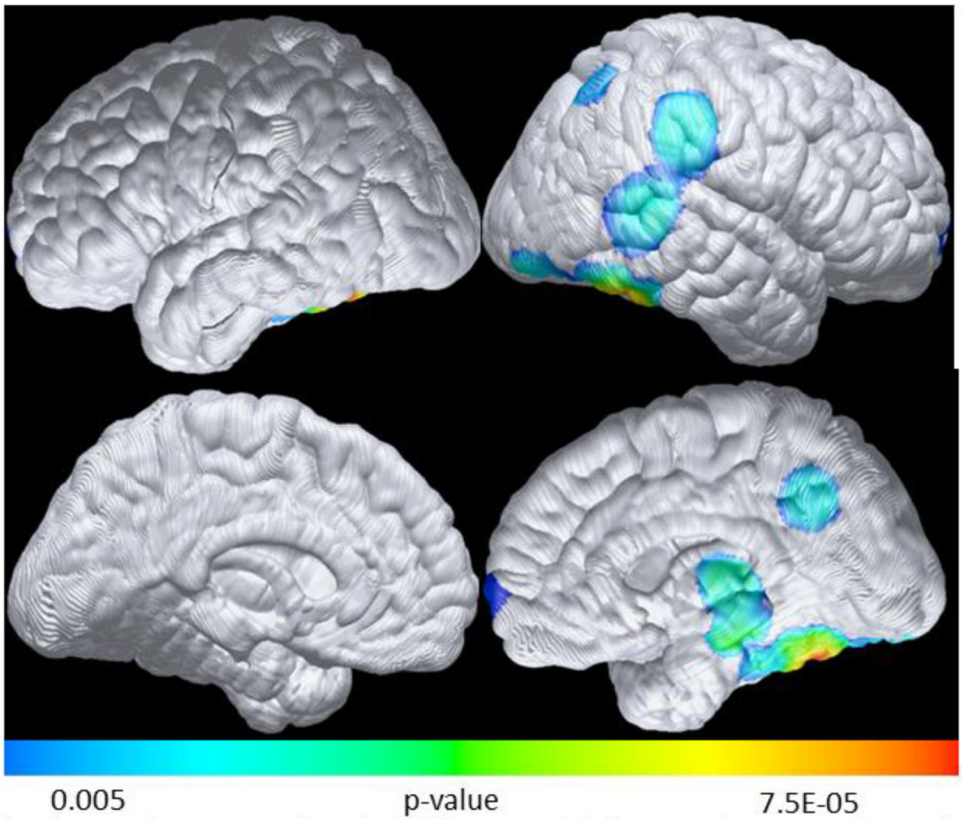


Figure 4:

Faster CMRgl decline in *BDNF* Met than *BDNF* Val/Val among *APOE*4c, as compared to same directional difference among *APOE*4nc over an average of 8 years' time

Colored areas indicate regions of significantly faster CMRgl decline in parahippocampus (p=0.002), precuneus (p=0.003), temporal (p=0.0001), and thalamus (p=0.0007) regions (uncorrected threshold of p=0.005) for *BDNF* Met than ßD/VF Val/Val among *APOE*4 carriers, as compared to *BDNF* Met versus *BDNF* Val/Val groups among *APOE*4 non-carriers, over an average of 8 years' time.

