Impact of *APOE* and *BDNF* Val66Met Gene Polymorphisms on Cognitive Functions in Patients with Amnestic Mild Cognitive Impairment

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Handling Associate Editor: Yen Ying Lim

Abstract. Apolipoprotein (*APOE*) ε4 is a well-known risk factor for late-onset Alzheimer's disease (AD), but other AD-related gene polymorphisms might also be important, such as the polymorphism within the brain-derived neurotrophic factor (*BDNF*) gene. Carriage of *BDNF* Val66Met has been associated with faster cognitive decline and greater hippocampal atrophy in cognitively normal elderly. Thus, we examined the effects of the concurrent presence of *APOE* and *BDNF* polymorphisms on cognitive functions and brain morphometry in amnestic mild cognitive impairment (aMCI) patients. 107 aMCI patients (mean age = 72.2) were recruited from the Czech Brain Aging Study and, based on *APOE* and *BDNF* genes polymorphisms, were divided into four groups: ε4$^-$*BDNF*$^{\text{Val/Val}}$ ($n = 37$), ε4$^-$*BDNF*$^{\text{Met}}$ ($n = 19$), ε4$^+$*BDNF*$^{\text{Val/Val}}$ ($n = 35$), and ε4$^+$*BDNF*$^{\text{Met}}$ ($n = 16$). All patients underwent clinical examination, magnetic resonance imaging, and complex neuropsychological battery. The combination of *APOE* ε4$^+$ and *BDNF* Met was associated with significantly worse memory performance in immediate and delayed recall compared to other polymorphism groups. We did not observe increased atrophy in areas related to memory function in the ε4$^+$*BDNF*$^{\text{Met}}$ group. Our findings suggest that carriage of ε4$^+$*BDNF*$^{\text{Met}}$ is associated with more pronounced memory dysfunction, a typical feature of early AD, but not with structural brain changes in aMCI patients. These findings

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suggest that in *APOE ε4/BDNF* Met carriers, synaptic dysfunction affecting memory may precede pronounced structural changes.

Keywords: Alzheimer's disease, amnestic mild cognitive impairment, Apolipoprotein E, brain-derived neurotrophic factor, cognition, gene polymorphism

INTRODUCTION

Apolipoprotein E (*APOE*) genotype is a main known genetic risk factor for late-onset Alzheimer's disease (AD), with individuals carrying the ε4 allele being at 3-4 fold higher risk than non-carriers [1]. However, recent studies have indicated that other genetic polymorphisms may also be related to the increased risk of AD [2]. One such polymorphism is within the brain-derived neurotrophic factor (*BDNF*) gene, which is a gene that encodes a neurotrophin well known for its role in hippocampal synaptic plasticity [3], hippocampal neurogenesis [4], and regulation of long-term potentiation (LTP) [5]. In the human *BDNF* gene, a single nucleotide polymorphism causes an amino acid substitution of valine (Val66Val) to methionine at amino acid residue 66 (Val66Met), which alters the secretion of the mature peptide. This alteration has been associated with cognitive deficits among its carriers [6].

Cross-sectional studies in cognitively healthy younger adults have shown that carriage of the Met allele is associated with poorer memory performance, reduced hippocampal volume, and lower hippocampal activation on functional magnetic resonance imaging (fMRI) [6–8]. In a longitudinal amyloid positron emission tomography (PET) study in older adults with 3 years of follow-up, cognitively unimpaired Met carriers with elevated levels of brain amyloid-β (Aβ) plaque deposits showed an accelerated decline in episodic memory ($d = 0.70$) and executive function ($d = 0.80$), and a faster rate of hippocampal atrophy ($d = 0.71$), compared to Val homozygotes who also manifested increased accumulation of amyloid binding ligand in the brain [9]. In brain morphometry studies using structural MRI scans, cognitively normal younger adults *BDNF* Met carriers have repeatedly shown smaller hippocampal volume, a brain region crucial for memory encoding, compared with Val homozygotes [10, 11]. Furthermore, inheritance of at least one Met allele has been associated with greater memory decline over 36 months compared to Val homozygotes ($d = 0.90$) in patients with mild cognitive impairment (MCI) but only in individuals with high Aβ [12]. Alto-

gether, these findings suggest that BDNF is crucial for hippocampal function and *BDNF* Met allele is associated with worse memory function. Because the first AD functional changes are observed in the hippocampus, Met carriers may be at increased risk for developing AD-related memory impairment. Nevertheless, in cross-sectional analyses performed in older individuals, it was difficult to observe these effects of *BDNF* on cognition and brain volume. One possible explanation is that the cross-sectional effect of *BDNF* polymorphism becomes more evident in non-demented older individuals who are at increased risk of developing dementia, as for example amnestic MCI (aMCI) patients *APOE* ε4 carriers.

APOE ε4 status is well known for its detrimental effect on episodic memory during aging [13], for lowering the age of AD onset [14], and for the higher possibility of having abnormal brain Aβ levels [15]. The risk of Aβ accumulation is significantly increased while the clearance of Aβ is reduced in *APOE* ε4 carriers [16, 17]. Also recently, it has been shown that the prevalence of *APOE* ε4 in Aβ positive subjects was 64% in MCI patients [18]. It is likely that aMCI patients carrying *APOE* ε4 are those with abnormal high Aβ load. Therefore, aMCI patients *APOE* ε4 carriers are likely those who have a greater chance to show the negative effect of *BDNF* Met carriage.

In a previous study, a negative effect of the combination of these risky alleles on episodic memory was observed, but only in cognitively normal older adults [19]. Also, in a longitudinal study, carriers of both *APOE* ε4 and *BDNF* Met developed clinically significant verbal memory impairment at a faster rate than individuals with *APOE* ε4/*BDNF* Val/Val, but still only in cognitively healthy older adults with elevated brain Aβ levels [20].

The question remains whether the co-occurrence of *APOE* and *BDNF* risk polymorphisms is associated to a different character of cognitive profile in individuals with cognitive impairment.

To elucidate this question, the aim of this cross-sectional study was to assess the effect of the combination of *BDNF* and *APOE* gene polymor-

phisms on cognitive performance in individuals with aMCI.

Based on previous findings, we expected worse episodic memory performance in carries of these risky polymorphisms. Since episodic memory impairment is correlated to alteration of specific brain areas, our secondary objective was to assess whether *APOE ε4/BDNF* Met carriers were characterized by structural changes in these brain regions. Specifically, we compared hippocampal volumes and parahippocampal and entorhinal cortices thickness between *APOE ε4/BDNF* Met carriers and non-carriers.

METHODS

Participants

Based on the predefined inclusion and exclusion criteria, 107 patients with aMCI were selected from the database of the Czech Brain Aging Study, a longitudinal, memory clinic-based study on aging and cognitive impairment [21]. All participants underwent neurological examination, laboratory evaluations (with *BDNF* Val66Met and *APOE* genotyping) and neuropsychological testing within 2 months of brain MRI. The aMCI participants met Petersen's criteria for MCI [22], with memory complaints reported by the patient or caregiver, an evidence of memory impairment on neuropsychological testing, generally intact activities of daily living, and absence of dementia. Memory impairment was established when the participant scored more than 1.5 standard deviations below the mean of age-adjusted and education-adjusted norms on any memory test [21]. Our aMCI group included both aMCI single-domain and aMCI multiple-domain phenotypes. Participants were stratified into four groups based on *APOE ε4* and *BDNF* Met carriage.

Participants were not included in the study if they reported a history of major neurological or psychiatric disorders, depression (≥ 6 points on the 15-item Geriatric Depression Scale) [23] or had significant vascular impairment on brain MRI (Fazekas scale more than 2) [24].

We did not include the *APOE ε4* homozygotes and *BDNF* Met homozygotes as they are consistently considered the most risky group and their frequency in our database was low ($n = 17$ and $n = 8$, respectively). We also did not include the *APOE ε2* carriers, both *APOE ε2/ε3* and *ε2/ε4*. Group-wise characteristics of four groups ($\varepsilon 4^- BDNF^{Val/Val}$, $\varepsilon 4^- BDNF^{Met}$, $\varepsilon 4^+ BDNF^{Val/Val}$, $\varepsilon 4^+ BDNF^{Met}$) are listed in Table 1. All participants involved in this study had signed written informed consent approved by the Motol University Hospital ethics committee.

Neuropsychological assessment

All participants were interviewed with the following questionnaires: Clinical Dementia Rating scale [25], activities of daily living, Hachinski ischemic scale [26], Beck Anxiety Inventory (BAI) [27], and Geriatric Depression Scale (GDS) [23]. The neuropsychological battery included the Mini-Mental State Examination (MMSE) [28], trials 1–5 and 30-minute delayed recall of the Rey Auditory Verbal Learning Test (RAVLT) [29], the immediate and 20-minute delayed trials of the Logical Memory test from the Wechsler Memory Scale-Third Edition [30], Digit Span forward and backward from the Wechsler Adult Intelligence Scale–Third Edition [31], Trail Making Tests (TMT) A and B [32], Prague Stroop test-colours [33], phonemic verbal fluency (PVF) [34], verbal fluency (VF) – animals + vegetables [34, 35], Boston naming test (BNT) [36], Rey-Osterrieth Complex Figure (ROCF) [37], and the Clock Drawing Test (CDT) [38].

Table 1
Characteristics of study participants; means (SD)

Variables	Entire sample ($n = 107$)	$\varepsilon 4^- BDNF^{Val/Val}$ ($n = 37$)	$\varepsilon 4^- BDNF^{Met}$ ($n = 19$)	$\varepsilon 4^+ BDNF^{Val/Val}$ ($n = 35$)	$\varepsilon 4^+ BDNF^{Met}$ ($n = 16$)	p	Effect sizes
Women/Men	49/58	18/19	8/11	15/20	8/8	0.93	0.01
Age in years; range	72.2 (7.1); 55–89	75.6 (6.4); 63–89	69.6 (8.0)*; 55–81	69.9 (6.8)*; 55–81	72.4 (5.7); 65–84	<0.01	0.12
Education in years	14.6 (3.5)	15.0 (4.0)	14.0 (3.4)	14.0 (3.1)	15.6 (3.2)	0.36	0.03
MMSE score	26.2 (2.7)	26.2 (2.3)	26.9 (2.5)	26.3 (3.2)	25.2 (2.3)	0.32	0.03
GDS score	2.8 (1.5)	3.0 (1.6)	2.7 (1.5)	2.6 (1.6)	2.8 (1.2)	0.82	0.01

Values are mean (SD) except for gender and age range. Effect sizes were calculated as Cramér's V for χ^2 (gender) and partial eta^2 for ANOVA. *$p < 0.05$ compared to the $\varepsilon 4^- BDNF^{Val/Val}$ group. $\varepsilon 4^- BDNF^{Val/Val}$, *APOE ε3/ε3*, BDNF homozygous for Valine group; $\varepsilon 4^- BDNF^{Met}$, *APOE ε3/ε3*, BDNF Val/Met carriers' group; $\varepsilon 4^+ BDNF^{Val/Val}$, *APOE ε3/ε4*, BDNF homozygous for Valine group; $\varepsilon 4^+ BDNF^{Met}$, *APOE ε3/ε4*, BDNF Val/Met carriers' group.

Genotyping

To determine the *APOE* genotype, DNA was isolated from blood samples (ethylenediaminetetraacetic acid; Qiagen extraction) and genotyping was performed according to Idaho-tech protocol (LunaProbes Genotyping Apolipoprotein [ApoE] Multiplexed Assay) for high-resolution melting analysis (HRM) [39, 40].

We developed HRM analysis for detection of rs6265(G196A) in BDNF gene. The PCR product of 59bp was amplified with primers: forward BDNFLSF1:5′-GCT TGA CAT CAT TGG CTG ACA CTT-3′ and reverse BDNFLSR1 5′GTC CTC ATC CAA CAG CTC TTC TAT-3′; Polymerase chain reaction (PCR) conditions: volume 10 μl: 1 μl/10 μM primers, 4 μl LightScanner Master Mix, 3 μl H_2O; 1 μl DNA sample; Cycling conditions: 95°C – 2 min; 95°C –30 s and 70°C –30 s for 40 cycles (cycler Biometra T3). Subsequent HRM analysis of PCR product was performed on Light Scanner (IdahoTech, USA:) HRM conditions: melting temperature range was 70°C–90°C.

Of the 107 patients, who completed neuropsychological assessment and underwent *APOE/BDNF* genotyping, MRI data were available for 75 individuals, of which 27 were *APOE* ε4⁻ $BDNF^{Val/Val}$, 14 *APOE* ε4⁻ $BDNF^{Met}$, 24 were *APOE* ε4⁺ $BDNF^{Val/Val}$, and 10 were *APOE* ε4⁺ $BDNF^{Met}$ carriers.

MRI acquisition and analysis

Individual brain MRIs were performed at 1.5T system (Siemenes, Erlangen, Germany). A T1 weighted, 3-dimensional high resolution magnetization-prepared rapid acquisition with gradient echo (MPRAGE) was acquired with TR/TE/TI = 2000/3.08/1100 ms, flip angle 15°, 192 continuous partitions, slice thickness 1.0 mm, and in-plane resolution 1 mm [21]. Individual scans were visually inspected to determine sufficient technical quality and to exclude participants with clinical radiologic findings that could interfere with cognitive functioning (i.e., cortical infarctions, tumors, subdural hematomas, hydrocephali or more extensive white matter hyperintensities equal to Fazekas scale above 2). To measure right- and left-sided hippocampal volume and entorhinal and parahippocampal cortical thickness, we used automated algorithm FreeSurfer, version 5.3. (http://surfer.nmr.mgh.harvard.edu), described in detail elsewhere [41, 42].

