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Polymorphism of brain derived neurotrophic factor influences β amyloid load in cognitively intact apolipoprotein E ϵ 4 carriers



Katarzyna Adamczuk^{a,b}, An-Sofie De Weer^{a,b}, Natalie Nelissen^{a,b}, Kewei Chen^{c,d,e}, Kristel Slegers^{g,h}, Karolien Bettens^{g,h}, Christine Van Broeckhoven^{g,h}, Mathieu Vandenbulckeⁱ, Pradeep Thiyyagura^{c,d}, Patrick Dupont^{a,b}, Koen Van Laere^j, Eric M. Reiman^{c,d,f}, Rik Vandenberghe^{a,b,k,*}

^a Laboratory for Cognitive Neurology, University of Leuven, Belgium

^b Alzheimer Research Centre KU Leuven, Leuven Institute for Neuroscience and Disease, Belgium

^c Banner Alzheimer's Institute, Phoenix, AZ, USA

^d The Arizona Alzheimer's Consortium, Phoenix, AZ, USA

^e Department of Radiology, University of Arizona, Tucson, AZ, USA

^f Department of Psychiatry, University of Arizona, Tucson, AZ, USA

^g Department of Molecular Genetics, Neurodegenerative Brain Diseases Group, VIB, Antwerp, Belgium

^h Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Belgium

ⁱ Psychiatry Department, University Hospitals Leuven, Belgium

^j Nuclear Medicine Department, University Hospitals Leuven, Belgium

^k Neurology Department, University Hospitals Leuven, Belgium

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ABSTRACT

Aside from apolipoprotein E (APOE), genetic risk factors for β amyloid deposition in cognitively intact individuals remain to be identified. Brain derived neurotrophic factor (BDNF) modulates neural plasticity, which has been implicated in Alzheimer's disease. We examined in cognitively normal older adults whether the BDNF codon 66 polymorphism affects β amyloid burden and the relationship between β amyloid burden and cognitive scores, and how this relates to the effect of APOE. Amyloid load was measured by means of ¹⁸F-flutemetamol PET in 64 community-recruited cognitively intact individuals (mean age 66, S.D. 5.1). Recruitment was stratified according to a factorial design with APOE (ϵ 4 allele present vs absent) and BDNF (*met* allele at codon 66 present vs absent) as factors. Individuals in the four resulting cells were matched by the number of cases, age, and gender. Among the APOE ϵ 4 carriers, BDNF *met* positive subjects had a significantly higher amyloid load than BDNF *met* negative subjects, while BDNF *met* carrier status did not have an effect in APOE ϵ 4 noncarriers. This interaction effect was localized to precuneus, orbitofrontal cortex, gyrus rectus, and lateral prefrontal cortex. In the APOE ϵ 4/BDNF *met* carriers, a significant inverse relationship existed between episodic memory scores and amyloid burden but not in any of the other groups. This hypothesis-generating experiment highlights a potential role of BDNF polymorphisms in the preclinical phase of β amyloid deposition and also suggests that BDNF codon 66 polymorphisms may influence resilience against β amyloid-related effects on cognition.

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1. Introduction

In a variable proportion of cognitively intact older subjects, in vivo amyloid imaging has revealed increased cerebral A β deposition, sometimes to a degree identical to that seen in patients with clinically probable Alzheimer's disease (AD) (Aizenstein et al., 2008; Mintun et al., 2006; Morris et al., 2010; Nelissen et al., 2007; Pike et al., 2007; Rowe et al., 2007, 2010). This finding has attracted a lot of interest as it may be a possible marker for preclinical Alzheimer's disease (Sperling et al., 2011). The only risk factor proven to be associated with increased amyloid load in a cognitively intact population is the apolipoprotein E (APOE) ϵ 4 allele (Fleisher et al., 2013; Morris et al., 2010; Reiman et al., 2009; Vemuri et al., 2010; Villemagne et al., 2011),

Abbreviations: BDNF, brain-derived neurotrophic factor; APOE, apolipoprotein E; SUVR, standardized uptake value ratio; SUVR_{comp}, SUVR in composite cortical volume of interest; *val*, valine; *met*, methionine; MRI, magnetic resonance imaging; PET, positron emission tomography; VOI, volume-of-interest; AD, Alzheimer's disease; PVC, partial volume correction.

* Corresponding author at: Neurology Department, University Hospitals Leuven, Herestraat 49 bus 7003, B-3000 Leuven, Belgium. Tel.: +32 16344280; fax: +32 16344285.

E-mail address: rik.vandenberghe@uz.kuleuven.ac.be (R. Vandenberghe).

which is also a strong risk factor for Alzheimer's disease (Corder et al., 1993; Rebeck et al., 1993). Beyond what can be explained by APOE alone, a family history of AD is associated with increased brain amyloid levels in cognitively intact older adults (Xiong et al., 2011). According to a recent study, polymorphisms of complement component (3b/4b) receptor-1 (*CR1*) modulate the effect of APOE $\epsilon 4$ on brain amyloid levels (Thambisetty et al., 2012). These findings suggest a complex interaction between genetic variants and brain amyloid deposition in preclinical AD (Sperling et al., 2011).

A failure of neural plasticity has been put forward as a unifying theme spanning across the multiple pathways that lead to clinical AD (Arendt, 2001; Ashford and Jarvik, 1985; Mesulam, 1999, 2000; Teter, 2004). Brain derived neurotrophic factor (BDNF), a neurotrophin with high affinity for tyrosine kinase B receptors (TrkB), has been implicated in neural plasticity (Gorski et al., 2003; Webster et al., 2006) as well as in memory, both in humans (Erickson et al., 2011) and in animal models (Li et al., 2008; Okuno et al., 1999; Osada et al., 2008). In humans, a common single nucleotide polymorphism (SNP) in the 5' prodomain of the BDNF gene which results in valine to methionine substitution at codon 66 (*val66met*), affects memory function (Chen et al., 2004; Dennis et al., 2011; Egan et al., 2003; Hariri et al., 2003; McAllister et al., 2012; Miyajima et al., 2008; Voineskos et al., 2011), hippocampal volume and fMRI responses (Egan et al., 2003; Pezawas et al., 2004). The *met* allele occurs in approximately 35% of the Caucasian population (Cheeran et al., 2008). Given the prominent role of BDNF in neuroplasticity and a failure of neural plasticity as a potential common theme in AD (Mesulam, 1999), the BDNF-TrkB pathway could theoretically be linked to a host of AD-related processes at the molecular, neuronal or systems level.

