NeuroImage: Clinical 2 (2013) 512–520

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Polymorphism of brain derived neurotrophic factor influences β amyloid load in cognitively intact apolipoprotein E ε4 carriers☆

Katarzyna Adamczuk [a,b], An-Sofie De Weer [a,b], Natalie Nelissen [a,b], Kewei Chen [c,d,e], Kristel Sleegers [g,h], Karolien Bettens [g,h], Christine Van Broeckhoven [g,h], Mathieu Vandenbulcke [i], 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

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 3 April 2013
Accepted 4 April 2013
Available online 11 April 2013

Keywords:
BDNF
APOE
Amyloid PET
Alzheimer
Flutemetamol

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 ^{18}F-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 (ε4 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 ε4 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 ε4 noncarriers. This interaction effect was localized to precuneus, orbitofrontal cortex, gyrus rectus, and lateral prefrontal cortex. In the APOE ε4/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.

1. Introduction

In a variable proportion of cognitively intact older subjects, in vivo amyloid imaging has revealed increased cerebral Aβ 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) ε4 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).

2213-1582/$ – see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nicl.2013.04.001

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 ε4 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 (*val*66*met*), 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 Aβ 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 (ε4 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 ε4 +ve, 16% BDNF *met* negative (−ve)/APOE ε4 +ve, 33% BDNF *met* +ve/APOE ε4 −ve, and 41% BDNF *met* −ve/APOE ε4 −ve. After genetic stratification, the genetic distribution of the final cohort (n = 64) was as follows: 25% were BDNF *met* +ve/APOE ε4 +ve, 23% BDNF *met* −ve/APOE ε4 +ve, 25% BDNF *met* +ve/APOE ε4 −ve, and 27% were BDNF *met* −ve/APOE ε4 −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.

Demographic data and neuropsychological test scores in the original dataset					
	Genetic groups				*P*
	BDNF *met* +ve	BDNF *met* −ve	BDNF *met* +ve	BDNF *met* −ve	
	APOE ε4 +ve	APOE ε4 +ve	APOE ε4 −ve	APOE ε4 −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

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 × 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 × 250 mm, 182 slices; voxel size 0.98 × 0.98 × 1.2 mm^3).

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 $2 \times 2 \times 2$ 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: $\varepsilon 4$ 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: $\varepsilon 4$ 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: $\varepsilon 4$ 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: $\varepsilon 4$ 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 $\varepsilon 4$ carriers had significantly higher ligand retention than APOE $\varepsilon 4$ 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 $\varepsilon 4$ +ve carriers had significantly higher ligand retention (mean $SUVR_{comp} = 1.37$, S.D. = 0.21) than BDNF *met* −ve/APOE $\varepsilon 4$ +ve carriers (mean $SUVR_{comp} = 1.23$, S.D. = 0.12) ($P = 0.01$), while amyloid ligand retention in APOE $\varepsilon 4$ noncarriers did not differ between BDNF *met* +ve (mean $SUVR_{comp} = 1.17$, S.D. = 0.06) and BDNF *met* −ve cases (mean $SUVR_{comp} = 1.24$, S.D. = 0.11) ($P = 0.2$) (Fig. 2A and B).

Non-parametric analysis confirmed these results. The four genetic groups differed significantly in $SUVR_{comp}$ ($H(\text{df } 3, \text{N } 64) = 11.35$, $P = 0.01$). The main effect of APOE was significant: APOE $\varepsilon 4$ carriers had significantly higher ligand retention than APOE $\varepsilon 4$ noncarriers ($H(\text{df } 1, \text{N } 64) = 5.25$, $P = 0.02$). The main effect of BDNF genotype was not significant ($H(\text{df } 1, \text{N } 64) = 0.04$, $P = 0.85$). Planned comparisons revealed that BDNF *met* +ve/APOE $\varepsilon 4$ +ve carriers exhibited higher ligand retention (mean rank = 19.1) than BDNF *met* −ve/APOE $\varepsilon 4$ +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 $\varepsilon 4$ 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 $\varepsilon 4$ carriers had significantly higher ligand retention than APOE $\varepsilon 4$ 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 $\varepsilon 4$ +ve carriers had significantly higher ligand retention (mean $SUVR_{comp} = 1.56$, S.D. = 0.39) than