Hippocampal volumes were normalized for the differences in head size by regressing the estimated total intracranial volume (eTIV) among subjects. The formula used was: $Volume_i(adjusted) = volume_i(observed) – \beta (eTIV_i – eTIV_{mean})$, where $eTIV_i$ is the ith subject's eTIV, $eTIV_{mean}$ is overall average eTIV and β is the slope of the regression line of the ith brain structure, regressed on eTIV [43]. Entorhinal and parahippocampal cortical thicknesses were not eTIV adjusted. Morphometric characteristics of the participants are listed in Table 3.

Statistical analyses

Composite cognitive scores were computed by standardizing the raw scores for each neuropsychological test to z-scores using the mean and standard deviation for the entire group, and subsequently averaged to create single composite scores for attention and working memory (Digit Span forward and backward, TMT A), immediate verbal memory (RAVLT trials 1–5, Logical Memory test immediate recall), delayed verbal memory (RAVLT 30-minute delayed recall trial, Logical Memory test 20-minute delayed trial), executive function (TMT B, Prague Stroop test – colours, PVF), language (VF, BNT), and visuospatial function (ROCF copy and CDT). Scores of TMT A and B, Stroop test, and BNT errors were reversed before transformation to z-scores.

To evaluate between-group differences in age, years of education, and global cognitive functioning as assessed by MMSE, we used one-way analysis of variance (ANOVA) with *post hoc* Tukey's honestly significant differences (HSD) test. The χ^2 test was used to evaluate gender frequency differences across groups.

To assess the study aims, two separate analyses were performed to examine the effect of *APOE* and *BDNF* gene polymorphisms on (1) cognitive performance and (2) brain volume and cortical thickness. First, to investigate whether different combinations of *APOE/BDNF* alleles were associated with different levels of cognitive performance, we used one-way analysis of covariance (ANCOVA). Each ANCOVA model included the averaged cognitive domain performance z-score as the outcome (attention and working memory, immediate verbal memory, delayed verbal memory, executive function, language and visuospatial function), the polymorphism group (ε4⁻ $BDNF^{Val/Val}$, ε4⁻ $BDNF^{Met}$, ε4⁺ $BDNF^{Val/Val}$, and ε4⁺ $BDNF^{Met}$) as a between-subject factor, and covariates of age, sex, and years of education. Next,

to explore differences in hippocampal volume and cortical thickness of parahippocampal and entorhinal cortex, we fitted similar one-way ANCOVA models with each brain MRI marker, rather than a cognitive domain, as the dependent variable. In the *post hoc* analysis we compared the data of cognitive composite scores and brain volumes of the $\varepsilon 4^+$ $BDNF^{Met}$ group with those of these polymorphism groups: $\varepsilon 4^-$ $BDNF^{Val/Val}$, $\varepsilon 4^-$ $BDNF^{Met}$, $\varepsilon 4^+$ $BDNF^{Val/Val}$. The magnitude of difference from the $\varepsilon 4^+$ $BDNF^{Met}$ group was expressed using Cohen's *d*. A *p* value <0.05 was considered statistically significant. Analyses were performed using R statistical language environment [44].

RESULTS

Patients characteristics

One hundred and seven patients with aMCI were included in the final analysis. With respect to genotype distribution of *APOE/BDNF*, there were significantly more subjects with $\varepsilon 4^-$ $BDNF^{Val/Val}$ and $\varepsilon 4^+$ $BDNF^{Val/Val}$ than $\varepsilon 4^-$ $BDNF^{Met}$ or $\varepsilon 4^+$ $BDNF^{Met}$ ($\varepsilon 4^-$ $BDNF^{Val/Val}$: $n = 37$ [34%], $\varepsilon 4^-$ $BDNF^{Met}$: $n = 19$ [18%], $\varepsilon 4^+$ $BDNF^{Val/Val}$: $n = 35$ [33%], $\varepsilon 4^+$ $BDNF^{Met}$: $n = 16$ [15%]); χ^2 [3] = 13.04, $p = <0.01$). There were no differences in years of education, sex, MMSE scores, or GDS scores among *APOE/BDNF* groups. However, age differed significantly among the groups (F[3] = 4.84, $p = 0.003$, $\eta^2 = 0.12$). *Post hoc* revealed that $\varepsilon 4^-$ $BDNF^{Val/Val}$ were significantly older than $\varepsilon 4^-$ $BDNF^{Met}$ ($p = 0.002$) and $\varepsilon 4^+$ $BDNF^{Val/Val}$ ($p = 0.012$) (Table 1).

Cognitive domains

We compared the mean adjusted cognitive performance (composites domain z-score) between the four different genotype groups. The groups did not differ in global cognitive performance evaluated by MMSE ($p = 0.31$). Despite their similar global cognitive functioning, we found significant difference in delayed memory score (F[3] = 5.23, $p = 0.002$, $\eta^2 = 0.15$) among the groups, with the most risky group $\varepsilon 4^+$ $BDNF^{Met}$ having the worst score. They performed by 1.06 SD worse than the $\varepsilon 4^-$ $BDNF^{Met}$ group ($p < 0.001$). Also, the $\varepsilon 4^+$ $BDNF^{Met}$ group performed by 0.83 SD worse than $\varepsilon 4^-$ $BDNF^{Val/Val}$ group ($p = 0.002$). The difference between $\varepsilon 4^+$ $BDNF^{Met}$ and $\varepsilon 4^+$ $BDNF^{Val/Val}$ carriers almost reached the sig-

nificance level ($p = 0.053$) with $\varepsilon 4^+$ $BDNF^{Met}$ having the lower score. The immediate memory score also differed among the groups (F[3] = 4.68, $p = 0.004$, $\eta^2 = 0.14$). Again, $\varepsilon 4^+$ $BDNF^{Met}$ had the worst score. $\varepsilon 4^+$ $BDNF^{Met}$ carriers performed by 0.88 SD worse than the $\varepsilon 4^-$ $BDNF^{Met}$ group ($p < 0.001$). In addition, the $\varepsilon 4^+$ $BDNF^{Met}$ group performed by 0.83 SD worse than $\varepsilon 4^-$ $BDNF^{Val/Val}$ ($p = 0.002$). Compared to $\varepsilon 4^+$ $BDNF^{Val/Val}$ carriers, the $\varepsilon 4^+$ $BDNF^{Met}$ scored worse, although the results did not reach the level of significance ($p = 0.070$). We found a significant main effect in executive domain score among the groups (F[3] = 4.95, $p = 0.003$, $\eta^2 = 0.07$), but we did not observe group differences in *post hoc* analyses. There were no significant differences among the groups in attention and working memory, language and visuospatial function (Table 2).

Brain morphometry

Using ANCOVA adjusted for age, sex, and years of education, a significant *APOE/BDNF* polymorphism group effect was identified in the right hippocampal volume (F[3] = 4.88, $p = 0.004$, $\eta^2 = 0.12$). *Post-hoc* analyses revealed that the $\varepsilon 4^-$ $BDNF^{Met}$ group had significantly higher right hippocampus volume as compared to $\varepsilon 4^+$ $BDNF^{Met}$ group ($p = 0.032$). The group effects for the left hippocampal volume and entorhinal and parahippocampal cortical thickness were not significant (Table 3).

DISCUSSION

This study aimed to examine the concurrent impact of *APOE* and *BDNF* Val66Met gene polymorphisms on cognition and brain structures in aMCI patients. We found that, despite the similar MMSE score among the groups, the combination of *APOE* $\varepsilon 4$ and *BDNF* Met alleles was related to poorer memory performance in non-demented older adults at risk for AD dementia as compared to carriers of only one risky gene polymorphism. Specifically, we found that *APOE* $\varepsilon 4$/*BDNF* Met carriers performed worse than *APOE* $\varepsilon 3$/*BDNF* Met ($p < 0.001$) and *APOE* $\varepsilon 3$/*BDNF* Val carriers ($p < 0.01$) in both delayed and immediate memory scores. When compared to *APOE* $\varepsilon 4$/*BDNF* Val, the results almost reached the significance ($p = 0.053$ and 0.070, respectively). The greatest effect of these polymorphisms was identified within delayed recall, indicating episodic memory dysfunction, which is a typical early symptom of AD.

Table 2

Characteristics of cognitive domains and magnitudes of differences (Cohen's d) in cognitive composite scores compared to $\varepsilon4^+$ $BDNF^{Met}$ carriers

	Cognitive domains (z scores); means (SD)				Cohen's d (95% CIs) vs $\varepsilon4^+$ $BDNF^{Met}$ carriers		
	$\varepsilon4^-$ $BDNF^{Val/Val}$ (n=37)	$\varepsilon4^-$ $BDNF^{Met}$ (n=19)	$\varepsilon4^+$ $BDNF^{Val/Val}$ (n=35)	$\varepsilon4^+$ $BDNF^{Met}$ (n=16)	$\varepsilon4^-$ $BDNF^{Val/Val}$	$\varepsilon4^-$ $BDNF^{Met}$	$\varepsilon4^+$ $BDNF^{Val/Val}$
Attention and working memory	−0.212 (0.793)	0.243 (0.660)	0.027 (0.883)	0.212 (0.562)	0.18 (−0.32, 0.60)	0.02 (−0.47, 0.50)	0.13 (−060, 0.39)
Memory Immediate	−0.069 (0.900)	0.443 (0.749)	−0.335 (1.085)	−0.531 (0.885)	0.75 (−1.17, −0.13)**	1.11 (−1.43, −0.33)***	0.49 (−0.09, 0.88)
Memory Delayed	0.062 (0.830)	0.431 (0.623)	−0.166 (0.940)	−0.631 (0.951)	0.98 (−1.33, −0.32)**	1.34 (−1.61, −0.51)***	0.58 (−0.02, 1.09)
Executive function	−0.357 (0.860)	0.332 (0.652)	0.127 (0.864)	0.143 (0.700)	0.06 (−0.45, 0.55)	0.27 (−0.76, 0.33)	0.06 (−0.45, 0.55)
Language	−0.232 (0.953)	0.288 (0.778)	−0.004 (0.811)	0.316 (0.678)	0.33 (−0.24, 0.84)	0.20 (−0.44, 0.81)	0.44 (−0.88, 0.14)
Visuospatial	−0.253 (0.951)	0.337 (0.671)	0.124 (0.737)	−0.109 (0.976)	0.26 (−0.79, 0.32)	0.56 (−1.18, 0.14)	0.27 (−0.30, 0.73)

*$p < 0.05$ **$p < 0.01$ and ***$p < 0.001$ compared to the $\varepsilon4^+$ $BDNF^{Met}$ group; values are adjusted for age, sex, and years of education. SD, standard deviation; CI, confidence interval; $\varepsilon4^-$ $BDNF^{Val/Val}$, APOE ε3/ε3, BDNF homozygous for Valine group; $\varepsilon4^-$ $BDNF^{Met}$, APOE ε3/ε3, BDNF Val/Met carriers' group; $\varepsilon4^+$ $BDNF^{Val/Val}$, APOE ε3/ε4, BDNF homozygous for Valine group; $\varepsilon4^+$ $BDNF^{Met}$, APOE ε3/ε4, BDNF Val/Met carriers' group.

Table 3

MRI characteristics of study groups and magnitudes of differences (Cohen's d) in brain volumes compared to $\varepsilon4^+$ $BDNF^{Met}$ carriers

	Brain volumes (z scores); means (SD)				Cohen's d (95% CIs) versus $\varepsilon4^+$ $BDNF^{Met}$		
	$\varepsilon4^-$ $BDNF^{Val/Val}$ (n=27)	$\varepsilon4^-$ $BDNF^{Met}$ (n=14)	$\varepsilon4^+$ $BDNF^{Val/Val}$ (n=24)	$\varepsilon4^+$ $BDNF^{Met}$ (n=10)	$\varepsilon4^-$ $BDNF^{Val/Val}$	$\varepsilon4^-$ $BDNF^{Met}$	$\varepsilon4^+$ $BDNF^{Val/Val}$
Left hippocampal volume, eTIV adjusted (mm³)	3228.7 (652.3)	3726.2 (720.0)	3347.8 (754.7)	3209.5 (724.0)	0.05 (−490.30, 425.03)	0.63 (−1138.26, 187.24)	0.23 (−358.63, 669.51)
Right hippocampal volume, eTIV adjusted (mm³)	3168.7 (736.4)	3925.7 (745.7)	3457.3 (754.1)	3278.0 (798.7)	0.08 (−432.83, 532.04)	0.73 (−1394.63, 123.94)*	0.30 (−303.15, 701.72)
Left parahippocampal thickness (mm)	2.6 (0.4)	2.7 (0.3)	2.5 (0.3)	2.5 (0.3)	0.25 (−0.39, 0.19)	0.42 (−0.41, 0.14)	0.29 (−0.15, 0.34)
Right parahippocampal thickness (mm)	2.5 (0.3)	2.7 (0.3)	2.6 (0.3)	2.5 (0.2)	0.17 (−0.17, 0.28)	0.40 (−0.33, 0.12)	0.61 (−0.04, 0.33)
Left entorhinal thickness (mm)	2.9 (0.6)	3.1 (0.5)	2.8 (0.4)	2.8 (0.2)	0.32 (−0.53, 0.21)	0.40 (−0.64, 0.23)	0.24 (−0.21, 0.40)
Right entorhinal thickness (mm)	2.9 (0.5)	3.3 (0.4)	3.0 (0.4)	3.1 (0.4)	0.51 (−0.11, 0.56)	0.15 (−0.47, 0.33)	0.02 (−0.31, 0.32)

*$p < 0.05$ **$p < 0.01$ and ***$p < 0.001$ compared to the $\varepsilon4^+$ $BDNF^{Met}$ group; values are adjusted for age, sex, and years of education. SD, standard deviation; CI, confidence interval; $\varepsilon4^-$ $BDNF^{Val/Val}$, APOE ε3/ε3, BDNF homozygous for Valine group; $\varepsilon4^-$ $BDNF^{Met}$, APOE ε3/ε3, BDNF Val/Met carriers' group; $\varepsilon4^+$ $BDNF^{Val/Val}$, APOE ε3/ε4, BDNF homozygous for Valine group; $\varepsilon4^+$ $BDNF^{Met}$, APOE ε3/ε4, BDNF Val/Met carriers' group.

