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Multimodal comparison of plasma proteins associated with blood-brain barrier impairment in Alzheimer's disease

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Author contributions

The research was planned and designed by EG and WX. EG wrote the manuscript. EG and RC performed the experiments. JSL contributed to data analysis. JR collected and processed human VA data. EG, NM and WX provided the funding. NM and WX revised the manuscript. The final version of the manuscript is approved by all authors.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

AD, Alzheimer's disease; ARIA, amyloid-related imaging abnormalities; Aβ, amyloid-beta; CAA, cerebral amyloid angiopathy; BBB, blood-brain barrier; CNS, central nervous system; ECs, endothelial cells; TJs, thigh junctions; MMP9, matrix metalloproteinase 9; VA, Veterans Affairs; ICD, International Classification of Disease; HC, healthy control; PEA, Proximity Extension Assay; VEGFR-1, vascular endothelial growth factor receptor 1; FDR, false discovery rate; DAVID, The Database for Annotation, Visualization and Integrated Discovery; GO, Gene Ontology; KYNU, kynureninase.

Citation

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Abstract

Background: Vascular impairment is one of the major contributors to dementia. We aimed to identify blood biomarkers suggestive of potential impairment of the blood-brain barrier (BBB) in subjects with Alzheimer's disease (AD). **Methods:** We used administrative data from the VA Informatics and Computing Infrastructure Resource Center to study both inpatients and outpatients with AD. Plasma samples from healthy control and AD individuals were analyzed using enzyme-linked immunosorbent assay and proteomics approaches to identify differentially expressed proteins. Bioinformatic analysis was applied to explore significantly enriched pathways. **Results:** In the same cohort of patients with AD, we found twice number of subjects with cerebral amyloid angiopathy in the two-year period after the onset of AD, compared to the number of subjects with cerebral amyloid angiopathy in the two-year period prior to AD onset. Different pathways related to BBB, like cell adhesion, extracellular matrix organization and Wnt signaling, were activated and differentially expressed proteins such as ADAM22, PDGFR-α, DKK-4, Neucrin and RSOP-1 were identified. Moreover, matrix metalloproteinase-9, which is implicated in causing degradation of basal lamina and BBB disruption, was significantly increased in the plasma of AD patients. **Conclusions:** Alteration of proteins found in AD subjects could provide new insights into biomarkers regulating permeability and BBB integrity.

Keywords: Alzheimer's disease; blood-brain barrier; peripheral biomarkers; matrix metalloproteinases; cerebral amyloid angiopathy

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Background

Alzheimer's disease (AD) is a neurodegenerative disorder affecting 25 million people worldwide [1]. Extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain are the main features of the pathology. Two current antibody therapies, aducanumab [2] and lecanemab [3, 4], target amyloid in AD, utilizing brain imaging and cerebrospinal fluid biomarkers for diagnosis and prognosis. However, a significant concern with these therapies is the emergence of amyloid-related imaging abnormalities (ARIA), detectable through Magnetic Resonance Imaging [5]. Outcomes from clinical trials reveals that edema and micro hemorrhages associated with ARIA occurs among patients treated by all seven monoclonal antibodies that aim to reduce brain amyloid load: bapineuzumab [6], solanezumab [7], gantenerumab [8], crenezumab [9], donanemab [10], and the above two United States Food and Drug Administration approved aducanumab and lecanemab. The exact mechanisms underlying ARIA are not fully understood. Immunotherapies designed to target amyloid-beta (Aβ) plaques in the brain can lead to Aβ plaque solubilization or breakdown and when pathological conditions such as cerebral amyloid angiopathy (CAA) occurs, this may accelerate the development of ARIA [11]. CAA is closely associated with AD; in fact, post-mortem autopsies often reveal the presence of CAA in a high percentage (80% to 90%) of patients with AD; the relationship between CAA and AD suggests that both conditions may share underlying mechanisms and risk factors [12].

Dysfunction and deterioration of the blood-brain barrier (BBB) are implicated in AD and CAA [13–15]. BBB maintains a stable environment for neural function and keeps ionic composition optimal for synaptic signaling of the central nervous system (CNS). Endothelial cells (ECs) and tight junctions (TJs) play a critical role in preventing the movement of substances between the plasma and the brain or CNS [16, 17].

