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Association of *APOE* genotype with blood-brain barrier permeability in neurodegenerative disorders

Ilenia Libri [a], Chiara Silvestri [a], Salvatore Caratozzolo [b], Antonella Alberici [b], Andrea Pilotto [a,b], Silvana Archetti [c], Laura Trainini [c], Barbara Borroni [a,d], Alessandro Padovani [a,b,e], Alberto Benussi [a,f,*]

[a] *Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy*
[b] *Neurology Unit, Department of Continuity of Care and Frailty, ASST Spedali Civili, Brescia, Italy*
[c] *Biotechnology Laboratory and Department of Diagnostics, ASST Spedali Civili, Brescia, Italy*
[d] *Cognitive and Behavioral Neurology Unit, Department of Continuity of Care and Frailty, ASST Spedali Civili, Brescia, Italy*
[e] *Brain Health Center, University of Brescia, Brescia, Italy*
[f] *Neurology Clinic, Department of Medicine, Surgery and Health Sciences, Trieste University Hospital, Trieste, Italy*

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ABSTRACT

Apolipoprotein E (APOE) is recognized for its role in modulating blood-brain barrier (BBB) permeability *in vitro*, which may have significant implications for the pathogenesis and progression of neurodegenerative disorders. However, evidence *in vivo* is contrasting. This study explores the impact of *APOE* genotypes on BBB integrity among 230 participants experiencing cognitive impairment, encompassing cases of Alzheimer's disease (AD) as well as various non-AD neurodegenerative conditions. To assess BBB integrity, we utilized cerebrospinal fluid (CSF)/serum albumin ratios and CSF/serum kappa and lambda free light chains (FLCs) as indirect markers. Our findings show a dose-dependent increase in BBB permeability in individuals carrying the *APOE* ε4 allele, marked by elevated CSF/serum albumin and FLCs ratios, with this trend being especially pronounced in AD patients. These results highlight the association of *APOE ε4* with BBB permeability, providing valuable insights into the pathophysiology of neurodegenerative diseases.

1. Introduction

The blood-brain barrier (BBB), a highly dynamic interface between the central nervous system and the peripheral circulation, plays a critical role in maintaining brain homeostasis (Abbott et al., 2010). The integrity of the BBB is fundamental to proper brain function, and its breakdown facilitates entry into the brain of neurotoxic blood-derived products, cells and pathogens and is associated with inflammatory and immune responses, which can initiate multiple neurodegenerative pathways (Sweeney et al., 2018).

Apolipoprotein E (*APOE*) is a key protein in lipid metabolism, with three major isoforms (ε2, ε3, and ε4) encoded by different alleles of the *APOE* gene. Notably, the ε4 variant is strongly associated with an elevated risk of developing late-onset Alzheimer's disease (AD) (Roses, 1996).

Multiple experimental studies have confirmed that BBB breakdown causes capillary leakage in Alzheimer's disease (AD) models of β-amyloidosis (Park et al., 2017; Sagare et al., 2013) and in *APOE* ε4 transgenic mice (Alata et al., 2015; Bell et al., 2012; Nishitsuji et al., 2011). However, while *in vitro* and animal *in vivo* studies have shed valuable light on the potential role of *APOE* ε4 in BBB permeability, there is a paucity of human *in vivo* evidence supporting this association (Bell et al., 2012; Blanchard et al., 2020; Cicognola et al., 2023; Deane et al., 2008; Freeze et al., 2020, 2017; Kurz et al., 2022; Methia et al., 2001; Montagne et al., 2020; Moon et al., 2021; Nishitsuji et al., 2011; Riphagen et al., 2020; Teng et al., 2017).

Evaluating BBB permeability *in vivo* poses a significant challenge due to the protected location and complex structure of the brain. Nonetheless, indirect markers such as the cerebrospinal fluid (CSF)/serum albumin ratio has been employed as proxy for BBB integrity (Reiber, 2001). Albumin, synthesized exclusively by the liver, can cross the BBB mainly when its integrity is compromised. Thus, an elevated CSF/serum

* Correspondence to: Neurology Clinic, Department of Medicine, Surgery and Health Sciences, Trieste University Hospital, Strada di Fiume, 447, Trieste 34149, Italy.
E-mail address: benussialberto@gmail.com (A. Benussi).

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albumin ratio is indicative of increased BBB permeability (Tibbling et al., 1977). Likewise, both kappa and lambda free light chains (FLCs), components of immunoglobulins, are synthesized by plasma cells and a change in their CSF/serum ratio may also reflect changes in BBB permeability, when an intrathecal synthesis has been excluded (Berven et al., 2007). Although *in vitro* models indicate an impact of *APOE* on BBB integrity in AD, studies using the CSF/serum albumin ratio *in vivo* have yielded inconsistent results (Janelidze et al., 2017; Lin et al., 2021; Montagne et al., 2020; Riphagen et al., 2020).

