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Associations of CSF PDGFRβ With Aging, Blood-Brain Barrier Damage, Neuroinflammation, and Alzheimer Disease Pathologic Changes

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Abstract

Background and objectives

Injured pericytes in the neurovascular unit release platelet-derived growth factor β (PDGFRβ) into the cerebrospinal fluid (CSF). However, it is not clear how pericyte injury contributes to Alzheimer's disease (AD)-related changes and blood brain barrier (BBB) damage. We aimed to test if CSF PDGFRβ was associated with different AD- and age-associated pathological changes leading to dementia.

Methods

PDGFRβ was measured in the CSF of 771 cognitively unimpaired (CU, n=408), mild cognitive impairment (MCI, n=175) and dementia subjects (n=188) from the Swedish

BioFINDER-2 cohort. We then checked association Aβ-PET and tau-PET SUVR, *APOE* ε4 genotype and MRI measurements of cortical thickness, white matter lesions (WML) and cerebral blood flow (CBF). We also analysed the role of CSF PDGFRβ in the relationship between aging, BBB dysfunction (measured by CSF/plasma albumin ratio, QAlb) and neuroinflammation (i.e., CSF levels of YKL-40 and glial fibrillary acidic protein [GFAP], preferentially expressed in reactive astrocytes).

Results

The cohort had a mean age of 67 years (CU=62.8, MCI=69.9, dementia=70.4) and 50.1% were male (CU=46.6%, MCI=53.7%, dementia=54.3%). Higher CSF PDGFRβ concentrations were related to higher age (b=19.1, β=0.5, 95% CI=16-22.2, p<0.001), increased CSF neuroinflammatory markers of glial activation YKL-40 (b=3.4, β=0.5, 95% CI=2.8-3.9, p<0.001) and GFAP (b=27.4, β=0.4, 95% CI=20.9-33.9, p<0.001), and worse BBB integrity measured by QAlb (b=37.4, β=0.2, 95% CI=24.9-49.9, p<0.001). Age was also associated with worse BBB integrity, and this was partly mediated by PDGFRβ and neuroinflammatory markers (16-33% of total effect). However, PDGFRβ showed no associations with *APOE* ε4 genotype, PET imaging of Aβ and tau pathology or MRI measures of brain atrophy and white matter lesions (p>0.05).

Discussion

In summary, pericyte damage, reflected by CSF PDGFRβ, may be involved in age-related BBB disruption together with neuroinflammation, but is not related to Alzheimer-related pathological changes.

Introduction

The neurovascular unit (NVU) is an anatomical and functional complex that includes neurons, glial cells (astrocytes, oligodendrocytes, microglia) and vascular cells (endothelium, pericytes and vascular smooth muscle cells) [1]. All these structures, and especially the vascular cells, concur in maintaining the integrity of the blood-brain barrier (BBB), a selective diffusion barrier responsible for the homeostasis of the central nervous system, which allows optimal synaptic and neuronal function [1]. According to the "two-hit" hypothesis of Alzheimer's disease (AD) pathogenesis, mid-life cardiovascular and metabolic risk factors (e.g., hypertension and diabetes) trigger the pathological disease cascade by causing damage to the NVU [1, 2]. It has been hypothesized that this damage to the NVU causes disruption of the BBB and reduction of cerebral blood flow (first hit), that ultimately leads to reduced amyloid β (Aβ) clearance and formation of Aβ-containing plaques (second hit) [1]. One of the key structural and functional elements of the NVU are pericytes, which are cells that adhere to the endothelium and are involved in maintaining the BBB, while regulating cerebral blood flow in the brain [1]. The platelet-derived growth factor receptor β (PDGFRβ) is expressed in brain pericytes during cell migration and angiogenesis, and it has also been found in minor part on the surface of vascular smooth muscle cells, but not on neurons, astrocytes, endothelium, microglia or oligodendroglia [3]. When the BBB is damaged, PDGFRβ is released in CSF from pericytes, but not from vascular smooth muscle cells, making it a CSF marker specific for pericyte injury [4]. In studies where AD was diagnosed not only based on clinical symptoms, but also with support of CSF biomarkers, higher levels of CSF PDGFRβ were associated with the severity of clinical symptoms and brain vascular damage [3, 5]. Further, it has been proposed that CSF PDGFRβ predicts subsequent cognitive decline in *APOE* ε4 carriers [5, 6]. We also know that BBB damage increases with age and that aging is

the strongest risk factor for AD dementia [7, 8]. However, it is still unclear how CSF PDGFRβ relates to aging in general and aging and key pathologic changes of AD in particular: different studies show varying associations of CSF PDGFRβ with age and Aβ and tau CSF biomarkers [3, 5, 9, 10]. Large-scale clinical studies are needed to determine its association with aging, fibrillar Aβ and tau aggregates, brain atrophy, blood flow, as well as neuroinflammation and BBB integrity.