The disruption of BBB integrity can trigger an inflammatory response, involving the release of pro-inflammatory cytokines and the infiltration of immune cells into the brain [18]. This condition can also induce the expression of matrix metalloproteinase 9 (MMP9) [19] cell types [20]. When released, MMP9 can degrade components of the TJs that connect adjacent ECs in the BBB and lead to an increase of BBB permeability, allowing the entry of several molecules contributing to neuronal damage and dysfunction [21]. MMP9 has been found increased in neurons, senile plaques, tangles, within the vascular wall and plasma, hippocampus, and cerebral cortex of AD patients [22, 23].

Leaky BBB can lead to the sharing of certain protein profiles between the peripheral system and the CNS, therefore certain biomarkers in the peripheral blood may reflect pathological changes occurring in the brain. Many neurological disorders are associated with elevated levels of proteins in the cerebrospinal fluid, indicative of abnormalities or disruption in the BBB.

The goal of this study was to compare plasma protein profiles between AD patients and non-AD subjects and identify a panel of proteins linked to BBB leakage. We speculate that biomarkers indicative of BBB leakage might emerge among AD patients who likely exhibit ARIA upon anti-amyloid therapies.

Methods

Study populations

Patient health records were obtained from the Department of Veterans Affairs (VA) corporate data warehouse. This included inpatient and outpatient visits, vital status, and patients' prescriptions during the study period in fiscal year 2018. We only included patients whose ages were ≥ 65 and < 90 years old at the end of the study period. Patients with AD were identified using International Classification of Disease (ICD)-10 diagnosis codes [24], which are standardized medical codes for the classification of diseases and medical conditions. Subjects who received at least one AD outpatient visit or one inpatient diagnosis (ICD-10 (G30.x) code) were defined as AD patients with the first date of an AD diagnosis as the date of AD onset. Subjects who received at least one outpatient visit or one inpatient diagnosis of sporadic CAA (ICD-10 (I68.0) code) were defined as CAA patients with the first date of a CAA diagnosis as the date of CAA onset. The control or comparator group was a random sampling of 10% of the subjects with records in VA Informatics and Computing Infrastructure without a claims diagnosis of AD during the study period.

Clinical characteristics of subjects and their plasma samples

The human plasma samples of healthy control (HC) (n = 49, average age 68.4 years) and AD patients (n = 41, average age 78.8 years) were obtained from the tissue bank at the Bedford VA Healthcare System. Enrolled subjects were evaluated using Montreal Cognition Assessment, and HC subjects were scored over 27. The protocol was approved by the Bedford VA Hospital Institutional Review Board and written informed consent for each participant was obtained before initiation of the study.

Protein quantification by Proximity Extension Assay (PEA)

For plasma proteins quantification 90 samples were randomly allocated into a 96-well plate using 1 μL of plasma from each patient. Proteins levels were measured using panels based on the PEA technology (Olink Proteomics Inc., Waltham, MA, USA) that combines antibody-epitope recognition and binding with quantitative polymerase chain reaction. Circulating plasma proteins are specifically bound by DNA-tagged antibodies upon epitope recognition. Pairs of complementary hybridized DNA tags are then amplified via quantitative polymerase chain reaction. Data were pre-processed using the Olink NPXManager software and presented as Normalized Protein Expression values. Three quality control samples were included. Our statistical analysis included 87 proteins after Olink QC measures.

Quantification of target proteins by enzyme-linked immunosorbent assay

Plasma samples were analyzed using commercial R-Plex kits from Meso Scale Discovery (Gaithersburg, MD, USA). Biotinylated antibodies against matrix metalloproteinase 2, 7, 9 (MMP2, MMP7, MMP9) and vascular endothelial growth factor receptor 1 (VEGFR-1) were pre-coated to plates for capture of target proteins before the detection. Assays were carried out following the manufacturer instructions.

Statistical analysis

Statistical analyses of proteins was performed using R (version 4.3). By comparing the experimental groups (AD vs HC) in each protein, differentially expressed proteins (e.g., significantly up- and down-regulated) were identified by simultaneous *t*-tests. The *P* values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) method to account for the multiple comparisons, with the adjusted P values < 0.05 indicating statistical significance. The list of differentially regulated proteins obtained were submitted to the Database for Annotation, Visualization and Integrated Discovery software (DAVID, v6.8) to extract biological features and meaning associated with large gene lists. Gene Ontology (GO) biological process, GO molecular function, and GO cellular components were obtained. Significant differences ($P < 0.05$) were identified using an Expression Analysis Systematic Explorer score, which was provided by DAVID. Metascape (http://metascape.org) was used for annotation and visualization and to obtain the biological functions and signaling pathways in which the proteins were involved 25.