In this study we explore the potential role of the *APOE* genotype in modulating BBB permeability in patients with dementia, utilizing the CSF/serum albumin ratio and the assessment of CSF/serum kappa and lambda FLCs ratio as indirect markers of BBB integrity. By shedding light on the influence of *APOE* on BBB permeability, we aim to enhance our understanding of the gene's role in neurodegenerative disorders.

2. Materials and methods

2.1. Participants

This cohort study involved a consecutive sample of 230 participants recruited from the Neurology Unit of the Department of Clinical and Experimental Sciences at the University of Brescia, Italy. Participants met current clinical criteria for probable AD (McKhann et al., 2011), dementia with Lewy bodies (DLB) (McKeith et al., 2017), fronto-temporal dementia (FTD) (Gorno-Tempini et al., 2011; Rascovsky et al., 2011) or vascular dementia (VaD) (Sachdev et al., 2014). Regarding AD, patients presented an insidious onset with a clear-cut history of worsening of cognition, and a CSF AD-like profile (Jack et al., 2018) (see below "Fluid biomarkers"). For DLB, patients presented fluctuating cognition with pronounced variations in attention and alertness, recurrent visual hallucinations, REM sleep behaviour disorder and at least one spontaneous cardinal feature of parkinsonism, associated with a positive SPECT DaTScan. Regarding FTD, patients were classified based on the initial and most prominent clinical features in the behavioural or language domains, associated with fronto-insular/temporal atrophy at magnetic MRI or hypometabolism at 18 F-fluorodeoxyglucose PET (FDG-PET). VaD diagnosis was based on criteria accounting for cerebrovascular events and neuroimaging findings indicative of vascular brain injury. Moreover, participants with subjective cognitive decline (SCD) (Jessen et al., 2014) were also enrolled.

Exclusion criteria included increased bleeding risk (such as thrombocytopenia, coagulopathies, or use of anticoagulant drugs), history of spinal operations, inability to obtain a sufficient amount of CSF, and a traumatic or bloody lumbar puncture. Additionally, individuals with mixed neurodegenerative pathology, specifically the coexistence of AD and VaD, were explicitly excluded. This approach ensured the clear categorization of participants into distinct AD (n=156) and non-AD groups (n=74).

All patients underwent an extensive standardised evaluation, following standard procedures. This assessment encompassed various tests, including the mini-mental state examination (MMSE) (Magni et al., 1996), for global cognitive function, the clinical dementia rating (CDR) global score (Morris, 1993) to evaluate disease severity, and the basic activities of daily living (BADL) (Katz et al., 1963) and instrumental activities of daily living (IADL) (Lawton and Brody, 1969) questionnaires to determine the level of functional independence. Brain MRI scans were performed on all patients. The diagnosis for each patient was supported by CSF biomarkers, including total tau (t-Tau), phosphorylated tau at threonine 181 (p-Tau$_{181}$) and amyloid-β_{1-42} (see "Fluid biomarkers" below).

Full written informed consent was obtained from all subjects according to the Declaration of Helsinki. The Brescia Ethics Committee approved the study protocol (NP 1471, DMA, Brescia).

2.2. Fluid biomarkers

CSF was obtained during routine diagnostic lumbar puncture according to a standardized protocol, in the outpatient clinic, at fasting, from 09:30–10:30 h, after informed written consent had been obtained (Fornari et al., 2022). CSF was collected in sterile polypropylene tubes and gently mixed to avoid gradient effects. Routine chemical measures were determined. The remaining CSF was centrifuged for 3 min at 3000 rpm and aliquots (0.5 mL) were immediately stored at −80 °C or in liquid nitrogen for subsequent analysis. CSF t-Tau, p-Tau$_{181}$ and amyloid-β_{1-42} concentrations were measured by Lumipulse (Fujirebio) by a single experienced technician blinded to diagnosis or *APOE* genotype. According to our laboratory standards, a CSF AD-like profile was defined by the following cut-off values: amyloid-β_{1-42} < 650 pg/mL, t-tau > 400 pg/mL and p-Tau$_{181}$ > 60 pg/mL (Fornari et al., 2022).

