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Linking APOE-ε4, blood-brain barrier dysfunction, and inflammation to Alzheimer's pathology

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ABSTRACT

The APOE-ε4 genotype is a risk factor for late-onset Alzheimer's disease (AD) as well as vascular pathology. Given the increased risk of blood-brain barrier (BBB) dysfunction and inflammation among APOE-ε4 carriers, we aimed to examine whether BBB dysfunction and inflammation contribute to the relationship between APOE and AD key pathologies, as measured in the cerebrospinal fluid (CSF). We applied bootstrapped regression and path analyses involving Q-albumin CSF/plasma ratio (a BBB/blood-CSF barrier function marker), interleukins (IL-1β, IL-6, and IL-12p70; inflammation markers), and CSF p-Tau$_{181}$ and amyloid-β$_{1-42}$ (AD pathology markers) of 97 participants (aged 38–83 years) from a university memory clinic. Our results showed that relationship between BBB dysfunction and AD pathology is modulated by IL-6 and these associations appear to be driven by the APOE-ε4 genotype. This suggests that APOE-ε4-related vascular factors are also part of the pathway to AD pathology, in synergy with an elevated immune response, and could become targets for trials focused on delaying AD.

1. Introduction

Late-onset Alzheimer's disease (AD) is the most common form of dementia and is characterized by extracellular accumulation of the amyloid-beta (Aβ) protein–forming senile plaques, and intracellular aggregation of hyperphosphorylated tau into neurofibrillary tangles. These pathological events start approximately 20–30 years before dementia onset (Jansen et al., 2015). *APOE-ε4* is the strongest non-Mendelian genetic risk factor for AD, apart from aging, and individuals with one or two *ε4* alleles typically develop cerebral amyloidosis at an earlier age than those with no e4 allele. As such, individuals with the *APOE-ε4* genotype are more likely to have elevated levels of Aβ on positron emission tomography and lower levels in cerebrospinal fluid (CSF) (Jansen et al., 2015; Kok et al., 2009). Recent work in animal models has shown that APOE-*ε4* is also related to tau pathogenesis and tau-mediated neurodegeneration, independently of Aβ pathology (Shi et al., 2017).

The ApoE protein is a critical regulator of cholesterol and lipid metabolism in the body, and within the central nervous system, ApoE is expressed by astrocytes, pericytes, and smooth muscle (Bruinsma et al., 2010), but it is strongly upregulated by microglia in the context of Aβ pathology and can influence the activation state of microglia (Krasemann et al., 2017; Uchihara et al., 1995) and higher systemic inflammation levels in both humans and animals (de la Torre, 1994; Iadecola, 2013; Zlokovic, 2011). Chronic microglial activation is considered to be a prominent part of AD and can reduce Aβ clearance and increase production of Aβ in animal and in vitro models (Shi and Holtzman, 2018). It has been suggested that

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chronic glial activation and the resulting immune response precedes and possibly drives tau pathology (Leyns and Holtzman, 2017; Yoshiyama et al., 2007). Moreover, an imbalance between innate and adaptive immune systems is associated with AD in the general population (van der Willik et al., 2019). In addition to microglial activation, APOE-ε4 is associated with increased vascular stiffness and higher vascular Aβ deposition, which may be related to a decreased CSF flow and lower Aβ clearance (Hughes et al., 2014). A downstream pathological process underlying the association between vascular damage and AD pathology could be blood-brain barrier (BBB) dysfunction (Iadecola, 2015). This barrier is thought to be affected in most neurodegenerative diseases (Obermeier et al., 2013), and BBB damage occurs independently from, and might precede both Aβ and tau pathology (Chalbot et al., 2011; Freeze et al., 2017; Montagne et al., 2017; Nation et al., 2019). Given these findings, we tested whether BBB dysfunction and/or the elevated immune response (Hansson and Hermansson, 2011; Shi and Holtzman, 2018) could contribute to the relationship between the APOE genotype and AD pathology. We examined the complex relationships between APOE genotype, inflammation markers that have been associated with AD in literature, BBB dysfunction, and AD pathology (Wyss-Coray and Rogers, 2012) using path analyses, in a memory clinic population varying from subjective cognitive complaints to mild AD dementia, providing a broad range in biomarker levels. In the present study, we used the ratio of CSF/plasma albumin: Q-Alb, a metric for BBB dysfunction, which also encompasses blood-CSF barrier dysfunction.

In addition, CSF biomarkers for AD pathology (p-Tau, Aβ42) and plasma markers inflammation (IL-1β, IL-6, IL-12p70) were used. We focused on plasma (rather than CSF) IL-1β and IL-6 as they have been associated with chronic neuroinflammation and are increased in AD (Swardfager et al., 2010) as well as implicated in vascular risk, and IL-12p70 as an inducer of IFN-γ (Mastrangelo et al., 2009; Trinchieri, 1995).