Results

Enrichment of patients with CAA after onset of AD

We have identified 51,832 Veterans with AD in fiscal year 2018 and identified those with CAA using ICD-10 code I68.0 (Table 1). In two years prior to the first evidence of AD, there were 70 patients with

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either CAA or amyloidosis. On average patients had 5 codes; of the 70 patients, 14 patients had both conditions of CAA and amyloidosis. Importantly, patients with CAA or amyloidosis in the prior 2 years have the same distribution of amount of evidence as patients who did not have those conditions. However, there were 135 patients with either condition of CAA and amyloidosis, with an average of 5 codes, in the 2 years after the first evidence of AD (Table 1). Therefore, the number of AD patients with the evidence of CAA doubled in the 2-year period after the onset of AD.

Enrichment of candidate plasma proteins from AD patients that may associate with leaky BBB

We performed target proteomic profiling using the Neuro Exploratory panel from OLink and quantified plasma proteins from our 49 HC subjects and 41 AD patients. A list of 87 proteins were obtained and log2 raw data were transformed in normalized protein expression values (Figure 1). Among the proteins analyzed, 35 were found significantly up-regulated, and one protein, kynureninase (KYNU), was down-regulated in AD compared to HC plasma samples (Table 2).

Table 1 Prevalence of CAA or amyloidosis among patients with onset of AD

Condition	N (# per 1,000 pt)
Total	51,823
2 years prior to onset of AD	
Either condition present	70 (1.4)
CAA only	23 (0.4)
Amyloidosis only	33 (0.6)
Both CAA & amyloidosis	14 (0.3)
Neither condition	51,753 (998.6)
2 years after onset of AD	
Either condition present	135 (2.6)
CAA only	33 (0.6)
Amyloidosis only	65 (1.3)
Both CAA & amyloidosis	37 (0.7)
Neither condition	51,688 (997.4)

Among 51,832 veterans with AD, 70 patients with CAA or amyloidosis in the prior 2 years of diagnosis were found and this number doubled in 2 years after the first evidence of AD. AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy.