Kappa and lambda FLCs, and albumin concentrations in CSF and serum samples were analysed using the turbidimetric analyser SPAplus® (The Binding Site Group Ltd, Birmingham, UK) with the serum free light chain immunoassay Freelite® (The Binding Site Group Ltd, Birmingham, UK) according to the manufacturer's instructions. To exclude an intrathecal synthesis of kappa and lambda FLCs, we used the following formulas considering serum FLC concentrations and blood-CSF barrier function (Leurs et al., 2020):

$$kappaFLCindex = \frac{kappaFLCs_{CSF}/kappaFLCs_{serum}}{albumin_{CSF}/albumin_{serum}}$$

$$lambdaFLCindex = \frac{lambdaFLCs_{CSF}/lambdaFLCs_{serum}}{albumin_{CSF}/albumin_{serum}}$$

The following cut-offs were used to define the presence of an intrathecal kappa FLC (≥6.39) and lambda synthesis (≥5.5) (Leurs et al., 2020).

2.3. APOE genotyping

Genomic DNA was extracted from whole peripheral blood using Maxwell® 16 Blood DNA Purification Kit with Maxwell® 16 Instrument (both Promega) (Benussi et al., 2022). The regions encompassing *APOE* rs429358 and rs7412 were amplified by polymerase chain reaction (PCR) using GoTaq® Hot Start Polymerase (Promega). PCR products were purified with Amicon® Ultra 0.5 mL Centrifugal Filters (Merck Millipore). Cycle sequencing was performed with the AB Prism Big Dye Terminator Sequencing kit 3.1 (Life Technologies), following the manufacturer's instructions. Sequences were subsequently purified using MicroSEQ™ ID Sequencing Clean-up Cartridges (Life Technologies) and then loaded on a 3500 Genetic Analyzer (Life Technologies). All sequences were analysed using the Chromas software (Technelysium Pty Ltd).

2.4. Statistical analysis

Continuous variables are reported as medians (interquartile range), and categorical variables are reported as numbers and percentages (n, %). We conducted comparisons of baseline demographic and clinical variables across groups using the Kruskal-Wallis *H* test for continuous variables and Fisher's exact test for categorical variables, as appropriate. To compare CSF/serum albumin ratios, along with both kappa and lambda CSF/serum ratios among groups, we employed a non-parametric analysis of covariance (ANCOVA) using Quade's test, adjusting for several variables which have been associated with increased BBB permeability, including sex, age, disease duration, disease severity, diagnosis, and comorbidities (including hypertension, dyslipidaemia, diabetes, heart disease, stroke and smoking status) (Bors et al., 2018; Janelidze et al., 2017; Lin et al., 2021; Miners et al., 2019; Montagne et al., 2015; Parrado-Fernández et al., 2018; Skillbäck et al., 2021; Taheri et al., 2011; Wada, 1998). Disease duration, expressed in years, was quantified from the time of diagnosis to the date of participation in the study. Disease severity was assessed using the global CDR score. Groups were differentiated based on the number of *APOE* ε4 alleles

(0/nullizygous, 1/heterozygous, or 2/homozygous).

All tests were corrected for multiple comparisons using FDR correction (Benjamini and Hochberg, 1995).

Statistical significance was set at $p<0.05$. Data analyses were performed using IBM SPSS version 29.0 and GraphPad Prism version 10.0.

3. Results

3.1. Participants

The study included a total of 230 participants (median [IQR] age, 71.0 [65.8–75.0] years; 118 females [51.3%]). Of these, 156 were classified as AD (median [IQR] age, 72.0 [67.0–75.8] years; 90 females [57.7%]), and 74 as non-AD (median [IQR] age, 72.0 [67.0–75.8] years; 90 females [57.7%]), the latter group including 36 participants with FTD, 11 with DLB, 11 with VaD and 16 with SCD. Participants were subdivided into three *APOE* subgroups based on the number of *APOE* ε4 alleles. Demographic and neuropsychological characteristics of included participants are reported in Table 1. Significant differences between groups were observed only in sex and *APOE* genotype, with a higher representation of females ($p=0.007$) and a different distribution of *APOE* alleles ($p=0.003$) in the AD group compared to the non-AD group.

3.2. Fluid biomarker assessment

Fluid biomarker levels are detailed in Table 2, categorized by the number of *APOE* ε4 alleles. In the whole group, after adjusting for sex, age, disease duration, disease severity, diagnosis, and comorbidities (including hypertension, dyslipidaemia, diabetes, heart disease, stroke and smoking status), CSF total protein levels were slightly elevated in homozygous *APOE* ε4 participants compared to non-carriers ($p=0.027$), while the number of free cells in the CSF was not significantly different across groups.