The original hypothesis underlying the design of our study was that BDNF polymorphisms might influence the resilience against β amyloid related changes (Nelissen et al., 2007). We hypothesized that BDNF *val* carriers would be able to compensate for the presence of β amyloid in a better way than *met* carriers. Such resilience might be manifest as a difference between genetic strata in how $A\beta$ load affects cognitive scores and also as a difference in compensatory mechanisms at the brain systems level (Nelissen et al., 2007). As a first step, we examined whether there were any direct effects of the BDNF codon 66 polymorphism on amyloid retention in cognitively intact older adults, measured by means of ^{18}F -flutemetamol positron emission tomography (PET) (Koole et al., 2009; Nelissen et al., 2009; Vandenberghe et al., 2010, 2013).

2. Subjects and methods

2.1. Subjects

The main cohort consisted of 64 community-recruited older adults between 50 and 75 years of age (mean age = 66, S.D. = 5.1, range 53–74) (Table 1). The study exclusion criteria were a Mini Mental State Examination (Folstein et al., 1975) score lower than 27, a Clinical Dementia Rating score (Morris et al., 1997) higher than 0, significant neurological or psychiatric history, significant brain lesions on structural MRI, and below-normal test scores on conventional neuropsychological assessment (Table 1). Inclusion was stratified per age bin (50–59, 60–64, 65–69, 70–75) for two genetic factors: BDNF (*met* allele present or absent) and APOE ($\epsilon 4$ allele present or absent). The cells of this 2×2 factorial design were prospectively matched for number of cases, gender, age, education and handedness (Edinburgh Handedness Inventory) (Table 1). BDNF and APOE variants were genotyped by sequencing at the Genetic Service Facility (GSF, www.vibgeneticservicefacility.be) of the VIB Department of Molecular Genetics.

The genetic distribution among all subjects who underwent screening, was 10% BDNF *met* positive (+ve)/APOE $\epsilon 4$ +ve, 16% BDNF *met* negative (-ve)/APOE $\epsilon 4$ +ve, 33% BDNF *met* +ve/APOE $\epsilon 4$ -ve,

Table 1

Demographic and neuropsychological characteristics of subjects in the original dataset. Values represent means and standard deviations (in parenthesis) unless stated otherwise; gender is expressed in number of individuals. Abbreviations: M = male; F = female; MMSE = Mini Mental State Examination; CDR = Clinical Dementia Rating global score; AVLT = Rey Auditory Verbal Learning Test; DR = delayed recall; TL = total learning; TMT = Trail Making Test part B divided by part A; BNT = Boston Naming Test; AVF = Animal Verbal Fluency Test; LVF = Letter Verbal Fluency Test; RPM = Raven's Progressive Matrices; +ve = positive; -ve = negative.

	Genetic groups				P
	BDNF <i>met</i> +ve	BDNF <i>met</i> -ve	BDNF <i>met</i> +ve	BDNF <i>met</i> -ve	
	APOE $\epsilon 4$ +ve	APOE $\epsilon 4$ +ve	APOE $\epsilon 4$ -ve	APOE $\epsilon 4$ -ve	
Gender (M/F)	7/9	9/6	8/8	10/7	0.8
Age	65.4 (5.5)	66.5 (4.3)	65.3 (5.5)	65.8 (5.4)	0.9
Education (years)	13.3 (3.0)	12.5 (2.2)	13.9 (2.2)	14.7 (3.6)	0.2
Handedness	95.0 (14.5)	100.0 (0.0)	96.7 (7.7)	100.0 (0.0)	0.2
MMSE (/30)	29.0 (0.9)	28.7 (1.1)	29.3 (0.6)	28.9 (0.9)	0.3
AVLT DR (/15)	11.4 (2.4)	10.3 (3.6)	11.3 (2.8)	10.6 (2.2)	0.6
AVLT TL (/75)	48.9 (7.8)	50.1 (8.6)	51.1 (12.3)	49.0 (9.1)	0.9
BNT (/60)	53.1 (5.4)	51.9 (6.5)	52.6 (4.8)	54.2 (3.2)	0.6
AVF	18.6 (4.7)	19.9 (5.3)	21.8 (5.5)	21.2 (4.2)	0.3
LVF	33.5 (11.8)	31.1 (8.1)	33.6 (9.9)	37.4 (9.8)	0.4
RPM	39 (8.6)	42.1 (9.2)	44.3 (7.3)	46.5 (7.5)	0.07
TMT B/A	2.9 (1.0)	2.5 (0.6)	2.6 (1.0)	2.5 (1.1)	0.5

and 41% BDNF *met* -ve/APOE $\epsilon 4$ -ve. After genetic stratification, the genetic distribution of the final cohort (n = 64) was as follows: 25% were BDNF *met* +ve/APOE $\epsilon 4$ +ve, 23% BDNF *met* -ve/APOE $\epsilon 4$ +ve, 25% BDNF *met* +ve/APOE $\epsilon 4$ -ve, and 27% were BDNF *met* -ve/APOE $\epsilon 4$ -ve.

The protocol was approved by the Ethics Committee University Hospitals Leuven (EudraCT: 2009-014475-45) and written informed consent was obtained from all subjects in accordance with the Declaration of Helsinki.