The aim of this paper was to determine whether CSF PDGFRβ is indeed associated with aging and key AD pathological changes (measured with Aβ-PET and tau-PET) and *APOE* ε4 genotype in the deeply phenotyped BioFINDER-2 cohort. Further, the relationship of CSF PDGFRβ to MRI measurements of cortical thickness, white matter lesions (WML) and cerebral blood flow (CBF) were studied. Finally, we analysed the role of CSF PDGFRβ in the relationship between aging, BBB dysfunction (measured by CSF/plasma albumin ratio, QAlb [11]) and neuroinflammation (i.e., CSF levels of YKL-40 and glial fibrillary acidic protein [GFAP], preferentially expressed in reactive astrocytes).

Methods

Standard protocol approvals, registrations, and patient consent

All participants gave written informed consent. Ethical approval was given by the Regional Ethical Committee in Lund, Sweden.

Study cohort

The cohort included participants from the Swedish BioFINDER-2 study (NCT03174938). All participants were recruited at Skåne University Hospital and the Hospital of Ängelholm,

Sweden. The cohort covers the full spectrum of AD, ranging from adults with intact cognition or subjective cognitive decline, mild cognitive impairment (MCI), to dementia. The main inclusion criteria, as described previously[12], were to be 40 years and older, being fluent in Swedish, having Mini-Mental State Examination (MMSE) between 27 and 30 for cognitively unimpaired (CU) participants, between 24 and 30 for MCI, and equal to or above 12 for AD dementia patients. MCI diagnosis was established if participants performed below 1.5 standard deviation from norms on at least one cognitive domain from an extensive neuropsychological battery examining verbal fluency, episodic memory, visuospatial ability, and attention/executive domains. Patients with AD dementia, vascular dementia (VaD), behavioural variant of frontotemporal dementia (bvFTD) and dementia with Lewy bodies (DLB) fulfilled the respective criteria of the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition [13]. Semantic and non-fluent variants of primary progressive aphasia (svPPA, nfvPPA) were defined according to the Gorno-Tempini criteria [14]. All patients were genotyped for *APOE*. Exclusion criteria included severe somatic disease, and current alcohol/substance misuse. CSF sampling and imaging investigations were done at the time of enrolment, in conjunction with the clinical examination and cognitive tests. The study was approved by the Regional Ethics Committee in Lund, Sweden. All participants gave written informed consent to participate.

CSF sampling and analysis

CSF was collected by lumbar puncture and stored at − 80 °C in polypropylene tubes following the Alzheimer's Association flow chart for lumbar puncture and CSF sample processing [15]. PDGFRβ was measured with the Human Total PDGFRβ DuoSet IC enzyme-linked immunosorbent assay (ELISA; R&D Systems Europe) with few adaptations. Briefly, the standard curve followed a 1:3 dilution, starting from 12000 pg/mL. Capture antibody was

diluted in PBS. 1% MSD Blocker A buffer (cat#R93BA-4, MesoScale Diagnostics) in PBS was used to dilute standards and as blocking buffer. Detection antibody and streptavidin were diluted in 20 mM Tris, 137 mM NaCl, Tween 0.05%, 0.1% BSA, pH 7.2-7.4. Inter-assay variability (CV%) measured over 14 runs was 7.3%. For a detailed description of the protocol, see supplementary material (eMethods). Aβ42, Aβ40, p-tau181, YKL-40 and GFAP were measured with NeuroToolKit (Roche Diagnostics International Ltd., Germany). Cut-off for an Aβ-positive (Aβ+) status was calculated with Youden index in the cohort, based on CSF Aβ42/40 (cutoff=0.08) [16].

Brain imaging

Aβ-PET images were acquired on digital GE Discovery MI scanners 90-110 min after the injection of ~185 MBq [18F]flutemetamol. Standardized uptake value ratio (SUVR) was calculated with pons as reference region. For the analysis, Aβ PET measures were considered both as continuous SUVR and as binarized data using a cut-off derived from mixture modelling in the BioFINDER-2 cohort (0.53 SUVR) [16]. A neocortical meta-ROI for Aβ-PET (prefrontal, lateral temporal, parietal, anterior cingulate, and posterior cingulate/precuneus) was calculated, as previously described [16, 17]. According to the enrolment protocol, Aβ-PET was not performed in the dementia group.

Tau-PET images were acquired on digital GE Discovery MI scanners 70-90 min post injection of ~370 MBq [18F]RO948. Tau PET SUVR were created using the inferior cerebellar cortex as reference region [16]. A temporal meta-ROI for tau-PET (entorhinal cortex, inferior and middle temporal cortices, fusiform gyrus, parahippocampal cortex and amygdala) was created, as previously described [18].