A significant increase in the CSF/serum albumin ratio was observed in homozygous *APOE* ε4 participants compared to nullizygous or heterozygous *APOE* ε4 carriers ($p<0.001$), as shown in Fig. 1 (for individual values, according to diagnosis, see Supplementary Figure 1). Additionally, CSF/serum kappa and lambda FLCs were significantly higher in homozygous *APOE* ε4 carriers compared to nullizygous and heterozygous carriers ($p<0.001$ for kappa FLCs, $p=0.001$ for lambda FLCs), as depicted in Fig. 2.

To exclude that the increase in the CSF/serum kappa and lambda FLCs ratio was secondary to and intrathecal antibody synthesis, we evaluated kappa and lambda indexes, which resulted within published cut-offs (<6.39 for kappa and <5.5 for lambda). Moreover, in keeping with this assumption, the CSF/serum albumin ratio was strongly correlated with the CSF/serum ratio of kappa ($r=0.51$, $p<0.001$) and lambda FLCs ($r=0.46$, $p<0.001$) (see Fig. 3), also controlling for the number of *APOE* ε4 alleles (kappa FLCs: $r=0.31$, $p<0.001$; lambda FLCs: $r=0.23$, $p=0.003$).

Focusing solely on participants with AD, there was a slight increase in CSF total protein levels in heterozygous *APOE* ε4 carriers ($p=0.026$) and a notable increase in the CSF/serum albumin ratio ($p<0.001$) (see Fig. 1 and Supplementary Table 1). The CSF/serum kappa and lambda FLCs ratios were also significantly elevated in homozygous *APOE* ε4 participants ($p<0.001$).

In the non-AD participants, comparable results were observed, primarily an increase in both the CSF/serum albumin ratio ($p=0.002$) and the CSF/serum kappa FLCs ratio ($p=0.017$) among homozygous compared to nullizygous and heterozygous *APOE* ε4 carriers (see Fig. 2 and Supplementary Table 2), but not in the CSF/serum lambda FLCs ratio ($p=0.130$), possibly due to the smaller number of *APOE* ε4 carriers.

If participants with SCD were excluded from the non-AD group, we still observed an increase in the CSF/serum albumin ratio ($p=0.026$) among homozygous compared to nullizygous and heterozygous *APOE* ε4 carriers.

Results were comparable if covariates were not considered in all of the analyses.

Table 1
Baseline demographic and clinical characteristics of included participants.

	AD (n=156)	Non-AD (n=74)	*p*-value*
Age, y	72.0 (67.0–75.8)	71.0 (64.5–75.0)	0.213
Sex, female, n (%)	90 (57.7)[†]	28 (37.8)	**0.007**
Disease duration, y	2.0 (1.0–3.0)	1.0 (1.0–3.0)	0.291
APOE distribution			**0.003**
***APOE* ε2/ε2, n (%)**	0 (0.0%)	0 (0.0%)	n.s.
***APOE* ε2/ε3, n (%)**	1 (0.6%)	2 (2.7%)	n.s.
***APOE* ε2/ε4, n (%)**	1 (0.6%)	1 (1.4%)	n.s.
***APOE* ε3/ε3, n (%)**	69 (44.2%)	50 (67.6%)	**0.001**
***APOE* ε3/ε4, n (%)**	67 (42.9%)	19 (25.7%)	**0.011**
***APOE* ε4/ε4, n (%)**	18 (11.5%)	2 (2.7%)	**0.026**
Education, y	8.0 (5.0–13.0)	8.0 (5.0–13.0)	0.512
MMSE score	25.0 (22.0–27.0)	24.0 (21.0–27.0)	0.252
BADL, lost	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.145
IADL, lost	0.0 (0.0–2.0)	1.0 (0.0–2.0)	0.485
CDR-Global	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.378
Hypertension, n (%)	94 (60.3%)	36 (48.6%)	0.117
Dyslipidemia, n (%)	62 (39.7%)	29 (39.2%)	1.000
Diabetes, n (%)	21 (13.5%)	9 (12.2%)	0.837
Heart disease, n (%)	14 (9.0%)	6 (8.1%)	1.000
Stroke, n (%)	0 (0.0%)	6 (8.1%)	**<0.001**
Smoking status			**0.018**
Non-smoker	109 (69.9%)	37 (50.7%)	**0.005**
Current smoker	17 (10.9%)	14 (18.9%)	n.s.
Former smoker	30 (19.2%)	22 (30.1%)	n.s.
CSF total tau, pg/mL	677.5 (470.2–963.0)	330.0 (218.0–396.0)	**<0.001**
CSF p-tau$_{181}$, pg/mL	98.0 (73.0–132.9)	44.5 (33.0–57.0)	**<0.001**
CSF Aβ$_{1–42}$, pg/mL	533.5 (449.3–625.0)	994.0 (672.5–1270.5)	**<0.001**

Data are reported as median (IQR) or n (%).y = years; AD = Alzheimer's disease; non-AD = non-Alzheimer's disease; n.s. = non-significant.
*Kruskal Wallis H test or Chi-squared test, as appropriate, applying FDR correction for multiple comparisons (Benjamini and Hochberg, 1995).

