Distinct Patterns Link the BDNF Val66Met Polymorphism to Alzheimer's Disease Pathology

Journal of Alzheimer's Disease 88 (2022) 447–453
DOI 10.3233/JAD-215353
IOS Press

Short Communication

Distinct Patterns Link the *BDNF* Val66Met Polymorphism to Alzheimer's Disease Pathology

Joost M. Riphagen[a,b,c,d,*], Roy W.E. van Hooren[a,d], Gunter Kenis[d], Frans R.J. Verhey[a] and Heidi I.L. Jacobs[a,c,d]

[a]*Alzheimer Center Limburg, School for Mental Health and Neuroscience (MHeNS), Maastricht University, Maastricht, the Netherlands*

[b]*Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Department of Radiology, Charlestown, MA, USA*

[c]*Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA*

[d]*Faculty of Health, Medicine and Life Sciences; School for Mental Health and Neuroscience, Department of Psychiatry and Neuropsychology, Maastricht University, Maastricht, the Netherlands*

Accepted 3 May 2022
Pre-press 28 May 2022

Abstract. The brain-derived neurotropic growth factor (*BDNF*) gene has been linked to dementia, inflammation, and Apolipoprotein E (*APOE*) ε4 status. We used cerebrospinal fluid (CSF) amyloid-β ($A\beta)_{42}$ and phosphorylated tau (p-tau) to investigate associations with *BDNF* polymorphisms and modifications by *APOE* ε4 or inflammation in a memory clinic population ($n = 114$; subjective cognitive decline, mild cognitive impairment, Alzheimer's disease). We found distinct pathways to Alzheimer's disease pathology: Val-Met displayed lower CSF-$A\beta_{42}$ in *APOE* ε4+ carriers, independent of p-tau, while Val-Val displayed greater p-tau at higher IL-6 and sub-threshold $A\beta_{42}$. This may contribute to resolving some inconsistencies in the BDNF literature and provide possible inroads to specific Aβ and tau interventions depending on *BDNF* polymorphism.

Keywords: Alzheimer's disease, amyloid-β, brain-derived neurotropic growth factor, inflammation, interleukin 6, phosphorylated tau

INTRODUCTION

Brain-derived neurotropic growth factor (BDNF), a protein encoded by the *BDNF* gene, plays an important role in neuronal maintenance, neuronal survival, neurotransmitter regulation, and in long term potentiation and plasticity, especially in the hippocampus, a structure that plays a critical role in memory formation and Alzheimer's disease (AD) [1–3]. The single nucleotide polymorphisms (SNP) of *BDNF* Val66Met may be a genetic risk factor for dementia. Previous research has linked *BDNF* SNPs with AD and observed worse cognitive performance in Met allele carriers diagnosed with autosomal dominantly inherited AD, caused by mutations in the amyloid precursor protein, presenilin-1, and presenilin-2 [4].

*Correspondence to: Joost M. Riphagen, MD, PhD, Faculty of Health, Medicine and Life Sciences; School for Mental Health and Neuroscience, Department of Psychiatry and Neuropsychology, Alzheimer Center Limburg, Maastricht University, Dr. Tanslaan 12, 6229 ET Maastricht, the Netherlands. E-mail: jriph@yahoo.co.uk.

ISSN 1387-2877/$35.00

Likewise, in a large middle-aged cognitively healthy cohort enriched for AD risk, carriage of the Met-allele was associated with steeper decline in episodic memory and executive function [5].

However, so far studies linking *BDNF* to AD pathophysiology remain inconclusive. Previous studies have shown an association of lower serum BDNF to widespread $A\beta_{42}$ in PET studies [6] and faster disease progression in Met carriers, and this effect is magnified by the presence of an Apolipoprotein E (*APOE)* ε4 allele [7, 8]. On the other hand, clinically healthy Val-Val individuals showed lower hippocampal volume compared to heterozygotes and with a dose-response effect with the number of *APOE* ε4 alleles [9]. Older meta-analyses in both healthy and neuropsychiatric populations found no effects of Val66Met on brain volume. It should be noted that the role of amyloid-β (Aβ) positivity was not considered in the meta-analyses, and one recurring observation across several recent studies is that $A\beta_{42}$ interacts with Met carriership on cognitive decline and hippocampal volume [10, 11].