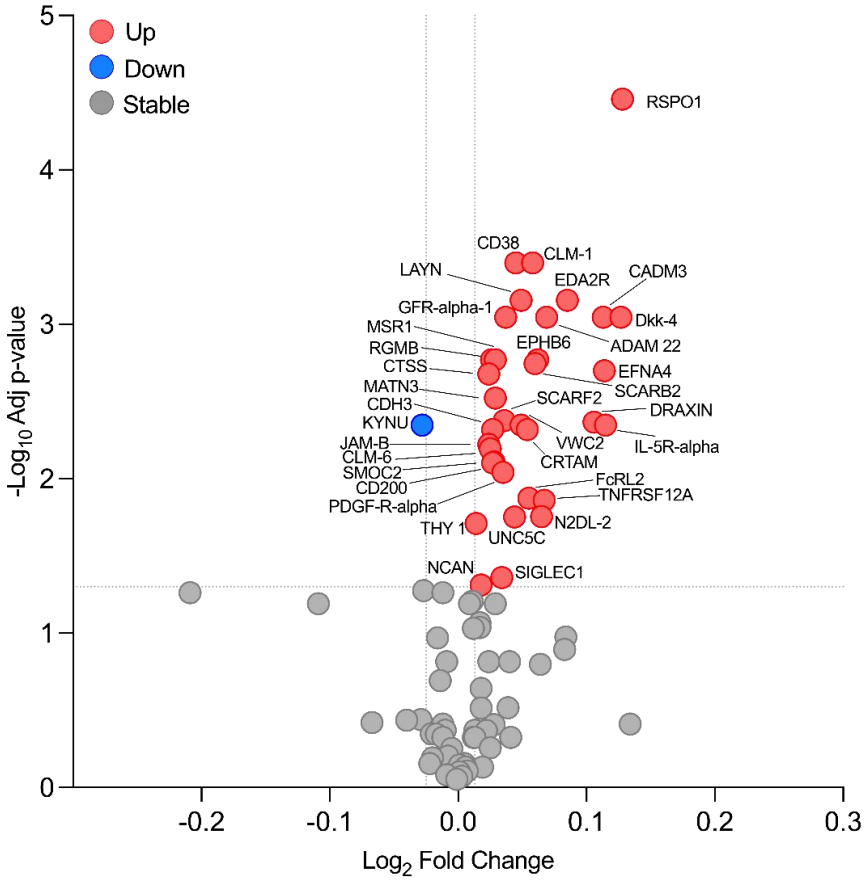

Figure 1 Volcano plot of up- and down-regulated proteins in plasma of AD patients compared to HC subjects. Volcano plot displaying the Log2 of the fold change (x-axis) against –Log10 of the adjusted *P* value (y-axis) for all proteins differentially express in AD plasma samples compared to HC plasma samples. AD, Alzheimer's disease; HC, healthy control.

Table 2 Significant alteration of plasma proteins from AD patients compared to HC subjects

Accession	Protein	Description	–Log10 (Adj-p)
Q2MKA7	RSPO1	R-spondin-1	4.46
P28907	CD38	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1	3.40
Q8TDQ1	CLM-1	CMRF35-like molecule 1	3.40
Q6UX15	LAYN	Layilin	3.15
Q9HAV5	EDA2R	Tumor necrosis factor receptor superfamily member 27	3.15
P56159	GFR-alpha-1	GDNF family receptor alpha-1	3.05
Q9P0K1	ADAM 22	Disintegrin and metalloproteinase domain-containing protein 22	3.05
Q8N126	CADM3	Cell adhesion molecule 3	3.05
Q9UBT3	Dkk-4	Dickkopf-related protein 4	3.05
P21757	MSR1	Macrophage scavenger receptor types I and II	2.77
Q6NW40	RGMB	Repulsive guidance molecule B	2.77
O15197	EPHB6	Ephrin type-B receptor 6	2.77
Q14108	SCARB2	Lysosome membrane protein 2	2.74
P52798	EFNA4	Ephrin-A4	2.70
P25774	CTSS	Cathepsin S	2.68
O15232	MATN3	Matrilin-3	2.52
Q96GP6	SCARF2	Scavenger receptor class F member 2	2.38
Q8NBI3	DRAXIN	Draxin	2.37
Q16719	KYNU	Kynureninase	2.35
Q2TAL6	VWC2	Brain-specific chordin-like protein	2.35
Q01344	IL-5R-alpha	Interleukin-5 receptor subunit alpha	2.35
P22223	CDH3	Cadherin-3	2.32
O95727	CRTAM	Cytotoxic and regulatory T-cell molecule	2.32
P57087	JAM-B	Junctional adhesion molecule B	2.22
Q08708	CLM-6	CMRF35-like molecule 6	2.19
Q9H3U7	SMOC2	SPARC-related modular calcium-binding protein 2	2.11
P41217	CD200	OX-2 membrane glycoprotein	2.10
P16234	PDGF-R-α	Platelet-derived growth factor receptor alpha	2.04
Q96LA5	FcRL2	Fc receptor-like protein 2	1.87
Q9NP84	TNFRSF12A	Tumor necrosis factor receptor superfamily member 12A	1.86
Q9BZM5	N2DL-2	UL16-binding protein 2	1.75
O95185	UNC5C	Netrin receptor UNC5C	1.75
P04216	THY1	Thy-1 membrane glycoprotein	1.71
Q9BZZ2	SIGLEC1	Sialoadhesin	1.36
O14594	NCAN	Neurocan core protein	1.31

A total of 34 proteins were found statistically upregulated and one protein, kynureninase downregulated in plasma from AD compared to HC subjects. AD, Alzheimer's disease; HC, healthy control.

