BDNF Val66Met predicts cognitive decline in the Wisconsin Registry for Alzheimer's Prevention

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ABSTRACT

Objective: To examine the influence of the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism on longitudinal cognitive trajectories in a large, cognitively healthy cohort enriched for Alzheimer disease (AD) risk and to understand whether β-amyloid (Aβ) burden plays a moderating role in this relationship.

Methods: One thousand twenty-three adults (baseline age 54.94 ± 6.41 years) enrolled in the Wisconsin Registry for Alzheimer's Prevention underwent *BDNF* genotyping and cognitive assessment at up to 5 time points (average follow-up 6.92 ± 3.22 years). A subset (n = 140) underwent ^{11}C-Pittsburgh compound B (PiB) scanning. Covariate-adjusted mixed-effects regression models were used to elucidate the effect of *BDNF* on cognitive trajectories in 4 cognitive domains, including verbal learning and memory, speed and flexibility, working memory, and immediate memory. Secondary mixed-effects regression models were conducted to examine whether Aβ burden, indexed by composite PiB load, modified any observed *BDNF*-related cognitive trajectories.

Results: Compared to *BDNF* Val/Val homozygotes, Met carriers showed steeper decline in verbal learning and memory ($p = 0.002$) and speed and flexibility ($p = 0.017$). In addition, Aβ burden moderated the relationship between *BDNF* and verbal learning and memory such that Met carriers with greater Aβ burden showed even steeper cognitive decline ($p = 0.033$).

Conclusions: In a middle-aged cohort with AD risk, carriage of the *BDNF* Met allele was associated with steeper decline in episodic memory and executive function. This decline was exacerbated by greater Aβ burden. These results suggest that the *BDNF* Val66Met polymorphism may play an important role in cognitive decline and could be considered as a target for novel AD therapeutics. ***Neurology*® 2017;88:2098–2106**

GLOSSARY

Aβ = β-amyloid; **AD** = Alzheimer disease; **BDNF** = brain-derived neurotrophic factor; **DVR** = distribution volume ratio; **Met** = methionine; **PiB** = ^{11}C-Pittsburgh compound B; **RAVLT** = Rey Auditory Verbal Learning Test; **SNP** = single nucleotide polymorphism; **Val** = valine; **WRAP** = Wisconsin Registry for Alzheimer's Prevention.

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Preclinical Alzheimer disease (AD) is thought to be a critical period for intervention therapies that could potentially delay or prevent AD onset.[1] Considerable focus has been placed on genetic and environmental risk factors that may play a role in progression to AD and are possibly targetable for intervention, including *APOE* ε4,[2,3] physical activity,[4,5] and cognitive reserve.[6,7] Increasing evidence suggests that brain-derived neurotrophic factor (*BDNF*) may be a genetic risk factor for AD. BDNF is a neurotrophin known to play roles in synaptic plasticity, neurogenesis, neuronal survival, and cognitive health.[8–10] Additional research suggests that BDNF may moderate β-amyloid (Aβ) accumulation, a hallmark feature of AD,[1] by reducing Aβ-mediated cell death,[11] decreasing Aβ formation,[12] and repairing Aβ-induced damage.[13]

Supplemental data at Neurology.org

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A polymorphism within the *BDNF* gene (rs6265) causes a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met). Carriage of 1 or 2 Met alleles is associated with lower BDNF production,[14] decreased hippocampal volume,[15] and cognitive decline.[16–19] However, null[20,21] and opposite findings[22,23] have been documented, making the relationship between *BDNF* and cognition in aging populations unclear.

Our primary objective was to investigate whether *BDNF* is associated with longitudinal cognitive trajectories within a large cohort of middle-aged, cognitively healthy individuals enriched for AD risk, a target population for interventional therapies. Our secondary objective was to determine whether Aβ burden moderates the aforementioned relationship. We hypothesized that *BDNF* Met carriers would exhibit comparatively steeper cognitive decline in all cognitive domains and that Aβ burden would exacerbate this cognitive vulnerability.

METHODS

Standard protocol approvals, registrations, and patient consents. The University of Wisconsin Institutional Review Board approved all study procedures, and all participants provided signed informed consent before participation.

Participants. Study participants were enrolled in the Wisconsin Registry for Alzheimer's Prevention (WRAP), a longitudinal study of persons 40 to 65 years of age and cognitively healthy at study entry. Details about WRAP have been previously described.[24] For this study, 1,410 participants were selected on the basis of available *BDNF* and *APOE* data. One hundred five were subsequently excluded because of self-reported neurologic diagnosis (multiple sclerosis, Parkinson disease, etc). To preclude the influence of sibling clusters, only the first enrolled sibling from a family was included, excluding 277 individuals. Five participants were excluded because of missing covariate data. Thus, 1,023 individuals were included in the study. This sample is enriched for AD risk, with 64.2% of participants having at least one parent with AD as defined by National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association research criteria and 38.0% being *APOE* ε4 positive. Table 1 gives participant characteristics.