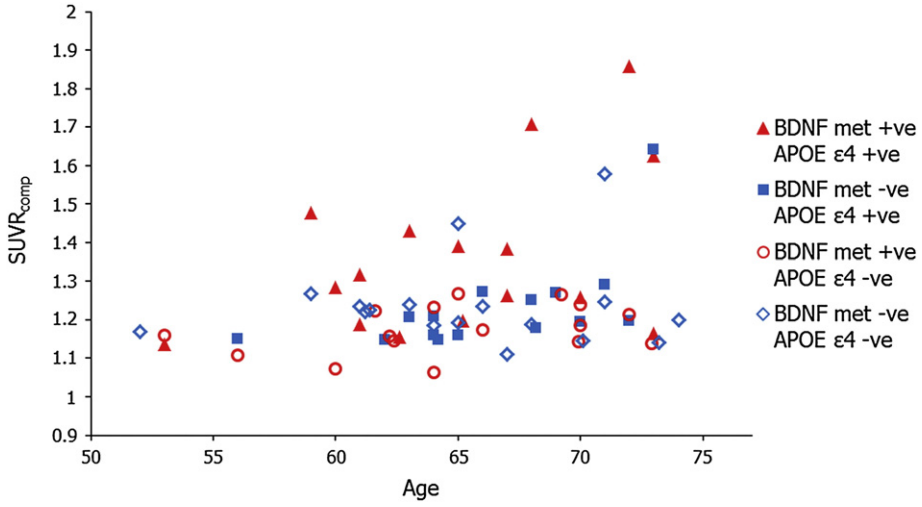

Fig. 1. Distribution of ^{18}F-flutemetamol retention levels for the different genetic groups. X axis: age in years; Y axis: $SUVR_{comp}$.

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 ^{18}F-flutemetamol retention than APOE ε4 noncarriers in the posterior cingulate (cluster peak −14, −20, 40, Z = 4.26, extent of voxels (ext.) 178 mm^3, 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 mm^3, corr. cluster-level P = 0.0001), left orbitofrontal cortex (cluster peak −8, 64, −14, Z = 4.69, ext. 387 mm^3, corr. cluster-level P = 0.0003), right orbitofrontal cortex (cluster peak 16, 68, −2, Z = 4.37, ext. 240 mm^3, corr. cluster-level P = 0.006), left gyrus rectus (cluster peak −8, 22, −18, Z = 4.62, ext. 252 mm^3, corr. cluster-level P = 0.004), right gyrus rectus (cluster peak 16, 28, −28, Z = 4.35, ext. 209 mm^3, corr. cluster-level P = 0.012), right middle frontal gyrus (cluster peak 34, 46, 28, Z = 4.14, ext. 316 mm^3, corr. cluster-level P = 0.001) and right inferior frontal sulcus (cluster peak 50, 38, 6, Z = 3.88, ext. 281 mm^3, 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 mm^3, corr. cluster-level P = 0.0003), gyrus rectus (cluster peak at −4, 34, −26, Z = 4.44, ext. 196 mm^3, corr. cluster-level P = 0.016), insula (cluster peak at 36, 8, −12, Z = 4.89, ext. 311 mm^3, corr. cluster-level P = 0.001) and posterior middle temporal cortex (cluster peak at 62, −62, 4, Z = 4.23, ext. 168 mm^3, 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).

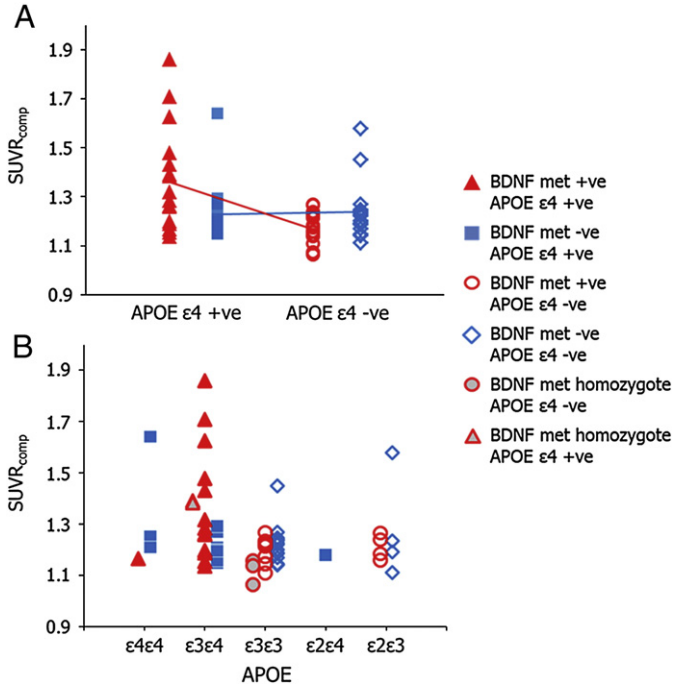

Fig. 2. Effects of BDNF and APOE genotypes on amyloid deposition measured by $SUVR_{comp}$. (A) $SUVR_{comp}$ in the different genetic subgroups. (B) Further differentiation depending on APOE and BDNF subgroups.