A better understanding of the processes underlying the link between APOE-ε4 and AD pathology could facilitate the identification of modifiable risk factors and plan preventative interventions in individuals at high risk for AD dementia in the pre-symptomatic phase.

2. Methods

2.1. Participants

A convenience sample of 97 patients from the memory clinic of the Maastricht University Medical Center including individuals diagnosed with subjective cognitive decline (SCD), mild cognitive impairment (MCI), and AD dementia (see Table 1 for demographics). Diagnoses were made by experienced physicians based on the core clinical criteria for MCI (Albert et al., 2011) and AD dementia (McKhann et al., 2011). Criteria for SCD diagnosis included the self-reported cognitive complaints and the endorsement of the question "Do you think your memory is becoming worse?" (Geerlings et al., 1999; Jessen et al., 2010). Patients with SCD did not have without having objective impairments on cognitive tests (defined as a score below −1.5SD of the age-, sex-, and education-adjusted mean).

As APOE-ε4 carriers have a higher risk for dementia but also exhibit higher Aβ and tau pathology at a younger age (Kok et al., 2009), and APOE-ε4 carriers with self-assessed cognitive concerns appear to have worse memory, and possibly accelerated memory decline (Samieri et al., 2014), we decided not to place an age cutoff on the SCD group. Exclusion criteria were major neurological disease, clinical diagnosis of other neurodegenerative disorders (e.g., frontotemporal dementia), recent transient ischemic attack or stroke (<2 years), history of severe psychiatric disorders, and alcohol or drug abuse. All patients provided informed consent and the study protocols were approved by the local Medical Ethics Committee of the Maastricht University Medical Center.

2.2. CSF and blood analyses

CSF was collected via puncture in the L3-4 or L4-5 interspaces and directly stored at -80°C in polypropylene tubes and processed

Table 1
Demographics

A	Total	APOE-ε4+	APOE-ε4−	t- test[a] or χ^2
Age				
Mean (SD)	63 (9.23)	62.9 (9.61)	63.5 (8.87)	t = 0.36, $p = 0.71$
Range (min-max)	(38–83)	(38–79)	(43–83)	
Sex				
% male (n)	69 (70)	27 (41)	41 (60)	$\chi 2$, $p = 0.03$[a]
APOE-ε4				
SCD/MCI/AD	ε4+= 47/ε4− = 51	(30/16/5)	(23/13/11)	
Educational level				
SCED 1997	3.8 (1.9)	3.8 (1.87)	3.8 (1.95)	(t = −0.02, $p = 0.98$)
MMSE score	27.44 (2.67)	27.49 (2.9)	27.43 (2.45)	(t = −0.03, $p = 0.97$)
CSF Aβ-42	939 (358) (315–1898)	803 (335) (315–1898)	1064 (333) (459–1898)	(t = −3.77, $p < 0.001$)
CSF p-Tau	63.66 (35.14) (22–188)	70.9 (35) (23–152)	56.94 (33.9) (22–188)	(t = −1.74, $p = 0.08$)
Interleukin-1b	0.54 (0.32)	0.553 (0.37)	0.558 (0.26)	(t = 0.46, $p = 0.64$)
Interleukin-12p70	0.26 (0.45)	0.23 (0.26)	0.29 (0.57)	(t = 0.81, $p = 0.41$)
Interleukin-6	1.36 (0.91)	1.236 (0.84)	1.489 (0.95)	(t = 2.24, $p = 0.02$)[a]
Q-Alb	5.6 (2.31)	5.2 (2.09)	5.95 (2.74)	(t = 1.62, $p = 0.10$)
Systolic BP				
Mean (SD/range)	146 (19.3/110–204)	146 (19.4/120–204)	143 (27.1/120–185)	(t = −0.28, $p = 0.77$)
Diastolic BP				
Mean (SD/range)	85 (9.2/60–116)	84 (9.8/70–116)	97 (8.4/60–105)	(t = 1.46, $p = 0.14$)

Demographics: SD, standard deviation; SCED 1997 (UNESCO) International Standard Classification of Education; BP, blood pressure (mmHg); MMSE, Mini–Mental State Examination; CSF, cerebrospinal fluid; Welsh two-sample *t*-test.
Key: AD, Alzheimer's disease; Aβ, amyloid-beta; SCD, subjective cognitive decline; MCI, mild cognitive impairment.
[a] Threshold of significance $p < 0.05$.

for biochemical analysis of CSF $A\beta_{1-42}$ and p-$Tau_{181p,}$ (Innotest ELISA, Innogenetics, Ghent, Belgium) according to a standardized protocol designed for biobanking. We opted to focus on p-Tau as it is more closely related to AD pathology and highly correlated to t-Tau (r = 0.92). In this study, both CSF $A\beta_{1-42}$ and p-Tau_{181p} are treated as continuous measures as accumulating evidence indicates that subthreshold CSF levels can be predictive of future clinical progression and do not exclude incident AD (Tijms et al., 2017). APOE genotyping was determined on genomic DNA using polymerase chain reaction. We also calculated the p-Tau/Aβ ratio, as this has been shown to track well with cognitive decline (Fagan et al., 2007; Landau et al., 2010) and cumulative AD pathology (Racine et al., 2016).