Structural MRI was performed using a Siemens 3T MAGNETOM Prisma scanner (Siemens Medical Solutions), with high resolution T1-weighted anatomical magnetization-prepared

rapid gradient echo (MPRAGE) images (1mm isotropic voxels). T1-images underwent volumetric segmentation and parcellation using FreeSurfer (v.6.0, https://surfer.nmr.mgh.harvard.edu). Cortical thickness was measured as the distance from the gray matter–white matter boundary to the perpendicular pial surface, as previously described [19]. The AD-specific cortical thickness meta-ROI (AD signature) was measured in regions with known susceptibility to atrophy in AD (entorhinal, fusiform, inferior temporal and middle temporal regions), adjusted for cortical surface area. Automated segmentation of WML using the LST toolbox implemented in SPM8 (https://www.applied-statistics.de/lst.html) generated a total lesion volume (mL), which was then normalized for intracranial volume (ICV), as previously described [20]. Total grey matter CBF was measured in a smaller cohort of subjects in the AD continuum (CU, MCI, AD dementia, n=392) with arterial spin labelling (ASL), see [21] for full method description.

Statistics

Statistical analysis and data visualization were performed with SPSS v. 26 (IBM). P values <0.05 were considered significant. Group differences were assessed in univariate general linear models, with post hoc least significant difference (LSD) tests for pairwise group comparisons. Biomarker values were LOG10 transformed prior to this analysis. Linear regression models were used to determine the associations between aging, biomarkers and imaging measures to PDGFRβ and to test for interaction between variables. For each linear model, subjects were excluded if they had one or more missing data in the variables included in the individual model. Mediation analysis was performed in SPSS with the PROCESS v3.5 extension (https://www.processmacro.org/index.html) with a bootstrap method for the confidence intervals (CI) of the mediated effect (n iterations=5000). Mediation effect was considered significant if the 95% CI did not include 0. Unless described otherwise, analyses

were adjusted for age, sex, diagnosis and ventricular volume. Numbers after the decimal point were rounded to the first significant figure.

Data availability

Anonymized data will be shared by request from any qualified investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation.

Results

Study cohort

The study cohort consisted of 771 subjects diagnosed as CU (n=408), MCI (n=175) or dementia (n=188) patients (Table 1). Disorders in the dementia group included AD (n=124), DLB (n=28), bvFTD (n=13), svPPA (n=6), nfvPPA (n=3) and VaD (n=14). There were, as expected, significant differences in age, *APOE* status, Mini Mental State Examination (MMSE) score, Aβ status and Aβ- and tau-PET SUVR between the CU, MCI and dementia groups ($p<0.001$) (Table 1). Men had higher CSF levels of PDGFRβ ($p<0.001$, eFigure 1). There were no differences in CSF concentrations of PDGFRβ between *APOE* ε4 carriers (one or two alleles) and non-carriers ($p>0.05$; Table 1, eFigure 2). CSF PDGFRβ concentrations did not differ between CU, MCI and dementia groups ($p>0.05$; eFigure 3A).

Associations between PDGFRβ and age

CSF PDGFRβ was overall significantly associated with age (b=19.1, β=0.5, 95% CI=16-22.2, $p<0.001$; Figure 1). There was an interaction effect between age and diagnosis on CSF PDGFRβ (b=5.3, β=0.6, 95% CI=0.6-10, $p=0.03$), but significant associations between age and CSF PDGFRβ survived in the diagnostic subgroups (CU: b=18.7, β=0.5, 95% CI=15-

22.4, p<0.001; MCI: b=22.2, β=0.4, 95% CI=13.1-31.2, p<0.001; dementia: b=26.5, β=0.3, 95% CI=15.3-37.7, p<0.001).

To better understand the relationship between age and PDGFRβ, we next studied whether this pericyte-injury marker was associated with other age-related pathological brain changes including key AD pathologies (Aβ and tau aggregates), small-vessel disease expressed as white matter lesions (WML), neuroinflammation and blood-brain barrier dysfunction.

Associations between PDGFRβ and AD-related pathological changes

CSF levels of PDGFRβ did not differ within diagnostic groups divided according to Aβ status or according to type of dementia (AD and non-AD dementias) (p>0.05 for all pairwise comparisons, eFigure 3B). Further, no associations were observed between CSF PDGFRβ and Aβ-PET SUVR (n=553, p>0.05; Figure 2A) or between CSF PDGFRβ and tau-PET SUVR (n=743, p>0.05; Figure 2B). Finally, the association between age and CSF PDGFRβ was not weakened when adjusting for Aβ- and tau-PET (n=544; b=18.9, β=0.5, 95% CI=15.2-22.1, p<0.001). Interaction between diagnosis and Aβ- or tau-PET had no significant effect on CSF PDGFRβ (p>0.05).

Associations between PDGFRβ and MRI measures

There were no associations between CSF PDGFRβ and cortical thickness in the temporal AD signature regions (n=749, p>0.05; Figure 2C). The WML volume (n=693, Figure 2D) and total grey matter CBF (n=392) were not associated with the CSF levels of PDGFRβ (p>0.05). The group sizes of the smaller cohort that underwent CBF analysis were consistent with those of the whole cohort (CU: n=236 vs. 408 in the whole cohort; MCI: n=84 vs. 175; dementia:

n=72 vs. 188). Interaction between diagnosis and measures of cortical thickness, WML volume or CBF had no significant effect on CSF PDGFRβ ($p>0.05$).