Gene ontology and pathway analysis of the differentially expressed proteins

To get a general overview of changes of plasma proteins in AD and search for the most affected processes and potential interactions among the candidate proteins, GO term enrichment analysis and pathways were performed. DAVID functional enrichment analysis was carried out, and the differentially expressed plasma proteins in AD and HC subjects were classified into three groups according to biological process, molecular function, and cell components (Figure 2). Plasma proteins from AD patients exhibited significant alteration in biological process of cell-cell adhesion (P = 3.13E-04), axon guidance (P = 0.004) and Wnt signaling (P = 0.043) (Figure 2). Proteins enriched in cell-cell adhesion included: JAM-B, CD200, SIGLEC1, NCAN, THY1, CDH3, RGMB, TFNRS12A and ADAM22. Protein enriched in Wnt signaling pathway were RSPO1, DRAXIN, DKK-4. DRAXIN was also involved in axon guidance, together with EPHB6, UNC5C, EFNA4. Enrichment of extracellular matrix component included MATN3, PDGF-R-α and SMOC2. PDGF-R-α was present in the external side of plasma membrane as an integral component of receptor complex. Several proteins of cellular component were differentially expressed, such as EPHB6, UNC5C, CRTAM, THY 1, PDGF-R-α, MSR1, CDH3, CD38, CD200, EFNA4, GFR-α-1, JAM-B, IL-5R-α, CLM-6, SCARB2, RGMB, CADM3, CLM-1, FcRL2, N2DL-2, SIGLEC1, EDA2R, TNFRSF12A, ADAM22. Most of these proteins were present in integral component of plasma membrane, cell surface, and receptor complex

like EPHB6 and PDGF-R-α. Analysis of the molecular function revealed that 11% of proteins were associate with integrin binding, e.g., CD38, IL-5R-α and RGMB. Function of transmembrane signaling was illustrated by their presence in the receptor complex enriched by FcRL2, CLM-1 and SCARB2, as previously identified in the cellular components. Reactome pathway analysis revealed that 17% of these proteins were enriched for extracellular matrix organization (P = 0.04), which is closely related to BBB leakage. In addition, 11% of the proteins were enriched in axon guidance (P = 0.012), 17% in nervous system development (P = 0.014), and 17% in immunoregulatory interactions between Lymphoid and non-Lymphoid cells (P = 3.50E-04) (Table 3).

Network analysis of up and down regulated proteins confirmed that the majority of differentially expressed proteins enriched cell-cell adhesion, extracellular matrix organization and regulation of canonical Wnt signaling pathway (Figure 3).

MMP9 and VEGFR-1 were increased in AD

The enzyme-linked immunosorbent assay analysis of MMPs and VEGFR-1 didn't reveal any changes in MMP2 and MMP7 (unpaired t-test: P = 0.2652, t = 1.122 and P = 0.8042, t = 0.2487) (Figure 4 A, 4B). Instead, MMP9 levels were increased in plasma from AD subjects compared to HC (unpaired t-test: P = 0.0198, t = 2.38) (Figure 4C). Similarly, VEGFR-1 plasma levels were also increased in plasma from AD patients compared to HC subjects (Figure 4D) (unpaired t-test: P = 0.0095, t = 2.654).

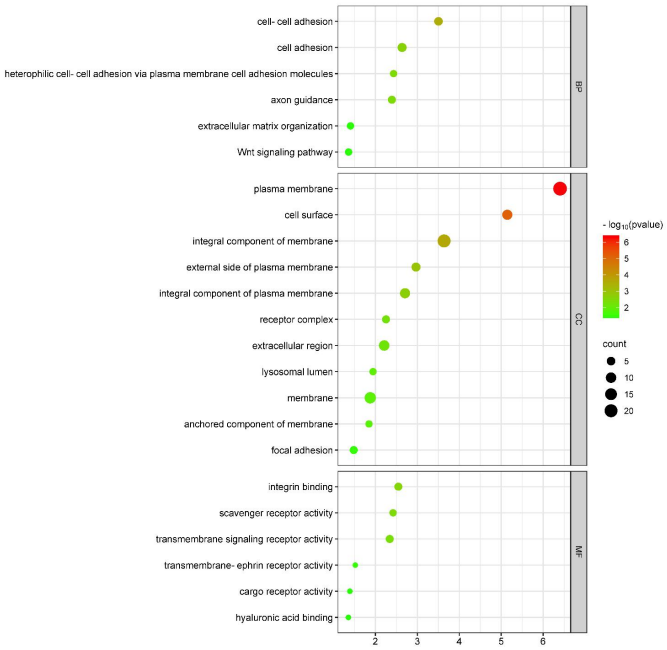

Figure 2 Gene ontology enrichment analysis. BP, biological process (upper panel), CC, cellular component (middle panel) and MF, molecular function (lower panel). The –log10 of the P value is reported on the horizontal axis and the single term enriched by the proteins is reported in the vertical axis. The color scale is used to visually represent the statistical significance of enrichment and the size of the dots represents the number of proteins associated with each pathway.