2.2. Image acquisition

^{18}F -flutemetamol PET imaging was performed at the University Hospitals Leuven. The acquisition procedure has been described before (Koole et al., 2009; Nelissen et al., 2009; Vandenberghe et al., 2010, 2013). Images were acquired on a 16-slice Siemens Biograph PET/CT scanner (Siemens, Erlangen, Germany). The PET tracer was injected intravenously as a bolus (mean activity 150.6 MBq, S.D. 8 MBq, range 137.9–192.5 MBq) in an antecubital vein. Image acquisition started 90 min after tracer injection and lasted for 30 min. Prior to the PET scan, a low-dose computed tomography (CT) scan was performed for attenuation correction. Random and scatter corrections were also applied. Images were reconstructed using Ordered Subsets Expectation Maximization (OSEM; 4 iterations \times 16 subsets).

A high-resolution T1-weighted structural MRI was obtained on a 3 T Philips Intera system equipped with an 8-channel receive-only head coil (Philips SENSitivity Encoding head coil), using a 3D turbo field echo sequence (coronal inversion recovery prepared 3D gradient-echo images, inversion time 900 ms, TR = 9.6 ms, TE = 4.6 ms, flip angle 8°, field of view = 250 \times 250 mm, 182 slices; voxel size 0.98 \times 0.98 \times 1.2 mm³).

2.3. Image analysis

All analyses were performed using Statistical Parametric Mapping 8 (SPM8, <http://www.fil.ion.ucl.ac.uk/spm>). The PET data were reconstructed as 6 frames of 5 min and realigned to the first frame to correct for potential head motion. Subsequently, the 6 frames were summed to

create one summed image. The individual's T1-weighted structural image was then co-registered to his/her PET summed image. This MR image was subsequently normalized to the SPM8 T1 template in Montreal Neurological Institute (MNI) space using a unified segmentation approach. Next, this normalization matrix was applied to the individual's co-registered PET summed image.

From the spatially normalized images (voxel size $2 \times 2 \times 2 \text{ mm}^3$) standardized uptake value ratios (SUVR) were calculated with cerebellar gray matter as reference region, resulting in SUVR images. The cerebellar gray matter reference region was defined as areas 91 to 108 of the Automated Anatomical Labelling atlas (AAL) (Tzourio-Mazoyer et al., 2002). The cerebellar reference region was resliced to each individual's normalized PET summed image. In order to exclude white matter (WM) content, it was masked by the normalized and modulated subject-specific gray matter (GM) map, with the threshold for masking set at 0.3.

We also defined a composite cortical volume of interest. This was composed of 5 bilateral cortical areas, i.e. frontal, parietal, anterior cingulate, precuneus/posterior cingulate and lateral temporal defined as AAL areas 3–10, 13–16, 23–28, 31–32, 35–36, 57–70, 81–82, 85–90. The composite cortical VOI was resliced to each individual's normalized PET summed image. In order to exclude WM content, it was masked by the normalized and modulated subject-specific GM map, with the threshold for masking set at 0.3.

In one of the subjects the structural T1 image was missing due to a contraindication for MRI. This individual's PET summed image was normalized to the group mean normalized PET summed image created from the 63 remaining subjects. The AAL-derived cerebellar and composite cortical VOIs were co-registered to this normalized PET summed image and were masked with the mean normalized modulated GM map (thresholded at 0.3) created out of the 63 normalized modulated GM maps. This individual's SUVR image was calculated based on this normalized PET summed image with the cerebellar gray matter reference region.

As a secondary measure we also worked on partial volume corrected (PVC) data. PVC was based on the MRI using the modified Müller-Gärtner method (Müller-Gärtner et al., 1992). This method determines tracer concentration per unit volume of GM. In the modified method, we use probabilistic segmentation instead of binary maps. The normalized unmodulated GM and WM segmentations were used to estimate different tissue fractions per voxel. PVC was applied to the normalized PET summed images. The remaining procedures were identical to those outlined above.

We also tested for any group differences in tracer retention in the cerebellar reference region, as this could cause spurious differences in cortical SUVR values. We calculated the standardized uptake values (SUV) in the cerebellar gray matter region. SUV values were defined as the ratio of mean activity concentration in cerebellar gray matter in the normalized PET summed image [MBq] to the injected activity concentration [MBq/kg] per total body weight [kg].

2.3.1. Statistical analysis

We statistically analyzed all SUVR images using two approaches: one was based on the global composite cortical VOI, the other was voxel-based. In the global composite cortical analysis our primary outcome measure was mean SUVR value calculated in the composite cortical VOI ($SUVR_{comp}$). We used $SUVR_{comp}$ as the dependent variable and performed a factorial ANOVA with BDNF (2 levels: *met* allele present vs absent) and APOE (2 levels: $\epsilon 4$ allele present vs absent) as between-subjects factors. In addition, we performed a confirmatory non-parametric Kruskal–Wallis ANOVA on the 4 genetic groups with $SUVR_{comp}$ as the dependent variable.

In the voxel based analysis, using SPM8, we analyzed SUVR images by means of a factorial ANOVA with BDNF (2 levels: *met* allele present vs absent) and APOE (2 levels: $\epsilon 4$ allele present vs absent) as between-subjects factors. The significance threshold was set at a cluster-level

of $P < 0.05$ family-wise error (FWE) corrected for the entire brain search volume, with the voxel-level threshold set at uncorrected $P < 0.001$.

The partial-volume corrected data were analyzed using a factorial ANOVA with BDNF (2 levels: *met* allele present vs absent) and APOE (2 levels: $\epsilon 4$ allele present vs absent) as between-subjects factors.

Differences in the SUV values in the cerebellar gray matter VOI were analyzed by a factorial ANOVA with 2 between-subjects factors: BDNF (2 levels: *met* allele present vs absent) and APOE (2 levels: $\epsilon 4$ allele present vs absent).

2.4. Relationship to episodic memory test scores

To assess the relationship between ^{18}F -flutemetamol retention and episodic memory measures, we conducted a linear regression analysis with either AVLT delayed recall score (DR) or total learning score (TL) as dependent variable and $SUVR_{comp}$ as independent variable. This analysis was performed across the entire group as well as within each genetic group separately. To evaluate whether the relationship was specifically observed with episodic memory scores, we also performed a linear regression analysis with other tests from our cognitive battery: Boston Naming Test (BNT), Animal Verbal Fluency Test (AVF) and total score on Raven's Progressive Matrices (RPM).