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

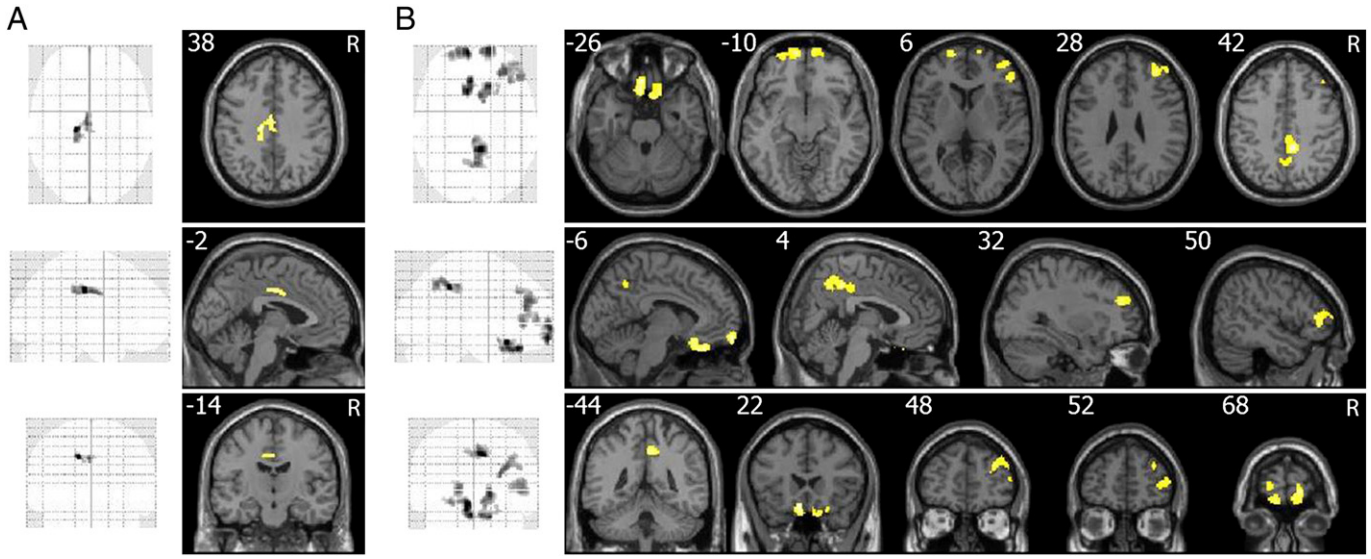

Fig. 3. Statistical parametric map of the main effect of APOE (A) and the interaction effect between APOE and BDNF (B). (A) Main effect of APOE genotype: increased ^{18}F-flutemetamol retention in APOE $\varepsilon4$ +ve carriers compared to APOE $\varepsilon4$ −ve carriers in the posterior cingulate. (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 Aβ load and episodic memory exists in BDNF *met*/APOE $\varepsilon4$ 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 BNDF *met* +ve/APOE $\varepsilon4$ +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 BNDF *met* and APOE $\varepsilon4$ carriers.

Most of the subjects with raised amyloid were APOE $\varepsilon4$ 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 Aβ 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 Aβ 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 Aβ 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 Aβ deposition and episodic memory. We found a strong negative correlation between Aβ deposition and episodic memory encoding and recall in the BDNF *met* +ve/APOE $\varepsilon4$ +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