Associations between PDGFRβ and markers of BBB dysfunction and neuroinflammation

CSF PDGFRβ was overall associated to the CSF/plasma albumin ratio (QAlb) (n=738, b=37.4, β=0.2, 95% CI=24.9-49.9, $p<0.001$; Figure 3A). There was a significant interaction effect between QAlb and diagnosis on the levels of CSF PDGFRβ (b=-25.2, β=-0.3, 95% CI=-48.5- -1.9 , p=0.002). Association with QAlb was not significant in the dementia subgroup ($p>0.05$). CSF PDGFRβ levels also showed overall strong associations to the neuroinflammatory markers YKL-40 (n=729, b=3.4, β=0.5, 95% CI=2.8-3.9, $p<0.001$; Figure 3B) and GFAP (n=732, b=27.4, β=0.4, 95% CI=20.9-33.9, $p<0.001$; Figure 3C). The effect of the interaction between inflammatory markers and diagnosis on CSF PDGFRβ was not significant ($p>0.05$).

Analysis of the effects of age on BBB dysfunction mediated by PDGFRβ-related changes and neuroinflammation

Since age, CSF PDGFRβ and CSF markers reflecting neuroinflammation (YKL-40, GFAP) were associated with QAlb (eTable 1) and R^2 for the models with combined effects of predictors was higher than that for individual effects (eTable 2), we performed a sequential statistical mediation analysis to determine if neuroinflammation and pericyte damage affect the relationship between age and QAlb. We observed that CSF PDGFRβ fully mediated the effect of YKL-40 on QAlb (b=0.01, β=0.05, 95% CI=0.01-0.02, $p<0.05$; sequential mediation shown by blue arrows in Figure 4A) since direct effect of YKL-40 on QAlb was not significant ($p>0.05$, red arrows in Figure 4A). The indirect mediation effect of CSF

PDGFRβ accounted for 16.6% of the total effect (b=0.01, β=0.02, 95% CI=0.002-0.01, p<0.05; green arrows in Figure 4A). The indirect mediation effect of GFAP on QAlb accounted for 33.3% of the total effect (b=0.02; β=0.08, 95% CI=0.01-0.04, p<0.05; red arrows in Figure 4B). In this model, CSF PDGFRβ showed a similar-sized (16.6%) indirect mediation effect on the total effect of age on QAlb (b=0.01, β=0.04, 95% CI=0.01-0.02, p<0.05; green arrows in Figure 4B). When considering the mediators individually (not corrected for each other in the same model), they all showed a significant mediation of the effects of age on QAlb (b=0.2-0.03, β=0.1, p<0.001), accounting for 33-50% of the total effect (eFigure 4, A–C).

Discussion

In this study, we have consistently shown that CSF PDGFRβ, a pericyte-specific marker, increases with age and is associated to BBB dysfunction (CSF/plasma albumin ratio, QAlb) and glial activation/neuroinflammation (CSF YKL-40 and GFAP). We also found that both age and the glial biomarkers are associated with QAlb. Interestingly, the effects of age on the BBB integrity were partially mediated by pericyte damage and neuroinflammation. CSF PDGFRβ was not related to other age-related pathologies such as AD pathological changes, as reflected by the lack of association with *APOE* ε4 genotype or with accumulation Aβ and tau aggregates as measured with PET imaging. Levels of CSF PDGFRβ were also not related to presence of WML or changes in CBF.

Aging is associated with morphological and functional changes in BBB and preclinical evidence indicates that age-related pericyte degeneration and reduced pericyte coverage could cause BBB breakdown, impairment of protein transcytosis, vascular damage, and alterations in blood flow (reviewed in [22, 23]). Although a prior study in living people indicated

that BBB integrity loss (measured at dynamic contrast-enhanced MRI [DCE-MRI]) was age-dependent and correlated with CSF levels of PDGFRβ levels, overall investigations in clinical cohorts are few, biased by a small samples size and often reporting conflicting results [24]. For instance, some (but not all) studies have shown correlations of PDGFRβ with age as well as with QAlb [3, 5, 9, 10, 24, 25]. Here we report that in a large cohort of well-characterized participants, older age was consistently associated with higher CSF levels of PDGFRβ and that the association was unaffected by clinical diagnosis and possible concomitant AD pathology. QAlb was also consistently associated with PDGFRβ in CU and MCI and at whole cohort level, with the exception of the dementia subgroup. Taken together, these findings provide support that age-related pericyte injury is associated with BBB dysfunction, and not with AD pathology.