Regarding structural brain data, the *APOE ε4/BDNF* Met group displayed pronounce atrophy of right hippocampus compared to *APOE ε3/BDNF* Met ($p < 0.05$). Although all aMCI patients may display mild brain atrophy [45], we did not observe increased atrophy in other brain areas related to episodic memory function in the *APOE ε4/BDNF* Met group. These findings suggest that in *APOE ε4/BDNF* Met carriers episodic memory dysfunction may be more pronounced despite similar structural brain changes.

Our data support the hypothesis that, in aMCI patients, carriage of *APOE ε4/BDNF* Met is associated with poorer memory performance, which is in agreement with previous data in cognitively healthy older individuals [19, 20, 46]. It was shown that Met carriers display worse memory function compared to *BDNF* Val homozygotes in cognitively healthy individuals [47], although studies in MCI patients found no effect in cross-sectional analysis of *BDNF* Met allele on memory performance [48]. Our data suggest that the negative effect of *BDNF* Met may be exacerbated by the carriage of *APOE ε4* in aMCI patients, who are closer to developing a dementia. Since MCI patients *APOE ε4* carriers are also those likely to have abnormal Aβ levels [17, 18], it cannot be excluded that the negative effects seen in *APOE ε4/BDNF* Met on memory is mediated by an abnormal load of Aβ. Indeed, in previous studies the negative effect of *APOE ε4* and *BDNF* Met on memory in cognitively healthy individuals was seen only in Aβ positive individuals [12, 20], and based on those findings, it was proposed that *BDNF* Met influences the ability of the brain to tolerate Aβ toxicity [20]. Recently, it was also demonstrated that *BDNF* Met carriage enhances the brain vulnerability to the toxic effects of Aβ [49]. Thus, it is possible that the presence of *BDNF* Met in *APOE ε4* carriers further amplifies the negative effects of the impaired Aβ clearance and worsen the memory impairment in early AD stage. In a previous longitudinal investigation with mixed sample of MCI and AD patients, the carriage of both detrimental alleles (*APOE ε4/BDNF* Met) was associated with a steeper memory decline when compared to non-carriers [50]. Instead, *BDNF* Val seems to have a neutral effect on memory function [11], which was not exacerbated by *APOE ε4* carriage in our sample cohort, even though *APOE ε4* carriers scored slightly worse than non-carriers. In a previous study, older adults who were either *APOE ε3/BDNF* Val homozygotes or *APOE ε4/BDNF* Val homozygotes displayed stable cognitive performance across the 4.5 year follow-up period even in Aβ

positive individuals [20]. Altogether these findings suggest that *BDNF* Met and *APOE ε4* aMCI carriers may have increased memory dysfunctions and this effect may be due to either a decrease in BDNF function or to the exacerbation of Aβ toxicity in the hippocampus.

In the executive domain, we did not find significant differences in *APOE ε4/BDNF* Met compared to other polymorphism groups. Some previous reports have found positive effects of *BDNF* Met polymorphism on executive function performance in cognitively healthy individuals [51], but not in aMCI patients [52]. Recently, it has been shown that *BDNF* Val homozygosity may be disadvantageous when inhibitory mechanisms are required, indicating that Met allele may confer some benefit to its carriers for the executive functions processed in the fronto-striatal pathways [53]. However, we could not find any beneficial effect of *BDNF* Met polymorphism among *APOE ε4* carriers, suggesting that ε4 allele may contribute to abolish the positive effect of *BDNF* Met polymorphism on executive functions. Supporting this hypothesis, Gomar et al. [50] failed to find effect of any *APOE/BDNF* combination on executive functions in healthy subject and in prodromal AD patients. Executive functions represent a set of heterogenous cognitive processes that are evaluated by different cognitive tests. The inconsistent of findings concerning the effect of Met allele on executive functions could be also due to differences in neuropsychological batteries across studies.

Our study failed to reveal any effect of *APOE* and *BDNF* polymorphisms related to attention or visuospatial functions. This is consistent with data of a previous study in cognitively unimpaired individuals, where no increased rate of decline in attention composite over time was observed among *APOE ε4/BDNF* Met carriers [20]. Moreover, in other studies, healthy carriers of *BDNF* Met did not perform differently from *BDNF* Val homozygotes in attention [54] or visuospatial tests [55]. One of the likely explanations is that BDNF is crucial factor for hippocampal functioning, and thus the main effect of its polymorphism is found in memory domain.

Regarding structural data, despite the finding that *APOE ε4/BDNF* Met group displayed the worse memory performance in both immediate and delayed recall, we observed increased atrophy only in right hippocampus compared to *APOE ε3/BDNF* Met ($p < 0.05$), but not in any other key regions for episodic memory formation. Consistently with our results, Gomar et al. [50] did not observe increased

atrophy at baseline in risky carriers, but over 3 years among *APOE* ε4 carriers, and *BDNF* Met showed greater atrophy compared to Val homozygotes in the entorhinal cortex in a mixed sample of MCI and AD patients. However, this group of patients was in an advanced stage of disease (MMSE = 22.6) compared to our sample. The fact that in *APOE* ε4/*BDNF* Met carriers brain atrophy increases over time suggests that the effects of the two polymorphisms may accumulate in an ongoing process with detrimental consequences in later stages of the disease [50]. Previous studies have shown greater hippocampal atrophy in *BDNF* Met carriers compared with Val homozygotes [10, 12, 56], but only in cognitively healthy individuals. Despite these data, a meta-analysis did not find any significant association between *BDNF* Val66Met polymorphism and hippocampal volume reduction [57]. Lim et al. concluded that the effect of *BDNF* Met on hippocampal volume is conditioned by the presence of Aβ [9], which was not measured in our study sample.

The exact mechanism of interaction between these two genes remains uncovered. Previous studies showed that *BDNF* Val66Met polymorphism alters activity-dependent secretion of BDNF protein, causing Met carriers to have a reduced secretion of BDNF in the hippocampus [6, 58], thus altering its function at a synaptic level [59, 60]. Interestingly, recent evidence has suggested that different ApoE isoforms may also regulate the cleavage and secretion of BDNF. Sen et al. [61] showed that ApoE3 treated hippocampal astrocytes secreted 38.4-fold more BDNF compared to control cells, whereas ApoE4 treated astrocytes produce a negligible amount of this protein. In addition, it has been shown that ApoE4 increased the nuclear translocation of histone deacetylases, which leads to histone deacetylation and causes a negative effect on BDNF gene transcription [62]. Therefore, it is possible that ApoE4 may exacerbate neurodegenerative processes by a mechanism that includes inhibition of BDNF synthesis. Thus, one possible explanation for the memory decline seen in *APOE* ε4/*BDNF* Met group is that ApoE4 further reduces BDNF synthesis in Met carriers. Lower BDNF levels in the hippocampus have deleterious effects, including loss of synaptic and neural integrity [63], leading to memory impairment [64] and increasing the risk of developing dementia [65, 66]. However, more studies are necessary to confirm this hypothesis.

The strengths of the study are the fact that we applied very stringent exclusion criteria, making our sample very homogenous. In addition, to the best of our knowledge this is the only study that evaluated the impact of *APOE* and *BDNF* Val66Met gene polymorphisms on a well-defined cohort of patients with aMCI, while other recent studies focused mostly on influence of *APOE* and *BDNF* in healthy elderly and one included a mixed sample of patients with MCI and AD dementia (MCI/AD group). There are few limitations that should be mentioned. First, the cross-sectional design does not allow tracking the changes in cognitive domains over time, although a longitudinal study is ongoing and will be presented subsequently. In addition, the assessment of specific AD biomarkers (Aβ$_{42}$, tau, p-tau$_{181}$) was performed only in a subset of participants, so we cannot confirm the AD-pathology in all aMCI patients. We also did not measure the circulating BDNF levels, however, the collection of blood samples is ongoing. Thus, this study can be considered as a pilot study and further longitudinal and cross-sectional studies in larger sample cohorts are required to validate our findings.

Conclusions

In conclusion, this study indicates that the concurrent presence of *APOE* ε4 and *BDNF* Met genetic polymorphisms in aMCI patients is associated with deficits in episodic memory. Notably, this memory dysfunction was not associated with increased atrophy in memory-related brain structures. The carriers of both polymorphisms appear to be at greater risk of memory deficits as compared to the other polymorphism groups. Thus, it is likely that they will progress faster to more severe disease stages. This information might be clinically relevant, as aMCI represents a heterogenic group of patients with variation in prognostic outcome. If our results are confirmed by longitudinal study, genotyping may be a complementary method to identify individuals at increased risk of developing dementia (for disease progression or conversion to dementia). Genetic determination seems to be a useful analytical method for identification of people at risk, particularly when CSF or PET biomarkers are unavailable, due to fact that this method is fast, inexpensive and minimally invasive for the patient. Thus, any indication for prediction of disease course at aMCI disease stage could be relevant to patient management, counselling, planning and patient selection in clinical trials or secondary prevention/therapeutic strategies.

ACKNOWLEDGMENTS

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The Combined Effect of *APOE* and *BDNF* Val66Met Polymorphisms on Spatial Navigation in Older Adults

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Abstract.

Background: The apolipoprotein E (*APOE*) $\varepsilon 4$ allele is associated with episodic memory and spatial navigation deficits. The brain-derived neurotrophic factor (*BDNF*) Met allele may further worsen memory impairment in *APOE* $\varepsilon 4$ carriers but its role in *APOE* $\varepsilon 4$-related spatial navigation deficits has not been established.
Objective: We examined influence of *APOE* and *BDNF* Val66Met polymorphism combination on spatial navigation and volumes of selected navigation-related brain regions in cognitively unimpaired (CU) older adults and those with amnestic mild cognitive impairment (aMCI).
Methods: 187 participants (aMCI [$n = 116$] and CU [$n = 71$]) from the Czech Brain Aging Study were stratified based on *APOE* and *BDNF* Val66Met polymorphisms into four groups: $\varepsilon 4^-/BDNF^{\text{Val/Val}}$, $\varepsilon 4^-/BDNF^{\text{Met}}$, $\varepsilon 4^+/BDNF^{\text{Val/Val}}$, and $\varepsilon 4^+/BDNF^{\text{Met}}$. The participants underwent comprehensive neuropsychological examination, brain MRI, and spatial navigation testing of egocentric, allocentric, and allocentric delayed navigation in a real-space human analogue of the Morris water maze.
Results: Among the aMCI participants, the $\varepsilon 4^+/BDNF^{\text{Met}}$ group had the least accurate egocentric navigation performance ($p < 0.05$) and lower verbal memory performance than the $\varepsilon 4^-/BDNF^{\text{Val/Val}}$ group ($p = 0.007$). The $\varepsilon 4^+/BDNF^{\text{Met}}$ group had smaller hippocampal and entorhinal cortical volumes than the $\varepsilon 4^-/BDNF^{\text{Val/Val}}$ ($p \le 0.019$) and $\varepsilon 4^-/BDNF^{\text{Met}}$ ($p \le 0.020$) groups. Among the CU participants, the $\varepsilon 4^+/BDNF^{\text{Met}}$ group had less accurate allocentric and allocentric delayed navigation performance than the $\varepsilon 4^-/BDNF^{\text{Val/Val}}$ group ($p < 0.05$).
Conclusion: The combination of *APOE* $\varepsilon 4$ and *BDNF* Met polymorphisms is associated with more pronounced egocentric navigation impairment and atrophy of the medial temporal lobe regions in individuals with aMCI and less accurate allocentric navigation in CU older adults.