DNA collection, genotyping, and quality assurance. DNA was extracted from whole-blood samples with the PUREGENE DNA Isolation Kit (Gentra Systems, Inc, Minneapolis, MN). DNA concentrations were quantified with ultraviolet spectrophotometry (DU 530 Spectrophotometer; Beckman Coulter, Fullerton, CA). Single nucleotide polymorphisms (SNPs) for *BDNF* (rs6265) and *APOE* (rs429358, rs7412) were genotyped by LGC Genomics (Beverly, MA) using competitive allele-specific PCR-based KASP genotyping assays. For quality assurance, duplicate quality control samples from 102 individuals were

Table 1 Participant characteristics split by *BDNF* Val66Met polymorphism in the full sample and in the PiB subsample

Characteristic	Total sample	Val/Val	Met carriers	p Value	PiB-PET sample	Val/Val	Met carriers	p Value
Total, n (%)	1023 (100.0)	693 (67.7)	330 (32.3)		140 (100.0)	91 (65.0)	49 (35.0)	
Val/Met			296 (28.9)				44 (31.4)	
Met/Met			34 (3.3)				5 (3.6)	
Baseline age, y	54.94 ± 6.41	54.85 ± 6.47	55.13 ± 6.27	0.521	55.43 ± 5.82	55.08 ± 5.91	56.07 ± 5.67	0.342
Female, n (%)	713 (69.7)	479 (69.1)	234 (70.9)	0.560	91 (65.0)	58 (63.7)	33 (67.3)	0.669
Education, y	16.36 ± 2.82	16.42 ± 2.83	16.24 ± 2.81	0.340	16.86 ± 2.79	17.01 ± 2.76	16.59 ± 2.86	0.399
Race/ethnicity, n (%)								
White	907 (88.7)	598 (86.3)	309 (93.6)	<0.001	132 (94.3)	85 (93.4)	47 (95.9)	0.073
Black	82 (8.0)	73 (10.5)	9 (2.7)		5 (3.6)	5 (5.5)	0 (0.0)	
Hispanic	21 (2.1)	14 (2.0)	7 (2.1)		1 (0.7)	1 (1.1)	0 (0.0)	
Other	13 (1.3)	8 (1.2)	5 (1.5)		2 (1.4)	0 (0.0)	2 (4.1)	
Parental history positive, n (%)	657 (64.2)	453 (65.4)	204 (61.8)	0.268	95 (67.9)	60 (65.9)	35 (71.4)	0.507
APOE ε4 carrier, n (%)	389 (38.0)	262 (37.8)	127 (38.5)	0.835	51 (36.4)	33 (36.3)	18 (36.7)	0.956
Study visits completed, n	3.14 ± 1.08	3.11 ± 1.10	3.19 ± 1.05	0.280	3.76 ± 0.64	3.82 ± 0.57	3.65 ± 0.75	0.168
Length of follow-up, y	6.92 ± 3.22	6.85 ± 3.27	7.07 ± 3.12	0.313	8.39 ± 1.83	8.45 ± 1.61	8.29 ± 2.19	0.628
Time between PiB scan and baseline visit, y					6.08 ± 1.68	6.12 ± 1.57	6.02 ± 1.89	0.749
PiB-PET DVR					1.17 ± 0.16	1.15 ± 0.12	1.21 ± 0.22	0.072

Abbreviations: *BDNF* = brain-derived neurotrophic factor; DVR = distribution volume ratio; Met = methionine ; PiB = ^{11}C-Pittsburgh compound B; Val = valine.
Values are mean ± SD unless otherwise stated. Group comparisons were made by independent-samples *t* test for continuous variables and χ^2 for categorical variables.

Table 2 Trajectories of change in cognition as a function of the *BDNF* Val66Met polymorphism

Cognitive outcome	β (SE)	*t* Value	*p* Value
Verbal learning and memory			
BDNF	0.153 (0.061)	2.496	0.013
Time	0.002 (0.004)	0.475	0.635
BDNF × time	−0.021 (0.007)	−3.036	0.002
RAVLT trials 3–5 total			
BDNF	0.877 (0.332)	2.639	0.008
Time	0.002 (0.023)	0.103	0.918
BDNF × time	−0.114 (0.040)	−2.822	0.005
RAVLT delayed recall			
BDNF	0.312 (0.176)	1.774	0.076
Time	0.022 (0.012)	1.775	0.076
BDNF × time	−0.058 (0.021)	−2.719	0.007
Immediate memory			
BDNF	0.018 (0.063)	0.280	0.780
Time	−0.001 (0.009)	−0.157	0.876
BDNF × time	−0.001 (0.009)	−0.087	0.931
Speed and flexibility			
BDNF	0.187 (0.061)	3.044	0.002
Time	0.002 (0.004)	0.485	0.628
BDNF × time	−0.016 (0.007)	−2.392	0.017
Trail-Making Test A[a]			
BDNF	−0.844 (0.560)	−1.507	0.132
Time	−0.060 (0.043)	−1.408	0.160
BDNF × time	0.060 (0.075)	0.792	0.428
Trail-Making Test B[a]			
BDNF	−4.150 (1.884)	−2.203	0.028
Time	0.003 (0.117)	0.023	0.981
BDNF × time	0.366 (0.205)	1.783	0.075
Stroop Color-Word Interference Trial			
BDNF	3.768 (1.313)	2.870	0.004
Time	−0.088 (0.065)	−1.368	0.172
BDNF × time	−0.178 (0.113)	−1.570	0.117
Working memory			
BDNF	0.097 (0.066)	1.456	0.146
Time	0.004 (0.003)	1.455	0.146
BDNF × time	−0.003 (0.005)	−0.539	0.590