In addition, analyses of serum for interleukin (IL-1b, IL-6, IL12p70) (BD cytometric bead array, BD-biosciences, Franklin Lakes, NJ, USA) were performed and the CSF/plasma albumin ratio (Q-Alb) was determined using nephelometry.

Because our samples were taken from a biobank (aliquoted to avoid freeze thaw cycles) obtained at different times and it is known that prolonged storage, even at −80°C, can differentially affect the level of cytokines (de Jager et al., 2009), we statistically corrected for storage times calculated from the day of entry in the biobank to the date of analysis.

Blood pressure was measured using a standardized automated oscillometric cuff method.

2.3. Statistical analyses

Statistical analyses were conducted in R version 3.4.2 (www.R-project.org). Demographics are reported using the mean, standard deviation, and range. First, we examined the zero-order correlations between all pathologies by using Pearson's correlation analyses for continuous variables and point biserial correlations for dichotomous variables and continuous variables, and phi correlation for two dichotomous variables.

Second, multivariable linear regression models were applied to test the additive and interactive association of BBB dysfunction and inflammation markers on AD pathology (p-Tau, Aβ, and p-Tau/aβ ratio). Given the non-normality of the distributions and model residuals, the estimates were bootstrapped with 10,000 replicates, generating bias-corrected and accelerated 95% confidence intervals. All regression analyses were corrected for age, sex, sample storage time, and diagnosis. No correction for multiple comparisons was used. Blood pressure is reported to have a relation with IL-6 and is a vascular risk factor. Blood pressure derivatives such as systolic pressure, mean arterial pressure (MAP = (Systole + 2*Diastole)/3) and peak pressure (Systole-Diastole) are predictors of cardiovascular disease (Sesso et al., 2000). Therefore, we assessed the inclusion systolic, mean arterial pressure and peak pressure as covariate into our models and chose the most parsimonious model.

Third, the inflammation marker(s) that showed statistically robust relations in the above regression analyses was used in our path analyses, in which we added APOE status and tested possible associations between BBB dysfunction, inflammation, p-tau/Aβ, and APOE genotype. Based on the literature, we tested three potential models against the null model by comparing model fit parameters.

Model 0 assumes that there are independent associations of *APOE-ε4* on interleukins and Q-Alb and subsequently on AD pathology (p-Tau/Aβ), this is the simplest model and hence, null model.

Model 1 also assumes independent associations of *APOE-ε4* status on IL-6 and Q-Alb, but posits that IL-6 levels moderate the relation between Q-Alb and p-Tau/Aβ, that is, that BBB dysfunction is associated with higher levels of AD pathology when systemic inflammation is higher.

In model 2, we assume that IL-6 modulates the relationship between *APOE-ε4* status and Q-Alb and between Q-Alb and p-Tau/Aβ. Finally, in model 3, we reversed the order between Q-Alb and p-Tau/Aβ (AD pathology positively correlates with Q-Alb), but still assume that IL-6 modulates the association of *APOE-ε4* and of p-Tau/Aβ.

Model fit was acceptable at a χ^2 p-value >0.05, a comparative fit index (CFI) of >0.90, a root mean square error of approximation (RMSEA) of less than 0.08, and a standardized root mean square approximation residual (SRMR) <0.08. The Akaike information criterion (AIC) is used to quantify the relationship between goodness of fit and model complexity. AIC allows the comparison of nonnested models with identical degrees of freedom (lower AIC is preferred). The standard errors of the estimates in the models were bootstrapped over 10,000 replicates, generating bias-corrected and accelerated 95% confidence intervals.

2.4. Sample size and power

As commonly used rules of thumb for sample size in SEM are not model specific, they will severely overestimate or underestimate the required number of participants needed, as the level of communality across the variables, sample size, and degree of factor determinacy all affect the accuracy of the parameter estimates and model fit statistics (MacCallum et al., 1999; Wolf et al., 2013). Therefore, we used Monte Carlo simulations (10.000 draws, alpha 0.05, two-sided) (Muthén and Muthén, 2002; Wolf et al., 2013) to take into account these factors and assess the effects of varying sample size and determine power using Simsem (simsem.org) (Jorgensen et al., 2018). This simulation showed that for a sample size of 97 the most complex model yields a model power of 0.83 for a moderate effect size.