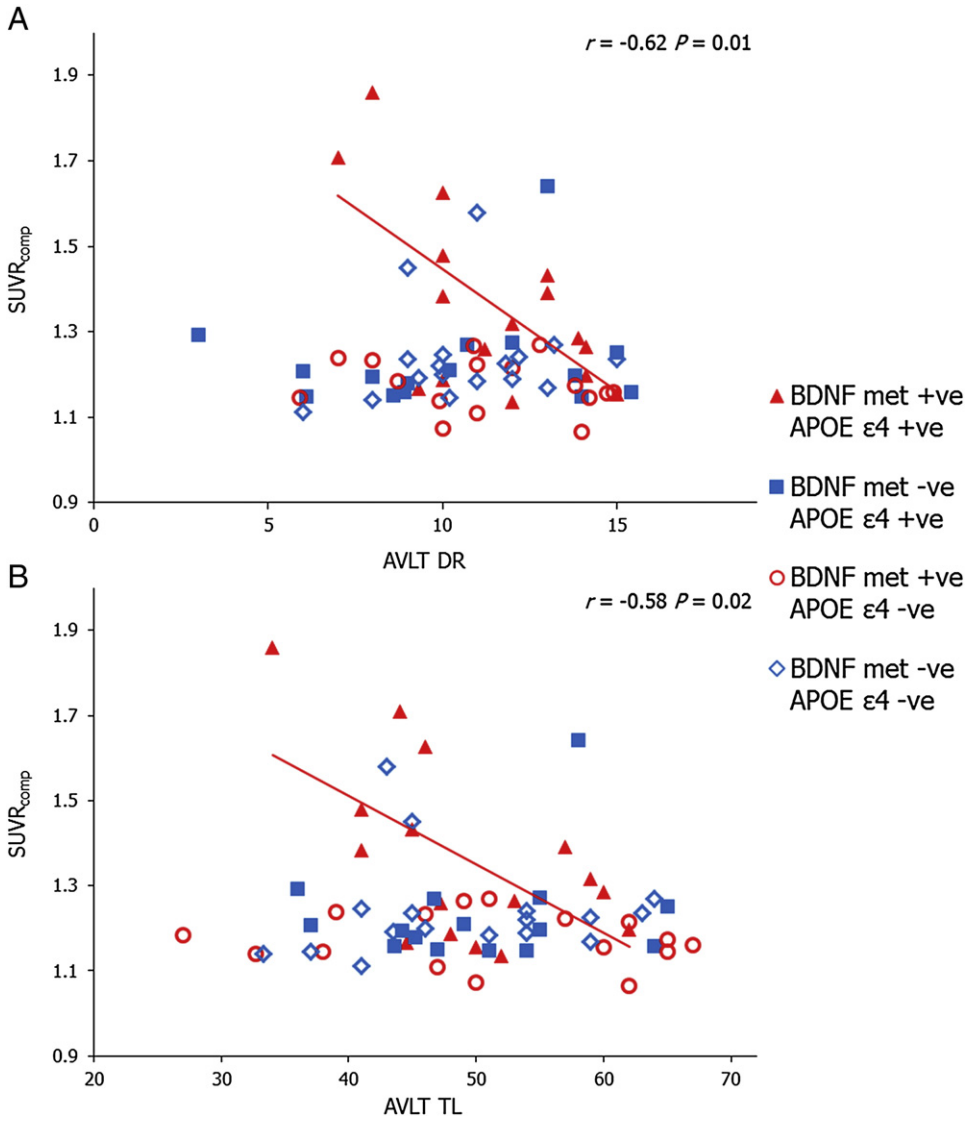

Fig. 4. Correlation between amyloid ligand retention and AVLT delayed recall (DR) and total learning (TL) scores. (A) Negative correlation between $SUVR_{comp}$ values and AVLT DR scores in the BDNF *met* +ve/APOE ε4 +ve group (red line). (B) Negative correlation between $SUVR_{comp}$ values and AVLT TL scores in the BDNF *met* +ve/APOE ε4 +ve (red line). $SUVR_{comp}$ (Y axis) plotted by AVLT DR or TL scores (X axis): BDNF *met* +ve/APOE ε4 +ve (red triangles), BDNF *met* −ve/APOE ε4 +ve (blue squares), BDNF *met* +ve/APOE ε4 −ve (red circles) and BDNF *met* −ve/APOE ε4 −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 *met* +ve		BDNF *met* −ve		BDNF *met* +ve		BDNF *met* −ve	
	APOE ε4 +ve		APOE ε4 +ve		APOE ε4 −ve		APOE ε4 −ve	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
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

Aβ 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 Aβ 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 Aβ load on episodic memory scores in the BDNF *met* + ve/APOE ε4 + 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; Szeszko 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 *val*66*met* 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 Aβ 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 Aβ(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 Aβ 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 ε4 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.

Acknowledgments

This study was funded by Foundation for Alzheimer Research (SAO-FRMA) grant 09013, 11020, Research Foundation Flanders (FWO, G.0660.09), and KU Leuven (OT/08/056). ^{18}F-flutemetamol was provided by GE Healthcare free of cost for this academic investigator-driven trial. Research of the Antwerp group was funded in part by the Inter-University Attraction Poles program of the Belgian Science Policy Office the Foundation for Alzheimer Research (SAO-FRMA), a Methusalem Excellence Grant of the Flemish Government, the Research Foundation Flanders and the Special Research Fund of the University of Antwerp. RV and KV are senior clinical investigators of the Research Foundation Flanders; NN and KB are postdoctoral fellows of the Research Foundation Flanders; and KA is a doctoral fellow of the Research Foundation Flanders. We would like to thank the staff of the Nuclear Medicine and Radiology Departments at the University Hospitals Leuven, as well as Carine Schildermans, and Tine De Cuyper for their help.

References

Aizenstein, H.J., Nebes, R.D., Saxton, J.A., Price, J.C., Mathis, C.A., Tsopelas, N.D., Ziolko, S.K., James, J.A., Snitz, B.E., Houck, P.R., Bi, W., Cohen, A.D., Lopresti, B.J., DeKosky, S.T., Halligan, E.M., Klunk, W.E., 2008. Frequent amyloid deposition without significant cognitive impairment among the elderly. Archives of Neurology 65, 1509–1517.

Arendt, T., 2001. Disturbance of neuronal plasticity is a critical pathogenetic event in Alzheimer's disease. International Journal of Developmental Neuroscience 19, 231–245.

Ashford, J.W., Jarvik, L., 1985. Alzheimer's disease: does neuron plasticity predispose to axonal neurofibrillary degeneration? The New England Journal of Medicine 313, 388–389.

Braak, H., Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathologica 82, 239–259.

Buckner, R.L., Sepulcre, J., Talukdar, T., Krienen, F.M., Liu, H., Hedden, T., Andrews-Hanna, J.R., Sperling, R.A., Johnson, K.A., 2009. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. Journal of Neuroscience 29, 1860–1873.

Bueller, J.A., Aftab, M., Sen, S., Gomez-Hassan, D., Burmeister, M., Zubieta, J.-K., 2006. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biological Psychiatry 59, 812–815.