Abbreviations: *BDNF* = brain-derived neurotrophic factor; RAVLT = Rey Auditory Verbal Learning Test.
The analyses reported here were adjusted for age at baseline, sex, years of education, parental history of Alzheimer disease, *APOE* ε4 allele carriage, *BDNF*, and time in years from baseline visit.
BDNF denotes the estimated mean difference in cognition between Val/Val homozygotes and Met carriers at baseline; time indicates the estimated annual rate of change in cognition for Val/Val homozygotes; and BDNF × time indicates the estimated difference in annual rate of change in cognition between Val/Val homozygotes and Met carriers (this estimate has to be added to the estimate for time to determine the estimated annual rate of change for Met carriers).
For each cognitive factor, given a significant *BDNF* × time term, follow-up analyses were conducted to elucidate which component tests of the factor drove the observed associations. Results of the component test analyses are listed under their respective cognitive factor when applicable.
[a] Higher scores on this test indicate worse performance.

placed randomly throughout each of the 96-well plates. Further quality assurance was conducted with PLINK version 1.07.[25] *BDNF* and *APOE* SNPs did not deviate from Hardy-Weinberg equilibrium with the use of a Bonferroni-adjusted global significance level of $p = 0.05$, with accordant allele call rates ≥95%.

Cognitive evaluation. The WRAP neuropsychological test battery[24] comprises measures that assess multiple cognitive domains. Prior factor analyses of these measures indicated they map onto 6 cognitive factors; details of this method have been previously described.[26] Four of these factor scores were included in the present study because of their representation of cognitive abilities implicated in AD.[27] These factors and their constituent tests are as follows: immediate memory—Rey Auditory Verbal Learning Test (RAVLT) Learning Trials 1 and 2; verbal learning and memory—RAVLT Learning Trials 3 through 5 and Delayed Recall; working memory—Digit Span and Letter-Number Sequencing subtests from the Wechsler Adult Intelligence Scale, third edition; and speed and flexibility—Stroop Color-Word Test Interference Trial and Trail-Making Tests A and B. Participants undergo cognitive evaluation at each study visit, with up to 5 visits completed and a maximum of 13.12 years of follow-up at the time of these analyses (table 1).

^{11}C-Pittsburgh compound B-PET neuroimaging protocol. A subset of participants (n = 140) underwent 3-dimensional ^{11}C-Pittsburgh compound B (PiB)-PET scanning on a Siemens EXACT HR+ scanner (Siemens AG, Erlangen, Germany) through participation in a WRAP-affiliated study. Detailed methods of the acquisition and postprocessing of PiB-PET data have been described previously.[4] Briefly, imaging entailed a 70-minute dynamic scan on bolus injection and a 6-minute transmission scan. Image postprocessing used an in-house automated pipeline.[28] We created distribution volume ratio (DVR) maps of ^{11}C-PiB binding using the time-activity curve in the gray matter of the cerebellum as a reference region.[29] Then, using an anatomic atlas,[30] we extracted quantitative DVR data from 8 bilateral regions of interest sensitive to Aβ accumulation, including the precuneus, posterior cingulate, orbitofrontal cortex, anterior cingulate, angular gyrus, supramarginal gyrus, middle temporal gyrus, and superior temporal gyrus. DVR data from these regions of interest were combined to form a continuous, composite measure of Aβ accumulation.[31] The mean time interval between PiB-PET scan and baseline WRAP visit was 6.08 ± 1.68 years (table 1).

Statistical analyses. We used linear mixed models to investigate differences in the 4 cognitive trajectories as a function of *BDNF* Val66Met polymorphism. Models were fit by use of the maximum likelihood estimation and were constructed in the following progressive steps (assuming normality in the random effects): random intercept only, random intercept and random slope (for time, measured in years since baseline visit) without correlation between the 2 (i.e., variance components covariance matrix), and random intercept and slope with correlation between the 2 (i.e., unstructured covariance matrix). The last was selected for all subsequent analyses on the basis of superior model fit (Akaike information criterion). After the covariance structure was decided, we then added fixed-effects covariates, which were determined a priori from known associations with cognition or AD. They included age at baseline visit, sex, years of education, *APOE* ε4 status (0 = no ε4 allele, 1 = 1 or 2 ε4 alleles), parental family history of AD (0 = negative parental history, 1 = positive parental history), time, and *BDNF* (0 = Val/Val homozygotes, 1 = Met carriers; table 1). Our term of interest was *BDNF* × time because it would indicate whether rates of decline in cognition across time differ between *BDNF* Val/Val and Met carriers. Primary analyses were adjusted for multiple comparisons with a false discovery rate correction.[32] In addition, raw data visualization of each primary analysis confirmed that linearity was a reasonable representation of the data (figure e-1 at Neurology.org).

Given a significant *BDNF* × time term, follow-up analyses were conducted to elucidate which cognitive tests contributing to the factor scores drove the observed associations. We fitted the same mixed-effects models as above, except outcomes were the individual cognitive test scores rather than factor scores. Our term of interest remained the *BDNF* × time interaction.

In addition, we performed secondary mixed-effects models to determine whether Aβ burden, as indexed by the continuous composite PiB-PET measure, modified any *BDNF* × time effect observed in our primary analyses. For this purpose, we incorporated terms for Aβ, *BDNF* × Aβ, Aβ × time, and Aβ × *BDNF* × time into our original models, with Aβ × *BDNF* × time being the term of interest. All follow-up analyses to our primary analyses were not corrected for multiple comparisons because of their exploratory nature. Unless specified above, SPSS default mixed-model specifications were used for all analyses. Details on mixed-modeling procedures and assumptions are described elsewhere.[33] Only findings with values of $p \leq 0.05$ (2-tailed) were considered significant. Analyses were conducted with IBM SPSS, version 21.0.