3. Results

The four genetic groups did not differ in age, gender, years of education or neuropsychological test scores (Table 1).

3.1. Effect of APOE and BDNF polymorphisms on SUVR

3.1.1. Global composite cortical analysis

Our primary outcome measure, $SUVR_{comp}$, differed significantly between the four genetic groups ($F(3,60) = 5.37, P = 0.002$) (Fig. 1). The main effect of APOE was significant: APOE $\epsilon 4$ carriers had significantly higher ligand retention than APOE $\epsilon 4$ noncarriers ($F(1,60) = 7.14, P = 0.01$). The main effect of BDNF genotype was not significant ($F(1,60) = 1.02, P = 0.32$). The interaction between BDNF and APOE on ligand retention was significant ($F(1,60) = 7.94, P = 0.007$): BDNF *met* +ve/APOE $\epsilon 4$ +ve carriers had significantly higher ligand retention (mean $SUVR_{comp} = 1.37, \text{S.D.} = 0.21$) than BDNF *met* –ve/APOE $\epsilon 4$ +ve carriers (mean $SUVR_{comp} = 1.23, \text{S.D.} = 0.12$) ($P = 0.01$), while amyloid ligand retention in APOE $\epsilon 4$ noncarriers did not differ between BDNF *met* +ve (mean $SUVR_{comp} = 1.17, \text{S.D.} = 0.06$) and BDNF *met* –ve cases (mean $SUVR_{comp} = 1.24, \text{S.D.} = 0.11$) ($P = 0.2$) (Fig. 2A and B).

Non-parametric analysis confirmed these results. The four genetic groups differed significantly in $SUVR_{comp}$ ($H(\text{df } 3, N \text{ } 64) = 11.35, P = 0.01$). The main effect of APOE was significant: APOE $\epsilon 4$ carriers had significantly higher ligand retention than APOE $\epsilon 4$ noncarriers ($H(\text{df } 1, N \text{ } 64) = 5.25, P = 0.02$). The main effect of BDNF genotype was not significant ($H(\text{df } 1, N \text{ } 64) = 0.04, P = 0.85$). Planned comparisons revealed that BDNF *met* +ve/APOE $\epsilon 4$ +ve carriers exhibited higher ligand retention (mean rank = 19.1) than BDNF *met* –ve/APOE $\epsilon 4$ +ve carriers (mean rank = 12.6) ($P = 0.048$). Amyloid ligand retention tended to be lower in BDNF *met* +ve (mean rank = 13.8) than in BDNF *met* –ve (mean rank = 19.9) APOE $\epsilon 4$ noncarriers ($P = 0.07$).

Analysis of partial volume corrected data confirmed these results. PVC $SUVR_{comp}$ was significantly different between the four genetic groups ($F(3,59) = 4.64, P = 0.006$). The main effect of APOE was significant: APOE $\epsilon 4$ carriers had significantly higher ligand retention than APOE $\epsilon 4$ noncarriers ($F(1,59) = 6.96, P = 0.01$). The main effect of BDNF genotype was not significant ($F(1,59) = 0.55, P = 0.46$). The interaction between BDNF and APOE on ligand retention was significant ($F(1,59) = 6.7, P = 0.01$): BDNF *met* +ve/APOE $\epsilon 4$ +ve carriers had significantly higher ligand retention (mean $SUVR_{comp} = 1.56, \text{S.D.} = 0.39$) than

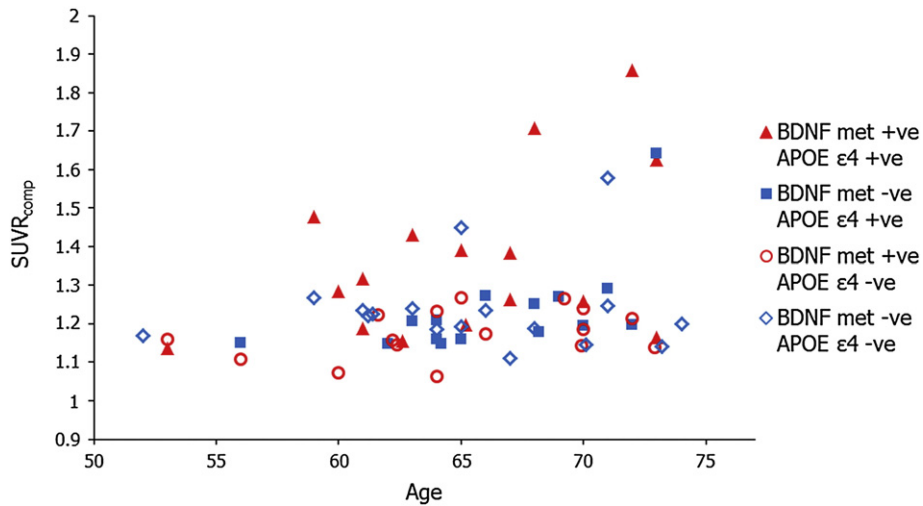


Fig. 1. Distribution of ¹⁸F-flutemetamol retention levels for the different genetic groups. X axis: age in years; Y axis: SUVR_{comp}.

BDNF *met* -ve/APOE ε4 +ve carriers (mean SUVR_{comp} = 1.36, S.D. = 0.24) ($P = 0.02$), while amyloid ligand retention in APOE ε4 noncarriers did not differ between BDNF *met* +ve (mean SUVR_{comp} = 1.24, S.D. = 0.09) and BDNF *met* -ve cases (mean SUVR_{comp} = 1.35, S.D. = 0.18) ($P = 0.19$).

SUV values in cerebellar gray matter did not differ between genetic groups ($F(3,60) = 0.54, P = 0.66$): There was no difference between APOE ε4 carriers and noncarriers ($F(1,60) = 0.21, P = 0.65$), between BDNF *met* carriers and noncarriers ($F(1,60) = 0.55, P = 0.46$) and neither was there any interaction ($F(1,60) = 0.79, P = 0.38$).