We hypothesize that these inconsistencies may also be partly due to variability in immune responses. Higher immune response in Met carriers was associated with higher depression symptoms within the cognitive dimension [12]. Interleukin 6 (IL-6) is a pleiotropic cytokine with both pro- and anti-inflammatory actions [13] that in animal models also plays a role in memory [14] and neuronal differentiation and maintenance [15]. Plasma IL-6 has been associated with chronic neuroinflammation, is elevated in AD [16], plays a central role in the activation of microglia, negatively affects the clearance system of $A\beta_{42}$ [17, 18], and modulates the relationship between AD pathology and vascular/blood brain barrier damage [19]. IL-6 was shown *in vitro* to have a reciprocal relation supporting neuronal survival in combination with endogenous BDNF [20]. Animal models showed that chronically increasing IL-6 markedly decreases neurogenesis [21].

Inflammation is associated with, and possibly exacerbates, AD pathology, and based on the above observations, we posit that *BDNF* Val66Met may moderate this association [19, 22–24]. Thus, we sought to examine the relationship of the *BDNF* polymorphism with $A\beta_{42}$ versus phosphorylated tau (p-tau), and whether these associations were modified by *APOE* ε4, or inflammation (specifically IL-6) in a memory clinic population.

MATERIALS AND METHODS

A convenience sample of 114) subjects was recruited from the memory clinic of the Maastricht University Medical Center (MUMC) for this cross-sectional study. This group includes individuals diagnosed with subjective cognitive decline (SCD), mild cognitive impairment (MCI), and AD dementia. Diagnoses were made by experienced physicians based on the Petersen core clinical criteria for MCI [25] and AD dementia. Criteria for SCD included self-reported presence of subjective cognitive complaints and endorsing the question "Do you think your memory is becoming worse" and absence of impairments on cognitive tests (defined as a score below –1.5 SD of the age-, sex-, and education-adjusted mean) [26]. Exclusion criteria were major neurological disease, clinical diagnosis of other neurodegenerative disorders (e.g., frontotemporal dementia), recent transient ischemic attack or cerebrovascular accident (< 2 years), history of psychiatric disorders, and alcohol or drug abuse. All patients provided informed written consent and the study protocols were approved by the Medical Ethics Committee of the MUMC in confirmation with the declaration of Helsinki.

CSF and blood analyses

CSF was collected via a lumbar puncture in the L3 to L5 vertebral interspaces, centrifuged, aliquoted, and stored at –80°C in polypropylene tubes. Biochemical analysis of CSF $A\beta_{42}$, total tau, and p-tau_{181p} (Innotest ELISA, Innogenetics, Ghent, Belgium) was done following standardized protocol and blinded to diagnostic information. *APOE* ε4 and *BDNF* SNP genotyping was determined on genomic DNA using polymerase chain reaction and restriction fragment length polymorphism at the MUMC. *BDNF* allele distribution did not deviate from the Hardy-Weinberg equilibrium [27]. *Met-Met* homozygotes ($n = 3$) in this sample were pooled with the Val-Met. Cytokine interleukin IL-6 was analyzed from serum using multiplex BD cytometric bead array (BD-biosciences, Franklin Lakes, NJ, USA). Samples were collected between 2011 and 2017 and stored at –80°C in a biobank during this time (aliquoted to avoid freeze thaw cycles). Prolonged storage, even at –80°C, can differentially affect the level of cytokines [28] and therefore we linearly adjusted for storage times calculated from the day of entry in the biobank to the date of analysis [19].

Table 1
Demographics stratified by BDNF genotype

		BDNF Val66met polymorphism		*p*
		Val-Met	Val-Val	
N = 114		45	69	
Sex = Female	*N* (%)	13 (28.9)	20 (29.0)	1.000[†]
Age	(Mean (SD))	63.49 (8.47)	62.64 (9.44)	0.625
range		47–78	38–89	
APOE ε4+	*N* (%)	21(46.6)	34(49.2)	0.935[†]
IL-6	(Mean (SD))	1.43 (0.85)	1.35 (0.92)	0.645
CSF $A\beta_{1\text{-}42}$	(Mean (SD))	911.07 (356.73)	930.91 (365.80)	0.775
CSF p-tau $_{181}$	(Mean (SD))	56.73 (27.79)	65.83 (36.71)	0.136
Education		3.91 (1.93)	3.88 (1.95)	0.934
CDR total				0.317[‡]
0		1	7	
0.5		41	55	
1		3	7	
Diagnosis	(% of total)			0.866[‡]
AD		7 (6.1)	12 (10.5)	
MCI		16 (14.0)	22 (19.3)	
SCD		22 (19.2)	35 (30.7)	
	Amyloid status[##] (%)	CSF $A\beta_{1\text{-}42}$ Positive	CSF $A\beta_{1\text{-}42}$ Negative	
Polymorphism				
Met-Met		0	2.63	
Val-Mat		5.26	31.57	
Val-Val		5.26	55.26	