Keywords: Alzheimer's disease, apolipoproteins E, brain-derived neurotrophic factor, entorhinal cortex, episodic memory, gene polymorphism, magnetic resonance imaging, mild cognitive impairment, Morris water maze, spatial navigation

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INTRODUCTION

Spatial navigation is a complex and multi-modal cognitive process essential for everyday functioning. It encompasses two basic strategies, egocentric (self-centered) and allocentric (world-centered), using different types of spatial reference frames to develop internal representations of surrounding environment. Egocentric navigation is a navigation strategy, where spatial information about locations and objects is encoded from the viewpoint of the navigator to form a self-centered spatial reference frame (self-to-object representations). Allocentric navigation is a navigation strategy, where locations and objects are encoded in relation to one another independently of the position of the navigator to form a world-centered spatial reference frame (object-to-object representations). Previous research has shown that distinct brain regions underlie egocentric and allocentric navigation strategies. Egocentric navigation is associated with the level of function of the posterior parietal cortex [1] including the precuneus [2] and the posterior cingulate cortex [3]. Allocentric navigation is associated with the level of function of the hippocampus [1] and related medial temporal lobe structures including the entorhinal cortex [4]. Recently, more attention has been focused on spatial navigation impairment as a promising early cognitive marker of Alzheimer's disease (AD) [5, 6]. Allocentric navigation deficits have been observed in individuals with preclinical AD [7], while impairment of both navigation strategies (i.e., egocentric and allocentric) have been observed in individuals with mild cognitive impairment (MCI) [8], especially in those with amnestic MCI (aMCI) [2, 9–11], who are at higher risk of conversion to AD dementia [12]. Spatial navigation impairment observed in the early stages of AD can be explained by the fact that allocentric and egocentric spatial navigation is associated with the level of function of brain regions impaired very early in AD including the hippocampus and entorhinal cortex, and the posterior parietal cortex including precuneus and posterior cingulate cortex, respectively [13–15]. Recently, it has been shown that spatial navigation is a cognitive marker of early AD that shares only limited variance with other cognitive functions and is well distinguishable as a separate cognitive function [16].

Spatial navigation is influenced by genetic background, where the apolipoprotein E gene (*APOE*) is one of the most important indicators. The *APOE* ε4 allele is the strongest genetic risk factor for sporadic AD dementia [17] that lowers its age at onset [18]. The *APOE* ε4 allele is associated with increased amyloid-β (Aβ) accumulation on positron emission tomography (PET) [19], increased tau load in the entorhinal cortex on PET [20], hippocampal atrophy on MRI [21], posterior cingulate and parietal hypometabolism on fluorodeoxyglucose (FDG) PET [22], and greater cognitive decline in older adults [23]. The *APOE* ε4 allele also increases the risk of progression from MCI to dementia [24] probably due to the fact that individuals with aMCI who are carriers of the *APOE* ε4 allele are more likely to have Aβ pathology [25]. Studies found that the *APOE* ε4 allele is associated with worse allocentric navigation performance in cognitively normal older adults [26] and less accurate egocentric and allocentric navigation in individuals with MCI [27–29]. In addition to the *APOE* gene, other genetic polymorphisms associated with AD dementia and impairment of cognitive functions including spatial navigation have been identified. One of these polymorphisms is the very long poly-T variant at rs10524523 of the *TOMM40* gene that modulates risk and onset age of AD dementia [30]. This polymorphism has been associated with worse memory performance in late middle-aged and older adults [31, 32] and also less accurate allocentric navigation in individuals with aMCI [33]. The other polymorphism is the rs17070145 polymorphism of the *KIBRA* gene, where non-carriers of the T-allele have had increased risk of AD dementia [34], worse memory performance [35] and less accurate spatial navigation in a study of cognitively normal older adults [36].

Recent studies indicated that among other genetic polymorphisms the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism may also be associated with an increased risk of AD dementia [37] and more pronounced cognitive impairment [38, 39]. BDNF is a critical neurotrophic factor for synaptic plasticity [40], dendritic arborization [41], and facilitation of long-term potentiation [42], especially in the hippocampus and entorhinal cortex where the place cells and grid cells important for spatial navigation are located [43]. BDNF expression is profoundly reduced in the entorhinal cortex, hippocampus, and parietal cortex of individuals with AD dementia [44, 45], that is, in the regions where the earliest pathophysiological changes of AD have been identified [46]. Lower BDNF levels have been found in *BDNF* Met carriers [47]. The *BDNF* Met allele has been associated with reduced entorhinal [48] and parietal cortical thickness [49] and smaller hippocampal volume on MRI [50], reduced hippocampal activation

on functional MRI [51], lower memory performance [52], and increased reliance on stimulus response rather than allocentric strategy in a human virtual navigation task [53] in cognitively normal younger adults. Next, the *BDNF* Met allele has been associated with accelerated memory decline and a greater rate of hippocampal volume reduction in cognitively normal older adults but only in those with high Aβ levels on PET [54]. The association between the *BDNF* Met allele and accelerated memory decline has also been reported in individuals with aMCI and high Aβ levels on PET [55]. The high risk combination of the *APOE* ε4 and *BDNF* Met alleles has shown the most pronounced negative effect on memory in cognitively normal older adults [56]. The later study has found that the combination of the *APOE* ε4 and *BDNF* Met alleles is associated with most accelerated memory decline in cognitively normal older adults but only in those with high Aβ levels on PET (i.e., those with preclinical AD) [57]. The combination of the *APOE* and *BDNF* Val66Met polymorphisms has also been associated with lowest memory performance in individuals with aMCI [58], prodromal AD and AD dementia [25]. The combined effect of the *APOE* and *BDNF* Val66Met polymorphisms on spatial navigation has not been studied.

Our group has demonstrated that egocentric and allocentric navigation is impaired in early clinical AD [11, 27] and can be influenced by genetic risk factors [33], especially by the *APOE* ε4 allele [28, 29]. Next, the *BDNF* Met allele is associated with structural changes in the navigation-related brain regions (i.e., in the medial temporal lobe structures and parietal cortex [48–50]) and the recent study has indicated that the *BDNF* Val66Met polymorphism may influence spontaneous navigational strategies in younger adults [53]. Building on this research, we assessed the combined effects of the *APOE* and *BDNF* Val66Met polymorphisms on spatial navigation performance and atrophy of brain regions associated with spatial navigation in older adults.

First, we evaluated the effect of combination of the *APOE* and *BDNF* Val66Met polymorphisms on two spatial navigation strategies, egocentric and allocentric, in a real-space version of the human analogue of the Morris Water Maze task (hMWM) in cognitively unimpaired (CU) and aMCI individuals. The effect of combination of the polymorphisms on other cognitive functions was also assessed. Second, we assessed the influence of combination of the *APOE* and *BDNF* Val66Met polymorphisms on volumes of the selected brain regions associated with spatial navigation (the

hippocampus, entorhinal cortex, precuneus, inferior parietal cortex, and posterior cingulate cortex) in CU and aMCI individuals. The association between each spatial navigation strategy and volumes of the relevant brain regions was also tested.

We hypothesized that CU and aMCI participants with the high risk combination of the *APOE* ε4 and *BDNF* Met alleles would exhibit the least accurate spatial navigation performance in the allocentric and both egocentric and allocentric navigation strategies, respectively. The high risk combination of both alleles would also be associated with the worst episodic memory performance, especially in the participants with aMCI. Further, we hypothesized that the high risk participants, especially those with aMCI, would have the most pronounced atrophy of the brain regions associated with spatial navigation and that less accurate spatial navigation performance would be associated with lower volumes of these brain regions, especially in the participants with aMCI.

METHODS

Participants

A total of 187 participants were recruited from the Czech Brain Aging Study (http://cbas.cz/) cohort at the Memory Clinic of the Charles University, Second Faculty of Medicine and Motol University Hospital in Prague, Czech Republic and signed an informed consent approved by the local ethics committee [59]. The participants were referred to the Memory Clinic by general practitioners, psychiatrists, and neurologists for memory complaints reported by themselves and/or by their informants. All participants underwent clinical and laboratory evaluations, *APOE* and *BDNF* Val66Met genotyping, comprehensive neuropsychological assessment, brain magnetic resonance imaging (MRI), and spatial navigation assessment in a real-space version of the hMWM. Data from various modalities were collected within 60 days of each other for every participant.

The participants with aMCI ($n = 116$) met the clinical criteria for aMCI outlined in recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for AD [60] including memory complaints, evidence of memory impairment (i.e., score lower than 1.5 standard deviations below the age- and education-adjusted norms in any memory test), generally intact activities of daily living (i.e., Clinical Dementia Rating [CDR] global score not greater than 0.5) and

absence of dementia. The aMCI group included participants with isolated memory impairment (single domain aMCI [aMCIsd]; $n = 31$) and those with memory impairment and additional impairment in any other non-memory cognitive domain (multiple domain aMCI [aMCImd]; $n = 85$). The CU participants ($n = 71$) had cognitive performance within the normal range (i.e., score higher than 1.5 standard deviations below the age- and education-adjusted norms in any cognitive test). Participants with depressive symptoms (≥ 6 points on the 15-item Geriatric Depression Scale [GDS-15]) [61], moderate to severe white matter vascular lesions on MRI (Fazekas score > 2 points) [62] or other primary neurological or psychiatric disorders were not included in the study. The CU and aMCI participants were further stratified into 4 groups each based on the *APOE* and *BDNF* Val66Met polymorphisms: *APOE* $\varepsilon 4$ and *BDNF* Met noncarriers ($\varepsilon 4^-/BDNF^{Val/Val}$; aMCI [$n = 29$], CU [$n = 27$]), *APOE* $\varepsilon 4$ noncarriers and *BDNF* Met carriers ($\varepsilon 4^-/BDNF^{Met}$; aMCI [$n = 11$], CU [$n = 15$]), *APOE* $\varepsilon 4$ carriers and *BDNF* Met noncarriers ($\varepsilon 4^+/BDNF^{Val/Val}$; aMCI [$n = 52$], CU [$n = 18$]), and *APOE* $\varepsilon 4$ and *BDNF* Met carriers ($\varepsilon 4^+/BDNF^{Met}$; aMCI [$n = 24$], CU [$n = 11$]). We did not include the *APOE* $\varepsilon 2$ carriers ($\varepsilon 2/\varepsilon 2$, $\varepsilon 2/\varepsilon 3$ and $\varepsilon 2/\varepsilon 4$), as the $\varepsilon 2$ allele is considered protective and its frequency among our participants was low. Group-wise characteristic are listed in Tables 1 and 2.

Neuropsychological assessment

The neuropsychological battery comprised the following tests for each cognitive domain: 1) verbal memory measured with the Logical Memory I – Immediate and 20-minute Delayed Recall conditions and the Rey Auditory Verbal Learning Test – trials 1–5 and 30-minute Delayed Recall trial; 2) nonverbal memory measured with the Rey-Osterrieth Complex Figure Test (ROCFT) – the Recall condition after 3 minutes; 3) executive function measured with the Trail Making Test B, Prague Stroop Test – colors and Controlled Oral Word Association Test – Czech version with letters N, K, and P; 4) attention and working memory measured with the Forward and Backward Digit Spans and Trail Making Test A; 5) language measured with the Boston Naming Test – 60-item version and Category Fluency Test – animals and vegetables; and 6) visuospatial function measured with the ROCFT – the Copy condition and the Clock Drawing Test. The Mini-Mental State Examination (MMSE) was administered to measure global cognitive function. The GDS-15 was used to assess depressive symptoms among participants. Group-wise neuropsychological characteristics are listed in Tables 1 and 2.

APOE and BDNF Val66Met genotyping

To determine the *APOE* genotype, DNA was isolated from blood samples (ethylenediaminetetraacetic acid; Qiagen extraction) and genotyping was performed according to Idaho-tech protocol (Luna-Probes Genotyping Apolipoprotein [ApoE] Multiplexed Assay) for high-resolution melting (HRM) analysis [28, 63].

The *BDNF* rs6265 (Val66Met) polymorphism was analyzed with a novel HRM method using polymerase chain reaction (PCR). The PCR product of 59bp was amplified with primers. Subsequent HRM analysis of PCR product was performed on Light Scanner (IdahoTech, USA) [58].

MRI acquisition and analysis

Brain scans were performed on a 1.5 T scanner (Siemens AG, Erlangen, Germany) using the T1-weighted 3-dimensional high resolution magnetization-prepared rapid gradient-echo (MP-RAGE) sequence with the following parameters: TR/TE/TI = 2000/3.08/1100 ms, flip angle 15°, 192 continuous partitions, slice thickness = 1.0 mm, and in-plane resolution = 1 mm. Scans were visually inspected by a neuroradiologist to ensure appropriate data quality and to exclude patients with a major brain pathology that could interfere with cognitive functioning such as cortical infarctions, tumor, subdural hematoma, and hydrocephalus.