3.1.2. Voxel-based analysis

The whole-brain voxel-wise analysis confirmed the findings from the composite cortical analysis. There was a significant main effect of APOE: APOE ε4 carriers had significantly higher ¹⁸F-flutemetamol retention than APOE ε4 noncarriers in the posterior cingulate (cluster

peak -14, -20, 40, $Z = 4.26$, extent of voxels (ext.) 178 mm³, corr. cluster-level $P = 0.025$) (Fig. 3A). There was no main effect of BDNF genotype (corr. cluster-level $P > 0.7$). The interaction effect of APOE and BDNF was significant in precuneus (cluster peak 10, -40, 42, $Z = 4.71$, ext. 437 mm³, corr. cluster-level $P = 0.0001$), left orbitofrontal cortex (cluster peak -8, 64, -14, $Z = 4.69$, ext. 387 mm³, corr. cluster-level $P = 0.0003$), right orbitofrontal cortex (cluster peak 16, 68, -2, $Z = 4.37$, ext. 240 mm³, corr. cluster-level $P = 0.006$), left gyrus rectus (cluster peak -8, 22, -18, $Z = 4.62$, ext. 252 mm³, corr. cluster-level $P = 0.004$), right gyrus rectus (cluster peak 16, 28, -28, $Z = 4.35$, ext. 209 mm³, corr. cluster-level $P = 0.012$), right middle frontal gyrus (cluster peak 34, 46, 28, $Z = 4.14$, ext. 316 mm³, corr. cluster-level $P = 0.001$) and right inferior frontal sulcus (cluster peak 50, 38, 6, $Z = 3.88$, ext. 281 mm³, corr. cluster-level $P = 0.002$) (Fig. 3B). Simple effects revealed that BDNF *met* +ve/APOE ε4 +ve carriers had higher ligand retention than BDNF *met* -ve/APOE ε4 +ve carriers in posterior cingulate (cluster peak at 2, -32, 42, $Z = 4.25$, ext. 388 mm³, corr. cluster-level $P = 0.0003$), gyrus rectus (cluster peak at -4, 34, -26, $Z = 4.44$, ext. 196 mm³, corr. cluster-level $P = 0.016$), insula (cluster peak at 36, 8, -12, $Z = 4.89$, ext. 311 mm³, corr. cluster-level $P = 0.001$) and posterior middle temporal cortex (cluster peak at 62, -62, 4, $Z = 4.23$, ext. 168 mm³, corr. cluster-level $P = 0.03$). There was no difference between BDNF *met* +ve/APOE ε4 -ve carriers and BDNF *met* -ve/APOE ε4 -ve carriers (corr. cluster-level $P > 0.9$).

3.1.3. Relationship between β amyloid load and episodic memory

Across the entire sample, SUVR_{comp} did not correlate with AVLT DR ($P = 0.38$) or TL ($P = 0.16$). When analyzed per genetic group, a highly significant and negative correlation was seen only in the group of BDNF *met* +ve/APOE ε4 +ve carriers (DR: $r = -0.62, P = 0.01$, and TL: $r = -0.58, P = 0.02$) (Fig. 4A and B, red lines). In the other groups the correlation remained far below significance ($P > 0.49$) (Table 2). A formal pairwise comparison however of the regression slopes between groups did not reach significance ($P > 0.05$) (Thöni, 1977).

None of the other cognitive test scores showed any correlation with β amyloid when tested across the entire sample ($P > 0.17$) or per group (Table 2).

4. Discussion

Our study revealed two key novel findings. First, APOE ε4 carriers exhibited a higher β amyloid load in the presence of one or two BDNF *met* alleles compared to BDNF *met* noncarriers (Figs. 1, 2). Second, an

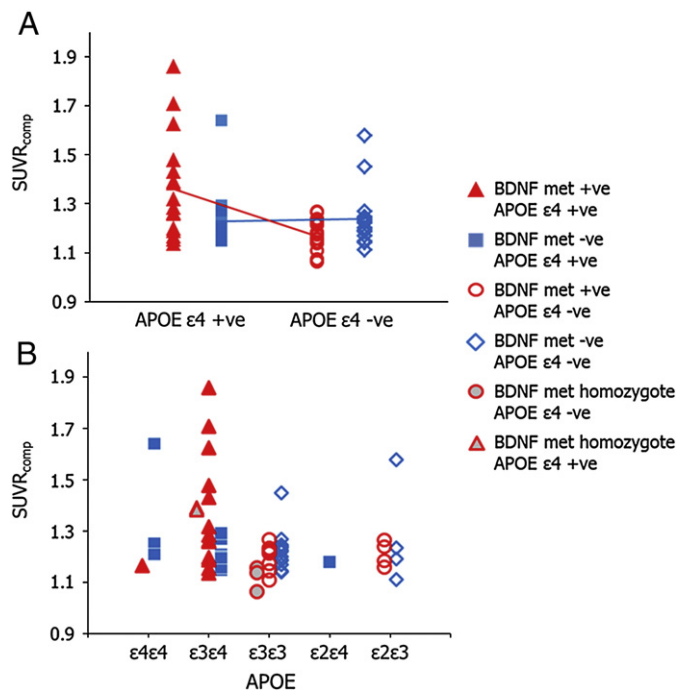


Fig. 2. Effects of BDNF and APOE genotypes on amyloid deposition measured by SUVR_{comp}. (A) SUVR_{comp} in the different genetic subgroups. (B) Further differentiation depending on APOE and BDNF subgroups.