Education is measured using an evaluation system based on Verhage (1964) and the Standard Classification of Education of the Dutch Central Bureau of Statistics (CBS, 2014). It is equivalent to the International Standard Classification of Education (UNESCO, 1997). [†]χ^{2-}test for dichotomous variables, *t*-test for continuous variables, [‡]Fisher Exact Test for count data; [##]$A\beta_{42}$ CSF positive clinical cutoff at < 500.

Statistical analyses

Statistical analyses were conducted in R version 3.6.2 (http://www.Rproject.org). Demographics are provided in Table 1. For each biomarker ($A\beta_{42}$, Tau, p-tau) we set up the following hierarchical multivariable regression models: 1) differences between *BDNF* Val-Val and Val-Met for each biomarker; 2) interaction between the *BDNF a*nd *APOE* ε4 genotype; 3) interaction between the *BDNF* genotype and IL-6 levels (as continuous variable). Each model was adjusted for age, sex, *APOE* ε4 and storage time (and diagnosis in a separate step); and 4) interaction models additionally adjusted for either $A\beta_{42}$ or p-tau to determine whether the effect was independent of the other pathology. *Post hoc*, we also interacted *BDNF* genotype by IL-6 by $A\beta_{42}$ on p-tau and performed Johnson-Neyman analyses [29, 30] using simple slopes to determine at which range of pathology the association was significant. To ensure effects where robust, linear models were bootstrapped (30,000 replications) and *p*-values were derived from the resulting distribution. In addition, FDR correction at alpha < 0.05 was implemented to adjust for multiple comparisons.

RESULTS

Demographics stratified by *BDNF* allele are listed in Table 1. Our sample had a mean age of 62.97 (SD = 9.04) years, 71% of the participants were male, 46.6% of Val-Met, and 49.2% Val-Val carried at least one *APOE* ε4 allele (no difference $p = 0.935$) There was no difference in demographics between *BDNF* Val-Val and Val-Met carriers (Table 1).

BNDF-Val66Met effects (covaried for APOE ε4, age, and sex)

There is no direct association between *BDNF* and $A\beta_{42}$ ($t_{109} = 0.22$, $p_{FDR} = 0.822$) or total tau ($t_{109} = 1.12$, $p_{FDR}0.265$). However, *BDNF* Val-Val carriers exhibited higher p-tau values ($t_{109} = 2.04$, $p_{FDR} = 0.043$). There is a trend level association between *BNDF* and p-tau ($t_{109} = 1.87$, $p_{FDR} = 0.08$) when $A\beta_{42}$ is added as a covariate ($t_{109} = 2.04$, $p = 0.04$ $p_{FDR} = 0.08$), suggesting shared variance in the relation of p-tau and $A\beta_{42}$. There are no differences in mean IL-6 level in the Val-Met group (1.430) and in the Val-Val group (1.352) ($t_{99} = 0.46211$, $p = 0.645$). There are no differences in IL-6 levels