FreeSurfer image analysis suite (version 5.3; http://surfer.nmr.mgh.harvard.edu) was used to compute regional brain volumes and estimated total intracranial volume (eTIV), the internal reference. The procedure was described in detail elsewhere [64–66]. We selected the regions that play a key role in spatial navigation [1, 4, 67] and are affected early in AD [68, 69] to limit the number of analyses to those that were aligned with our hypothesis. The selected regions included the hippocampus, entorhinal cortex, precuneus, inferior parietal cortex, and posterior cingulate cortex. Volumes were reported separately for the left and right hemisphere, since spatial navigation is thought to be lateralized to the right hemisphere

Table 1
Characteristics of study participants with amnestic mild cognitive impairment

Variables	$\varepsilon4^-/BDNF^{Val/Val}$ (n = 29)	$\varepsilon4^-/BDNF^{Met}$ (n = 11)	$\varepsilon4^+/BDNF^{Val/Val}$ (n = 52)	$\varepsilon4^+/BDNF^{Met}$ (n = 24)	p	Effect size
Demographic characteristics						
Women/Men	10/19	5/6	24/28	16/8	0.13	0.22[d]
aMCIsd/aMCImd	5/24	4/7	16/36	6/18	0.51	0.14[d]
Age in years; range	72.14 (5.23); 59–85	71.91 (8.87); 51–86	70.10 (6.97); 52–84	72.67 (6.29); 62–85	0.356	0.03[e]
Education in years	15.76 (3.80)	14.18 (2.96)	15.02 (3.09)	13.54 (3.36)	0.098	0.05[e]
MMSE score	26.90 (2.30)	27.91 (1.22)	27.12 (2.24)	26.08 (2.04)	0.099	0.05[e]
GDS-15 score	2.52 (1.95)	2.80 (1.55)	2.60 (1.94)	2.38 (1.41)	0.928	0.00[e]
Cognitive characteristics						
Memory verbal[a]	0.20 (0.84)*	0.31 (0.55)	−0.01 (0.79)	−0.35 (0.52)	0.026	0.08[e]
Memory nonverbal[a]	0.21 (1.11)	0.04 (0.90)	−0.03 (1.01)	−0.21 (0.89)	0.472	0.01[e]
Executive function[a]	−0.22 (1.03)	0.07 (0.51)	0.04 (0.70)	0.16 (0.61)	0.300	0.05[e]
Attention and working memory[a]	−0.19 (0.80)	0.20 (0.49)	−0.01 (0.75)	0.15 (0.70)	0.268	0.00[e]
Language[a]	−0.12 (1.03)	−0.12 (0.43)	0.06 (0.77)	0.07 (0.62)	0.674	0.02[e]
Visuospatial[a]	−0.23 (1.06)	0.25 (0.58)	0.06 (0.69)	0.03 (0.62)	0.270	0.04[e]
Navigational characteristics						
Egocentric navigation	28.45 (17.41)*	24.65 (13.50)*	34.49 (26.54)*	56.63 (40.04)	0.037	0.13[f]
Allocentric navigation	65.69 (27.43)	69.44 (34.56)	73.98 (36.65)	80.49 (40.51)	0.918	0.02[f]
Delayed navigation	76.08 (42.97)	71.41 (43.66)	77.65 (52.14)	100.73 (60.35)	0.692	0.04[f]
MRI characteristics[b]						
Left hippocampal volume[c] (mm^3)	3504.42 (474.37)*	3755.13 (429.41)**	3224.87 (647.65)	2877.19 (421.63)	<0.001	0.24[g]
Right hippocampal volume[c] (mm^3)	3419.31 (762.38)	3915.11 (592.66)*	3318.58 (635.68)	3151.07 (595.28)	0.020	0.14[g]
Left entorhinal volume[c] (mm^3)	1401.27 (232.61)**	1365.48 (383.70)*	1044.38 (277.14)	1004.16 (158.99)	<0.001	0.29[g]
Right entorhinal volume[c] (mm^3)	1053.15 (194.65)	1333.24 (392.48)*	1042.99 (266.38)	1036.85 (139.04)	0.002	0.19[g]
Left inferior parietal volume[c] (mm^3)	9020.13 (1242.56)	10047.24 (1879.06)	9252.97 (1163.27)	8991.20 (873.12)	0.210	0.07[g]
Right inferior parietal volume[c] (mm^3)	11550.19 (1380.26)	11297.88 (2921.69)	11226.68 (1295.92)	10337.68 (1198.49)	0.333	0.05[g]
Left posterior cingulate volume[c] (mm^3)	2532.60 (498.63)	2399.50 (594.13)	2698.89 (348.26)	2500.84 (491.74)	0.167	0.07[g]
Right posterior cingulate volume[c] (mm^3)	2646.09 (307.10)	2460.46 (642.13)	2739.80 (289.77)	2639.67 (398.46)	0.226	0.06[g]
Left precuneus volume[c] (mm^3)	7067.74 (913.77)	7119.46 (1337.66)	7232.81 (750.66)	6852.11 (507.84)	0.684	0.02[g]
Right precuneus volume[c] (mm^3)	7352.30 (846.09)	7455.15 (1501.38)	7695.30 (863.16)	7351.45 (669.35)	0.679	0.02[g]

Values are mean (SD) except for gender and age range. For p indicating the level of significance compared with the $\varepsilon4^+/BDNF^{Met}$ group are *$p < 0.05$ and **$p < 0.01$. [a]Values are presented in z-scores (SD). [b]Based on a sample restricted to those with brain imaging data (n = 85; $\varepsilon4^-/BDNF^{Val/Val}$ [n = 23], $\varepsilon4^-/BDNF^{Met}$ [n = 11], $\varepsilon4^+/BDNF^{Val/Val}$ [n = 38] and $\varepsilon4^+/BDNF^{Met}$ [n = 13]). [c]Adjusted for estimated total intracranial volume. [d]Effect size reported using Cramér's V (the χ^2 test). [e]Effect size reported using partial eta-squared (one-way ANOVA). [f]Effect size reported using partial eta-squared (linear mixed effects regression analyses). [g]Effect size reported using partial eta-squared (MANCOVA controlled for age, gender and education). $\varepsilon4^-/BDNF^{Val/Val}$, APOE $\varepsilon4$ and BDNF Met noncarriers' group; $\varepsilon4^-/BDNF^{Met}$, APOE $\varepsilon4$ noncarriers and BDNF Met carriers' group; $\varepsilon4^+/BDNF^{Val/Val}$, APOE $\varepsilon4$ carriers and BDNF Met noncarriers' group; $\varepsilon4^+/BDNF^{Met}$, APOE $\varepsilon4$ and BDNF Met carriers' group; aMCIsd, single domain amnestic mild cognitive impairment; aMCImd, multiple domain amnestic mild cognitive impairment; MMSE, Mini-Mental State Examination; GDS-15; 15-item Geriatric Depression Scale; MRI, magnetic resonance imaging.

Table 2
Characteristics of cognitively unimpaired study participants

Variables	ε4⁻/BDNF^Val/Val (n = 27)	ε4⁻/BDNF^Met (n = 15)	ε4⁺/BDNF^Val/Val (n = 18)	ε4⁺/BDNF^Met (n = 11)	p	Effect size
Demographic characteristics						
Women/Men	16/11	13/2	9/9	10/1	0.035	0.35[d]
Age in years; range	68.44 (6.39); 54–86	64.87 (8.08); 52–82	65.61 (7.30); 52–82	66.55 (5.56); 55–74	0.364	0.05[e]
Education in years	16.93 (2.62)	17.07 (2.87)	16.17 (2.26)	15.00 (2.83)	0.162	0.07[e]
MMSE score	29.04 (1.09)	28.80 (1.37)	29.06 (0.97)	28.91 (1.38)	0.911	0.01[e]
GDS-15 score	2.67 (3.28)	3.27 (3.61)	2.53 (2.32)	2.10 (1.51)	0.782	0.02[e]
Cognitive characteristics						
Memory verbal[a]	0.08 (0.82)	0.01 (0.82)	−0.05 (0.80)	0.06 (0.83)	0.944	0.00[e]
Memory nonverbal[a]	−0.07 (1.03)	−0.02 (1.18)	0.19 (0.85)	0.05 (1.03)	0.865	0.01[e]
Executive function[a]	0.00 (0.78)	−0.11 (0.69)	0.06 (0.48)	0.22 (0.99)	0.693	0.02[e]
Attention and working memory[a]	0.23 (0.77)	0.08 (0.57)	−0.25 (0.79)	−0.22 (0.57)	0.122	0.08[e]
Language[a]	−0.10 (0.86)	−0.22 (0.85)	0.23 (0.58)	0.24 (0.88)	0.252	0.06[e]
Visuospatial[a]	−0.08 (0.92)	−0.15 (0.61)	0.31 (0.60)	−0.10 (0.83)	0.279	0.06[e]
Navigational characteristics						
Egocentric navigation	19.99 (6.57)	21.09 (6.33)	21.71 (7.40)	22.24 (10.10)	0.732	0.02[f]
Allocentric navigation	19.00 (3.88)*	23.82 (5.50)	27.38 (8.22)	35.28 (16.67)	0.017	0.22[f]
Delayed navigation	15.72 (6.04)*	21.25 (5.32)	21.77 (8.89)	25.99 (12.78)	0.028	0.19[f]
MRI characteristics[b]						
Left hippocampal volume[c] (mm³)	3733.92 (657.66)	3941.28 (504.83)	3906.37 (339.6)	3862.18 (273.81)	0.206	0.09[g]
Right hippocampal volume[c] (mm³)	3697.89 (646.54)	4124.32 (418.13)	3888.16 (517.43)	4006.76 (378.62)	0.126	0.11[g]
Left entorhinal volume[c] (mm³)	1217.14 (195.58)	1375.90 (230.86)	1306.00 (275.89)	1404.36 (303.92)	0.112	0.12[g]
Right entorhinal volume[c] (mm³)	1139.21 (242.38)	1159.44 (179.72)	1193.61 (263.86)	1207.06 (344.16)	0.395	0.06[g]
Left inferior parietal volume[c] (mm³)	10193.91 (957.03)	10002.32 (1616.12)	9789.21 (1183.13)	10134.89 (1096.83)	0.702	0.03[g]
Right inferior parietal volume[c] (mm³)	11903.11 (1197.12)	11780.80 (1386.19)	11734.89 (995.51)	12038.19 (1533.04)	0.758	0.02[g]
Left posterior cingulate volume[c] (mm³)	2755.31 (320.93)	2798.20 (382.01)	2767.77 (215.05)	2767.62 (420.88)	0.544	0.04[g]
Right posterior cingulate volume[c] (mm³)	2729.43 (329.39)	2789.67 (383.44)	2849.55 (407.77)	2786.89 (293.95)	0.170	0.10[g]
Left precuneus volume[c] (mm³)	7733.91 (595.22)	8350.33 (895.09)	7673.38 (793.67)	7870.21 (576.64)	0.245	0.08[g]
Right precuneus volume[c] (mm³)	8225.50 (682.58)	8563.94 (816.38)	8068.20 (786.82)	8344.45 (477.81)	0.733	0.03[g]

Values are mean (SD) except for gender and age range. For *p* indicating the level of significance compared with the ε4⁺/BDNF^Met group is *$p < 0.05$. [a]Values are presented in z-scores (SD). [b]Based on a sample restricted to those with brain imaging data (n = 85; ε4⁻/BDNF^Val/Val [n = 18], ε4⁻/BDNF^Met [n = 12], ε4⁺/BDNF^Val/Val [n = 18] and ε4⁺/BDNF^Met [n = 9]). [c]Adjusted for estimated total intracranial volume. [d]Effect size reported using Cramér's V (the χ² test). [e]Effect size reported using partial eta-squared (one-way ANOVA). [f]Effect size reported using partial eta-squared (linear mixed effects regression analyses). [g]Effect size reported using partial eta-squared (MANCOVA controlled for age, gender, and education). ε4⁻/BDNF^Val/Val, *APOE* ε4 and *BDNF* Met noncarriers' group; ε4⁻/BDNF^Met, *APOE* ε4 noncarriers and *BDNF* Met carriers' group; ε4⁺/BDNF^Val/Val, *APOE* ε4 carriers and *BDNF* Met noncarriers' group; ε4⁺/BDNF^Met, *APOE* ε4 and *BDNF* Met carriers' group; MMSE, Mini-Mental State Examination; GDS-15; 15-item Geriatric Depression Scale; MRI, magnetic resonance imaging.
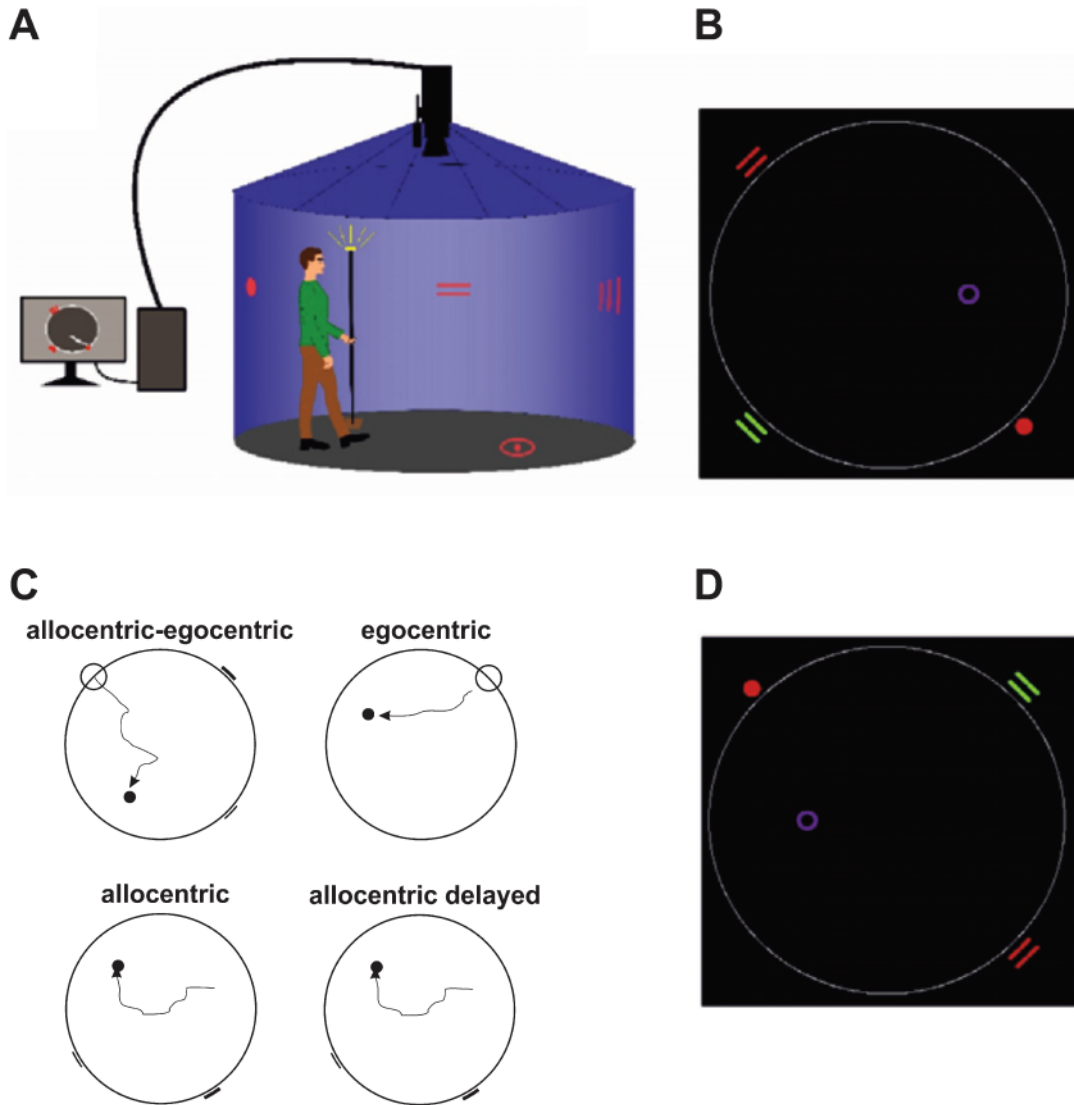
Fig. 1. Human analogue of the Morris Water Maze task. A) The real-space navigation setting. B) The scheme of the task showing an aerial view of the arena (large white circle) with starting point (red filled circle), orientation cues (red and green lines), and goal (purple circle). C) The scheme of 4 individual tasks: allocentric-egocentric (i.e., training; designed to use the starting point and 2 orientation cues for navigation), egocentric (designed to use the starting point for navigation), allocentric (designed to use 2 orientation cues for navigation) and allocentric delayed (identical to the allocentric task administered 30 minutes after its completion). D) An aerial view of the arena rotated 180 degrees from the previous trial shown in B.