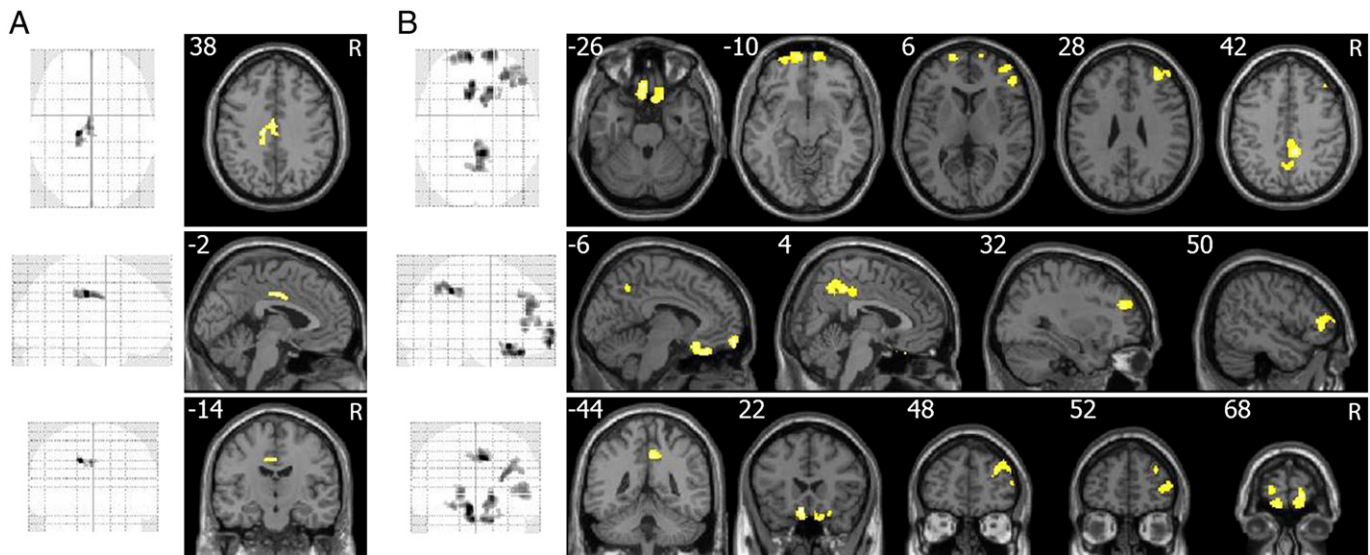


Fig. 3. Statistical parametric map of the main effect of APOE (A) and the interaction effect between APOE and BDNF (B). (A) Main effect of APOE genotype: increased ^{18}F -flutemetamol retention in APOE $\epsilon 4 +ve$ carriers compared to APOE $\epsilon 4 -ve$ carriers in the posterior cingulate. (B) Interaction effect between BDNF and APOE: increased ^{18}F -flutemetamol retention in the precuneus, orbitofrontal cortex, gyrus rectus, middle frontal gyrus, and inferior frontal sulcus. Images are displayed at voxel-level $P < 0.001$, only clusters that reached corrected cluster-level $P < 0.05$ are shown. SPM t maps are projected onto axial, sagittal, and coronal sections of the SPM8 standard single subject T1 template and as SPM8 glass brain views.

inverse relationship between $A\beta$ load and episodic memory exists in BDNF *met*/APOE $\epsilon 4$ carriers but not in any of the other subgroups.

The interaction effect between BDNF and APOE on β amyloid load was unexpected and needs further confirmation by independent studies. The genetic stratification for APOE and BDNF prior to recruitment for scanning is a unique feature of our cohort that was probably critical for obtaining this novel finding. In the group of individuals volunteering for this study, after applying all non-genetic criteria for in/exclusion, the proportion of BDNF *met +ve*/APOE $\epsilon 4 +ve$ subjects was only 10%. After stratification this was raised to 25%. It is this genetic combination that drives the interaction. Had we not strived for a balanced factorial design with cells matched for numbers at recruitment, the interaction effect would almost certainly have been missed. This means that we had to genotype much higher numbers of subjects than were actually able to enter the scanning phase of the study. Replication of the interaction effect will probably also have to be based on samples enriched for the naturally least frequent combination of BDNF *met* and APOE $\epsilon 4$ carriers.

Most of the subjects with raised amyloid were APOE $\epsilon 4$ carriers with BDNF *met* allele at codon 66 (Fig. 1). The number of subjects with raised amyloid is relatively low but impacts relatively strongly on the statistical outcome. Non-parametric testing confirmed that BDNF *met* carriage affected $A\beta$ load. Our findings were obtained in a cohort stratified at recruitment for APOE and BDNF polymorphisms according to a balanced factorial design with demographically matched subjects. The stratification, the balanced design and the fact that we only tested these two gene polymorphisms, reduces the risk of a false-positive. As a relatively low number of the 64 healthy subjects had a raised $A\beta$ load, the power of our study to determine an influence of genetics is limited and any negative findings should be interpreted with caution. The proportion of subjects with raised amyloid increases with age (Morris et al., 2010). Further studies in a higher age range will be needed to confirm whether the interaction is present also in an older population.

We used the volumetric MRI scan for normalization and the segmented gray matter maps for definition of the cerebellar gray matter and the composite cortical volume. The method we applied differs from the purely PET-based method applied in the phase 2 study of ^{18}F -flutemetamol (Vandenberghe et al., 2010). As a consequence, we

cannot simply use the cut-off from the phase 2 study to discriminate positive from negative cases in a binary manner. Furthermore, in a cognitively intact population of older adults, a significant minority exhibits intermediary values. While categorical discrimination between positive and negative individuals is relevant for clinical purposes, a binary division in positive and negative cases is not essential for the current scientific question.

The interaction effect was seen in frontobasal cortex, precuneus, and lateral prefrontal cortex, which are well-established areas of predilection for amyloid deposition in the initial phase of the disease (Aizenstein et al., 2008; Fleisher et al., 2011, 2013; Mintun et al., 2006; Pike et al., 2007; Rowe et al., 2007). For posterior cingulate and precuneus this has been linked to their status as a network hub (Buckner et al., 2009; Mormino et al., 2011), including their centrality in the connections from the hippocampal formation to parietal cortex (Mesulam et al., 1977; Seltzer and Pandya, 1994). Why orbitofrontal cortex regularly recurs as an area of predilection, is less clear. In any case, numerous studies of the earliest changes in amyloid load in AD have reported increased amyloid load in this region (Braak and Braak, 1991; Chételat et al., 2010, 2011; Fleisher et al., 2011, 2013). Orbitofrontal cortex also shows hypometabolism on ^{18}F -deoxyglucose PET early in the AD disease course (Herholz et al., 2002).