With aging increases also the neuroinflammatory activity in astrocytes, cells whose processes directly connect to the BBB in the NVU (reviewed in [22, 23, 26]). Pericytes themselves can both respond to and themselves secrete inflammatory cytokines, sustaining the local inflammation in the NVU and contributing to BBB disruption [27-29]. Our study is the first to investigate the effect of the complex relationship between age, neuroinflammation and pericyte damage on the integrity of the BBB in a large clinical cohort. Although we cannot prove causality through statistical mediation analysis, we lift the hypothesis that both neuroinflammation (as partly reflected by the astrocytic markers YKL-40 and GFAP) and pericyte damage mediate the effects of age on the BBB. We also propose a model where age triggers increase in neuroinflammation and pericyte damage, which are both involved in the disruption of the BBB. Further, we suggest that, based on their individual and combined effects, neuroinflammation and pericyte damage interact in the disruption of the neurovascular unit.

In contrast to our findings, increases in CSF PDGFRβ concentrations have been observed in AD defined clinically or by A/T/N classification [3, 9, 10, 24, 30]. The lack of association between CSF PDGFRβ and Aβ status and Aβ or tau biomarkers was observed previously [3, 5, 9, 10, 24], although one study showed that Aβ burden modulated the association of PDGFRβ with Tau-PET [31]. Other authors also did not find an association between PDGFRβ and small vessel disease in cerebral amyloid angiopathy (CAA) subjects [9]. The existing literature has important differences from our study that need to be considered. Ours is the largest PDGFRβ clinical study to date and was conducted in a cohort characterized with not only CSF but also imaging measures. Previously, clinical groups were mostly defined based on clinical diagnosis, and the only differences in CSF PDGFRβ in groups defined by biomarkers were between A+/T+/N+ and A-/T-/N- (i.e., a difference was only seen when amyloid, tau and neurodegeneration CSF biomarkers were pathological, but not when only core AD biomarkers were abnormal) or within cohorts defined by A/T/N that only included preclinical AD [3, 5, 9, 10, 24]. Most importantly, this is one of the few and the largest study using PET imaging and not only CSF biomarkers. PET imaging accurately defines the load of the core AD pathological changes, i.e., the amount and spread of insoluble Aβ and tau aggregates, which is not influenced by possible CSF dynamics that can affect biomarker concentration [32, 33]. Method-wise, some of these studies used a western blot method for detection of PDGFRβ in CSF instead of ELISA, which might have led to lower accuracy in the measurements [5, 6, 24, 31]. The studies where ELISA was used had a smaller sample size than ours [3, 9, 10, 25]. In the only study that compared the western blot and ELISA methods in parallel, the authors suggest that the two techniques measure different species of PDGFRβ, which might have led to discrepancies in the results between different studies [9]. Another possible limitation of the study is the use of QAlb to measure integrity of the BBB, which raised questions on whether this is the best method [34]. QAlb has been shown to perform satisfactorily in this sense,

especially in dementia studies [11, 35]; however, more sensitive methods for detecting BBB dysfunction using MRI neuroimaging have been used in other studies [36], showing that BBB permeability is affected differently by AD pathology and cardiovascular risk factors. This warrants adjustment for cardiovascular risk scores in future studies.

Despite convincing evidence of the interplay between age, pericyte injury, neuroinflammation and BBB damage, the actual extent of their role in aging and disease remains unclear. Targeted longitudinal studies in clinical cohorts and *in vivo* models are needed to confirm these observations and investigate the relationship between microglia, pericytes and BBB in the aging brain.

In conclusion, we observed that the levels of CSF PDGFRβ increase with age and are associated with neuroinflammation and BBB dysfunction, but not with other age-related pathologies such as AD pathological changes or WMLs. We also propose that pericyte damage partially mediates the disruptive effects of age on the BBB, together with neuroinflammation. Further studies are however needed to clarify the role of pericyte injury in aging, BBB dysfunction, and neurodegenerative diseases.

Table 1. Characteristics of the study cohort

	CU	MCI	Dementia	p
N	408	175	188	
Mean age (min-max)	62.8 (20-88)	69.9 (43-84)	70.4 (52-87)	<0.001[1]
Sex (% M)	46.6%	53.7%	54.3%	0.1[2]
At least one *APOE* **ε**4 allele	42.4%	51.4%	59%	<0.001[2]
MMSE score (mean)	29	26.9	21.1	<0.001[1]
Aβ-PET (SUVR, mean)	0.5	0.7	0.8	<0.001[1]

Aβ+ status (based on Aβ-PET, % positive)	20.3%	58.1%	75%	<0.001[2]
Aβ+ status (based on CSF Aβ42/40, % positive)	21.1%	57.5%	80%	<0.001[2]
Tau-PET (SUVR, mean)	1.2	1.3	1.8	<0.001[1]
CSF PDGFRβ (pg/mL, mean)	1719.7	1754.4	1847.8	0.06[1]

P values for differences between diagnostic groups were measured with [1]ANOVA or [2]Chi square tests.

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Figure legends

Figure 1. Scatter-dot plot representing the correlation between CSF PDGFRβ and age in the whole sample (n=771).

CU, MCI and dementia subjects shown in blue, green and red, respectively. Regression line with 95% confidence intervals is not adjusted for covariates.