Table 1

Entire Cohort (n=114) Demographics

Baseline Characteristics	APOE4c/ BDNF Met (N=20)	APOE4c/ BDNF Val/Val (N= 39)	APOE4nc/ BDNF Met (N=20)	APOE4nc/ BDNF Val/Val (N= 35)	APOE4 effect p-value	BDNF effect p-value	APOE4×BDNF p-value
Sex male/female, (%)	6/14 (30%/ 70%)	14/25 (35.9%/ 64.1%)	11/9 (55%/ 45%)	12/23 (34.3%/ 65.7%)	0.11	0.65	0.17
APOE4 (HM/HT/NC)	11/9/0	12/27/0	0/0/20	0/0/35	NA	NA	NA
Education, years mean (SD)	16.1 (1.8)	15.5 (2.1)	58.3 (4.2)	57.0 (4.5)	0.25	0.31	0.58
Age, years mean (SD)	55.0 (4.0)	56.8 (4.5)	16.3 (2.1)	16.1 (2.1)	0.18	0.70	0.07
MMSE mean (SD)	29.6 (0.7)	29.9 (0.4)	29.7 (0.7)	29.6 (0.7)	0.16	0.21	0.18
AVLT STM mean (SD)	10.6 (2.8)	10.1 (2.7)	8.3 (1.9)	9.5 (2.5)	0.01*	0.42	0.07
AVLT LTM mean (SD)	9.9 (3.4)	9.8 (2.5)	7.3 (2.5)	8.9 (3.1)	0.01*	0.17	0.12
AVLT TL mean (SD)	51.3 (9.5)	48.6 (7.7)	43.9 (5.8)	47.0 (8.8)	0.02*	0.86	0.07
COWAT mean (SD)	45.8 (10.3)	44.9 (12.0)	43.6 (9.3)	43.4 (10.3)	0.39	0.82	0.88
Diabetes n (%)	0	1 (2.6%)	0	0	1.00	1.00	1.00
Hypertension n (%)	3 (15.0%)	11 (28.2%)	2 (10.0%)	3 (8.6%)	0.65	0.27	0.41
Smoking n (%)	3 (15.0%)	7 (17.9%)	6 (30.0%)	10 (28.6%)	0.26	0.78	0.77
Hyperlipidemia n (%)	3 (15.0%)	11 (28.2%)	2 (10.0%)	3 (8.6%)	0.38	0.24	0.05
Any cardiovascular n (%)	0	1 (2.6%)	0	0	1.00	1.00	1.00

Abbreviations: *APOE*4 HM: apolipoprotein e4 homozygote, *APOE*4/4; *APOE*4 HT: apolipoprotein e4 heterozygote, *APOE*3/4; *APOE*4 NC: apolipoprotein e4 non-carrier, *APOE* 3/3, 2/3; MMSE: Mini-Mental State Examination; AVLT: Auditory-Verbal Learning Test; STM: short term memory; LTM: long term memory; TL: total learning; COWAT: Controlled Oral Word Association Test. Note: COWAT tests executive function and language skills, on a scale with a lower limit of 0 and no upper limit, with higher scores indicating better performance. Data are given as mean +/− standard deviation unless otherwise indicated, p-values for continuous variables are from two-way ANOVA and p-values for categorical data are from logistics regression test as developed and reported in Hosmer & Lemeshow, 1989.[44]

Table 2

PiB-PET Cohort (N=58) Demographics

Baseline Characteristics	APOE4c/ BDNF Met (N=14)	APOE4c/ BDNF Val/Val (N= 20)	APOE4nc/ BDNF Met (N=10)	APOE4nc/ BDNF Val/Val (N= 14)	APOE4 effect p-value	BDNF Met effect p-value	APOE4×BDNF p-value
Sex male/female, (%)	4/10 (28.6%/ 71.4%)	8/12 (40%/ 60%)	3/7 (30%/ 70%)	5/9 (35.7%/ 64.3%)	0.94	0.49	0.83
APOE4 (HM/HT/NC)	8/6/0	5/15/0	0/0/10	0/0/14	NA	NA	NA
Education, years mean (SD)	15.8 (1.6)	15.7 (2.3)	16 (2.3)	15.7 (2.5)	0.78	0.87	0.87
Age, years mean (SD)	55.3 (5.5)	57.4 (4.4)	57.1 (5.3)	57.5 (4.1)	0.28	0.56	0.43
MMSE mean (SD)	29.6 (0.8)	29.8 (0.4)	29.7 (0.7)	29.6 (0.7)	0.64	0.96	0.50
AVLT STM mean (SD)	10.3 (2.9)	9.4 (3.1)	8.8 (2)	9.6 (2.4)	0.79	0.55	0.24
AVLT LTM mean (SD)	9.6 (3.5)	8.8 (3.3)	9 (2.4)	9.4 (2.9)	0.69	0.89	0.49
AVLT TL mean (SD)	51.8 (9.3)	46.5 (9.5)	46.7 (7.8)	48.3 (8.3)	0.30	0.66	0.16
COWAT mean (SD)	43.9 (7.1)	46.7 (11.4)	40.6 (7.9)	48.9 (13.1)	0.07	0.98	0.34
Diabetes (%)	0	0	0	0	1.00	1.00	1.00
Hypertension (%)	4 (20.0%)	6 (15.4%)	0	1 (2.9%)	0.99	0.93	0.99
Smoking (%)	4 (20.0%)	4 (10.3%)	3 (15.0%)	1 (2.9%)	0.94	0.56	0.40
Hyperlipidemia (%)	2 (10.0%)	6 (15.4%)	3 (15.0%)	0	0.36	0.30	0.99
Any cardiovascular (%)	1 (5.0%)	2 (5.1%)	1 (5.0%)	0	0.80	0.77	1.00