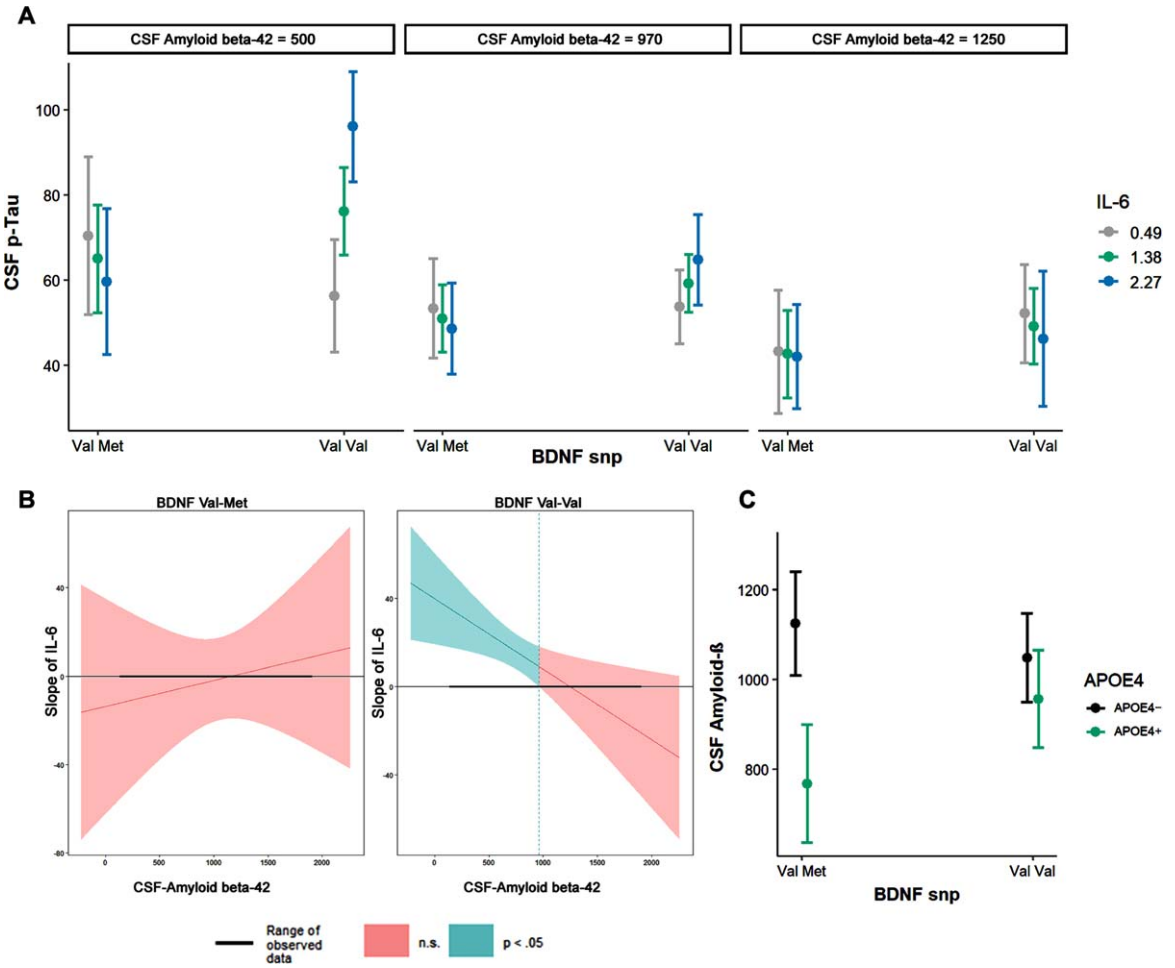

Fig. 1. A) *BDNF* polymorphism SNP show different associations between IL-6 and CSF p-tau, dependent on $A\beta_{42}$ (continuous variable, plotted marginal effects at mean ± 1SD). B) Johnson-Neyman plot showing the level of CSF $A\beta_{42}$ (< 963 pg/ml) where the interaction BDNF*IL6*$A\beta_{42}$ becomes significant. Clinical threshold for $A\beta_{42}$ is 500 pg/ml and p-tau (85 pg/ml). C) CSF $A\beta_{42}$ levels differ among *BDNF* by *APOE* ε4 SNPs.

for *APOE* ε4 groups (*APOE* ε4– 1.50/*APOE* ε4+ 1.25), ($t_{112} = 1.47$, $p = 0.14$). The differences of CSF $A\beta_{42}$ in *APOE* ε4 groups were significant (*APOE* ε4– 1049/*APOE* ε4+ 787), ($t_{112} = 4.15$, $p \leq 0.0001$).

BDNF by APOE ε4 interaction (covaried for age and sex)

When looking at the interaction of *BDNF* and *APOE* ε4, we found lower CSF $A\beta_{42}$ in Val-Met carriers who also carried the *APOE* ε4 allele ($t_{108} = 2.19$, $p = 0.03$ ($p_{FDR} = 0.03$), Fig. 1C), compared to Val-Val carriers with or without *APOE* ε4 carriage. When adding p-tau as covariate, the interaction between *BDNF* and *APOE* ε4 remained significant ($t_{107} = 2.62$, $p = 0.009$ ($p_{FDR} = 0.018$)), indicating that this effect was independent of p-tau. If the sample was *post hoc* restricted to MCI and SCD, the interaction between *BDNF* and *APOE* ε4 was not significant ($t_{85} = 1.83$, $p_{FDR} = 0.11$). The interaction between *BDNF* and *APOE* ε4 was not significant for t-tau ($t_{108} = 1.09$, $p = 0.275$) or p-tau ($t_{108} = 0.648$ $p_{FDR} = 0.56$).