Cheeran, B., Talelli, P., Mori, F., Koch, G., Suppa, A., Edwards, M., Houlden, H., Bhatia, K., Greenwood, R., Rothwell, J.C., 2008. A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. The Journal of Physiology 586, 5717–5725.

Chen, Z.-Y., Patel, P.D., Sant, G., Meng, C.-X., Teng, K.K., Hempstead, B.L., Lee, F.S., 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. Journal of Neuroscience 24, 4401–4411.

Chételat, G., Villemagne, V.L., Bourgeat, P., Pike, K.E., Jones, G., Ames, D., Ellis, K.A., Szoeke, C., Martins, R.N., O'Keefe, G.J., Salvado, O., Masters, C.L., Rowe, C.C., 2010. Relationship between atrophy and beta-amyloid deposition in Alzheimer disease. Annals of Neurology 67, 317–324.

Chételat, G., Villemagne, V.L., Pike, K.E., Ellis, K.A., Bourgeat, P., Jones, G., O'Keefe, G.J., Salvado, O., Szoeke, C., Martins, R.N., Ames, D., Masters, C.L., Rowe, C.C., and the Australian Imaging Biomarkers and Lifestyle Study of Ageing (AIBL) Research Group, L.S., 2011. Independent contribution of temporal beta-amyloid deposition to memory decline in the pre-dementia phase of Alzheimer's disease. Brain 134, 798–807.

Chiang, M.-C., Avedissian, C., Barysheva, M., Toga, A.W., McMahon, K.L., de Zubicaray, G.I., Wright, M.J., Thompson, P.M., 2009. Extending genetic linkage analysis to diffusion tensor images to map single gene effects on brain fiber architecture. Medical Image Computing and Computer Assisted Intervention 12, 506–513.

Christensen, R., Marcussen, A.B., Wörtwein, G., Knudsen, G.M., Aznar, S., 2008. Abeta(1–42) injection causes memory impairment, lowered cortical and serum BDNF levels, and decreased hippocampal 5-HT(2A) levels. Experimental Neurology 210, 164–171.

Connor, B., Young, D., Yan, Q., Faull, R.L., Synek, B., Dragunow, M., 1997. Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Molecular Brain Research 49, 71–81.

Cordell, H.J., 2009. Detecting gene–gene interactions that underlie human diseases. Nature Reviews Genetics 10, 392–404.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., Pericak-Vance, M.A., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921–923.