[70]. Volumes were normalized to eTIV using the previously published regression formula [71]. MRI data were available for 85 participants with aMCI ($\varepsilon4^{-}/BDNF^{Val/Val}$ [$n = 23$], $\varepsilon4^{-}/BDNF^{Met}$ [$n = 11$], $\varepsilon4^{+}/BDNF^{Val/Val}$ [$n = 38$], and $\varepsilon4^{+}/BDNF^{Met}$ [$n = 13$]) and 57 CU participants ($\varepsilon4^{-}/BDNF^{Val/Val}$ [$n = 18$], $\varepsilon4^{-}/BDNF^{Met}$ [$n = 12$], $\varepsilon4^{+}/BDNF^{Val/Val}$ [$n = 18$], and $\varepsilon4^{+}/BDNF^{Met}$ [$n = 9$]). Group-wise MRI characteristics are listed in Tables 1 and 2.

Spatial navigation assessment

For spatial navigation assessment, we used the real-space version of the hMWM that was performed in a real-space navigational setting, a fully enclosed cylindrical arena 2.8 m in diameter surrounded by a 2.9 m high dark blue velvet curtain (Fig. 1A). The task and the real-space navigational setting were described in detail elsewhere [9, 10]. The participants

located an invisible goal on the arena floor in 4 different tasks using a start position (egocentric) or 2 distal orientation cues on the wall (allocentric), respectively (Fig. 1B, 1C). The first, allocentric–egocentric, task was a training task designed to familiarize participants with the testing procedure and involved locating the goal using both the start position and 2 distal orientation cues on the arena wall. The second, egocentric, task involved using the start position to locate the goal with no distal orientation cues displayed. The third, allocentric, task involved using 2 distal orientation cues at the arena wall for navigation to the goal from the start position that was unrelated to the goal position. The fourth, allocentric delayed, task was identical to the allocentric task and was administered 30 minutes after its completion. The training (egocentric-allocentric), egocentric, and allocentric tasks had 8 trials and the correct position of the goal was shown after each trial to provide the feedback. The delayed task had 2 trials and no feedback through showing the position of the goal was provided. The relative positions (distances and directions) of the goal to the start position or to both orientation cues were constant across all trials in all tasks. After each trial, the goal position along with the start position and the positions of 2 distal orientation cues were rotated in a pseudorandom sequence and the participants were instructed to go to the new start position at each consecutive trial in all tasks (Fig. 1D). The tasks had no time limit. Spatial navigation performance derived as distance error in centimeters from the correct goal position was automatically recorded by in-house developed software. A TV camera with a sampling frequency of 25 frames per second located above the center of the arena was used to capture the position of an infrared light-emitting diode placed on top of a hat that was worn by the participants during the testing and indicated their location.

Statistical analyses

Data standardization and analyses were performed separately for the CU and aMCI participants. Scores for neuropsychological and spatial navigation tests and MRI data were standardized to z-scores calculated from the overall mean and SD of the sample to enable comparison across the measurements scored on different scales. The values from the Prague Stroop Test, Trail Making Tests A and B (seconds to completion), and the Boston Naming Test (number of errors) were reversed before the z-scores were generated. The scores for each cognitive domain were expressed as a unit-weighted composite z-score from the relevant neuropsychological tests. Higher z-scores reflected better neuropsychological performance, but less accurate spatial navigation performance (distance error in centimeters). All data were found to be adequate for parametric analysis. Spatial navigation data were log transformed prior to the standardization and analyses because of their right-skewed distribution. The transformation satisfactorily solved the skewness.

A one-way analysis of variance (ANOVA) with *post hoc* Sidak's test evaluated between-group differences in age, years of education, GDS and MMSE scores, and cognitive domains' composite scores. The χ^2 test evaluated differences in gender proportions. Differences in proportions of aMCI subtypes (aMCIsd versus aMCImd) were evaluated in the participants with aMCI.

In the spatial navigation analyses, scores from all trials in each spatial navigation task were entered into linear mixed effects regression models [72] that were used to properly account for the repeated measures structure of the spatial navigation data. The distance error in each spatial navigation task was the outcome and the polymorphism group was the independent variable. As in the one-way ANOVA, our primary interest was in the main effect for group and differences in spatial navigation performance across the individual polymorphism groups ($\varepsilon 4^-/BDNF^{Val/Val}$ versus $\varepsilon 4^-/BDNF^{Met}$ versus $\varepsilon 4^+/BDNF^{Val/Val}$ versus $\varepsilon 4^+/BDNF^{Met}$) that were evaluated by *post hoc* Sidak's test. We also report the main effect for trial (trials 1–8 for the egocentric and allocentric tasks and trials 1–2 for the allocentric delayed task) and the group-by-trial interaction, which reflect learning and differential learning by group, respectively. The intercept and trial were specified as random effects. Based on model fit, the final models used the unstructured covariance matrix. Gender distribution was not equal across the groups, so gender was added as a covariate to the analyses. This decreased the model fit and the main effects for gender and group-by-gender interactions were not significant. Therefore, gender was not used in the final analyses.

Next, we estimated a multivariate analysis of covariance (MANCOVA) to assess the between-group differences in volumes of the brain regions relevant for spatial navigation. Age, gender, and education were controlled in the analysis. Initially, we performed multivariate tests and when the Wilks' Lambda indicating overall differences across the four

groups in volumes of the brain regions was significant, we subsequently performed univariate tests with *post hoc* Sidak's test, where the polymorphism group was the independent variable and volumes of the relevant brain regions were separately entered as the outcome.

Finally, we calculated Pearson correlation coefficients to explore relationships between spatial navigation performance in the egocentric, allocentric, and allocentric delayed tasks and volumes of the selected brain regions. A Bonferroni correction for multiple comparisons was used, resulting in a p value of 0.005 (0.05/10 regions). The subsequent multivariate linear regression analysis was used to evaluate the associations between spatial navigation performance in each task and selected regional brain volumes controlling for age, gender, education, and polymorphism group membership. Volumes of the navigation-related brain regions that correlated with spatial navigation performance were separately entered as the independent variables. Spatial navigation performance was the dependent variable and was calculated as the distance error averaged across the trials for each spatial navigation task.

Statistical significance was set at 2-tailed (alpha) of 0.05. Effect sizes are reported using Cramér's V for the χ^2 test and partial eta-squared (η_p^2) for one-way ANOVA, MANCOVA, and linear mixed effects regression analyses [73]. Cramér's V of about 0.47 and partial eta-squared of 0.12 correspond to Cohen's d of about 1.0. All analyses were conducted with IBM SPSS 25.0 software.

RESULTS

Demographic and neuropsychological characteristics

The participants with aMCI

There were no significant differences in age, gender, years of education, aMCI subtypes, depressive symptoms (assessed by GDS-15), and global cognitive function (assessed by MMSE) between the polymorphism groups. The main effect of group on verbal memory performance was significant (F[3,112] = 5.18, $p = 0.026$, $\eta_p^2 = 0.08$). The *post hoc* analysis revealed that the $\varepsilon 4^+/BDNF^{\mathrm{Met}}$ group had lower verbal memory performance than the $\varepsilon 4^-/BDNF^{\mathrm{Val/Val}}$ ($p = 0.007$) and similar performance to the $\varepsilon 4^-/BDNF^{\mathrm{Met}}$ ($p = 0.071$) and $\varepsilon 4^+/BDNF^{\mathrm{Val/Val}}$ ($p = 0.240$) groups. There were no

significant differences in other cognitive domains including attention and working memory, nonverbal memory, executive function, language and visuospatial function between the polymorphism groups. The results are presented in detail in Table 1.

The CU participants

There were no significant differences in age, years of education, depressive symptoms, and global cognitive function between the polymorphism groups but there were more women in the $\varepsilon 4^-/BDNF^{\mathrm{Met}}$ and $\varepsilon 4^+/BDNF^{\mathrm{Met}}$ groups than in the $\varepsilon 4^-/BDNF^{\mathrm{Val/Val}}$ and $\varepsilon 4^+/BDNF^{\mathrm{Val/Val}}$ groups (13/2 and 10/1 versus 16/11 and 9/9; $\chi^2[3] = 3.09$, $p = 0.035$, Cramér's V = 0.35). There were no significant differences in cognitive performance in any cognitive domain including attention and working memory, verbal memory, nonverbal memory, executive function, language and visuospatial function between the polymorphism groups. The results are presented in detail in Table 2.

Spatial navigation performance

The participants with aMCI

Using the linear mixed models we found main effects for group on egocentric navigation performance (F[3,110] = 2.93; $p = 0.037$; $\eta_p^2 = 0.13$), where the $\varepsilon 4^+/BDNF^{\mathrm{Met}}$ group had less accurate egocentric navigation performance than the $\varepsilon 4^-/BDNF^{\mathrm{Val/Val}}$ ($p = 0.028$), $\varepsilon 4^-/BDNF^{\mathrm{Met}}$ ($p = 0.013$) and $\varepsilon 4^+/BDNF^{\mathrm{Val/Val}}$ ($p = 0.045$) groups. The estimated pairwise differences were around 0.5 SD ($\varepsilon 4^+/BDNF^{\mathrm{Met}}$ versus $\varepsilon 4^-/BDNF^{\mathrm{Val/Val}}$ and $\varepsilon 4^+/BDNF^{\mathrm{Met}}$ versus $\varepsilon 4^+/BDNF^{\mathrm{Val/Val}}$) and approached 1 SD ($\varepsilon 4^+/BDNF^{\mathrm{Met}}$ versus $\varepsilon 4^-/BDNF^{\mathrm{Met}}$) for these comparisons. There were no significant differences in egocentric navigation performance between the $\varepsilon 4^-/BDNF^{\mathrm{Val/Val}}$, $\varepsilon 4^-/BDNF^{\mathrm{Met}}$ and $\varepsilon 4^+/BDNF^{\mathrm{Val/Val}}$ groups (ps ≥ 0.654). There were no significant differences in allocentric (F[3,107] = 0.17; $p = 0.918$; $\eta_p^2 = 0.02$) and allocentric delayed (F[3,105] = 0.49; $p = 0.692$; $\eta_p^2 = 0.04$) navigation performance between the polymorphism groups. The between-group comparisons of spatial navigation performance in each task are listed in Tables 1 and 3. The main effects for trial were not significant in the egocentric (F[1,110] = 0.24; $p = 0.625$; $\eta_p^2 = 0.00$), allocentric (F[1,106] = 0.61; $p = 0.438$; $\eta_p^2 = 0.03$), and allocentric delayed (F[1,105] = 0.14; $p = 0.707$; $\eta_p^2 = 0.00$) tasks, indicating no signifi-

Table 3
Group-wise comparisons of adjusted mean error distances from the goal in spatial navigation tasks in the participants with amnestic mild cognitive impairment

(I) Groupcode	(J) Groupcode	Mean difference (I-J)	p	95% Confidence interval for difference	
				Lower bound	Upper bound
Egocentric task					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	**0.485**	**0.045**	**0.006**	**0.963**
	$\varepsilon4^-$/BDNF^{Met}	**0.812**	**0.013**	**0.120**	**1.505**
	$\varepsilon4^-$/BDNF$^{Val/Val}$	**0.586**	**0.028**	**0.043**	**1.129**
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	0.328	0.654	−0.296	0.951
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.101	0.992	−0.351	0.553
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	−0.226	0.938	−0.901	0.448
Allocentric task					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	0.084	0.998	−0.415	0.582
	$\varepsilon4^-$/BDNF^{Met}	0.240	0.935	−0.470	0.951
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.161	0.970	−0.401	0.723
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	0.157	0.986	−0.480	0.794
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.078	0.998	−0.388	0.543
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	−0.079	1.000	−0.768	0.609
Delayed task					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	0.264	0.808	−0.335	0.864
	$\varepsilon4^-$/BDNF^{Met}	0.450	0.651	−0.405	1.306
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.246	0.917	−0.441	0.933
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	0.186	0.987	−0.581	0.953
	$\varepsilon4^-$/BDNF$^{Val/Val}$	−0.018	1.000	−0.591	0.555
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	−0.204	0.987	−1.041	0.632

Linear mixed models. Mean differences are in standard deviation units. Values in bold indicate significant between-group differences ($p < 0.05$). $\varepsilon4^+$/BDNF^{Met}, *APOE* $\varepsilon4$ and *BDNF* Met carriers' group; $\varepsilon4^+$/BDNF$^{Val/Val}$, *APOE* $\varepsilon4$ carriers and *BDNF* Met noncarriers' group; $\varepsilon4^-$/BDNF^{Met}, *APOE* $\varepsilon4$ noncarriers and *BDNF* Met carriers' group; $\varepsilon4^-$/BDNF$^{Val/Val}$, *APOE* $\varepsilon4$ and *BDNF* Met noncarriers' group.

cant learning effect across consecutive trials in the sample overall. Finally, there were no significant group-by-trial interactions, suggesting no significant differences in learning between the polymorphism groups in the egocentric (F[3,110] = 0.38; p = 0.767; η_p^2 = 0.05), allocentric (F[3,106] = 1.48; p = 0.225; η_p^2 = 0.02), and allocentric delayed (F[3,105] = 0.09; p = 0.964; η_p^2 = 0.00) tasks.