BNDF by Il-6 interaction (covaried for APOE ε4, time in storage, age, and sex)

The interaction between BDNF and IL-6 showed no significant associations with $A\beta_{42}$ ($t_{106} = 0.74$, $p_{FDR} = 0.45$) and t-tau ($t_{106} = 1.78$, $p_{FDR} = 0.07$). Higher p-tau was associated with *BDNF* Val-Val and higher IL-6 ($t_{106} = 2.957$, $p_{FDR} = 0.01$). When

$A\beta_{42}$ was added as a covariate, this association did not change ($t_{106} = 2.79$, $p_{FDR} = 0.02$); likewise adding diagnosis as a covariate did not change results ($t_{96} = -3.037$ $p_{FDR} = 0.02$).

The 3-way interaction of *BDNF*, IL-6, and $A\beta_{42}$ was significant ($t_{102} = -3.00$, $p = 0.0033$, $p_{FDR} = 0.01$, adjusted R-square: 0.4825): Val-Val carriers with lower CSF $A\beta_{42}$ and higher IL-6 levels (Fig. 1A) exhibited higher p-tau levels. The Johnson-Neyman interval revealed that this three-way interaction became significant at $A\beta_{42} < 962.96$ pg/ml ($p_{FDR} < 0.05$, $t = 2.30$; Fig. 1B)

DISCUSSION

In this study we found distinct relationships of *BDNF* polymorphism to either $A\beta_{42}$ or p-tau. We observed that individuals with Val-Met genotype displayed lower CSF $A\beta_{42}$ when also carrying at least one *APOE* ε4 allele, independent of p-tau. However, Val-Val individuals exhibited higher p-tau under higher levels of IL-6, and subthreshold levels of $A\beta_{42}$ (< 963 pg/ml; clinical threshold for $A\beta_{42} = 500$). BDNF plays a crucial role in synaptic plasticity and long-term potentiation in the hippocampus and therefore SNPs of the *BNDF* gene have been of interest for dementia research. These findings could provide new inroads to specific interventions for $A\beta_{42}$ and p-tau depending on *BDNF* polymorphism and immune response.

While only few studies examined the effect of *BDNF* on AD pathology, the literature is consistent in that the relationship between *BDNF* and memory is dependent on amyloid, but potential effect-modification by *APOE* is less clear. In in Aβ-SCD participants, no increased risk of dementia was found, but combined Aβ+ and Val66Met+ did cognitively worse than Aβ+ only [8], with similar findings in unimpaired but increased AD risk [11] suggesting a synergistic effect of Met+ and Aβ. However, no moderating effect of *APOE* ε4 was found on cognition. In the AIBL study, Aβ+ *APOE* ε4+ and Val66Met+ had worse memory performance [7] but interaction effects of *APOE* by *BNDF* on Aβ are not described. Two studies took a similar approach as ours and examined the relationship between *BDNF* Val66Met on AD pathology in cognitively normal older individuals [31, 32] and reported higher PET $A\beta_{42}$ in *APOE* ε4+ in Val66Met+ [32]. In a combined autosomal dominant AD group, lower CSF $A\beta_{42}$ in *APOE* ε4+ participants but no interaction with *BDNF* was found [2]; however, Val66Met+ was associated with hippocampus-frontal connectivity in autosomal dominant AD and AD and stronger associations with Aβ in sporadic AD but not in SCD [33]. Reasons underlying these differences between sporadic AD, preclinical autosomal dominant AD, and SCD might be related to different ages of onset and disease progression.

It is increasingly accepted that inflammation plays an important role in linking concurrent pathologies in AD dementia [19, 34, 35]. IL-6 is a pleiotropic cytokine that has both pro- and anti-inflammatory effects, plays an important part in the innate immune system, can induce hyperphosphorylation of tau in animal models [36], and can interfere with $A\beta_{42}$ clearance [17, 18, 37]. Inflammation and microglial activation lead to production of multiple neurotrophic factors, including BDNF. Sustained immune activation, however, strongly reduces the generation of these neurotropic factors possibly interfering with neuroplasticity [38].