Dennis, N.A., Cabeza, R., Need, A.C., Waters-Metenier, S., Goldstein, D.B., LaBar, K.S., 2011. Brain-derived neurotrophic factor val66met polymorphism and hippocampal activation during episodic encoding and retrieval tasks. Hippocampus 21, 980–989.
Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.
Erickson, K.I., Prakash, R.S., Voss, M.W., Chaddock, L., Heo, S., McLaren, M., Pence, B.D., Martin, S.A., Vieira, V.J., Woods, J.A., McAuley, E., Kramer, A.F., 2010. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. Journal of Neuroscience 30, 5368–5375.
Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., Kramer, A.F., 2011. Exercise training increases size of hippocampus and improves memory. Proceedings of the National Academy of Sciences of the United States of America 108, 3017–3022.
Ferrer, I., Marín, C., Rey, M.J., Ribalta, T., Goutan, E., Blanco, R., Tolosa, E., Martí, E., 1999. BDNF and full-length and truncated TrkB expression in Alzheimer disease. implications in therapeutic strategies. Journal of Neuropathology and Experimental Neurology 58, 729–739.
Fleisher, A.S., Chen, K., Liu, X., Roontiva, A., Thiyyagura, P., Ayutyanont, N., Joshi, A.D., Clark, C.M., Mintun, M.A., Pontecorvo, M.J., Doraiswamy, P.M., Johnson, K.A., Skovronsky, D.M., Reiman, E.M., 2011. Using positron emission tomography and florbetapir F18 to image cortical amyloid in patients with mild cognitive impairment or dementia due to Alzheimer disease. Archives of Neurology 68, 1404–1411.
Fleisher, A.S., Chen, K., Liu, X., Ayutyanont, N., Roontiva, A., Thiyyagura, P., Protas, H., Joshi, A.D., Sabbagh, M., Sadowsky, C.H., Sperling, R.A., Clark, C.M., Mintun, M.A., Pontecorvo, M.J., Coleman, R.E., Doraiswamy, P.M., Johnson, K.A., Carpenter, A.P., Skovronsky, D.M., Reiman, E.M., 2013. Apolipoprotein E $\varepsilon 4$ and age effects on florbetapir positron emission tomography in healthy aging and Alzheimer disease. Neurobiology of Aging 34, 1–12.
Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. Journal of Psychiatry Research 12, 189–198.
Gorski, J.A., Zeiler, S.R., Tamowski, S., Jones, K.R., 2003. Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. Journal of Neuroscience 23, 6856–6865.
Hajek, T., Kopecek, M., Höschl, C., 2012. Reduced hippocampal volumes in healthy carriers of brain-derived neurotrophic factor val66met polymorphism: meta-analysis. The World Journal of Biological Psychiatry 13, 178–187.
Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F., Weinberger, D.R., 2003. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. Journal of Neuroscience 23, 6690–6694.
Herholz, K., Salmon, E., Perani, D., Baron, J.C., Holthoff, V., Frlich, L., Schnknecht, P., Ito, K., Mielke, R., Kalbe, E., ZÃ¼ndorf, G., Delbeuck, X., Pelati, O., Anchisi, D., Fazio, F., Kerrouche, N., Desgranges, B., Eustache, F., Beuthien-Baumann, B., Menzel, C., SchrÃ¶der, J., Kato, T., Arahata, Y., Henze, M., Heiss, W.D., 2002. Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. NeuroImage 17, 302–316.
Hock, C., Heese, K., Hulette, C., Rosenberg, C., Otten, U., 2000. Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. Archives of Neurology 57, 846–851.
Holsinger, R.M., Schnarr, J., Henry, P., Castelo, V.T., Fahnestock, M., 2000. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. Brain Research. Molecular Brain Research 76, 347–354.
Huang, R., Huang, J., Cathcart, H., Smith, S., Poduslo, S.E., 2007. Genetic variants in brain-derived neurotrophic factor associated with Alzheimer's disease. Journal of Medical Genetics 44, e66.
Koole, M., Lewis, D.M., Buckley, C., Nelissen, N., Vandenbulcke, M., Brooks, D.J., Vandenberghe, R., Van Laere, K., 2009. Whole-body biodistribution and radiation dosimetry of ^{18}F-GE067: a radioligand for in vivo brain amyloid imaging. Journal of Nuclear Medicine 50, 818–822.
Laske, C., Stransky, E., Leyhe, T., Eschweiler, G.W., Wittorf, A., Richartz, E., Bartels, M., Buchkremer, G., Schott, K., 2006. Stage-dependent BDNF serum concentrations in Alzheimer's disease. Journal of Neural Transmission 113, 1217–1224.
Li, Y., Luikart, B.W., Birnbaum, S., Chen, J., Kwon, C.-H., Kernie, S.G., Bassel-Duby, R., Parada, L.F., 2008. Trkb regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. Neuron 59, 399–412.
McAllister, T.W., Tyler, A.L., Flashman, L.A., Rhodes, C.H., McDonald, B.C., Saykin, A.J., Tosteson, T.D., Tsongalis, G.J., Moore, J.H., 2012. Polymorphisms in the brain-derived neurotrophic factor gene influence memory and processing speed one month after brain injury. Journal of Neurotrauma 29, 1111–1118.
Mesulam, M.M., 1999. Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. Neuron 24, 521–529.
Mesulam, M.M., 2000. A plasticity-based theory of the pathogenesis of Alzheimer's disease. Annals of the New York Academy of Sciences 924, 42–52.
Mesulam, M.M., Van Hoesen, G.W., Pandya, D.N., Geschwind, N., 1977. Limbic and sensory connections of the inferior parietal lobule (area PG) in the rhesus monkey: a study with a new method for horseradish peroxidase histochemistry. Brain Research 136, 393–414.
Mintun, M.A., Larossa, G.N., Sheline, Y.I., Dence, C.S., Lee, S.Y., Mach, R.H., Klunk, W.E., Mathis, C.A., DeKosky, S.T., Morris, J.C., 2006. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology 67, 446–452.
Miyajima, F., Ollier, W., Mayes, A., Jackson, A., Thacker, N., Rabbitt, P., Pendleton, N., Horan, M., Payton, A., 2008. Brain-derived neurotrophic factor polymorphism Val66Met influences cognitive abilities in the elderly. Genes, Brain, and Behavior 7, 411–417.