Table 3 Reactome pathway analysis of plasma proteins

Term	Description	%	P value
R-HSA-1280218	Adaptive immune system	14	3.50E-04
R-HSA-1474244	Extracellular matrix organization	17	0.01209
R-HSA-9675108	Nervous system development	17	0.014439
R-HSA-422475	Axon guidance	11	0.040282
R-HSA-198933	Interactions between lymphoid and non-lymphoid cells	17	0.043078

Reactome pathway enrichment analysis was performed using 35 differently expressed proteins in AD subjects compared to HC subjects. AD, Alzheimer's disease; HC, healthy control.

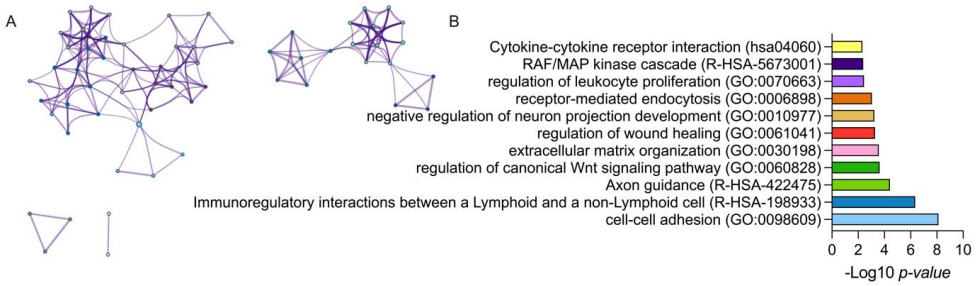

Figure 3 Network analysis of up- and down-regulated plasma proteins from AD patients compared to HC subjects. (A) Enriched terms are colored by individual cluster, the nodes that share the same cluster are connected to each other. (B) The list of clusters from AD patients (compared to HC subjects) are colored individually and represent different nodes in A. The x axis represents the –log (10) of P value. AD, Alzheimer's disease; HC, healthy control.

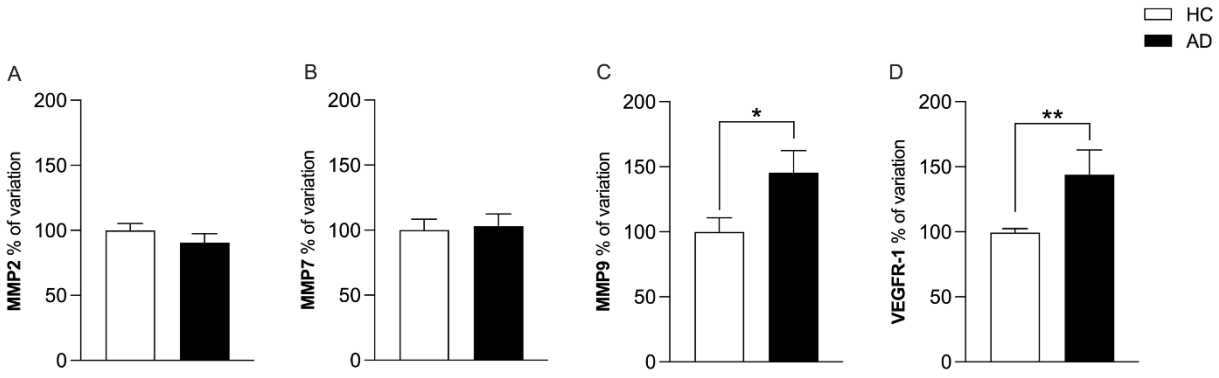

Figure 4 MMPs and VEGFR-1 plasma levels in AD patients compared to HC subjects. Percentage of variation of (A) MMP2, (B) MMP7, (C) MMP9 and (D) VEGFR-1 in AD compared to HC plasma samples. Values were normalized as percentage of variation compared to HC group ($^{*}P < 0.05$, $^{**}P < 0.01$). MMPs, matrix metalloproteinases; VEGFR-1, vascular endothelial growth factor receptor 1; AD, Alzheimer's disease; HC, healthy control.