3. Results

We included 97 participants (SCD n = 52; MCI n = 29; mild AD n = 16) who visited the university memory clinic. To obtain a broad range in pathologies, we combined all participants in our analyses. Demographics for the entire sample stratified by *ε4* allele are listed in Table 1. Our sample had a mean age of 63.0 (SD = 9.23) years, 70% of the participants were male and 48% of the individuals carried at least one *ε4* allele. The three individuals (all SCD) younger than 45 years, all carried at least one ε4 allele. The age of the *APOE-ε4* noncarriers was not significantly different from the carriers (t (91.59) = 0.36, df = 91.59, $p = 0.71$), but the proportion of males was larger in the noncarriers relative to the carriers ($\chi^2 = 4.48$, $p = 0.03$). In addition, Aβ42 (t (93.62) = −3.77, $p < 0.001$) levels were lower in the *APOE-ε4* carriers compared with the noncarriers. p-Tau, Q-Alb, educational level, Mini–Mental State Examination score, and cytokine levels were not different between *APOE-ε4* carrier and noncarrier groups.

In all our analyses, we treated CSF $A\beta_{1-42}$ and p-Tau_{181p} continuous measures as accumulating evidence indicates that subthreshold CSF levels can be predictive of future clinical progression and do not exclude incident AD (Tijms et al., 2017). We also calculated the p-Tau/Aβ ratio, as this has been shown to be a robust measure of cumulative AD pathology (Racine et al., 2016).

To understand the univariate relationships among our variables of interest, we performed correlation analyses between the measured pathologies, genotype, blood pressure, and demographic variables (Supplemental Table 1). Being an *APOE-ε4* carrier was associated with male sex, having lower Aβ42, and IL-6, and having a higher p-Tau/Aβ ratio. Q-Alb only correlated with sex (male sex). When we examined the inflammation markers, IL-6 correlated positively with age and IL12p70. IL-12p70 levels correlated positively with education, p-Tau, t-tau, IL-6, and the p-Tau/Aβ ratio, and IL1b only correlated negatively with the male sex.

3.1. Additive and interactive associations of BBB dysfunction and inflammation on the p-Tau/Aβ ratio

To guide our model building, we first investigated whether BBB dysfunction and inflammation contribute additively or synergistically to AD pathology by performing bootstrapped regression models. In these models, we corrected for age, sex, sample storage time, and diagnosis (to account for the cognitive heterogeneity).

We observed that IL-6 had a synergistic (but not additive) relationship with Q-Alb on the p-Tau/Aβ ratio (β = 0.01, t = (89) 3.15, $p = 0.002$ bootstrapped bias-corrected and accelerated confidence interval [0.003, 0.028]), in such a way that for a given level of Q-Alb, higher levels in IL-6 were associated with higher levels in p-Tau and lower Aβ (Fig. 1, Table 2). There were no additive or interaction associations between Q-Alb and IL-1b or IL-12p70. Adding blood pressure derivatives to the model as an additional covariate did not change the results (Supplemental Fig. 2).

We also examined the associations between IL-1b, IL-12p70, or IL-6 and Q-Alb with the Aβ and p-Tau markers separately. Because we observed similar synergistic relationships between IL-6 and Q-Alb when we used Aβ and p-Tau as dependent variables in our models (Table 2), we used the p-Tau/Aβ ratio as measure for AD pathology in subsequent analyses.

3.2. Path analyses

To further test the hypothesis that the innate immune system and BBB dysfunction can explain the relationship between APOE-ε4 and AD pathology, three biologically plausible hypothesized models were tested with different relations between *APOE-ε4* status, IL-6, Q-Alb, and p-Tau/Aβ using path analyses (Fig. 2, Table 3). Model fit measures determined which model described the data best (a CFI of >0.90, an RMSEA of <0.08, and an SRMR <0.08). Model 0 assumes that there are independent associations of *APOE-ε4* on interleukins and Q-Alb and subsequently on AD pathology (p-Tau/Aβ). Model 1 also assumes independent associations of *APOE-ε4* status on IL-6 and Q-Alb, but posits that IL-6 levels moderate the relation between Q-Alb and p-Tau/Aβ, that is, that BBB dysfunction is associated with higher levels of AD pathology when systemic inflammation is higher. In model 2, we assume that IL-6 modulates the relationship between *APOE-ε4* status and Q-Alb and between Q-Alb and p-Tau/Aβ. Finally, in model 3, we reversed the order between Q-Alb and p-Tau/Aβ, (AD pathology positively correlates with Q-Alb) but still assume that IL-6 modulates the association of *APOE-ε4* and of p-Tau/Aβ.

The null model showed inadequate fit parameters ($\chi^2 = 0.042$, CFI 0.938, RMSEA 0.150). Compared with the null model, model 1 showed inadequate fit parameters ($\chi^2 = 0.123$, CFI 0.972, RMSEA 0.106). Model 2 showed an adequate model fit ($\chi^2 = 0.238$, CFI 0.98, RMSEA 0.065, AIC 502.8, sample size adjusted BIC 493.4). All interactive relationships in the model were significant. Model 3 showed inadequate fit parameters ($\chi^2 < 0.001$, CFI 0.695, RMSEA 0.293). These results indicate that model 2 is most consistent with our data and suggests that inflammation response links APOE to AD pathology via BBB dysfunction.