Montag, C., Weber, B., Fliessbach, K., Elger, C., Reuter, M., 2009. The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. Psychological Medicine 39, 1831–1839.
Mormino, E.C., Kluth, J.T., Madison, C.M., Rabinovici, G.D., Baker, S.L., Miller, B.L., Koeppe, R.A., Mathis, C.A., Weiner, M.W., Jagust, W.J., 2009. Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. Brain 132, 1310–1323.
Mormino, E.C., Smiljic, A., Hayenga, A.O., Onami, S.H., Greicius, M.D., Rabinovici, G.D., Janabi, M., Baker, S.L., Yen, I.V., Madison, C.M., Miller, B.L., Jagust, W.J., 2011. Relationships between beta-amyloid and functional connectivity in different components of the default mode network in aging. Cerebral Cortex 21, 2399–2407.
Morris, J.C., Ernesto, C., Schafer, K., Coats, M., Leon, S., Sano, M., Thal, L.J., Woodbury, P., 1997. Clinical dementia rating training and reliability in multicenter studies: the Alzheimer's Disease Cooperative Study experience. Neurology 48, 1508–1510.
Morris, J.C., Roe, C.M., Xiong, C., Fagan, A.M., Goate, A.M., Holtzman, D.M., Mintun, M.A., 2010. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Annals of Neurology 67, 122–131.
Müller-Gärtner, H.W., Links, J.M., Prince, J.L., Bryan, R.N., McVeigh, E., Leal, J.P., Davatzikos, C., Frost, J.J., 1992. Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. Journal of Cerebral Blood Flow and Metabolism 12, 571–583.
Nagahara, A.H., Merrill, D.A., Coppola, G., Tsukada, S., Schroeder, B.E., Shaked, G.M., Wang, L., Blesch, A., Kim, A., Conner, J.M., Rockenstein, E., Chao, M.V., Koo, E.H., Geschwind, D., Masliah, E., Chiba, A.A., Tuszynski, M.H., 2009. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nature Medicine 15, 331–337.
Nelissen, N., Vandenbulcke, M., Fannes, K., Verbruggen, A., Peeters, R., Dupont, P., Van Laere, K., Bormans, G., Vandenberghe, R., 2007. Abeta amyloid deposition in the language system and how the brain responds. Brain 130, 2055–2069.
Nelissen, N., Van Laere, K., Thurfjell, L., Owenius, R., Vandenbulcke, M., Koole, M., Bormans, G., Brooks, D.J., Vandenberghe, R., 2009. Phase 1 study of the Pittsburgh compound B derivative ^{18}F-flutemetamol in healthy volunteers and patients with probable Alzheimer disease. Journal of Nuclear Medicine 50, 1251–1259.
Nemoto, K., Ohnishi, T., Mori, T., Moriguchi, Y., Hashimoto, R., Asada, T., Kunugi, H., 2006. The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. Neuroscience Letters 397, 25–29.
Okuno, H., Tokuyama, W., Li, Y.X., Hashimoto, T., Miyashita, Y., 1999. Quantitative evaluation of neurotrophin and trk mRNA expression in visual and limbic areas along the occipito-temporo-hippocampal pathway in adult macaque monkeys. The Journal of Comparative Neurology 408, 378–398.
Osada, T., Adachi, Y., Kimura, H.M., Miyashita, Y., 2008. Towards understanding of the cortical network underlying associative memory. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 363, 2187–2199.
Pezawas, L., Verchinski, B.A., Mattay, V.S., Callicott, J.H., Kolachana, B.S., Straub, R.E., Egan, M.F., Meyer-Lindenberg, A., Weinberger, D.R., 2004. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. Journal of Neuroscience 24, 10099–10102.
Phillips, H.S., Hains, J.M., Armanini, M., Laramee, G.R., Johnson, S.A., Winslow, J.W., 1991. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron 7, 695–702.
Pike, K.E., Savage, G., Villemagne, V.L., Ng, S., Moss, S.A., Maruff, P., Mathis, C.A., Klunk, W.E., Masters, C.L., Rowe, C.C., 2007. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. Brain 130, 2837–2844.
Rebeck, G.W., Reiter, J.S., Strickland, D.K., Hyman, B.T., 1993. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. Neuron 11, 575–580.
Reiman, E.M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., Ayutyanont, N., Keppler, J., Reeder, S.A., Langbaum, J.B.S., Alexander, G.E., Klunk, W.E., Mathis, C.A., Price, J.C., Aizenstein, H.J., DeKosky, S.T., Caselli, R.J., 2009. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America 106, 6820–6825.
Rentz, D.M., Locascio, J.J., Becker, J.A., Moran, E.K., Eng, E., Buckner, R.L., Sperling, R.A., Johnson, K.A., 2010. Cognition, reserve, and amyloid deposition in normal aging. Annals of Neurology 67, 353–364.
Resnick, S.M., Sojkova, J., Zhou, Y., An, Y., Ye, W., Holt, D.P., Dannals, R.F., Mathis, C.A., Klunk, W.E., Ferrucci, L., Kraut, M.A., Wong, D.F., 2010. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. Neurology 74, 807–815.
Rowe, C.C., Ng, S., Ackermann, U., Gong, S.J., Pike, K., Savage, G., Cowie, T.F., Dickinson, K.L., Maruff, P., Darby, D., Smith, C., Woodward, M., Merory, J., Tochon-Danguy, H., O'Keefe, G., Klunk, W.E., Mathis, C.A., Price, J.C., Masters, C.L., Villemagne, V.L., 2007. Imaging beta-amyloid burden in aging and dementia. Neurology 68, 1718–1725.
Rowe, C.C., Ellis, K.A., Rimajova, M., Bourgeat, P., Pike, K.E., Jones, G., Fripp, J., Tochon-Danguy, H., Morandeau, L., O'Keefe, G., Price, R., Raniga, P., Robins, P., Acosta, O., Lenzo, N., Szoeke, C., Salvado, O., Head, R., Martins, R., Masters, C.L., Ames, D., Villemagne, V.L., 2010. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiology of Aging 31, 1275–1283.
Seltzer, B., Pandya, D.N., 1994. Parietal, temporal, and occipital projections to cortex of the superior temporal sulcus in the rhesus monkey: a retrograde tracer study. The Journal of Comparative Neurology 343, 445–463.