Abbreviations: *APOE*4 HM: apolipoprotein e4 homozygote, *APOE*4/4; *APOE*4 HT: apolipoprotein e4 heterozygote, *APOE*3/4; *APOE*4 NC: apolipoprotein e4 non-carrier, *APOE* 3/3, 2/3; MMSE: Mini-Mental State Examination; AVLT: Auditory-Verbal Learning Test; STM: short term memory; LTM: long term memory; TL: total learning; COWAT: Controlled Oral Word Association Test. Note: COWAT tests executive function and language skills, on a scale with a lower limit of 0 and no upper limit, with higher scores indicating better performance. Data are given as mean +/− standard deviation unless otherwise indicated, p-values for continuous variables are from two-way ANOVA and p-values for categorical data are from logistics regression test as developed and reported in Hosmer & Lemeshow, 1989.[44]

Table 3

Rate of Cognitive Change over Time from Baseline for Entire Cohort (N=114) and *APOE*4c (N=59) Subgrouped by BDNF Status

	Total N=114			***Total N=114***			***Total N=59***			
	***APOE4c* (N=59)**	***APOE4nc* (N=55)**	**P-Value**	***BDNF* Met Carrier (N=40)**	***BDNF* Val/Val (N=74)**	**P-Value**	***APOE4c/BDNF* Met Carrier (N=20)**	***APOE4c/BDNF* Val/Val (N=39)**	**P-Value**	***APOE* by *BDNF* P-Value**
Time from Baseline, years Mean (SD)	15 (4.76)	15.04 (5.69)	0.97	13.93 (5.29)	15.61 (5.10)	0.10	13.4 (4.76)	15.82 (4.60)	0.06	0.62
FDG follow up time in years Mean (SD)	7.34 (3.60) N=50	8.56 (3.43) N=46	0.09	8.31 (3.52) N=32	7.73 (3.58) N=64	0.45	7.26 (3.99) N=17	7.38 (3.44) N=33	0.91	0.33
MMSE (SD)	−0.03 (0.04)	−0.01 (0.04)	0.06	−0.03 (0.04)	−0.02 (0.04)	0.21	−0.04 (0.05)	−0.02 (0.04)	0.10	0.28
AVLT TL (SD)	−0.45 (0.55)	−0.22 (0.48)	0.02*	−0.31 (0.58)	−0.36 (0.50)	0.64	−0.52 (0.75)	−0.43 (0.44)	0.65	0.22
AVLT STM (SD)	−0.14 (0.22)	−0.04 (0.13)	0.01*	−0.10 (0.19)	−0.09 (0.18)	0.72	−0.17 (0.25)	−0.12 (0.20)	0.43	0.13
AVLT LTM (SD)	−0.17 (0.25)	−0.06 (0.18)	0.01*	−0.09 (0.21)	−0.12 (0.23)	0.56	−0.15 (0.30)	−0.17 (0.23)	0.86	0.08
COWAT (SD)	0.11 (0.46)	0.19 (0.50)	0.34	0.16 (0.47)	0.15 (0.49)	0.93	0.21 (0.61)	0.08 (0.41)	0.44	0.54
# of subjects who developed incident MCI	17	4	0.0004*	7	14	0.85	6	11	0.89	0.89

Table 3: Rate of cognitive change over time from baseline for entire cohort (N=114) and *APOE*4c (N=59) subgrouped by *BDNF* status was determined in the framework of linear mixed effects model for each subject who had at least two visits. Abbreviations: *APOE*4c: apolipoprotein e4 carrier, *APOE*4/4, 3/4; *APOE*4nc: apolipoprotein e4 non-carrier, *APOE* 3/3, 2/3; MMSE: Mini-Mental State Examination; AVLT: Auditory-Verbal Learning Test; STM: short term memory; LTM: long term memory; TL: total learning; COWAT: Controlled Oral Word Association Test. Note: COWAT tests executive function and language skills, on a scale with a lower limit of 0 and no upper limit, with higher scores indicating better performance. Data are given as mean +/− standard deviation unless otherwise indicated, p-values were computed under the linear mixed effect modeling with *APOE*4 and *BDNF* interaction term (p≤0.05)