The CU participants

We found main effects for group on allocentric (F[3,127] = 3.83; p = 0.017; η_p^2 = 0.22) and allocentric delayed (F[3,69] = 3.35; p = 0.028; η_p^2 = 0.19) navigation performance where the $\varepsilon4^+$/*BDNF*^{Met} group had less accurate performance than the $\varepsilon4^-$/*BDNF*$^{Val/Val}$ group (p = 0.047 and p = 0.048, respectively). The estimated pairwise differences were around 0.5 and 0.7 SD, respectively, for these comparisons. There were no significant differences in egocentric navigation performance between the polymorphism groups (F[3,125] = 0.43; p = 0.732; η_p^2 = 0.02). The between-group comparisons of spatial navigation performance in each task are listed in Tables 2 and 4. The main effects for trial were not sig-

nificant in the egocentric (F[1,125] = 0.03; p = 0.999; η_p^2 = 0.00), allocentric (F[1,127] = 0.87; p = 0.534; η_p^2 = 0.02), and allocentric delayed (F[1,69] = 0.05; p = 0.826; η_p^2 = 0.00) tasks. There were no significant group-by-trial interactions in the egocentric (F[3,125] = 1.03; p = 0.423; η_p^2 = 0.05), allocentric (F[3,127] = 1.30; p = 0.173; η_p^2 = 0.09), and allocentric delayed (F[3,69] = 0.09; p = 0.968; η_p^2 = 0.01) tasks.

Brain MRI characteristics and their association with spatial navigation

The participants with aMCI

Using the MANCOVA adjusted for age, gender, and education, the multivariate tests showed the between-group differences in volumes of the navigation-related brain regions (Wilks' Lambda = 2.33; $p \leq 0.001$; η_p^2 = 0.29). In the subsequent univariate tests analyzing each brain region separately, the between-group differences were found for left and right hippocampal volumes (F[3,85] = 7.13; $p < 0.001$; η_p^2 = 0.24 and F[3,85] = 3.50; p = 0.020; η_p^2 = 0.14, respectively) and left and

Table 4
Group-wise comparisons of adjusted mean error distances from the goal in spatial navigation tasks in the cognitively unimpaired participants

(I) Groupcode	(J) Groupcode	Mean difference (I-J)	p	95% Confidence interval for difference	
				Lower bound	Upper bound
Egocentric task					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	−0.014	1.000	−0.477	0.449
	$\varepsilon4^-$/BDNF^{Met}	0.002	1.000	−0.478	0.482
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.120	0.975	−0.315	0.555
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	0.016	1.000	−0.407	0.439
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.134	0.911	−0.237	0.505
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.118	0.961	−0.274	0.510
Allocentric task					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	0.091	0.997	−0.412	0.594
	$\varepsilon4^-$/BDNF^{Met}	0.080	0.999	−0.451	0.611
	$\varepsilon4^-$/BDNF$^{Val/Val}$	**0.501**	**0.047**	**0.005**	**0.997**
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	−0.011	1.000	−0.482	0.460
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.410	0.070	−0.021	0.841
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.421	0.093	−0.043	0.884
Delayed task					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	0.223	0.949	−0.488	0.934
	$\varepsilon4^-$/BDNF^{Met}	0.108	0.999	−0.645	0.862
	$\varepsilon4^-$/BDNF$^{Val/Val}$	**0.705**	**0.048**	**0.004**	**1.406**
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	−0.115	0.998	−0.805	0.575
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.482	0.224	−0.150	1.114
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.596	0.113	−0.083	1.276

Linear mixed models. Mean differences are in standard deviation units. Values in bold indicate significant between-group differences ($p < 0.05$). $\varepsilon4^+$/BDNF^{Met}, *APOE* ε4 and *BDNF* Met carriers' group; $\varepsilon4^+$/*BDNF*$^{Val/Val}$, *APOE* ε4 carriers and *BDNF* Met noncarriers' group; $\varepsilon4^-$/*BDNF*^{Met}, *APOE* ε4 noncarriers and *BDNF* Met carriers' group; $\varepsilon4^-$/BDNF$^{Val/Val}$, *APOE* ε4 and *BDNF* Met noncarriers' group.

right entorhinal cortical volumes (F[3,85] = 9.29; $p < 0.001$; $\eta_p^2 = 0.29$ and F[3,85] = 5.34; $p = 0.002$; $\eta_p^2 = 0.19$, respectively). The between-group differences in volumes of other navigation-related brain regions were not significant (F[3,85] ≤ 1.74; $p \geq 0.167$; $\eta_p^2 \leq 0.07$). The *post hoc* tests showed that the $\varepsilon4^+$/*BDNF*^{Met} group had smaller left hippocampal and left entorhinal cortical volumes compared to the $\varepsilon4^-$/*BDNF*$^{Val/Val}$ ($p = 0.019$ and $p = 0.004$) and $\varepsilon4^-$/*BDNF*^{Met} ($p = 0.001$ and $p = 0.020$) groups and smaller right hippocampal and right entorhinal cortical volumes compared to the $\varepsilon4^-$/*BDNF*^{Met} group ($p = 0.038$ and $p = 0.030$). The $\varepsilon4^+$/BDN F$^{Val/Val}$ group had smaller left and right hippocampal and right entorhinal cortical volumes compared to the $\varepsilon4^-$/*BDNF*^{Met} group ($p = 0.006$, $p = 0.020$ and $p = 0.001$) and left entorhinal cortical volume compared to the $\varepsilon4^-$/*BDNF*$^{Val/Val}$ ($p < 0.001$) and $\varepsilon4^-$/*BDNF*^{Met} ($p = 0.007$) groups. The between-group comparisons of volumes of the navigation-related brain regions are listed in Tables 1 and 5.

In the correlational analyses (Supplementary Table 1), right hippocampal volume correlated with allocentric navigation performance ($r = -0.39$; $p = 0.001$) indicating that smaller volume was associated with greater distance error in the navigational task. Right hippocampal volume correlated with allocentric delayed ($r = -0.33$; $p = 0.006$) and egocentric ($r = -0.32$; $p = 0.006$) navigation performance, left hippocampal volume correlated with allocentric ($r = -0.28$; $p = 0.020$), allocentric delayed ($r = -0.30$; $p = 0.014$), and egocentric ($r = -0.28$; $p = 0.017$) navigation performance, right entorhinal cortical volume correlated with allocentric delayed navigation performance ($r = -0.27$; $p = 0.028$) and left posterior cingulate volume correlated with allocentric delayed navigation performance ($r = -0.27$; $p = 0.028$); however, these relationships did not surpass the threshold of Bonferroni-corrected p-value.

In the multivariate linear regression analyses, right hippocampal volume was associated with allocentric navigation performance ($\beta = -0.41$; $p = 0.001$) indicating that smaller volume was associated with greater distance error in the navigational task above and beyond demographic characteristics. Right hippocampal volume was associated with allocentric delayed ($\beta = -0.30$; $p = 0.014$) and

Table 5
Group-wise comparisons of hippocampal and cortical volumes in the participants with amnestic mild cognitive impairment

(I) Groupcode	(J) Groupcode	Mean difference (I-J)	p	95% Confidence interval for difference	
				Lower bound	Upper bound
Left hippocampal volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.376	0.655	−1.099	0.346
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.394**	**0.001**	**−2.345**	**−0.443**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	**−0.967**	**0.019**	**−1.823**	**−0.111**
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.018**	**0.006**	**−1.825**	**−0.211**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.590	0.123	−1.270	0.090
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.428	0.767	−0.500	1.355
Right hippocampal volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.093	1.000	−0.926	0.739
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.136**	**0.038**	**−2.232**	**−0.041**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.348	0.919	−1.334	0.638
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.043**	**0.020**	**−1.973**	**−0.113**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.255	0.944	−1.038	0.528
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.788	0.263	−0.280	1.856
Left entorhinal volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.055	1.000	−0.825	0.715
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.135**	**0.020**	**−2.148**	**−0.122**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	**−1.202**	**0.004**	**−2.114**	**−0.290**
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.079**	**0.007**	**−1.939**	**−0.219**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	**−1.147**	**0.000**	**−1.871**	**−0.422**
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.067	1.000	−1.055	0.921
Right entorhinal volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	0.166	0.989	−0.546	0.879
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.003**	**0.030**	**−1.940**	**−0.065**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.017	1.000	−0.827	0.861
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	**−1.169**	**0.001**	**−1.965**	**−0.373**
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.149	0.991	−0.820	0.521
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	**1.020**	**0.021**	**0.105**	**1.934**
Left inferior parietal volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.153	0.998	−1.041	0.735
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	−0.818	0.319	−1.987	0.351
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.014	1.000	−1.066	1.038
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	−0.665	0.368	−1.657	0.327
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.139	0.998	−0.697	0.974
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.804	0.311	−0.336	1.943
Right inferior parietal volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.459	0.655	−1.339	0.422
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	−0.600	0.661	−1.759	0.559
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.669	0.420	−1.713	0.374
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	−0.141	0.999	−1.125	0.843
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.211	0.983	−1.039	0.618
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.669	0.420	−0.374	1.713
Left posterior cingulate volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.499	0.619	−1.424	0.426
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	0.240	0.996	−0.977	1.458
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.096	1.000	−1.192	1.000
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	0.739	0.296	−0.295	1.772
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.403	0.764	−0.468	1.274
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.336	0.971	−1.523	0.851
Right posterior cingulate volume[a]					
$\varepsilon4^+$/BDNF$^{\text{Met}}$	$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	−0.293	0.950	−1.215	0.629
	$\varepsilon4^-$/BDNF$^{\text{Met}}$	0.487	0.861	−0.726	1.701
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.025	1.000	−1.117	1.068
$\varepsilon4^+$/BDNF$^{\text{Val/Val}}$	$\varepsilon4^-$/BDNF$^{\text{Met}}$	0.780	0.236	−0.250	1.811
	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	0.268	0.956	−0.600	1.136
$\varepsilon4^-$/BDNF$^{\text{Met}}$	$\varepsilon4^-$/BDNF$^{\text{Val/Val}}$	−0.512	0.815	−1.696	0.671

Table 5
Continued

(I) Groupcode	(J) Groupcode	Mean difference (I-J)	p	95% Confidence interval for difference	
				Lower bound	Upper bound
Left precuneus volume[a]					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	–0.400	0.809	–1.316	0.517
	$\varepsilon4^-$/BDNF^{Met}	–0.310	0.982	–1.517	0.896
	$\varepsilon4^-$/BDNF$^{Val/Val}$	–0.211	0.996	–1.297	0.875
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	0.089	1.000	–0.935	1.113
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.189	0.992	–0.674	1.052
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.100	1.000	–1.077	1.276
Right precuneus volume[a]					
$\varepsilon4^+$/BDNF^{Met}	$\varepsilon4^+$/BDNF$^{Val/Val}$	–0.296	0.941	–1.199	0.606
	$\varepsilon4^-$/BDNF^{Met}	–0.120	1.000	–1.308	1.068
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.031	1.000	–1.038	1.101
$\varepsilon4^+$/BDNF$^{Val/Val}$	$\varepsilon4^-$/BDNF^{Met}	0.176	0.998	–0.832	1.185
	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.328	0.882	–0.522	1.177
$\varepsilon4^-$/BDNF^{Met}	$\varepsilon4^-$/BDNF$^{Val/Val}$	0.151	1.000	–1.007	1.310

Analysis of covariance adjusted for age, gender and education. Mean differences are in standard deviation units. Values in bold indicate significant between-group differences ($p < 0.05$). [a]Adjusted for estimated total intracranial volume. $\varepsilon4^+$/BDNF^{Met}, *APOE* $\varepsilon4$ and *BDNF* Met carriers' group; $\varepsilon4^+$/BDNF$^{Val/Val}$, *APOE* $\varepsilon4$ carriers and *BDNF* Met noncarriers' group; $\varepsilon4^-$/BDNF^{Met}, *APOE* $\varepsilon4$ noncarriers and *BDNF* Met carriers' group; $\varepsilon4^-$/BDNF$^{Val/Val}$, *APOE* $\varepsilon4$ and *BDNF* Met noncarriers' group.

egocentric ($\beta = -0.27$; $p = 0.022$) navigation performance, left hippocampal volume was associated with allocentric ($\beta = -0.37$; $p = 0.007$), allocentric delayed ($\beta = -0.32$; $p = 0.020$), and egocentric ($\beta = -0.26$; $p = 0.048$) navigation performance and left posterior cingulate volume was associated with allocentric delayed navigation performance ($\beta = -0.23$; $p = 0.042$); however, these associations did not surpass the threshold of Bonferroni-corrected p-value.