This study has some limitations. This is a medium sized cross-sectional convenience sample from a referral-only university hospital memory clinic in northern Europe. The Val66Met prevalence is extremely dependent (0 to > 70%) on genetic background [39] and the prevalence of the Val66Met polymorphism is approximately 20% in Europe. The locale should be considered when comparing these results with studies conducted in a different genetic background. Both IL-6 and *BDNF* are not specific to any single disease complicating the interpretation of associations in various populations. Larger studies are needed to better model interactions between pathology, genetics, and the innate immune system. We tentatively hypothesize based on this data that trials targeting AD pathology (both Aβ and p-tau), especially using monoclonal antibodies, may have to consider *APOE* status, *BDNF* polymorphisms as well as innate immune system activation. Substantially larger cohorts are needed in other to be able to investigate the downstream effects of *BDNF* on cognition.

Conclusion

Both *BDNF* genotypes are associated with AD pathology, depending on the interaction with either *APOE* ε4 or inflammation. Elevated p-tau was associated with *BDNF* Val-Val carriers with elevated IL-6, with a dose response to $A\beta_{42}$ and starting at subthreshold levels, while lower CSF-$A\beta_{42}$ was observed in *BDNF* Val-Met/*APOE* ε4 carriers, independent of p-tau.

ACKNOWLEDGMENTS

This work was supported by a standard grant of Alzheimer Nederland [#15007, HILJ].

Authors' disclosures available (https://www.j-alz.com/manuscript-disclosures/21-5353r2).

REFERENCES

[1] Bathina S, Das UN (2015) Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci* **11**, 1164-1178.

[2] Lim YY, Hassenstab J, Cruchaga C, Goate A, Fagan AM, Benzinger TL, Maruff P, Snyder PJ, Masters CL, Allegri R, Chhatwal J, Farlow MR, Graff-Radford NR, Laske C, Levin J, McDade E, Ringman JM, Rossor M, Salloway S, Schofield PR, Holtzman DM, Morris JC, Bateman RJ (2016) BDNF Val66Met moderates memory impairment, hippocampal function and tau in preclinical autosomal dominant Alzheimer's disease. *Brain* **139**, 2766-2777.

[3] Leal G, Bramham CR, Duarte CB (2017) BDNF and hippocampal synaptic plasticity. *Vitam Horm* **104**, 153-195.

[4] Lim YY, Hassenstab J, Goate A, Fagan AM, Benzinger TLS, Cruchaga C, McDade E, Chhatwal J, Levin J, Farlow MR, Graff-Radford NR, Laske C, Masters CL, Salloway S, Schofield P, Morris JC, Maruff P, Bateman RJ (2018) Effect of BDNFVal66Met on disease markers in dominantly inherited Alzheimer's disease. *Ann Neurol* **84**, 424-435.

[5] Boots EA, Schultz SA, Clark LR, Racine AM, Darst BF, Koscik RL, Carlsson CM, Gallagher CL, Hogan KJ, Bendlin BB, Asthana S, Sager MA, Hermann BP, Christian BT, Dubal DB, Engelman CD, Johnson SC, Okonkwo OC (2017) *BDNF* Val66Met predicts cognitive decline in the Wisconsin Registry for Alzheimer's Prevention. *Neurology* **88**, 2098-2106.

[6] Hwang KS, Lazaris AS, Eastman JA, Teng E, Thompson PM, Gylys KH, Cole GM, Apostolova LG (2015) Plasma BDNF levels associate with Pittsburgh Compound B binding in the brain. *Alzheimers Dement (Amst)* **1**, 187-193.

[7] Lim YY, Villemagne VL, Laws SM, Pietrzak RH, Snyder PJ, Ames D, Ellis KA, Harrington K, Rembach A, Martins RN, Rowe CC, Masters CL, Maruff P (2015) APOE and BDNF polymorphisms moderate amyloid β-related cognitive decline in preclinical Alzheimer's disease. *Mol Psychiatry* **20**, 1322-1328.

[8] van den Bosch KA, Verberk IMW, Ebenau JL, van der Lee SJ, Jansen IE, Prins ND, Scheltens P, Teunissen CE, Van der Flier WM (2021) BDNF-Met polymorphism and amyloid-beta in relation to cognitive decline in cognitively normal elderly: The SCIENCe project. *Neurobiol Aging* **108**, 146-154.