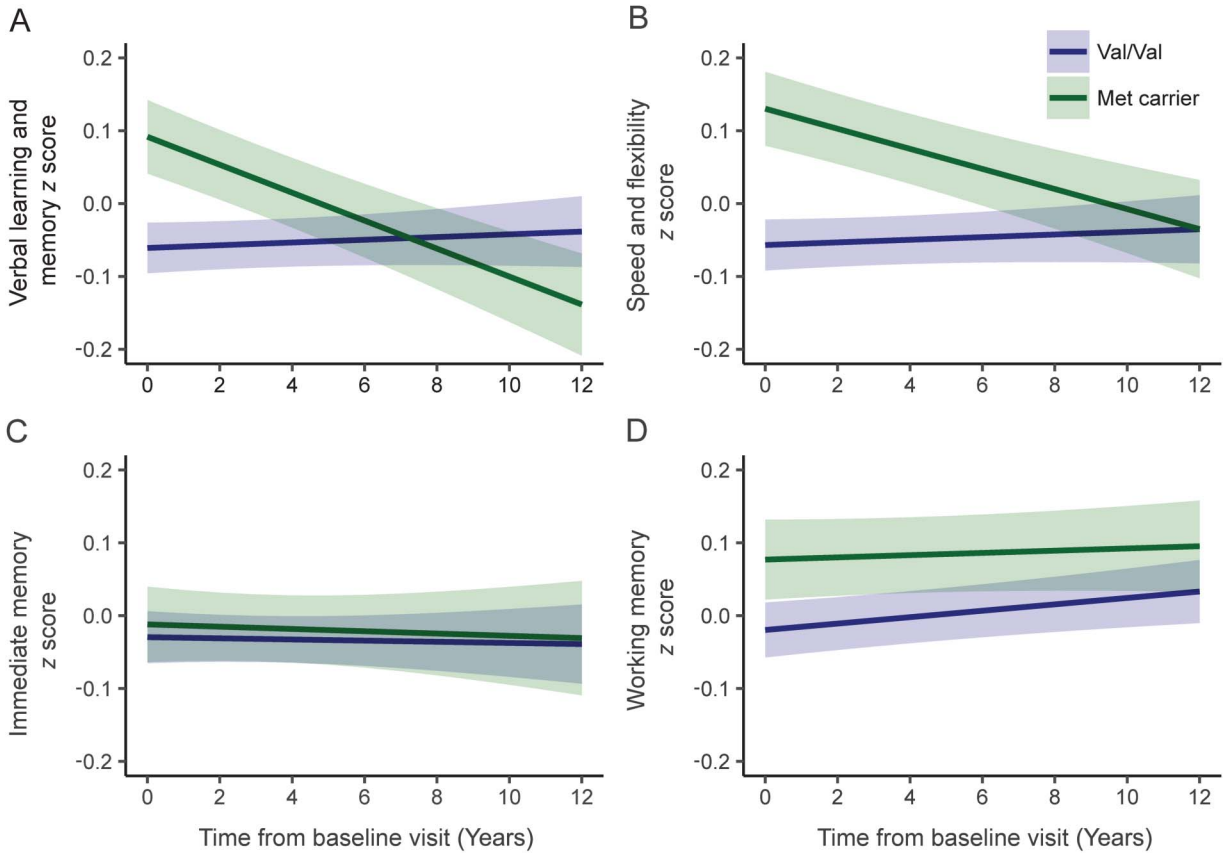
RESULTS

Background characteristics. Participant background characteristics are described in table 1. The mean ± SD age at baseline was 54.94 ± 6.41 years; women made up 69.7% of the sample. The group was highly educated (mean ± SD education 16.36 ± 2.82 years) and primarily white (88.7%, self-reported). In our sample, *BDNF* Met carriers encompassed 32.3% of the sample, which is lower than the reported white population frequency of 37.0% (http://www.alzgene.org). In a comparison of *BDNF* Val/Val homozygotes and *BDNF* Met carriers, there were no differences in demographic characteristics except for race/ethnicity ($p < 0.001$). There were no differences in demographic characteristics in the subset of 140 individuals with PiB-PET data.

Influence of BDNF on cognitive trajectories. Results of the linear mixed-effects models showed a *BDNF* × time interaction in the cognitive domains of verbal learning and memory ($p = 0.002$) and speed and flexibility ($p = 0.017$) such that *BDNF* Met carriers declined more steeply over time compared with *BDNF* Val/Val homozygotes (table 2 and figure 1). Differences in cognitive trajectories between Met carriers and Val/Val homozygotes were not seen in the cognitive domains of working memory ($p = 0.590$) and immediate memory ($p = 0.931$; table 2 and figure 1). Because of the difference in race/ethnicity between *BDNF* Met carriers and Val/Val homozygotes (table 1), we repeated the analyses while additionally including race/ethnicity as a covariate. Results were substantively unchanged.