The CU participants

The multivariate tests did not show significant differences between the polymorphism groups in volumes of the navigation-related brain regions (Wilks' Lambda = 0.96; $p = 0.532$; $\eta_p^2 = 0.20$). There was no significant correlation between spatial navigation performance in the egocentric, allocentric, and allocentric delayed tasks and volumes of the selected brain regions. The results are presented in detail in Table 2 and Supplementary Tables 2 and 3.

DISCUSSION

In this study, we examined for the first time the combined effect of *APOE* and *BDNF* Val66Met polymorphisms on spatial navigation and volume of brain regions associated with spatial navigation in CU and aMCI individuals. Our findings indicate that the combination of the *APOE* $\varepsilon4$ and *BDNF* Met alleles is associated with least accurate spatial navigation performance in individuals with aMCI. Specifically, we found that those with aMCI who carry both *APOE* $\varepsilon4$ and *BDNF* Met alleles, although similar to other polymorphism groups in demographic characteristics, aMCI subtypes, global cognitive function, or depressive symptoms, have the least accurate egocentric spatial navigation performance. Our findings further indicate that the combination of the *APOE* $\varepsilon4$ and *BDNF* Met alleles may influence spatial navigation in CU individuals. Specifically, we found that those CU individuals with the high risk combination of the *APOE* $\varepsilon4$ and *BDNF* Met alleles compared to those with the low risk combination of the *APOE* $\varepsilon3$ and *BDNF* Val/Val alleles have less accurate allocentric spatial navigation performance. Egocentric (self-centered) navigation, where spatial information about locations and objects is encoded from the viewpoint of the navigator, is associated with the level of function of the posterior parietal cortex including the precuneus [2]. The function of the parietal cortex is adversely affected by the *APOE* $\varepsilon4$ allele [74, 75], which is associated with less accurate egocentric navigation in individuals with aMCI [27–29]. In addition, levels of BDNF protein in this region are prominently decreased [45, 76] due to the presence of

the *BDNF* Met allele [52]. This may explain why the combination of the *APOE* ε4 and *BDNF* Met alleles was negatively associated with egocentric navigation in our participants with aMCI. Allocentric (world-centered) navigation, where locations and objects are encoded in relation to one another and are independent of the position of the navigator, is associated with the level of function of the medial temporal lobe structures, especially of the hippocampus [1]. BDNF expression is profoundly reduced in the hippocampus and adjacent entorhinal cortex [44] that are affected by *APOE* ε4-related changes [20, 21]. Next, the previous studies showed that the *APOE* ε4 allele is associated with less accurate allocentric navigation performance in cognitively normal older adults [26] and that the *BDNF* Met allele is associated with decreased use of allocentric spatial strategy in cognitively normal younger adults [53]. This may explain why the combination of the *APOE* ε4 and *BDNF* Met alleles was negatively associated with allocentric navigation in our CU participants. One would expect that the combination of the *APOE* ε4 and *BDNF* Met alleles could also negatively influence allocentric navigation in individuals with aMCI. However, in the current study we did not find the differences in allocentric spatial navigation performance between the polymorphism groups of participants with aMCI. This finding may be explained by the severity of cognitive impairment in our participants who were in the late stage of aMCI (with a mean MMSE score of 26.9 and predominant multiple domain cognitive impairment) and whose results could thus be affected by the floor effect. Similar findings were reported in our previous study, where the results for allocentric unlike egocentric navigation as a function of *APOE* ε4 categorization have not been significant in aMCI participants with a mean MMSE score of 26.4 [29]. Allocentric navigation is impaired in the course of aging [77, 78] and especially in preclinical AD and early aMCI [9, 79]. Impairment of egocentric navigation becomes increasingly more prominent later in the course of aMCI and in AD dementia [9, 10] and unlike impairment of allocentric navigation more accurately discriminates AD dementia from other types of dementia [80]. Therefore, aMCI participants with both *APOE* ε4 and *BDNF* Met alleles who have more pronounced egocentric navigation impairment may represent the more advanced stage of the disease compared to other polymorphism groups and may be more likely to have steeper cognitive decline and progress to AD dementia. The CU participants with both *APOE* ε4 and *BDNF* Met alleles who have

less accurate allocentric navigation performance may also be more prone to cognitive decline and progression to MCI. Since individuals with aMCI who are carriers of the *APOE* ε4 allele are more likely to have Aβ pathology (i.e., prodromal AD) [81], it is plausible that the negative effect of the *BDNF* Met allele on spatial navigation has been influenced by abnormally high levels of Aβ in our aMCI cohort. Indeed, the *BDNF* Met allele enhances the brain vulnerability to Aβ toxicity [82] and therefore it may further worsen Aβ-related egocentric navigation impairment in individuals with aMCI and *APOE* ε4 allele [27, 28]. In the current study, there was no evidence of a learning effect across the trials in individual spatial navigation tasks in the participants with aMCI, which is in line with previous studies showing impairment of spatial navigation learning in individuals with aMCI and early AD [9, 28, 29].

As expected, the combination of the *APOE* ε4 and *BDNF* Met alleles was associated with more pronounced memory dysfunction in participants with aMCI. Specifically, the carriers of both *APOE* ε4 and *BDNF* Met alleles had lower verbal memory performance compared to the non-carriers. These results are in agreement with previous findings in individuals with preclinical AD [57], aMCI [58], prodromal AD and AD dementia [25] and support the hypothesis that combination of the *APOE* ε4 and *BDNF* Met alleles may specifically interfere with memory function. Again, as suggested previously [55, 57, 83], the negative effect of the *BDNF* Met allele on memory in our aMCI cohort may be influenced by abnormally high levels of Aβ that are more frequent in *APOE* ε4 carriers [81]. However, neither the current nor the previous studies [25, 58] have found the differences in memory performance between the *BDNF* Met carriers and non-carriers within a group of individuals with aMCI and the *APOE* ε4 allele, that is, within the group at increased risk of progressing to AD dementia [84]. The noteworthy finding in the current study was that the *APOE* ε4/*BDNF* Met carriers with aMCI compared to all other polymorphism groups including those with *APOE* ε4/*BDNF* Val/Val alleles have less accurate egocentric spatial navigation performance. This result may indicate that spatial navigation testing could more reliably reflect the deleterious effect of the *BDNF* Met allele on cognition than commonly used episodic memory tests. Given that spatial navigation is distinguishable from other cognitive functions [16] strongly influenced by genetic polymorphisms [29, 33] and impaired very early in AD [5, 6], its assessment along with exam-

ination of other cognitive functions could be highly beneficial when characterizing the cognitive profile of individuals with aMCI and their risk of progression to AD dementia.

The combination of the *APOE ε4* and *BDNF* Met alleles was also associated with smaller volumes of the navigation-related brain regions in the participants with aMCI. Specifically, the carriers of both *APOE ε4* and *BDNF* Met alleles had lower volumes of the hippocampus and the entorhinal cortex, that is, of the brain regions affected very early in AD and known to be important for allocentric navigation [1, 4]. The results are consistent with previous research demonstrating more pronounced hippocampal and entorhinal cortical atrophy in *APOE ε4* [21, 85] and *BDNF* Met [48, 50] carriers and findings of the previous study showing lower right hippocampal volume in individuals with aMCI and combination of the *APOE ε4* and *BDNF* Met alleles [58]. These *APOE* and *BDNF* Val66Met polymorphism-related structural changes in participants with aMCI could be explained by increased vulnerability of *APOE ε4* carriers to AD-related pathological changes in this region [20] and by the fact that levels of BDNF protein that are decreased in *BDNF* Met carriers [47] are prominently reduced in the medial temporal lobe of individuals with AD [44]. This may negatively influence BDNF-induced neurogenesis [86] and dendritic arborization [41] and consequently lead to volumetric changes in this region. In our study, the *APOE ε4/BDNF* Met carriers with aMCI had lower volumes of the hippocampus and the entorhinal cortex compared to the *APOE ε4* non-carriers. However, lower volumes of these regions were also found in the *APOE ε4/BDNF* Val/Val carriers. Therefore, it seems that structural differences in the medial temporal lobe between our aMCI polymorphism groups may be mainly driven by the presence of the *APOE ε4* allele [21, 29]. Another noteworthy result was that right hippocampal volume was associated with allocentric navigation performance in participants with aMCI. This is in line with our previous findings in individuals with aMCI and AD dementia [16, 29, 70, 87] and underlines the important role of the right hippocampus in allocentric navigation. The combination of the *APOE ε4* and *BDNF* Met alleles in individuals with aMCI was not associated with volume reduction of the inferior parietal cortex, precuneus, and posterior cingulate cortex, that is, of the brain regions known to be important for egocentric navigation [1, 2]. These brain regions have been associated with lower BDNF expression in AD dementia [45] and

hypometabolism on FDG-PET in *APOE ε4* carriers [22, 88] but their structural changes related to the *APOE* and *BDNF* Val66Met polymorphisms have not been reported. This may be explained by the fact that AD-related pathological changes associated with tissue loss occur later in the parietal cortex than in the medial temporal lobe during the disease [89] and that functional changes in these areas associated with cognitive impairment precede structural abnormalities [90]. Therefore, more pronounced egocentric navigation impairment in individuals with aMCI and combination of the *APOE ε4* and *BDNF* Met alleles may not be reflected in volumetric changes of these navigation-related brain regions.

One of the strengths of the current study is the fact that this is the first study to date to focus on the influence of combination of the *APOE* and *BDNF* Val66Met polymorphisms on spatial navigation in a well-defined cohort of CU and aMCI participants. In addition, we used the real-space version of the hMWM, a well-established method mimicking navigation in the real world, to examine spatial navigation, a specific and neglected cognitive function, whose impairment is observed and frequently reported by patients in the early stages of AD [5, 91]. The real-space version allows the use of vestibular and proprioceptive information that is missing in virtual reality and therefore may better reflect real-world navigation [15]. Finally, we assessed the influence of these polymorphisms on volumes of specific brain regions relevant for spatial navigation and being affected very early in the course of AD [13, 14].

This study also has some limitations. First, the number of participants was relatively small, which may increase the chances of bias towards the Type II error. Next, our hypotheses required many statistical comparisons, which may increase the chances of bias towards the Type I error, although the *post hoc* tests and corrections for multiple comparisons were used in all analyzes. Because of these limitations our results should be interpreted with caution. Further studies with larger study cohorts are required to validate our findings. In addition, specific AD biomarkers ($A\beta_{1-42}$ and p-tau$_{181}$ in cerebrospinal fluid and amyloid PET imaging) were assessed only in a subset of the participants and therefore we could not evaluate the presence of AD pathology in all CU and aMCI participants. Because of the relatively small number of participants in some groups we could not evaluate the differences between the polymorphism groups in associations between spatial navigation performance and volumes of the brain regions, which should be a

focus of future studies. Spatial navigation tasks were always performed in the same order and therefore we were not able to control for changes in participants' performance across the tasks. Future studies randomly varying order of egocentric and allocentric tasks between participants may be needed to control for the practice effect. Finally, the cross-sectional design did not allow evaluating the combined effect of *APOE* and *BDNF* Val66Met polymorphisms on spatial navigation changes over time but longitudinal follow-up is ongoing.

Conclusion

In conclusion, the current study demonstrated that the high risk combination of the *APOE ε4* and *BDNF* Met alleles is associated with more pronounced egocentric spatial navigation impairment and smaller volumes of the medial temporal lobe structures in individuals with aMCI and allocentric spatial navigation deficits in CU individuals. This finding may indicate that aMCI individuals with both *APOE ε4* and *BDNF* Met alleles may have more advanced AD pathology and higher risk of progression to AD dementia and CU individuals may be at greater risk of cognitive decline and progression to MCI. Further, our findings, in line with previous research [56–58], suggest that the combination of the *APOE ε4* and *BDNF* Met alleles may interfere with memory function in individuals with aMCI. However, memory testing, unlike spatial navigation testing, did not distinguish *BDNF* Met carriers from non-carriers among the individuals with the *APOE ε4* allele. These findings may indicate that spatial navigation testing could more reliably assess the deleterious effect of the *BDNF* Met allele on cognition than traditionally used episodic memory tests. The focus of future studies should be to evaluate the effect of combination of *APOE* and *BDNF* Val66Met polymorphisms on longitudinal spatial navigation changes in individuals with aMCI and those in the earlier stage of the disease, such as individuals with subjective cognitive decline.

ACKNOWLEDGMENTS

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-0615r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-200615.

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