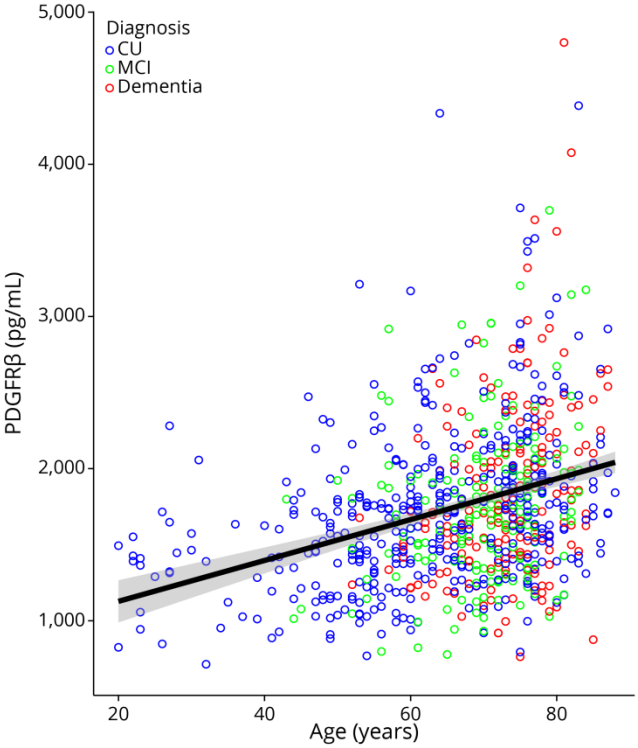


Figure 2. CSF PDGFRβ and AD imaging measures.

Scatter-dot plot representing the correlation between CSF PDGFRβ and Aβ-PET SUVR in the neocortical meta-ROI (A), tau-PET SUVR in the temporal meta-ROI (B), weighted cortical thickness in the AD signature meta-ROI (entorhinal, fusiform, inferior temporal and middle temporal (C) and volume of white matter lesions (D) in the whole sample. CU, MCI and dementia subjects shown in blue, green and red, respectively. According to the study protocol, Aβ-PET was not performed in dementia subjects. Regression lines with 95% confidence intervals are not adjusted for covariates.