4. Discussion

The present study investigated the association of the *APOE* genotype on BBB permeability in AD and other non-AD neurodegenerative disorders. We observed that the *APOE* ε4 allele was associated with an increased BBB permeability in a dose-dependent relationship. Traditionally, BBB integrity has been assessed *in vivo* using the CSF/serum albumin ratio. This ratio is a reliable indicator of BBB permeability because albumin, a relatively large protein (~67 kDa) synthesized by the liver, does not readily cross the intact BBB (Pardridge et al., 1985). Under normal physiological conditions, the concentration of albumin in the CSF is much lower than in the serum. However, in instances where the BBB is compromised, as seen in individuals with the *APOE* ε4 allele, the barrier's permeability to substances like albumin increases, leading to a higher CSF concentration relative to serum and thus elevating the CSF/serum albumin ratio. This elevation serves as a biomarker of increased BBB permeability and an indirect yet effective measure of the barrier's integrity. However, the complexities surrounding albumin's transport across the BBB, including aspects of its metabolism, cleavage, and uptake by macrophages, along with its turnover and clearance dependent on CSF production and flow, are not fully understood (Jin et al., 2023; Reiber, 2001). Additionally, factors such as the severity of ongoing inflammation or damage in the CNS may independently influence CSF albumin levels, further complicating the interpretation of these markers in neurodegenerative conditions (Akaishi et al., 2015). Thus, a novel aspect of this study is the assessment of the CSF/serum FLCs ratio to corroborate our findings on BBB integrity. FLCs, encompassing both kappa and lambda types, are typically produced by plasma cells and are present in both blood and CSF (Fischer et al., 2004; Hegen et al., 2022;

Table 2
Biological markers in all participants.

	Nullizygous *APOE* ε4 (n=122)	Heterozygous *APOE* ε4 (n=85)	Homozygous *APOE* ε4 (n=23)	*p*-value*
CSF protein, mg/L	400.0 (311.5–510.3)[§]	428.5 (375.3–518.5)	453.0 (367.5–627.3)[†]	**0.027**
CSF cells/μL	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (0.0–2.0)	0.083
CSF/serum albumin ratio × 10^3	5.7 (4.4–7.5)[‡§]	6.4 (5.6–7.9)[†§]	7.5 (7.0–10.4)[‡†]	**<0.001**
CSF/serum κ FLCs ratio × 10^2	1.08 (0.73–1.53)[§]	1.30 (1.0–1.58)[§]	2.7 (1.8–3.0)[‡†]	**<0.001**
CSF/serum λ FLCs ratio × 10^2	1.34 (1.01–1.80)[§]	1.65 (1.2–1.9)[§]	2.3 (1.62–4.22)[‡†]	**0.001**
CSF total tau, pg/mL	507.0 (323.0–804.3)	534.5 (389.8–840.1)	468.0 (372.8–846.0)	0.506
CSF p-tau$_{181}$, pg/mL	72.0 (44.0–116.0)	84.5 (61.8–127.1)	69.8 (61.0–139.3)	0.712
CSF Aβ$_{1–42}$, pg/mL	675.5 (516.8–997.8)[‡§]	560.5 (460.0–675.0)[†§]	465.5 (368.6–514.0)[‡†]	**<0.001**

Data are reported as median (IQR); n = number of patients; *APOE* ε4 (0, 1, 2) = number of *APOE* ε4 alleles; CSF = cerebrospinal fluid; FLCs = free light chains. Pairwise comparisons after significant difference at the *non-parametric ANCOVA (Quade's test) for [†]$p<0.05$ vs nullizygous *APOE* ε4, [‡]$p<0.05$ vs heterozygous *APOE* ε4, [§]$p<0.05$ vs homozygous *APOE* ε4, applying FDR correction for multiple comparisons (Benjamini and Hochberg, 1995).