Sperling, R.A., Aisen, P.S., Beckett, L.A., Bennett, D.A., Craft, S., Fagan, A.M., Iwatsubo, T., Jack Jr., C.R., Kaye, J., Montine, T.J., Park, D.C., Reiman, E.M., Rowe, C.C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M.C., Thies, B., Morrison-Bogorad, M., Wagster, M.V., Phelps, C.H., 2011. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 7, 280–292.

Sublette, M.E., Baca-Garcia, E., Parsey, R.V., Oquendo, M.A., Rodrigues, S.M., Galfalvy, H., Huang, Y.-Y., Arango, V., Mann, J.J., 2008. Effect of BDNF val66met polymorphism on age-related amygdala volume changes in healthy subjects. Progress in Neuropsychopharmacology and Biological Psychiatry 32, 1652–1655.

Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R.M., Kane, J.M., Goldman, D., Malhotra, A.K., 2005. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Molecular Psychiatry 10, 631–636.

Teter, B., 2004. ApoE-dependent plasticity in Alzheimer's disease. Journal of Molecular Neuroscience 23, 167–179.

Thambisetty, M., An, Y., Nalls, M., Sojkova, J., Swaminathan, S., Zhou, Y., Singleton, A.B., Wong, D.F., Ferrucci, L., Saykin, A.J., Resnick, S.M., for the Baltimore Longitudinal Study of Aging and, the Alzheimer's Disease Neuroimaging Initiative, 2012. Effect of Complement CR1 on brain amyloid burden during aging and its modification by APOE genotype. Biological Psychiatry 73, 422–428.

Thöni, H., 1977. Testing the difference between two coefficients of correlation. Biometrical Journal 19, 355–359.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15, 273–289.

Vandenberghe, R., Van Laere, K., Ivanoiu, A., Salmon, E., Bastin, C., Triau, E., Hasselbalch, S., Law, I., Andersen, A., Korner, A., Minthon, L., Garraux, G., Nelissen, N., Bormans, G., Buckley, C., Owenius, R., Thurfjell, L., Farrar, G., Brooks, D.J., 2010. ^{18}F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: a phase 2 trial. Annals of Neurology 68, 319–329.

Vandenberghe, R., Nelissen, N., Salmon, E., Ivanoiu, A., Hasselbalch, S., Andersen, A., Korner, A., Minthon, L., Brooks, D.J., Van Laere, K., Dupont, P., 2013. Binary classification of (18)F-flutemetamol PET using machine learning: comparison with visual reads and structural MRI. NeuroImage 64, 517–525.

Vemuri, P., Wiste, H.J., Weigand, S.D., Knopman, D.S., Shaw, L.M., Trojanowski, J.Q., Aisen, P.S., Weiner, M., Petersen, R.C., Jack Jr., C.R., 2010. Effect of apolipoprotein E on biomarkers of amyloid load and neuronal pathology in Alzheimer disease. Annals of Neurology 67, 308–316.

Villemagne, V.L., Pike, K.E., Chételat, G., Ellis, K.A., Mulligan, R.S., Bourgeat, P., Ackermann, U., Jones, G., Szoeke, C., Salvado, O., Martins, R., O'Keefe, G., Mathis, C.A., Klunk, W.E., Ames, D., Masters, C.L., Rowe, C.C., 2011. Longitudinal assessment of Aβ and cognition in aging and Alzheimer disease. Annals of Neurology 69, 181–192.

Voineskos, A.N., Lerch, J.P., Felsky, D., Shaikh, S., Rajji, T.K., Miranda, D., Lobaugh, N.J., Mulsant, B.H., Pollock, B.G., Kennedy, J.L., 2011. The brain-derived neurotrophic factor Val66Met polymorphism and prediction of neural risk for Alzheimer disease. Archives of General Psychiatry 68, 198–206.

Webster, M.J., Herman, M.M., Kleinman, J.E., Shannon Weickert, C., 2006. BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. Gene Expression Patterns 6, 941–951.

Xiong, C., Roe, C.M., Buckles, V., Fagan, A., Holtzman, D., Balota, D., Duchek, J., Storandt, M., Mintun, M., Grant, E., Snyder, A.Z., Head, D., Benzinger, T.L.S., Mettenburg, J., Csernansky, J., Morris, J.C., 2011. Role of family history for Alzheimer biomarker abnormalities in the adult children study. Archives of Neurology 68, 1313–1319.

Xu, C., Wang, Z., Fan, M., Liu, B., Song, M., Zhen, X., Jiang, T., and Alzheimer's Disease Neuroimaging Initiative, 2010. Effects of BDNF Val66Met polymorphism on brain metabolism in Alzheimer's disease. NeuroReport 21, 802–807.

Yang, X., Liu, P., Sun, J., Wang, G., Zeng, F., Yuan, K., Liu, J., Dong, M., von Deneen, K.M., Qin, W., Tian, J., 2012. Impact of brain-derived neurotrophic factor val66met polymorphism on cortical thickness and voxel-based morphometry in healthy chinese young adults. PLoS One 7, e37777.