[9] Vilor-Tejedor N, Operto G, Evans TE, Falcon C, Crous-Bou M, Minguillón C, Cacciaglia R, Milà-Alomà M, Grau-Rivera O, Suárez-Calvet M, Garrido-Martín D, Morán S, Esteller M, Adams HH, Molinuevo JL, Guigó R, Gispert JD (2020) Effect of BDNF Val66Met on hippocampal subfields volumes and compensatory interaction with APOE-ε4 in middle-age cognitively unimpaired individuals from the ALFA study. *Brain Struct Funct* **225**, 2331-2345.

[10] Lim YY, Villemagne VL, Laws SM, Ames D, Pietrzak RH, Ellis KA, Harrington KD, Bourgeat P, Salvado O, Darby D, Snyder PJ, Bush AI, Martins RN, Masters CL, Rowe CC, Nathan PJ, Maruff P (2013) BDNF Val66Met, Aβ amyloid, and cognitive decline in preclinical Alzheimer's disease. *Neurobiol Aging* **34**, 2457-2464.

[11] Boots EA, Schultz SA, Clark LR, Racine AM, Darst BF, Koscik RL, Carlsson CM, Gallagher CL, Hogan KJ, Bendlin BB, Asthana S, Sager MA, Hermann BP, Christian BT, Dubal DB, Engelman CD, Johnson SC, Okonkwo OC (2017) BDNF Val66Met predicts cognitive decline in the Wisconsin Registry for Alzheimer's Prevention. *Neurology* **88**, 2098-2106.

[12] Dooley LN, Ganz PA, Cole SW, Crespi CM, Bower JE (2016) Val66Met BDNF polymorphism as a vulnerability factor for inflammation-associated depressive symptoms in women with breast cancer. *J Affect Disord* **197**, 43-50.

[13] Rothaug M, Becker-Pauly C, Rose-John S (2016) The role of interleukin-6 signaling in nervous tissue. *Biochim Biophys Acta* **1863**, 1218-1227.

[14] Balschun D, Wetzel W, Del Rey A, Pitossi F, Schneider H, Zuschratter W, Besedovsky HO (2004) Interleukin-6: A cytokine to forget. *FASEB J* **18**, 1788-1790.

[15] Kummer KK, Zeidler M, Kalpachidou T, Kress M (2021) Role of IL-6 in the regulation of neuronal development, survival and function. *Cytokine* **144**, 155582.

[16] Swardfager W, Lanctot K, Rothenburg L, Wong A, Cappell J, Herrmann N (2010) A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry* **68**, 930-941.

[17] Hagihara K, Nishikawa T, Isobe T, Song J, Sugamata Y, Yoshizaki K (2004) IL-6 plays a critical role in the synergistic induction of human serum amyloid A (SAA) gene when stimulated with proinflammatory cytokines as analyzed with an SAA isoform real-time quantitative RT-PCR assay system. *Biochem Biophys Res Commun* **314**, 363-369.

[18] Miida T, Yamada T, Seino U, Ito M, Fueki Y, Takahashi A, Kosuge K, Soda S, Hanyu O, Obayashi K, Miyazaki O, Okada M (2006) Serum amyloid A (SAA)-induced remodeling of CSF-HDL. *Biochim Biophys Acta* **1761**, 424-433.

[19] Riphagen JM, Ramakers I, Freeze WM, Pagen LHG, Hanseeuw BJ, Verbeek MM, Verhey FRJ, Jacobs HIL (2020) Linking APOE-epsilon4, blood-brain barrier dysfunction, and inflammation to Alzheimer's pathology. *Neurobiol Aging* **85**, 96-103.

[20] Murphy PG, Borthwick LA, Altares M, Gauldie J, Kaplan D, Richardson PM (2000) Reciprocal actions of interleukin-6 and brain-derived neurotrophic factor on rat and mouse primary sensory neurons. *Eur J Neurosci* **12**, 1891-1899.

[21] Vallières L, Campbell IL, Gage FH, Sawchenko PE (2002) Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci* **22**, 486-492.

[22] Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. *Nat Rev Neurol* **9**, 106-118.

[23] Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, Warner DS, Laskowitz DT (2003) APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* **278**, 48529-48533.