4. Discussion

In the present study, it was our goal to examine whether APOE genotype impacts AD pathology via inflammation and BBB dysfunction in a memory-clinic population. Our results demonstrate that a combination of modestly elevated levels of IL-6 and BBB dysfunction are associated with greater AD pathology in a population at risk for vascular problems (*ε4* carriers). This adds to the large body of work pointing toward both vascular and immune factors contributing to AD pathology. Given the recent disappointments in several clinical trials targeting Aβ in AD (Egan et al., 2018; Honig et al., 2018; Salloway et al., 2014), these observations provide more support to target multiple etiologies or to conduct preventive trials in at-risk populations focused at maintaining optimal cardiovascular health, potentially in conjunction with monitoring the immune system. In fact, recent large scale studies targeting vascular risk such as the SPRINT trial showed that treatment to a lower blood pressure target not only markedly reduced cardiac risk, but also lowered the risk for MCI and possibly dementia (SPRINT MIND Investigators for the SPRINT Research Group, 2019), while an upregulated innate immune system was associated with increased risk of developing dementia in the Rotterdam study (van der Willik et al., 2019).

Although a multitude of animal and neuropathology studies established associations between the *APOE-ε4* genotype and BBB integrity (Bell et al., 2012; Halliday et al., 2016; Nishitsuji et al., 2011; Utter et al., 2008), a direct relationship has been more elusive in human populations (Janelidze et al., 2017). APOE was known for its role in cardiovascular risk (20, 30) before it was recognized as a major risk factor in early- and late-onset AD. In

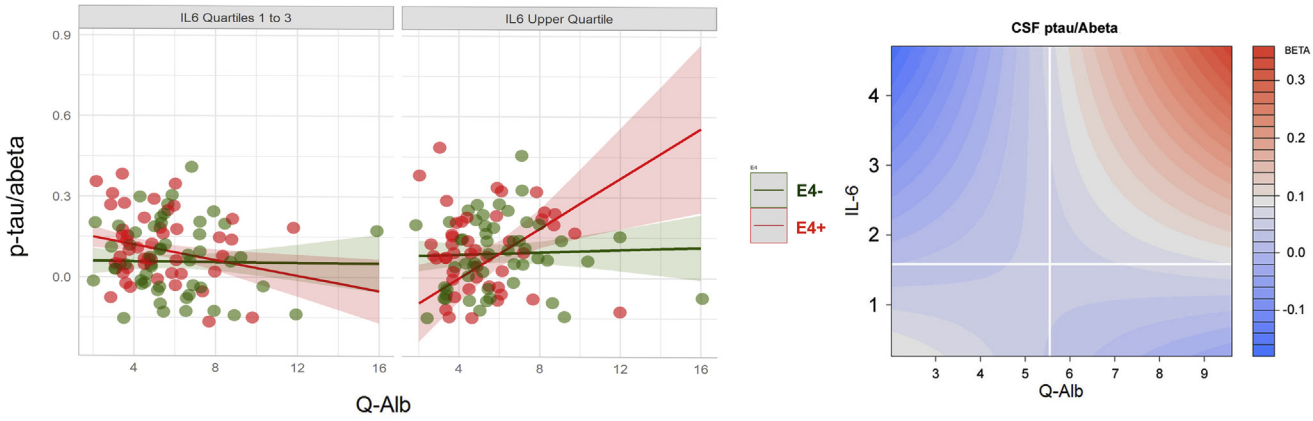

Fig. 1. Left panels show the predicted values for the relationship between Q-Alb and p-Tau/Aβ, corrected for covariates at specific values of IL-6 (lower 3 quartiles [level 0 under 1.59(ng/l)] and the upper quartile [IL-6 level 1 over 1.59 (ng/l)]). Shaded regions show 95% confidence intervals. APOE-ϵ4+ are marked red and APOE-ϵ4- green. Note: these plots in quartiles are provided to show the general topology of the raw data. All analyses (regression and path analyses) were conducted on continuous data and bootstrapped. Right panel shows the general topography of the relation between AD pathology, Q-Alb, and IL-6 in the entire group (adjusted for covariates), demonstrating that high IL-6 and high BBB dysfunction is associated with high p-Tau/ Aβ ratio.