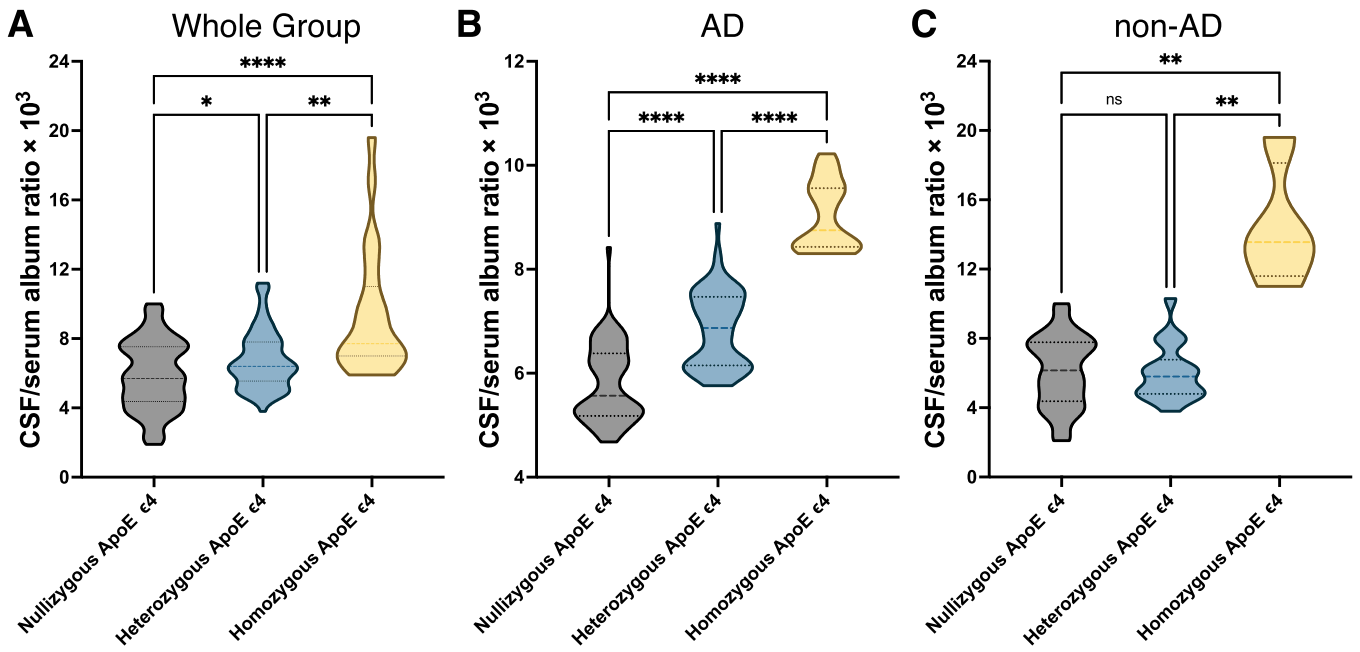

Fig. 1. CSF/serum albumin ratio in A) whole group, B) in AD and C) non-AD participants, according to the number of *APOE* ε4 alleles. AD = Alzheimer's disease; non-AD = non-Alzheimer's disease; ns = non-significant; *$p<0.050$; **$p<0.005$; ***$p<0.001$.

Lo Sasso et al., 2019; Reiber, 2001). Like albumin, FLCs do not easily cross an intact BBB due to their size and the barrier's selective permeability. In our study, the observed increase in the CSF/serum FLCs ratio in individuals with the *APOE* ε4 allele add a substantial dimension to the current understanding of BBB permeability and its implications in neurodegenerative disorders, suggesting that the increased permeability extends beyond albumin to other large molecules. While our findings of an elevated CSF/serum FLC ratio in individuals carrying the *APOE* ε4 allele align with the concept of increased BBB permeability, it is important to consider that this elevation could also suggest increased intrathecal production of FLCs, possibly reflecting an immune response within the central nervous system (Berek et al., 2021; Hegen et al., 2022). Nevertheless, the absence of a notable increase in the kappa or lambda index and their strong correlation with the CSF/serum albumin ratio points towards a primary association with BBB permeability (Gudowska-Sawczuk et al., 2021; Leurs et al., 2020; Senel et al., 2019). The simultaneous increase in both kappa and lambda FLCs supports this interpretation, suggesting that BBB permeability changes in *APOE* ε4 carriers affect multiple large molecules.