Follow-up analyses were conducted to determine which cognitive tests in verbal learning and memory

Figure 1 ***BDNF*** **Met carriage is associated with decline in memory and executive function**



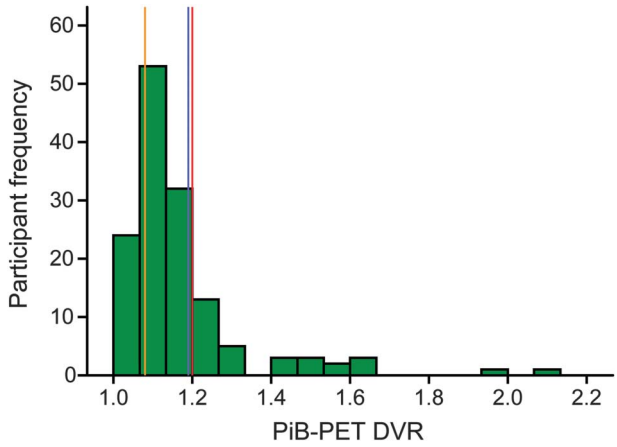
(A–D) Estimated trajectories of change in *BDNF* Val/Val homozygotes (blue) vs *BDNF* Met carriers (green) in cognitive domains of (A) verbal learning and memory, (B) speed and flexibility, (C) immediate memory, and (D) working memory. All models were adjusted for age at baseline, sex, years of education, parental history of Alzheimer disease, *APOE* ε4 allele carriage, *BDNF*, and time in years from baseline visit. Trajectories were plotted by calculating the regression equation lines for *BDNF* Val/Val homozygotes and Met carriers using the mean values for each of the covariates. Shaded regions represent standard errors. *BDNF* = brain-derived neurotrophic factor; Met = methionine; Val = valine.

and speed and flexibility drove the associations noted above. For verbal learning and memory, both measures, RAVLT Learning Trials 3 through 5 and Delayed Recall, showed associations wherein *BDNF* Met carriers declined more steeply over time compared with *BDNF* Val/Val homozygotes ($p = 0.005$ and 0.007, respectively; table 2). For speed and flexibility, none of the 3 constituent tests showed associations with *BDNF*.

Influence of Aβ and *BDNF* on cognitive trajectories. Given the *BDNF* × time interactions observed in verbal learning and memory and speed and flexibility in our primary analyses, we performed secondary analyses investigating whether the relationships observed were modified by Aβ burden. Results showed that there was an Aβ × *BDNF* × time interaction for verbal learning and memory (β [SE] = −0.218 [0.101], $t = -2.164$, $p = 0.033$) but not for speed and flexibility (β [SE] = −0.007 [0.086], $t = -0.078$, $p = 0.938$). When race/ethnicity also was included as a covariate, results remained substantively unchanged. A frequency distribution of DVRs is depicted in figure 2, with previously determined Aβ positivity cut points indicated.[34,35] Figure 3 depicts the change trajectories in verbal learning and memory, while accounting for covariates, across the following 4 prototypical groups: Val/Val Aβ+, Met carrier Aβ+, Val/Val Aβ−, and Met carrier Aβ−. Although Aβ burden, indexed with the PiB-PET composite DVR, was included in the analysis as a continuous variable, for graphing purposes, we chose 2 anchor points, the minimum (1.002) and maximum (2.073) DVR values, to represent Aβ+ vs Aβ−. As depicted in figure 3, Aβ+ Met carriers showed steeper cognitive decline over time compared with Aβ+ Val/Val homozygotes, who exhibited normal cognitive performance over time. These results indicate that the adverse influence of Met carriage on cognitive trajectory is further exacerbated by Aβ burden, whereas Val/Val genotype protects cognitive function even in the context of coexisting Aβ burden.

Of note, refitting our original *BDNF* × time analyses within this PiB subsample did not yield

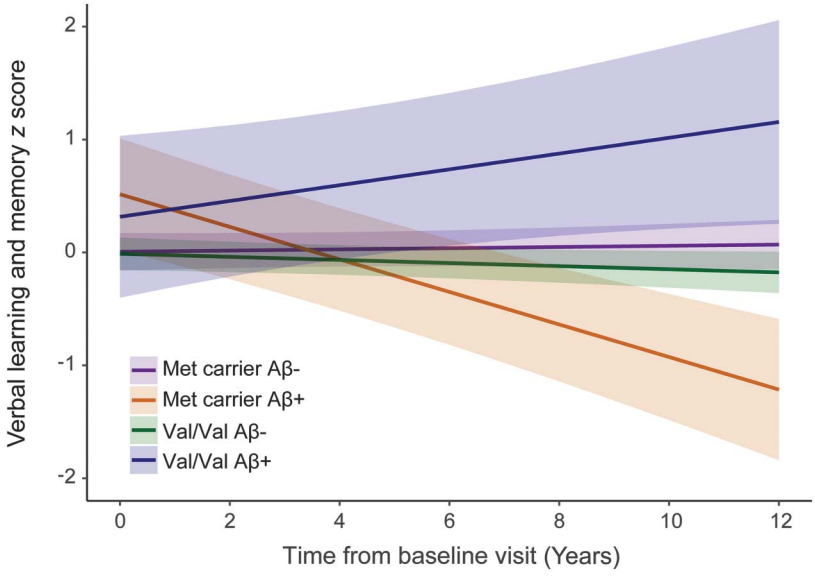
Figure 2 Frequency distribution of PiB-PET DVR values



Histogram depicts the DVR distribution for the subset of participants with PiB-PET data (n = 140). Three cut points for β-amyloid positivity are indicated: (1) a low-threshold DVR cut point of 1.08 (orange),[35] (2) a high-threshold DVR cut point of 1.20 (red),[35] and (3) an in-house cut point of 1.19 (blue).[34] DVR = distribution volume ratio; PiB = ^{11}C-Pittsburgh compound B.

significant results, although effects were in the same direction as in the full sample (verbal learning and memory: β [SE] = −0.023 [0.017], t = −1.330, p = 0.186; speed and flexibility: β [SE] = −0.006 [0.015], t = −0.390, p = 0.697).