Table 2
Synergistic associations of Q-Alb and interleukins on AD pathology

p-Tau/amyloid-β		Q-Alb [95% CI]	Interleukin (IL) [95% CI]	Q-Alb*IL [95% CI]
IL-6	Additive	B = −0.0007 [−0.004 | 0.005]	B = 0.008 [−0.007 | 0.037]	
	Synergistic	**B = −0.01 [−0.033 | −0.006]**	**B = −0.07 [−0.140 | −0.01]**	**B = 0.014 [0.003 | 0.028] *p* = 0.002**
IL-1b	Additive	B = −0.0006 [−0.004 | 0.005]	B = 0.027 [−0.005 | 0.072]	
	Synergistic	B = −0.006 [−0.018 | 0.004]	B = −0.036 [−0.185 | 0.113]	B = −0.010 [−0.010 | 0.038]
IL12p70	Additive	B = −0.005 [−0.004 | 0.004]	B = 0.041 [−0.014 | 0.061]	
	Synergistic	B = −0.0025 [−9.20e-03 | 0.004]	B = −0.021 [−0.163 | 0.098]	B = 0.009 [−1.e-02 | 0.033]
Amyloid-β				
IL-6	Additive	B = 2.83 [−23.52 | 24.01]	B = 3.83 [−81.52 | 112.32]	
	Synergistic	**B = 67.86 [9.33 | 131.95]**	**B = 291.43 [19.26 | 594.61]**	**B = -51.72 [−100.46 | −7.64] *p* = 0.037**
IL-1b	Additive	B = −3.45 [−22.23 | 25.11]	B = −31.21 [−201.03 | 216.42]	
	Synergistic	B = 29.24 [−23.39 | 89.15]	B = 237.36 [−387.78 | 934.94]	B = −48.53 [−167.31 | 49.76]
IL12p70	Additive	B = 3.78 [−20.08 | 24.85]	B = −106.73 [−318.144 | 55.83]	
	Synergistic	B = 1.77 [−29.02 | 30.22]	B = −170.25 [−693.18 | 439.69]	B = 9.79 [−81.79 | 90.51]
p-Tau		Q-Alb	Interleukin (IL)	Q-Alb*IL
IL-6	Additive	B = −0.34 [−1.78 | 1.90]	B = 4.34 [−2.99 | 17.56]	
	Synergistic	**B = −7.34 [−14.79 | −2.09]**	**B = -26.58 [−57.30 | −4.45]**	**B = 5.46 [1.00 | 12.15] *p* = 0.006**
IL-1b	Additive	B = −0.22 [−1.72 | 2.04]	B = 6.19 [−7.04 | 23.61]	
	Synergistic	B = −4.10 [−9.55 | 0.20]	B = −34.25 [−101.91 | 13.76]	B = 7.30 [−0.78 | 19.85]
IL12p70	Additive	B = −0.32 [−1.71 | 2.12]	B = 15.07 [−19.53 | 25.18]	
	Synergistic	B = −1.63 [−5.25 | 0.90]	B = −28.46 [−95.60 | 17.83]	B = 6.76 [−2.98 | 18.92]

Significant regressions are denoted in bold taking into consideration of the 95% bootstrapped CI (10,000 draws).

addition, *APOE* affects lipid metabolism and transport, has been shown to modulate the BBB in an isoform-dependent manner in in vitro (Nishitsuji et al., 2011) and postmortem human studies (Halliday et al., 2016), and the *APOE-ε4* genotype is associated with an upregulated innate immune system. We observed that of the cytokines tested, only IL-6 modulated the relationship between Q-Alb and p-Tau/Aβ in our study sample. IL-6 is a pleiotropic cytokine linked to not only to innate immunity, vascular health and increased cardiac risk, BBB permeability, and activation of microglia (Held et al., 2017; Kitazawa et al., 2005), but is also associated with cognitive impairment (Weaver et al., 2002).

The involvement of IL-6 in the pathophysiology of AD within the proposed framework is conceivable for several reasons. First, IL-6 is involved in the remodeling of microvasculature, causing thickening of endothelium and decreasing lumen available for blood flow (Ricard et al., 2014), as well as defective angiogenesis (Gopinathan et al., 2015). Second, the relationship between IL-6 and AD pathology is consistent with meta-analyses of cytokines showing involvement of IL-6 in AD and an increased risk of all cause dementia (Koyama et al., 2013; Swardfager et al., 2010). Third, greater levels of IL-6 increase levels of serum amyloid A, which especially for the APOE-ε4+ genotype, negatively affects the transport mechanism involved in the clearance of Aβ (Hagihara et al., 2004; Miida et al., 2006) in addition to affecting atherosclerosis.