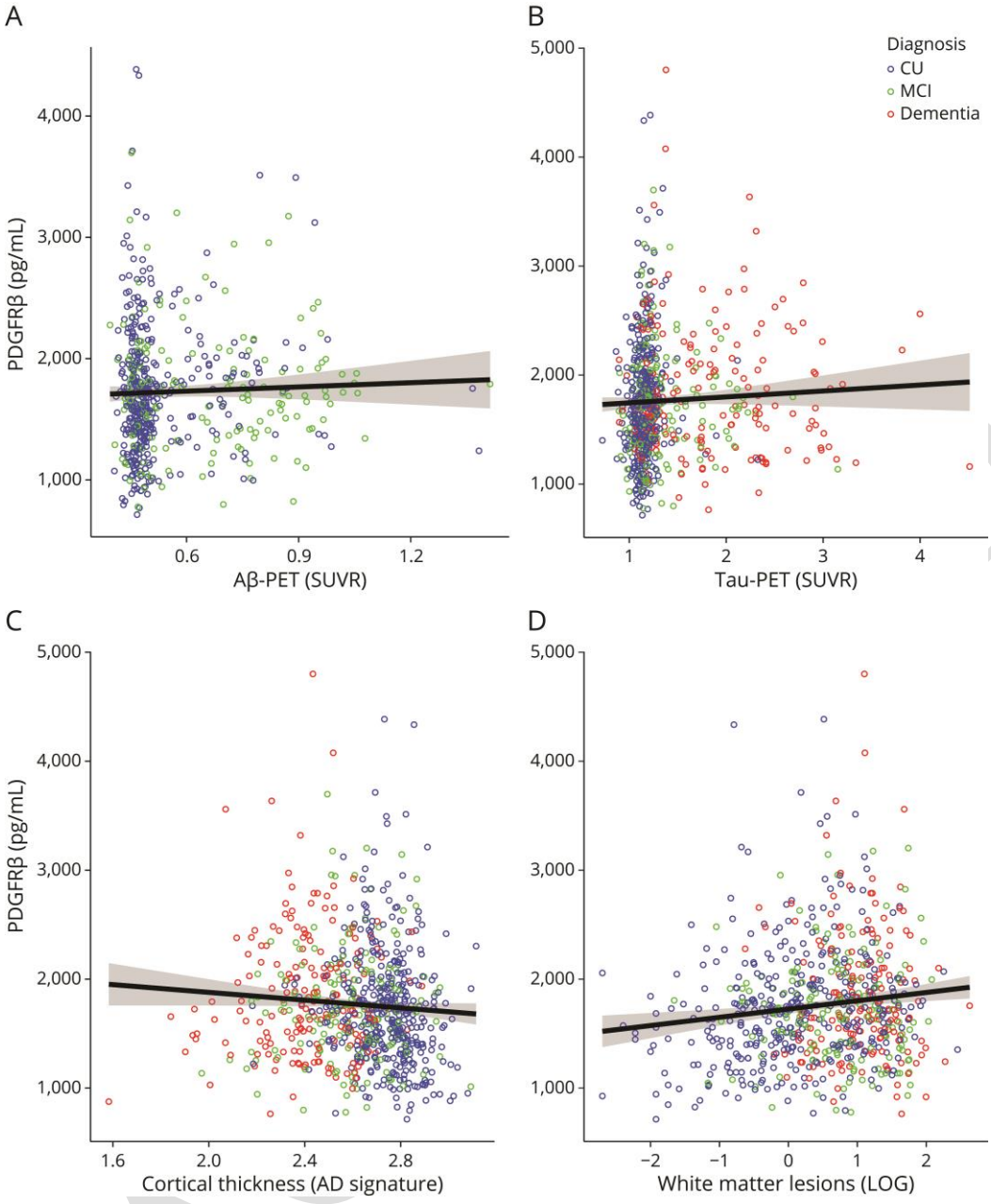


Figure 3. Scatter-dot plot representing the correlation between CSF PDGFRβ and the CSF/plasma albumin ratio (QAlb, A), YKL-40 (B) and GFAP (C).

CU, MCI and dementia subjects shown in blue, green and red, respectively. Regression lines with 95% confidence intervals are not adjusted for covariates.