The original purpose of our factorial design was to evaluate the effect of BDNF on functional reorganization and plasticity (Nelissen et al., 2007) in response to β amyloid related injury. We predicted that compared to BDNF *met* carriers BDNF *val* status would confer a higher compensatory capacity for functional reorganization in the face of increased $A\beta$ amyloid (Nelissen et al., 2007). Given ample evidence for a relationship between BDNF polymorphism and episodic memory (Chen et al., 2004; Dennis et al., 2011; Egan et al., 2003; Hariri et al., 2003; McAllister et al., 2012; Miyajima et al., 2008; Voineskos et al., 2011) and given the prominent role of episodic memory decline in the earliest clinical stages of Alzheimer's disease, we primarily evaluated whether BDNF affected the relationship between $A\beta$ deposition and episodic memory. We found a strong negative correlation between $A\beta$ deposition and episodic memory encoding and recall in the BDNF *met +ve*/APOE $\epsilon 4 +ve$ carriers but not in any of the three other groups (Fig. 4, Table 2). This finding is in line with our a priori hypothesis that BDNF polymorphism may influence resilience against

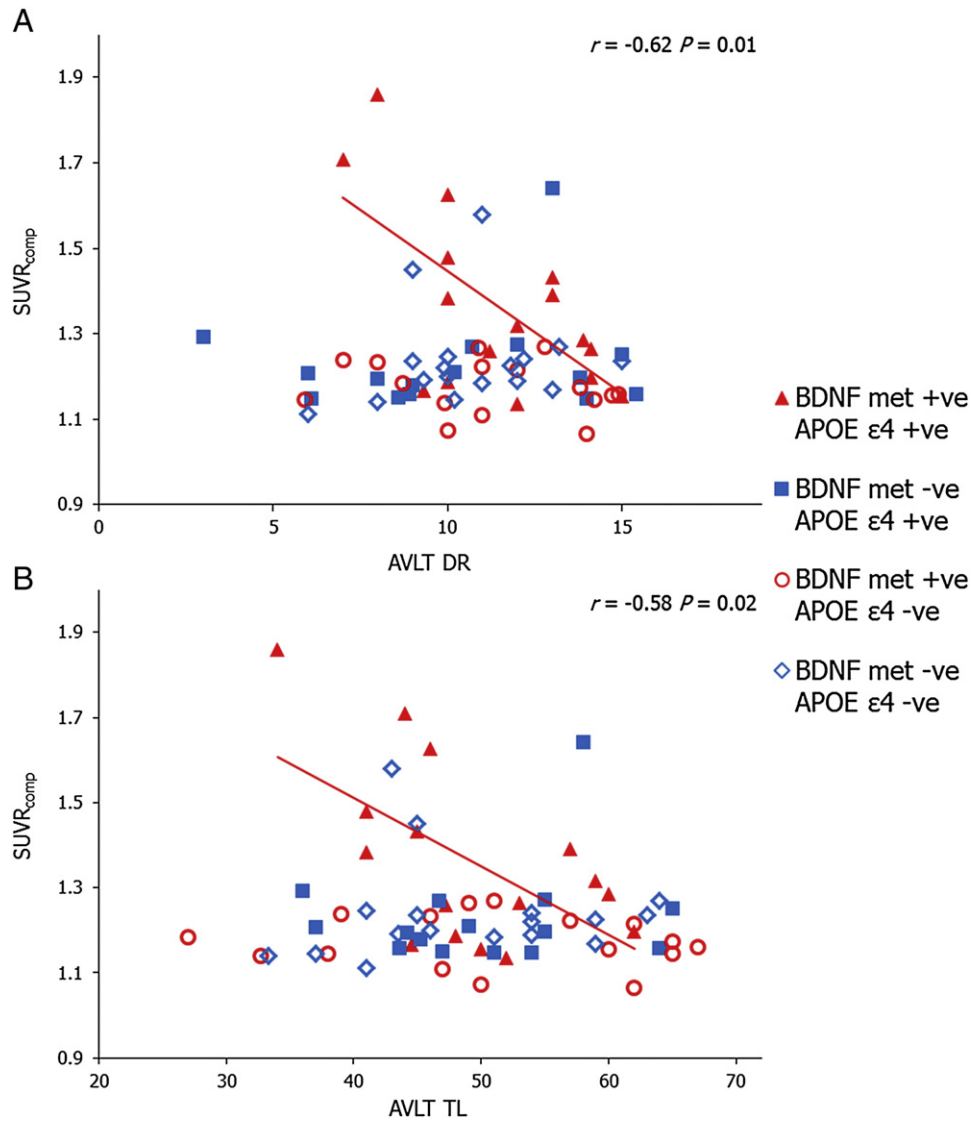


Fig. 4. Correlation between amyloid ligand retention and AVLT delayed recall (DR) and total learning (TL) scores. (A) Negative correlation between $SUVR_{comp}$ values and AVLT DR scores in the BDNF *met +ve*/APOE $\epsilon 4 +ve$ group (red line). (B) Negative correlation between $SUVR_{comp}$ values and AVLT TL scores in the BDNF *met +ve*/APOE $\epsilon 4 +ve$ (red line). $SUVR_{comp}$ (Y axis) plotted by AVLT DR or TL scores (X axis): BDNF *met +ve*/APOE $\epsilon 4 +ve$ (red triangles), BDNF *met -ve*/APOE $\epsilon 4 +ve$ (blue squares), BDNF *met +ve*/APOE $\epsilon 4 -ve$ (red circles) and BDNF *met -ve*/APOE $\epsilon 4 -ve$ (blue diamonds). When the correlation does not reach significance, no regression line is shown.

Table 2

Linear regression analysis between $SUVR_{comp}$ and cognitive test scores in each of the four genetic groups. Values represent correlation coefficient (*r*) and statistical significance (*P*). Abbreviations: AVLT = Rey Auditory Verbal Learning Test; DR = delayed recall; TL = total learning; BNT = Boston Naming Test; AVF = Animal Verbal Fluency Test; RPM = Raven's Progressive Matrices.