Several previous studies have already shown that AD is characterized by an increased BBB permeability, even in the preclinical and prodromal stages of disease (Halliday et al., 2013; Janelidze et al., 2017; Montagne et al., 2015; Nation et al., 2019; Nishitsuji et al., 2011; Skillbäck et al., 2017; Skoog et al., 1998; Sweeney et al., 2015; van de Haar et al., 2016). However, studies assessing the effect of *APOE* on BBB permeability in vivo have yielded inconsistent findings (Bruno et al., 2023; Janelidze et al., 2017; Montagne et al., 2020; Toniolo et al., 2023). Recent insights have clarified that that the *APOE* ε4 allele affects BBB integrity through several mechanisms. This includes the interaction between *APOE* ε4 and low-density lipoprotein receptor-related protein 1 (LRP1) on pericytes, which are pivotal in maintaining BBB stability (Nehra et al., 2022). The low binding affinity between human *APOE* ε4 and LRP1 increases intracellular Cyclophilin A in pericytes, leading to NFκB activation and the subsequent release of matrix metalloproteinase-9 (MMP9), which degrades essential components of the BBB (Bell et al., 2012; Ma et al., 2018; Machida et al., 2015; Zlokovic, 2013). *APOE* ε4 is associated with accelerated pericyte loss and enhanced activation of the LRP1-dependent Cyclophilin A-MMP9 pathway in pericytes and endothelial cells, potentially leading to more extensive BBB damage in AD *APOE* ε4 carriers (Halliday et al., 2016). Furthermore, in endothelial cells, *APOE* ε4 redirects amyloid-β clearance from LRP1 to the very low-density lipoprotein receptor. This redirection slows down amyloid-β clearance, causing its accumulation, which then contributes to the degradation of the endothelial basement membrane and tight junctions via MMP9 (Kirchner et al., 2023; Rempe et al., 2016). Additionally, the *APOE* ε4 allele causes a reduction in LRP1 on endothelial cells, leading to the loss of crucial endothelial tight junction proteins and further compromising BBB integrity (Ma et al., 2018; Yamazaki et al., 2019).

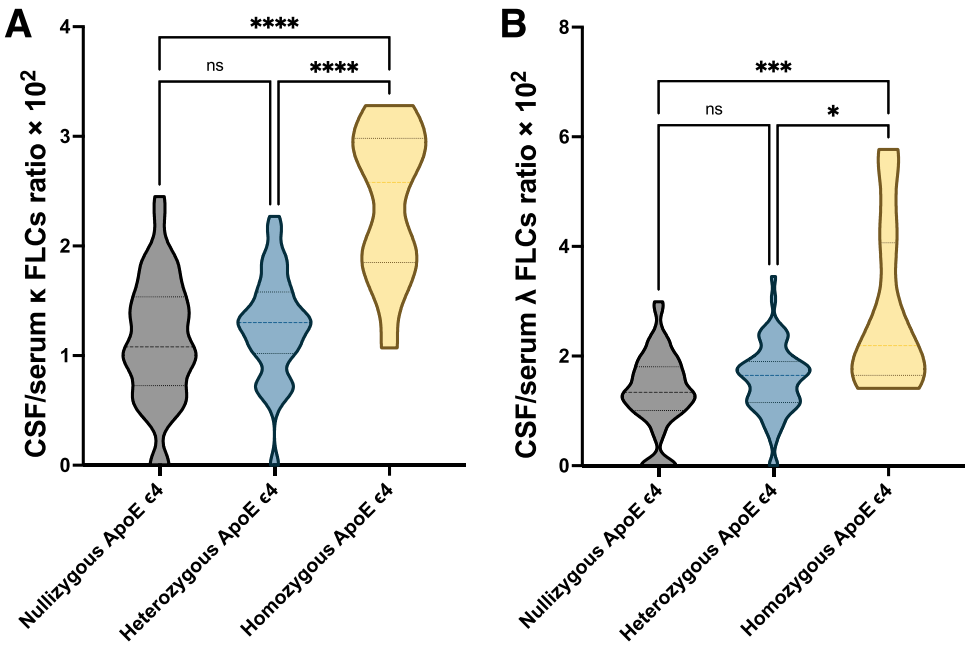

Fig. 2. CSF/serum A) kappa and B) lambda free light chain ratio according to the number of *APOE* ε4 alleles. FLCs = free light chains; ns = non-significant; *$p<0.050$; **$p<0.005$; ***$p<0.001$.

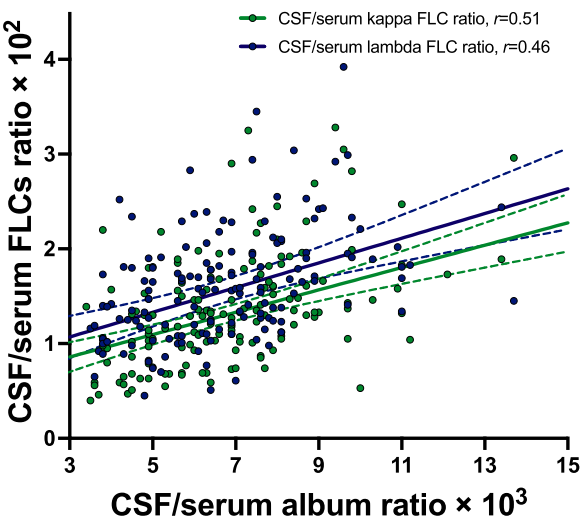

Fig. 3. Correlations between CSF/serum albumin ratio and both CSF/serum kappa and lambda free light chain ratios. FLCs = free light chains.