[24] McCarrey AC, Pacheco J, Carlson OD, Egan JM, Thambisetty M, An Y, Ferrucci L, Resnick SM (2014) Interleukin-6 is linked to longitudinal rates of cortical thinning in aging. *Transl Neurosci* **5**, 1-7.

[25] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on

Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.

[26] Geerlings MI, Jonker C, Bouter LM, Ader HJ, Schmand B (1999) Association between memory complaints and incident Alzheimer's disease in elderly people with normal baseline cognition. *Am J Psychiatry* **156**, 531-537.

[27] Edwards AW (2008) G. H. Hardy (1908) and Hardy–Weinberg equilibrium. *Genetics* **179**, 1143-1150.

[28] de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V (2009) Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol* **10**, 52.

[29] Johnson PO, Fay LC (1950) The Johnson-Neyman technique, its theory and application. *Psychometrika* **15**, 349-367.

[30] Bauer DJ, Curran PJ (2005) Probing interactions in fixed and multilevel regression: Inferential and graphical techniques. *Multivariate Behav Res* **40**, 373-400.

[31] Stonnington CM, Velgos SN, Chen Y, Syed S, Huentelman M, Thiyyagura P, Lee W, Richholt R, Caselli RJ, Locke DEC, Lu B, Reiman EM, Su Y, Chen K (2020) Interaction between BDNF Val66Met and APOE4 on biomarkers of Alzheimer's disease and cognitive decline. *J Alzheimers Dis* **78**, 721-734.

[32] Adamczuk K, Weer A-S, Nelissen N, Chen K, Sleegers K, Bettens K, Van Broeckhoven C, Vandenbulcke M, Thiyyagura P, Dupont P, Laere K, Reiman E, Vandenberghe R (2013) Polymorphism of brain derived neurotrophic factor influences β amyloid load in cognitively intact apolipoprotein E ε4 carriers. *Neuroimage Clin* **2**, 512-520.

[33] Franzmeier N, Ren J, Damm A, Monté-Rubio G, Boada M, Ruiz A, Ramirez A, Jessen F, Düzel E, Rodríguez Gómez O, Benzinger T, Goate A, Karch CM, Fagan AM, McDade E, Buerger K, Levin J, Duering M, Dichgans M, Suárez-Calvet M, Haass C, Gordon BA, Lim YY, Masters CL, Janowitz D, Catak C, Wolfsgruber S, Wagner M, Milz E, Moreno-Grau S, Teipel S, Grothe MJ, Kilimann I, Rossor M, Fox N, Laske C, Chhatwal J, Falkai P, Perneczky R, Lee JH, Spottke A, Boecker H, Brosseron F, Fliessbach K, Heneka MT, Nestor P, Peters O, Fuentes M, Menne F, Priller J, Spruth EJ, Franke C, Schneider A, Westerteicher C, Speck O, Wiltfang J, Bartels C, Araque Caballero M, Metzger C, Bittner D, Salloway S, Danek A, Hassenstab J, Yakushev I, Schofield PR, Morris JC, Bateman RJ, Ewers M (2021) The BDNF(Val66Met) SNP modulates the association between beta-amyloid and hippocampal disconnection in Alzheimer's disease. *Mol Psychiatry* **26**, 614-628.

[34] Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimers Dement* **12**, 719-732.

[35] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP (2015) Neuroinflammation in Alzheimer's disease. *Lancet Neurol* **14**, 388-405.

[36] Quintanilla RA, Orellana DI, González-Billault C, Maccioni RB (2004) Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. *Exp Cell Res* **295**, 245-257.

[37] Marsh SE, Abud EM, Lakatos A, Karimzadeh A, Yeung ST, Davtyan H, Fote GM, Lau L, Weinger JG, Lane TE, Inlay MA, Poon WW, Blurton-Jones M (2016) The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. *Proc Natl Acad Sci U S A* **113**, E1316-E1325.

[38] Ising C, Heneka MT (2018) Functional and structural damage of neurons by innate immune mechanisms during neurodegeneration. *Cell Death Dis* **9**, 120.

[39] Petryshen TL, Sabeti PC, Aldinger KA, Fry B, Fan JB, Schaffner SF, Waggoner SG, Tahl AR, Sklar P (2010) Population genetic study of the brain-derived neurotrophic factor (BDNF) gene. *Mol Psychiatry* **15**, 810-815.