	Correlation between amyloid tracer retention and cognitive test scores							
	BDNF <i>met +ve</i>		BDNF <i>met -ve</i>		BDNF <i>met +ve</i>		BDNF <i>met -ve</i>	
	APOE $\epsilon 4 +ve$		APOE $\epsilon 4 +ve$		APOE $\epsilon 4 -ve$		APOE $\epsilon 4 -ve$	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
AVLT DR	0.62	0.01	0.14	0.61	0.19	0.49	0.15	0.56
AVLT TL	0.58	0.02	0.18	0.51	0.12	0.65	0.02	0.95
BNT	0.18	0.49	0.03	0.91	0.06	0.81	0.07	0.78
AVF	0.04	0.88	0.21	0.44	0.11	0.69	0.42	0.09
RPM	0.36	0.17	0.00	1.00	0.03	0.92	0.10	0.72

A β related injury. A formal pairwise comparison however of the regression slopes of β amyloid load versus episodic memory scores between the groups did not reach significance, possibly due to the relatively small sample size. The relationship between cognitive test scores in cognitively intact individuals and A β deposition is still a topic of active research, with some studies reporting an inverse relationship and others an absence of any relationship (Chételat et al., 2011; Mormino et al., 2009; Pike et al., 2007; Rentz et al., 2010; Resnick et al., 2010). Our findings offer one possible explanation for this divergence between studies: if the genetic composition differs between cohorts, this may cause differences in how β amyloid load relates to episodic memory scores (Fig. 4 A, B). The effect of A β load on episodic memory scores in the BDNF *met +ve*/APOE $\epsilon 4 +ve$ group highlights the behavioral relevance of our findings.

Previous studies have shown the negative effect of BDNF codon 66 *val* to *met* substitution on a number of parameters: It is associated with worse memory function (Chen et al., 2004; Egan et al., 2003; Hariri et al., 2003; McAllister et al., 2012; Miyajima et al., 2008; Voineskos et al., 2011), lower fMRI responses in hippocampi during

both encoding and retrieval (Egan et al., 2003; Hariri et al., 2003), smaller volumes of hippocampi (Bueller et al., 2006; Hajek et al., 2012; Miyajima et al., 2008; Pezawas et al., 2004; Szeszek et al., 2005), lateral prefrontal cortex (Nemoto et al., 2006; Pezawas et al., 2004; Yang et al., 2012), temporal neocortex, cingulate, and insula (Yang et al., 2012), and amygdalae (Montag et al., 2009; Sublette et al., 2008), as well as age-related volume reduction of other cortical areas which may be gender-dependent (Nemoto et al., 2006). The BDNF codon 66 *met* allele also affects white matter connectivity (Chiang et al., 2009). Interestingly, a recent study reported effects of BDNF *val66met* polymorphism on brain metabolism in healthy controls, MCI and AD cases (Xu et al., 2010). To the best of our knowledge our study is the first to directly examine how the well-studied negative effects of BDNF *met* relate to subclinical A β amyloid and its relationship to episodic memory.

An interaction effect between BDNF and APOE on amyloid ligand retention has not been reported in previous genome-wide association studies (GWAS) of clinically probable Alzheimer's disease patients. In their conventional form, GWAS make use of massively univariate single-locus tests. Such tests are designed to search for single disease-associated polymorphisms (Huang et al., 2007) where each gene variant is tested individually for association with a specific phenotype (cases versus controls) (Cordell, 2009). GWAS in their classical form will miss interaction effects between genes.

Apart from amyloid load, it is currently unknown how the interaction between BDNF and APOE polymorphisms affects other contributors to the pathogenesis of AD, such as synapse loss, cholinergic depletion, or neurofibrillary tangle formation. For this reason, it remains to be seen whether the interaction effect between BDNF and APOE on amyloid burden we observed in cognitively intact subjects can be extrapolated to genetic comparisons between clinically probable AD and controls.

Studies in animal models of AD offer us with several putative mechanisms through which BDNF and the amyloid cascade may interact. Intra-hippocampal injections of A β (1–42) in rats reduce the expression of BDNF and also result in decreased BDNF levels in prefrontal cortex (Christensen et al., 2008). In amyloid precursor protein (APP) double transgenic mice with both the Swedish and the Indiana APP mutations, BDNF gene delivery to entorhinal cortex reverses synapse loss and improves cell signaling, partially normalizes APP-related alterations in hippocampal and entorhinal gene expression, and restores learning and memory (Nagahara et al., 2009). In AD postmortem samples, BDNF protein expression as well as mRNA levels are decreased in hippocampi and other cortical areas (Connor et al., 1997; Ferrer et al., 1999; Hock et al., 2000; Holsinger et al., 2000; Phillips et al., 1991). During the course of AD, BDNF serum concentration levels correlate with the severity of dementia: levels are increased in early stages of AD and decreased in the advanced stages (Erickson et al., 2010; Laske et al., 2006). It however is difficult to speculate on the exact mechanism through which BDNF may interact with APOE to increase amyloid burden as the relationship between APOE and increased amyloid aggregation itself is still relatively poorly understood. Both genes have been implicated in neural plasticity. This concept encompasses widely diverse processes (Mesulam, 1999). Possibly, the efficacy with which neurons dispose of toxic forms of A β may depend on pathways that are linked to lipid metabolism and neuronal survival in which APOE and BDNF, respectively, play pivotal roles.

In conclusion, we demonstrate an association between BDNF *met* allele and PET measures of amyloid deposition in cognitively normal older adult APOE ϵ 4 carriers. This finding provides empirical evidence for a role of the BDNF-TrkB pathway not only in neural plasticity but also in the pathogenesis of Alzheimer's disease (Mesulam, 1999). It underscores the opportunity to use brain imaging measurements to help characterize the individual and epistatic effects of putative genetic risk factors in the predisposition to AD. We however would

like to emphasize that the current study is hypothesis-generating and needs further replication.

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