It should be also considered that recent studies have shown that the CSF/serum albumin ratio may also be influenced by biomarkers of angiogenesis and endothelial dysfunction, particularly in patients with vascular diseases and diabetes (Janelidze et al., 2017; Lin et al., 2021; Miners et al., 2019; Taheri et al., 2011; Wada, 1998). These findings underline the much more complex interplay between neurodegenerative processes and cerebrovascular conditions. To account for these factors, in our statistical analysis we included covariates that have been demonstrated to correlate with differences in BBB permeability, including male sex, increased age, longer disease duration, higher disease severity, diagnosis, and comorbidities such as hypertension, dyslipidaemia, diabetes, heart disease, stroke, and smoking status (Bors et al., 2018; Janelidze et al., 2017; Lin et al., 2021; Miners et al., 2019; Montagne et al., 2015; Parrado-Fernández et al., 2018; Skillbäck et al., 2021; Taheri et al., 2011; Wada, 1998).

An interesting finding of our study is the observation that *APOE* ε4 significantly influenced BBB permeability in participants across both AD and non-AD groups. This suggests a broader role for *APOE* ε4 in neurodegeneration, extending beyond its established association with AD (Boccardi et al., 2004; Chen et al., 2016; Mehta et al., 2007; Seripa et al., 2011). The presence of BBB damage in non-AD conditions, particularly in the context of *APOE* ε4 carriers, raises crucial questions about the common underlying pathways in various neurodegenerative diseases (Moon et al., 2021). This observation may provide valuable insights into shared therapeutic targets, emphasizing the need for a more unified approach in treating neurodegeneration.

These findings could have significant implications in clinical contexts, particularly regarding blood-based biomarkers, which are markedly influenced by several factors, including BBB permeability (Deli et al., 2005; Kadry et al., 2020; Stocker et al., 2023). For instance, a recent study has revealed that BBB integrity affects plasma amyloid-$\beta_{42/40}$ levels, indicating that a decreased BBB integrity enhances the efficacy of plasma amyloid-$\beta_{42/40}$ in detecting brain amyloid pathology (Bellaver et al., 2023).

The results presented in this study should be interpreted considering certain limitations. Firstly, BBB permeability was inferred using CSF/serum albumin and FLCs ratios, which, while informative, are indirect measures. Furthermore, the reliance on clinical diagnoses, although supported by biomarkers, especially for conditions like DLB, introduces an element of uncertainty, as definitive diagnosis is often possible only post-mortem and may reveal mixed pathology. This limitation underscores the need for caution in interpreting our findings, particularly regarding the potential overlap and co-pathologies of neurodegenerative diseases like DLB and AD. Additionally, the study's cross-sectional nature limits our ability to infer causal relationships or the directionality of the observed associations. Future longitudinal studies with larger, more diverse cohorts, including also healthy controls, are essential to validate these findings and to understand the temporal dynamics of BBB permeability changes in relation to neurodegenerative disease progression.

In conclusion, our study provides evidence of the association between the *APOE* ε4 allele and BBB permeability in neurodegenerative disorders. This finding not only advances our understanding of the pathophysiological mechanisms underlying these diseases but also opens new avenues for diagnostic and therapeutic strategies. As the field

moves forward, integrating genetic, molecular, and clinical data will be crucial in developing a holistic approach to managing neurodegenerative diseases. Our study contributes to the understanding of the relationship between genetics and BBB integrity in neurodegeneration, providing new insights into this complex interplay.

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CRediT authorship contribution statement

Laura Trainini: Writing – review & editing, Methodology. **Silvana Archetti:** Writing – review & editing, Methodology, Data curation. **Andrea Pilotto:** Writing – review & editing, Data curation. **Antonella Alberici:** Writing – review & editing, Data curation. **Salvatore Caratozzolo:** Writing – review & editing, Data curation. **Chiara Silvestri:** Writing – review & editing, Data curation. **Ilenia Libri:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Alberto Benussi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessandro Padovani:** Writing – review & editing, Supervision, Data curation. **Barbara Borroni:** Writing – review & editing, Data curation.

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Potential conflicts of interest

Nothing to report.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neurobiolaging.2024.04.003.

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