As we are using plasma, it is relevant to know if these peripheral cytokines can cross the BBB. Evidence suggests this is the case: circulating IL-6 in the blood can cross the BBB by influencing tight junctions by upregulating the adhesion molecule VCAM-1 allowing T-cells to traverse the endothelial layer along with IL-6. This enables both IL-6 and T-cells to cross the BBB unchecked by transporters, where it can activate astrocytes and microglia cells in the central nervous system leading to secretion of more IL-6 within the brain (Oh et al., 1998; Rothaug et al., 2016). Although our work as well as recent work by Nation (Nation et al., 2019) did not find a direct relationship between CSF IL-6 and AD pathology or disease staging, our data now suggest that IL-6 is associated with greater AD pathology in the context of higher BBB dysfunction. This was especially the case in APOE-ϵ4+ carriers. In the APOE-ϵ4+ group, we found a lower mean level of IL-6 compared with APOE-ϵ4− group. Although we found lower levels of IL-6, these levels combined with comparatively low levels of Q-Alb were associated with higher levels of AD pathology in APOE-ϵ4+ carriers. Such a relationship was not observed in the APOE-ϵ4− group, who could withstand higher levels of IL-6. It has been shown that in APOE-ϵ4+ carriers, the IL-6 pathway is altered, in such a way that increased IL-6 receptor levels in APOE-ϵ4+ carriers may explain the greater effect of lower IL-6 levels Haddick et al., 2017. In the entire sample, the multiplicative effect of IL-6 and Q-Alb ratio on AD pathology is significant starting from low levels of IL-6 (>1.59 ng/L) and at a Q-Alb ratio of 5.6 or greater, well below the upper reference limit of 10.2 for individuals older than 45 years or 6.8 for <45 years (Blennow et al., 1993). Taken together, this suggests that IL-6 (probably as a proxy for an

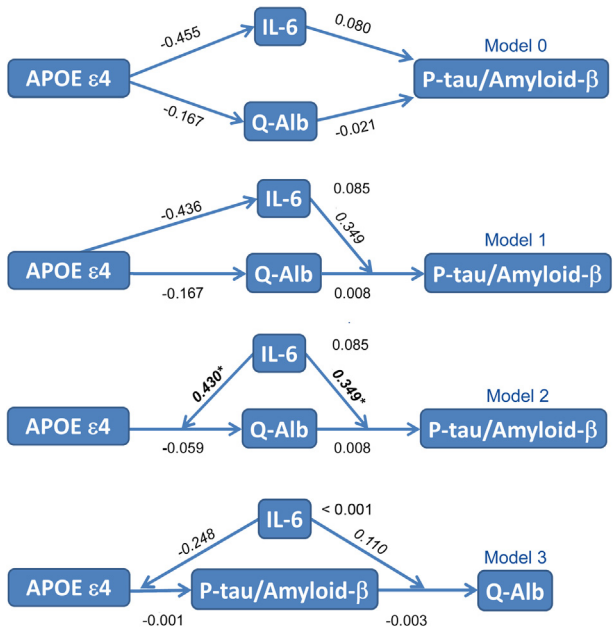

Fig. 2. Path analyses models describing the different examined relations (see text). The interactions of model 2 were significant (Q-Alb~IL-6*APOEε4 z = 2.06, *p* = 0.03; p-Tau/Aβ~Q-Alb*IL-6 z = 2.31, *p* = 0.02; see also Table 3). This indicates that both BBB dysfunction and inflammation contribute to the relationship between APOE and AD pathology. Interaction estimates are marked in italic, significant associations are marked in bold (*).

Table 3
Path analyses

	B	Wald Z	*p*-value	CI 95%
Model 0 fit parameters: (chi-square): <0.001, CFI: 0.923, RMSEA: 0.150, SRMR: 0.032 Akaike (AIC): 788				
APOE~IL6	−0.455	−2.246	**0.025**	**−0.878 to 0.088**
APOE~Q-Alb	−0.167	−0.850	0.397	−0.566 to 0.215
p-Tau/amyloid-β~Q-Alb	−0.021	−0.319	0.750	−0.130 to 0.140
p-Tau/amyloid-β~IL-6	0.080	0.664	0.507	−0.119 to 0.362
Model 1 fit parameters: (chi-square): 0.123, CFI: 0.972, RMSEA: 0.106, SRMR: 0.023 Akaike (AIC): 772				
Q-Alb ~APOE	−0.167	−0.840	0.391	−0.541 to 0.221
IL-6 ~ APOE	−0.436	−2.124	0.034	−0.059 to 0.436
p-Tau/amyloid-β~IL-6 * Q-Alb	0.349	2.663	0.088	−0.088 to 0.349
p-Tau/amyloid-β~IL-6	0.085	0.870	0.384	−0.104 to 0.285
P-Tau/amyloid-β~ Q-Alb	0.008	0.082	0.935	−0.124 to 0.262
Model 2 fit parameters: (chi-square): 0.238, CFI: 0.981, RMSEA: 0.065, SRMR: 0.020 Akaike (AIC): 502				
Q-Alb ~ IL-6 * APOE	0.430	2.013	**0.041**	**0.030 to 0.868**
Q-Alb ~ APOE	−0.059	−0.277	0.782	−0.480 to 0.352
Q-Alb ~IL-6	0.039	0.364	0.716	−0.159 to 0.258
p-Tau/amyloid-β~IL-6 *Q-Alb	0.349	2.280	**0.023**	**0.086 to 0.689**
p-Tau/amyloid-β~IL-6	0.085	0.869	0.385	−0.102 to 0.287
p-Tau/amyloid-β~Q-Alb	0.008	0.080	0.935	−0.125 to 0.257
Model 3 fit parameters: (chi-square): <0.001, CFI: 0.201, RMSEA: 0.73, SRMR: 0.093 Akaike (AIC): 515				
p-Tau/amyloid-β ~IL6 * APOE	−0.248	−1.131	0.258	−0.669 to 0.202
p-Tau/amyloid-β ~IL6	0.130	0.917	0.359	−0.094 to 0.476
p-Tau/amyloid-β ~APOE	−0.001	−0.009	0.993	−0.101 to 0.169
Q-Alb ~ p-Tau/amyloid-β * IL-6	0.110	1.228	0.219	−0.043 to 0.301
Q-Alb ~ IL-6	0.000	0.092	0.927	−0.062 to 0.333
Q-Alb ~ p-Tau/amyloid-β-0.00	−0.003	−1.071	0.284	−0.010 to 0.003