Figure 3 Aβ modifies *BDNF*-related trajectories of change in memory



Shown are estimated trajectories of change in the verbal learning and memory cognitive factor when both the *BDNF* Val66Met polymorphism and Aβ positivity as measured by PiB-PET imaging are examined. This analysis was adjusted for age at baseline, sex, years of education, parental history of Alzheimer disease, *APOE* ε4 allele carriage, *BDNF*, Aβ, and time in years from baseline visit. Trajectories were plotted by calculating the regression equation lines for the 4 groups (Val/Val Aβ+, Met carrier Aβ+, Val/Val Aβ−, and Met carrier Aβ−) using the mean values for each of the covariates. Although Aβ burden, as indexed with PiB-PET composite DVR, was included in the analysis as a continuous variable, for ease of display, we chose 2 anchor points, the minimum (1.002) and maximum (2.073) DVR values, to represent Aβ+ vs Aβ−. Shaded regions represent standard errors. Aβ = β-amyloid; *BDNF* = brain-derived neurotrophic factor; DVR = distribution volume ratio; Met = methionine; PiB = ^{11}C-Pittsburgh compound B; Val = valine.

Exploratory analyses. An "additive" genetic model (i.e., in which Val/Val = 0, Val/Met = 1, and Met/Met = 2, as opposed to our original "dominant" genetic model, in which Val/Val = 0, Met carriers = 1) was also considered for the cognitive analyses. Results from this additive model were similar to results of the original model, with Met/Met homozygotes exhibiting the steepest cognitive decline. However, because of the limited number of Met/Met homozygotes (n = 34), these results may have poor stability.

In addition, we examined whether *APOE* ε4 status moderated the relationship between *BDNF* and cognitive trajectories in verbal learning and memory and speed and flexibility. The findings were nonsignificant (p = 0.359 and 0.686, respectively).

DISCUSSION This study found that the *BDNF* Val66Met polymorphism is associated with cognitive decline in a large cohort of individuals with increased risk for AD. Specifically, compared with Val/Val homozygotes, Met carriers exhibited steeper decline in the cognitive domains of verbal learning and memory and speed and flexibility. In addition, we showed that Aβ accumulation adversely moderated the relationship between *BDNF* and verbal learning and memory such that Met carriers with greater Aβ burden had steeper memory decline compared to those with lesser Aβ burden. This is one of few longitudinal studies to report the association between the *BDNF* Val66Met polymorphism and cognitive decline over time. Our study also expands on previous findings regarding the moderating relationship between Aβ accumulation and Met carriership on cognitive decline.[16] Our research was conducted in a cognitively healthy cohort with risk factors for AD, highlighting the potential for early detection of cognitive decline and subsequent implementation of interventional therapies[36] during this preclinical AD phase.

Our study adds longitudinal evidence to a growing body of literature on the relationship between the *BDNF* Val66Met polymorphism and cognitive health. Initial cross-sectional studies on this polymorphism found that in younger adults, Met carriership was associated with worse episodic memory as measured by the Wechsler Memory Scale and an fMRI declarative memory paradigm.[14,19] More recently, a study in an aging population[18] (mean age 56 years) demonstrated cross-sectionally that Met carriers performed worse in both item memory and prospective memory with advancing age compared with Val/Val homozygotes. Item memory was assessed with the California Verbal Learning Test,[18] a psychometric measure similar to the RAVLT, which comprises our verbal learning and memory measure. Another cross-sectional study[17] showed that in addition to memory, processing speed was reduced in Met carriers compared

to Val/Val homozygotes (mean age 63 years). Our study adds evidence to these findings by demonstrating that in a longitudinal cohort at risk for AD, Met carriership was associated with steeper decline in the domains of verbal learning and memory and speed and flexibility over an average time period of 7 years.

However, other work examining the relationship between the *BDNF* Val66Met polymorphism and cognitive health has failed to find a relationship or has even observed findings that are opposite to the aforementioned. In one study, Val/Val genotype occurred with higher frequency in those with AD compared to healthy controls[22]; another study showed lower scores on the Frontal Assessment Battery in Val/Val homozygote patients with mild AD.[23] These discrepancies could possibly be explained by genetic differences between samples (i.e., lack of Hardy-Weinberg equilibrium), differences in disease stage at the time of analysis (probable AD vs preclinical AD), or cross-sectional design. In fact, in the present study, Met carriers performed better at baseline in the domains of verbal learning and memory ($p = 0.013$) and speed and flexibility ($p = 0.002$) (table 2) yet declined more steeply over time, emphasizing the importance of longitudinal design in studies of *BDNF* and cognition.

Other studies have failed to find a relationship between the Val66Met polymorphism and cognitive outcomes or AD incidence.[15,20,21] However, in one of these studies, the authors reported relationships between greater BDNF expression in the dorsolateral prefrontal cortex and slower cognitive decline.[21] Another group did not find associations with the polymorphism but noted that greater serum BDNF levels resulted in reduced dementia and AD incidence.[20] Finally, a third report did not find associations with the Val66Met polymorphism but did with other *BDNF* SNPs, specifically rs1157659, rs11030094, and rs11030108.[15] These discrepancies underscore the need for further understanding of the intricacies in the relationship between *BDNF* polymorphisms, BDNF expression, and cognitive function.