Discussion

In this study we explored potential biomarker proteins that could be associated to BBB dysfunction in AD. Using the PEA technology, we identified a large number of differentially expressed plasma proteins in AD subjects that could serve as potential markers of BBB damage. Statistically significant changes in plasma proteins and their involvement in several pathways and networks of protein interactions were investigated, and their implication in BBB leakage was explored.

Importantly, we found that MMP9 was significantly increased in plasma from AD patients as compared to HC subjects. Many others evidence support our findings about the increased level of MMP9 and its important relationship with AD pathology [26, 27]. The expression of MMP9 was found to be raised in both moderate and late stages of AD and confocal microscopy showed MMP9 and tau protein co-localization [26]. The recent research indicates that MMP9 has a specific influence on lipoprotein receptor shedding and the transit of Aβ across BBB, depending on the isoform. Inhibition of MMP9 overall enhances the clearance of Aβ across the BBB, suggesting a potential effective strategy to reduce Aβ levels in the brain and alleviate the AD phenotype [28]. Elevated plasma MMP9 levels in AD could potentially exacerbate neurodegeneration and contribute to cognitive decline [29].

A second protein, VEGFR-1, was significantly increased in plasma from AD patients compared to HC group. VEGFR-1 has been extensively investigated for its role in regulating cerebral blood flow and maintaining BBB integrity, especially in the context of AD pathogenesis [30]. VEGF-A, one of the VEGF family members, can bind to VEGFR-1 and activate downstream signaling pathways in brain ECs. This activation has been associated with increased BBB permeability and disruption of TJs [31]. The presence of Aβ in AD is linked to heightened permeability, disruption of TJs (specifically claudin-5), and increased expression of leukocyte adhesion molecules. These effects may be mediated by proinflammatory and vasoactive substances, including MMP9 and VEGF [15]. The balance between MMP9 and VEGFR-1 in the BBB is a critical factor in maintaining its integrity and function. Dysregulation of this balance can lead to BBB breakdown and increased permeability. The alterations we observed strongly suggested the involvement of metal-binding proteins in pathways that contribute to BBB perturbance indicating a possible correlation between these proteins and BBB leakage, particularly in patients with AD. This is an important finding that underscores the potential role of these proteins in the disease's pathogenesis.

Once we identified the up- and down-regulated proteins in AD group compared to HC group, we used DAVID to explore all proteins that may implicated in BBB leakage associated with the pathology. The cell-cell adhesion GO term was enriched and included several differentially expressed proteins, among which ADAM22 that was significantly up-regulated in plasma from AD patients. ADAM22 is another metalloproteinase, like MMP9, that plays a crucial role in synaptic transmission and plasticity, and it is present both in the peripheral system and CNS. While the reduction in ADAM22 expression may contribute to synaptic dysfunction and cognitive decline in AD, peripheral increase of ADAM22 may simply reflect a "sink" effect of collecting ADAM22 leaking from CNS [32]. Loss of key proteins in the CNS due to leaky BBB presents a potential mechanism for neuronal dysfunction. Our study revealed the increase of several proteins in plasma from AD patients, and these proteins play important role in synaptic function. Therefore, the peripheral increase in ADAM22 levels observed in AD patients may not directly reflect its activity in the CNS. It may, instead, be a result of BBB breakdown and the subsequent release of CNS-derived proteins into the peripheral circulation. Similarly, EPHB6 and EFNA4, that we found upregulated in AD, regulate synaptic plasticity by modulating the strength and stability of synaptic connections and interacting with other ephrin receptors promoting the formation of stable synapses. An increase of these proteins in peripheral system may suggest a loss of their presence in brains due to a potential BBB leakage in AD patients.

It has been established that Wnt/β-catenin pathway is involved in BBB formation, integrity, and function and its activation in ECs is a key factor for BBB formation and function [33, 34]. Functional analysis revealed that the regulation of canonical Wnt signaling pathway was enriched (Figure 2) by up-regulated proteins such as DKK-4 and RSOP-1 (Table 2). DKK-4 is a member of the Dickkopf family of proteins, which are well-known inhibitors of the Wnt pathway. DKK-4 plays a crucial role in cell proliferation, differentiation, neuronal survival and synaptic plasticity. While there are several evidence about others Dickkopf family members and their direct involvement in the pathology [35], there is no direct report of this protein in AD plasma samples.