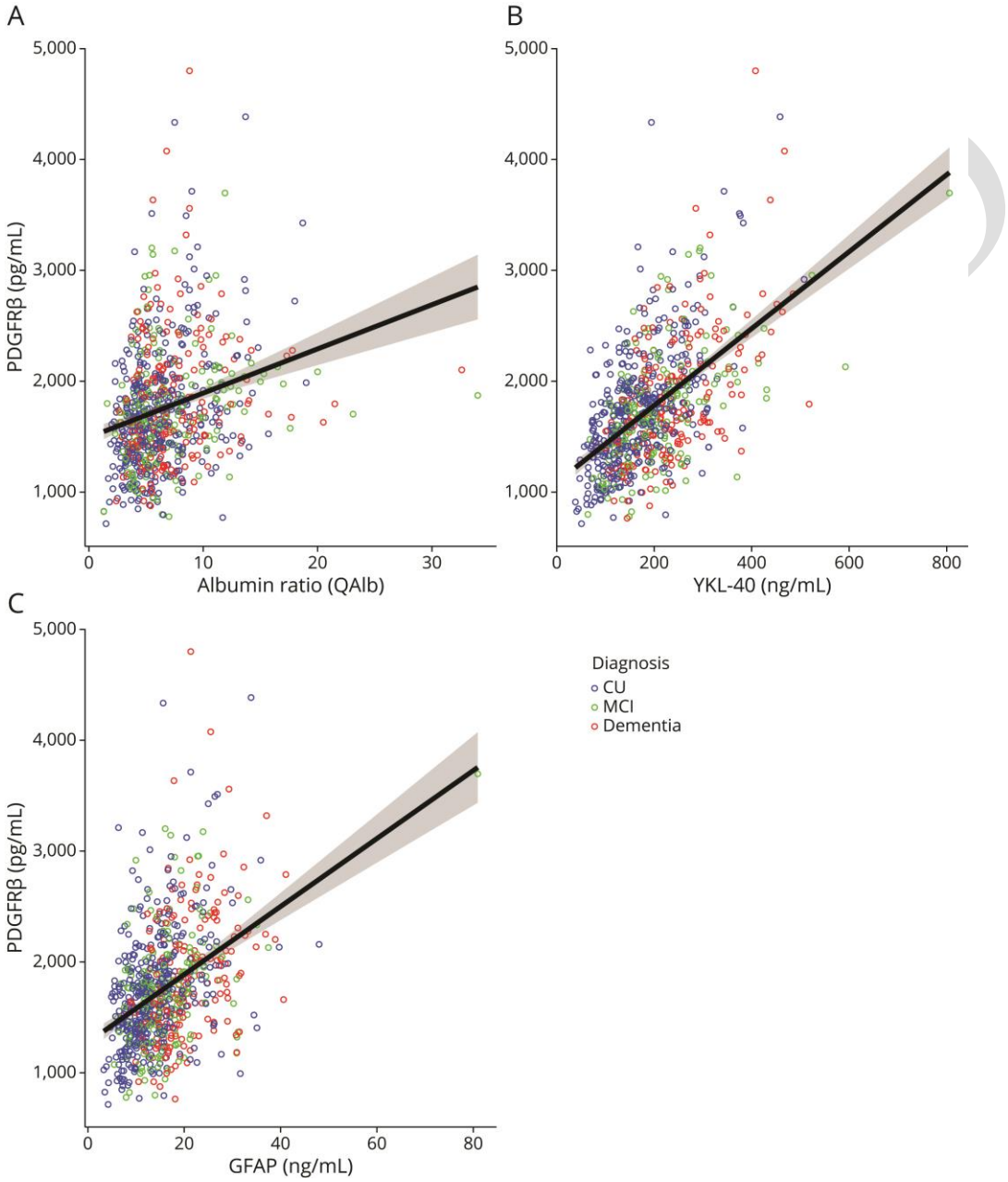
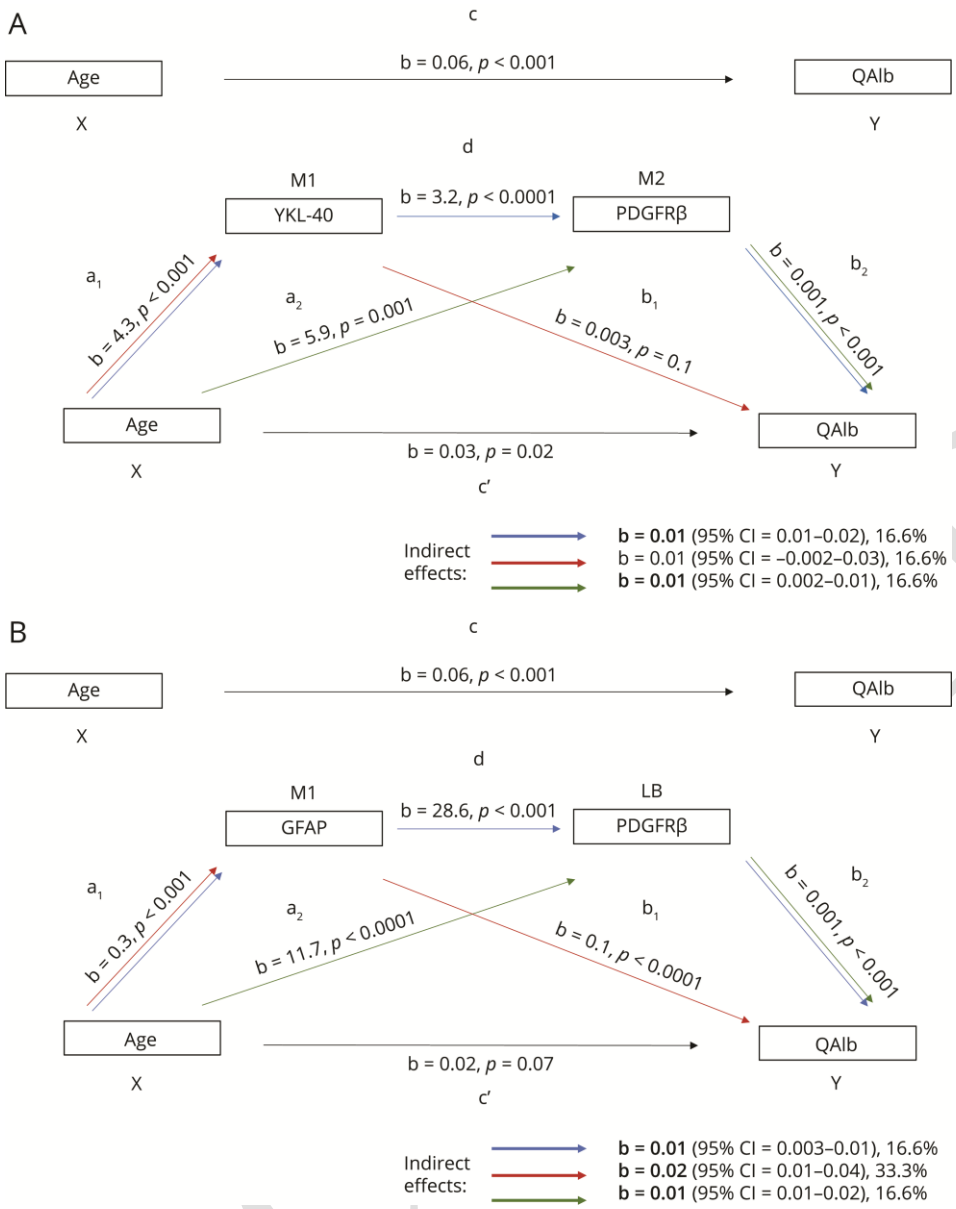


Figure 4. YKL-40, GFAP and PDGFRβ as mediators of the effect of age on BBB damage.

Sequential mediation analysis for neuroinflammation markers (YKL-40, A; GFAP, B) and PDGFRβ (A, B) as mediators of the relationship between age (X) and CSF/plasma albumin ratio (QAlb, Y). a_1: effect of X on M1; a_2: effect of X on M2 adjusted for M1; b_1: effect of M1 on Y adjusted for M2 and X; b_2: effect of M2 on Y adjusted for M1 and X; c': direct effect of X on Y; c: total effect of X on Y; d: effect of M1 on M2 adjusted for X. Blue arrow: indirect effect for model X→M1→M2→Y; red arrow: indirect effect for model X→M1→Y; green arrow: indirect effect for model X→M2→Y. Indirect effect (coefficient indicated with b) was considered significant if the 95% confidence intervals (95% CI) did not include 0 (shown in bold). Size of the indirect effect on the total effect shown as %.



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