We found that greater Aβ accumulation, as measured by PiB load in 8 bilateral regions of interest, moderated the relationship between *BDNF* and verbal learning and memory such that in Met carriers, those with greater Aβ accumulation had steeper cognitive decline. This finding aligns with a similar report[16] that found that in cognitively healthy older adults (mean age 71 years), Met carriers with high levels of Aβ exhibited steeper cognitive decline in episodic memory, executive function, and language over 36 months. The same group also observed that among persons with amnestic mild cognitive impairment, Met carriers with high levels of Aβ had worse episodic memory.[37] Other groups have noted similar moderating effects of Aβ and *BDNF* on cognition in older adults.[38]

This interaction between Aβ and the *BDNF* Val66Met polymorphism adds further support for the potential role the BDNF protein has in moderating Aβ production, thus protecting cognitive function. Animal studies have indicated that BDNF regulates sorting protein-related receptor with A-type repeats, a known modulator of Aβ precursor protein trafficking and processing, via extracellular signal-regulated protein kinase stimulation, suggesting that BDNF could deter amyloid production.[12] Other studies have found that BDNF delivery rescues memory impairment in mice injected with $A\beta_{1\text{-}42}$,[39] prevents neuronal loss,[11] and rescues cells from degeneration due to Aβ toxicity.[13] It is critical for future studies to further investigate the role that the *BDNF* gene and BDNF protein may have in Aβ accumulation because it could be a potential target for intervention against Aβ toxicity.

A major strength of this study is its scale. With 1,023 individuals and up to 13 years of follow-up, it is the one of the largest studies investigating the *BDNF* Val66Met polymorphism. In addition, conducting the study within the WRAP cohort provides further evidence that subtle cognitive changes can be detected early in the AD cascade, and intervention during this time period could be critical for delaying or preventing onset of AD. However, this study is not without limitations. Our sample consists of predominantly highly educated, white individuals, reducing the generalizability of our results. Generalizability might also be reduced by the discord between Met carriers in our sample and the white population frequency. Furthermore, the sample size of individuals with PiB-PET data was limited. In addition, we were able to conduct analyses using only one *BDNF* polymorphism, and no serum BDNF data were available for this research. Ongoing work in our group will further examine the interplay between *BDNF* polymorphisms, systemic and central BDNF levels, and neuroimaging and cognitive outcomes. We find this research to be of particular importance, especially given the body of research suggesting that BDNF levels can be increased with physical activity[5] and other modifiable lifestyle factors.[40]

This study provides evidence that the *BDNF* Val66Met polymorphism is associated with cognitive decline in a large sample of individuals at risk for AD and that Aβ plays a moderating role in this relationship. Our findings suggest that Met carriership could accelerate cognitive decline throughout the preclinical phase of AD, emphasizing the importance of investigating *BDNF* as a potential target for novel AD therapeutics in the future.[36]

AUTHOR CONTRIBUTIONS

Study concept or design: Boots, Okonkwo. Acquisition, analysis, or interpretation of data: Boots, Schultz, Clark, Racine, Darst, Koscik, Carlsson, Gallagher, Hogan, Bendlin, Asthana, Sager, Hermann, Christian, Dubal, Engelman, Johnson, Okonkwo. Drafting of manuscript: Boots, Okonkwo. Critical revision of manuscript: Boots, Schultz, Clark, Racine, Darst, Koscik, Carlsson, Gallagher, Hogan, Bendlin, Asthana, Sager, Hermann, Christian, Dubal, Engelman, Johnson, Okonkwo. Statistical analysis: Boots, Okonkwo. Obtaining funding: Carlsson, Asthana, Dubal, Johnson, Okonkwo. Administrative, technical, or material support: Boots, Schultz, Clark, Racine, Darst, Koscik, Gallagher, Hogan, Bendlin, Sager, Hermann, Christian, Engelman. Supervision: Okonkwo.

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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REFERENCES

1. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:280–292.
2. Pietrzak RH, Lim YY, Ames D, et al. Trajectories of memory decline in preclinical Alzheimer's disease: results from the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Ageing. Neurobiol Aging 2015;36:1231–1238.
3. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–923.
4. Okonkwo OC, Schultz SA, Oh JM, et al. Physical activity attenuates age-related biomarker alterations in preclinical AD. Neurology 2014;83:1753–1760.
5. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci USA 2011;108:3017–3022.
6. Boots EA, Schultz SA, Almeida RP, et al. Occupational complexity and cognitive reserve in a middle-aged cohort at risk for Alzheimer's disease. Arch Clin Neuropsychol 2015;30:634–642.
7. Stern Y. Cognitive reserve in ageing and Alzheimer's disease. Lancet Neurol 2012;11:1006–1012.
8. Song JH, Yu JT, Tan L. Brain-derived neurotrophic factor in Alzheimer's disease: risk, mechanisms, and therapy. Mol Neurobiol 2015;52:1477–1493.
9. Webster MJ, Herman MM, Kleinman JE, Shannon Weickert C. BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. Gene Expr Patterns 2006;6:941–951.
10. Diniz BS, Teixeira AL. Brain-derived neurotrophic factor and Alzheimer's disease: physiopathology and beyond. Neuromolecular Med 2011;13:217–222.
11. Nagahara AH, Mateling M, Kovacs I, et al. Early BDNF treatment ameliorates cell loss in the entorhinal cortex of APP transgenic mice. J Neurosci 2013;33:15596–15602.
12. Rohe M, Synowitz M, Glass R, Paul SM, Nykjaer A, Willnow TE. Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression. J Neurosci 2009;29:15472–15478.
13. Kimura N, Takahashi M, Tashiro T, Terao K. Amyloid beta up-regulates brain-derived neurotrophic factor production from astrocytes: rescue from amyloid beta-related neuritic degeneration. J Neurosci Res 2006;84:782–789.
14. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;112:257–269.
15. Honea RA, Cruchaga C, Perea RD, et al. Characterizing the role of brain derived neurotrophic factor genetic variation in Alzheimer's disease neurodegeneration. PLoS One 2013;8:e76001.
16. Lim YY, Villemagne VL, Laws SM, et al. BDNF Val66Met, Abeta amyloid, and cognitive decline in preclinical Alzheimer's disease. Neurobiol Aging 2013;34:2457–2464.
17. Miyajima F, Ollier W, Mayes A, et al. Brain-derived neurotrophic factor polymorphism Val66Met influences cognitive abilities in the elderly. Genes Brain Behav 2008;7:411–417.
18. Kennedy KM, Reese ED, Horn MM, et al. BDNF val66-met polymorphism affects aging of multiple types of memory. Brain Res 2015;1612:104–117.
19. Hariri AR, Goldberg TE, Mattay VS, et al. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 2003;23:6690–6694.
20. Weinstein G, Beiser AS, Choi SH, et al. Serum brain-derived neurotrophic factor and the risk for dementia: the Framingham Heart Study. JAMA Neurol 2014;71:55–61.
21. Buchman AS, Yu L, Boyle PA, Schneider JA, De Jager PL, Bennett DA. Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. Neurology 2016;86:735–741.
22. Feher A, Juhasz A, Rimanoczy A, Kalman J, Janka Z. Association between BDNF Val66Met polymorphism

and Alzheimer disease, dementia with Lewy bodies, and Pick disease. Alzheimer Dis Assoc Disord 2009; 23:224–228.
23. Nagata T, Shinagawa S, Nukariya K, Yamada H, Nakayama K. Association between BDNF polymorphism (Val66Met) and executive function in patients with amnestic mild cognitive impairment or mild Alzheimer disease. Dement Geriatr Cogn Disord 2012;33:266–272.
24. Sager MA, Hermann B, La Rue A. Middle-aged children of persons with Alzheimer's disease: APOE genotypes and cognitive function in the Wisconsin Registry for Alzheimer's Prevention. J Geriatr Psychiatry Neurol 2005;18:245–249.
25. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.
26. Koscik RL, La Rue A, Jonaitis EM, et al. Emergence of mild cognitive impairment in late middle-aged adults in the Wisconsin Registry for Alzheimer's Prevention. Dement Geriatr Cogn Disord 2014;38:16–30.
27. Schultz SA, Larson J, Oh J, et al. Participation in cognitively-stimulating activities is associated with brain structure and cognitive function in preclinical Alzheimer's disease. Brain Imaging Behav 2015;9:729–736.
28. Floberg JM, Mistretta CA, Weichert JP, Hall LT, Holden JE, Christian BT. Improved kinetic analysis of dynamic PET data with optimized HYPR-LR. Med Phys 2012;39: 3319–3331.
29. Price JC, Klunk WE, Lopresti BJ, et al. Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh compound-B. J Cereb Blood Flow Metab 2005;25:1528–1547.
30. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 2002;15:273–289.
31. Schultz SA, Boots EA, Almeida RP, et al. Cardiorespiratory fitness attenuates the influence of amyloid on cognition. J Int Neuropsychol Soc 2015;21:841–850.
32. Curran-Everett D. Multiple comparisons: philosophies and illustrations. Am J Physiol Regul Integr Comp Physiol 2000;279:R1–R8.
33. Singer JD, Willett JB. Applied Longitudinal Data Analysis. New York: Oxford University Press; 2003.
34. Racine AM, Clark LR, Berman SE, et al. Associations between performance on an Abbreviated CogState battery, other measures of cognitive function, and biomarkers in people at risk for Alzheimer's disease. J Alzheimers Dis 2016;54:1395–1408.
35. Villeneuve S, Rabinovici GD, Cohn-Sheehy BI, et al. Existing Pittsburgh compound-B positron emission tomography thresholds are too high: statistical and pathological evaluation. Brain 2015;138:2020–2033.
36. Lu B, Nagappan G, Guan X, Nathan PJ, Wren P. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. Nat Rev Neurosci 2013;14: 401–416.
37. Lim YY, Villemagne VL, Laws SM, et al. Effect of BDNF Val66Met on memory decline and hippocampal atrophy in prodromal Alzheimer's disease: a preliminary study. PLoS One 2014;9:e86498.
38. Adamczuk K, De Weer AS, Nelissen N, et al. Polymorphism of brain derived neurotrophic factor influences beta amyloid load in cognitively intact apolipoprotein E epsilon4 carriers. Neuroimage Clin 2013;2:512–520.
39. Zhang L, Fang Y, Lian Y, et al. Brain-derived neurotrophic factor ameliorates learning deficits in a rat model of Alzheimer's disease induced by abeta1-42. PLoS One 2015;10:e0122415.
40. Lee J, Seroogy KB, Mattson MP. Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. J Neurochem 2002;80:539–547.