Neucrin is another secreted Wnt antagonist that negatively regulates the canonical Wnt signaling pathway and, similar to DKK-4, it inhibits the activation of the canonical Wnt pathway [36]. On the contrary, RSPO-1 is a member of the R-spondin family that acts as Wnt agonists or enhancers; plasma levels of RSPO1 were increased in subjects with dementia [37] and it has been identified as an early biomarker for neurodegeneration [38]. The enrichment of the term "regulation of the canonical Wnt signaling pathway" suggests that the levels or activities of these secreted Wnt antagonists (DKK-4, Neucrin) and enhancer (RSPO-1) are increased in plasma of AD patients compared to HC subjects. The imbalance in these proteins may lead to dysregulation of the canonical Wnt pathway, which has been associated with various cellular processes, including BBB dysfunction, that could potentially contribute to the pathogenesis of AD [39–43].

The extracellular matrix term was also enriched with several proteins including PDGFR-α (Figure 2, Table 2). Extracellular matrix could be affected by alterations that lead to BBB disruption and contribute to the progression of neurological disease. PDGFR-α belongs to the family of tyrosine kinase receptors expressed

throughout various cell-types in the brain like astrocytes and ECs [44, 45]. While PDGFR-α is involved in the disruption of the BBB following stroke and cerebral hemorrhage [46, 47], its suppression significantly reduced MMP9 activity and the expression of MMP10 and MMP13, resulting in preserving the BBB integrity. We found an upregulation of PDGFR-α (Table 2) and this might be correlated with the increase of MMP9 in AD subjects. MMPs could be the direct downstream proteins of PDGFR-α/p38 pathway and direct mediators of the BBB impairment following intracerebral hemorrhage [48].

An undisputable role of neuroinflammation in BBB leakage has been extensively investigated. Among all proteins altered in plasma from AD patients, the down-regulated protein KYNU (Table 2) is a key enzyme that catalyzes the cleavage of kynurenine into anthranilic acid and the conversion of 3-hydroxy-L-kynurenin to the neurotoxic metabolite, quinolinic acid [49]. The kynurenine pathway is essential for peripheral and central catabolism of L-tryptophan and, together with other metabolites like quinolinic acid, seems to be involved in neurodegeneration and AD, and it plays an important role in neuroinflammation associated with oxidative stress regulation and neurotoxicity [50–54]. Overactivation of kynurenine pathway may lead to an increase in the production of excitotoxin quinolinic acid by activated microglia with perivascular and infiltrating macrophages. Instead, the downregulation of KYNU could lead to an increase of 3-hydroxy-kynurenine which has neurotoxic properties and is involved in oxidative stress regulation [55–57].

Another key contributor of neuroinflammation and oxidative stress is CD38 that cleaves NAD^+ to produce cyclic ADP-ribose, activates intracellular calcium release and stimulates Aβ production [58]. SIGLEC1 is similarly involved in neuroinflammation and phagocytosis of Aβ [59]. We found that both proteins are upregulated in AD subjects and they could be associated with BBB dysfunction. Moreover, the Reactome pathways enrichment analysis shows that the term “adaptive immune system” is enriched in AD patients (Table 3). Immune response, together with other pathways, could play a role in overall neuroprotection suggesting the importance of exploring these pathways in relationship with AD [60].

Others interesting terms were significantly enriched, such as “extracellular matrix organization”, and confirmed the findings highlighted by GO and networks analysis. Importantly, the dysregulated proteins in AD compared to HC, involved in “axon guidance” and “nervous system development”, may serve as potential indicators of attempted regeneration processes taking place in brains affected by AD [61, 62].

Addressing CAA and its associated complications is crucial as the aging population continues to grow. Research into prevention, early detection, and treatment of CAA is essential to reduce the burden of this condition on elderly individuals and the healthcare system. Additionally, strategies to manage the risk factors for CAA, such as Aβ accumulation, may have broader implications for addressing cognitive decline and dementia in the elderly.

Conclusions

Our results provide new insights into BBB deterioration in AD that could be related to CAA conditions. These markers in the plasma of AD patients could help understand the correlation between AD progression and BBB impairment, which in turn affects peripheral and CNS proteins involved in the pathology. Future studies are needed to validate the biomarkers identified in individuals with AD and CAA that may develop ARIA upon immunotherapy. This validation process can help identify candidate pathways to prevent or minimize the side effects of ARIA associated with immunotherapy.

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