All models are corrected for age, sex, and diagnosis.
Key: B, estimate; CI, confidence interval; CFI, comparative fit index; RMSEA, root mean square error of approximation; SRMR, standardized root mean square residual; AIC, Akaike information criterion; BIC, Bayesian information criterion.
Significant *p*-values are denoted in bold taking into consideration of the 95% bootstrapped CI (10,000 draws).

upregulated immune system) can link APOE-ϵ4, BBB dysfunction and AD pathology together.

Mechanistically, inflammation may drive hyperphosphorylation of tau (Collins-Praino and Corrigan, 2017; Grundke-Iqbal et al., 1986; Hampel et al., 2010; Kitazawa et al., 2005; Quintanilla et al., 2004). Tau itself is thought to induce blood vessel abnormalities (Bennett et al., 2018), possibly affecting BBB function and perpetuating the inflammatory response. When we reversed BBB and p-Tau/Aβ relationship in our path analyses (model 3), however, we found no evidence for this model.

4.1. Limitations and future perspectives

Q-Alb may reflect BBB permeability, but also reflects the function of the blood-CSF barrier at the choroid plexus (Janelidze et al., 2017), and therefore, we cannot differentiate between these two systems. The blood-CSF barrier might be more affected by vascular damage (Wolburg and Paulus, 2010). Using additional markers linked to pericyte function such as SPDGF- might be of interest in future research. Second, although IL-6 is relatively indifferent to pre-analytical factors and stable in storage for several years, absolute levels of cytokines can be affected by time in storage, even at −80°C. Linearly correcting for storage duration did not change our findings. Cytokines show a circadian rhythm which might affect sampled levels (Scheiermann et al., 2018). Future studies should take these sources of variation into account.

Unintentional biases such as survival bias could play a role as APOE-ϵ4 carriers, particularly those with higher levels of IL-6 and elevated p-Tau and Aβ, may struggle to maintain sufficient health to attend our outpatient clinic. The sample size of this study is adequate for the use of these path analyses; however, for future research using more complex models utilizing the diagnostic subgroup and cognition, a larger sample size is needed to ensure adequate power.

These models are based on cross-sectional data and do not infer causal relationships. Longitudinal studies with larger sample size with more emphasis on immune status are required to better probe these complex relationships as present large-scale studies such as ADNI do not have all required data.

According to the 2017 ACC/AHA guidelines, both the APOE carriers and noncarriers would be classified as stage-2 hypertension, and this possibly limits the generalizability of our findings. However, none of the blood pressure metrics significantly contributed to the explained variance when added as a covariate (either as systolic blood pressure, pulse pressure, or mean arterial pressure).

Finally, IL-6 represents a small part of highly complex system. However, its important role in innate, humoral, and cell-mediated immunity does provide an indication of the state of the immune system that could in turn affect pathways that affect vascular health as well as contribute to AD pathology.

In light of the failure of multiple amyloid clearance trails, a rethink might be in order. The direct approach to treating or even delaying AD has so far been disappointing notwithstanding massive efforts. However, given the extensive experience with the long-term treatment of vascular risk factors with acceptable side effects, an extra focus on this major comorbidity in specific subgroups may be important.

While vascular risk factors might not be the primary cause of AD, we provide some evidence that it might contribute to AD pathology possibly in concert with an upregulated immune system. An early intervention approach in individuals with increased risk such as APOE-ε4 carriers could be a valuable asset in what is bound to be a multisystem approach to the treatment of AD and dementias in general.

5. Conclusion

Our findings provide evidence that a combination of modestly elevated levels of IL-6 and BBB dysfunction are associated with greater AD pathology in APOE-ϵ4 carriers of a memory clinic population. This suggests that APOE-ε4-related vascular factors are also part of the pathway to AD pathology, in synergy with the immune

response. Early cardiovascular risk factors and immune modulation in APOE-ε4 carriers could be important reducing in AD pathology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.09.020.

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