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Growth-Associated Protein 43 Levels in the Cerebrospinal Fluid Correspond to the Cerebral Blood Flow Alterations in Alzheimer's Dementia Continuum: An Original Study

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ABSTRACT

Background and Aims: Alzheimer's disease (AD) is a widespread neurodegenerative condition that has a growing impact on a global scale. This study aims to examine the relationship between cerebral blood flow (CBF) and the synaptic biomarker growth-associated protein 43 (GAP-43) through the utilization of arterial spin labeling (ASL). The research identified noteworthy correlations between cerebrospinal fluid (CSF) GAP-43 levels, CBF, and cognitive composite scores, especially among participants with mild cognitive impairment (MCI) who possess the APOE-ε4 gene.

Methods: The study examined 92 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, including 36 cognitively normal (CN) and 56 MCI. The cognitive status of 42 participants was evaluated using ADNI composite scores. Independent t-tests and Mann-Whitney tests were used for the comparison of continuous variables between groups, and multiple linear regression analysis with adjustments for confounding factors was used to assess the relationship between GAP-43 and CBF values.

Results: Significant positive correlations were observed between GAP-43 levels and (A) the executive function composite score (ADNI_EF) in CN individuals, as well as (B) the language composite score (ADNI_LAN) in individuals with MCI. CSF biomarkers and ASL regions did not show statistical significance between diagnostic groups after correction for multiple comparisons. No significant differences in baseline characteristics were found between diagnostic groups. However, associations were observed between ROI CBF and Mini Mental State Examination in various subgroups.

Conclusion: The findings indicate a potential function for ASL perfusion in identifying early AD-related alterations and gaining insight into the pathophysiology of AD and mild cognitive impairment.

The study revealed associations between CBF, cognitive scores, and APOE-ε4 gene status. This study contributes to the comprehension of the correlation between CSF biomarkers, regional brain perfusion, and cognitive function in individuals with AD using ASL as a noninvasive approach.

1 | Introduction

AD, the most common neurodegenerative disorder, impacts over 40 million individuals globally [1], and its incidence continues to rise as a result of the aging population [2]. AD accounts for approximately 60% of all dementia cases and is a progressive neurodegenerative condition marked by a gradual decline in cognitive abilities, functional capacity, and behavior [3]. Many cases of dementia are ultimately classified as AD due to neurodegenerative processes occurring in specific brain regions. These processes result in decreased glucose metabolism and disrupted CBF, which are observable in neuroimaging studies [1]. The correlation between CBF and localized neuronal activity and metabolism referred to as neurovascular coupling, accounts as an indirect measure of brain function [4]. The clinical diagnosis of AD is challenging and requires comprehensive neuropsychological, clinical, and neuroimaging assessments.

The current method of assessing brain structure through neuroimaging primarily emphasizes general indicators such as atrophy, which are only detectable in the advanced stages of the disease. Imaging modalities such as positron emission tomography (PET) and magnetic resonance imaging (MRI) have identified specific changes in the brains of individuals afflicted with AD [5]. In recent decades, there has been a significant increase in the utilization of diverse neuroimaging modalities for assessing patients with AD, demonstrating the potential for expediting the diagnosis of AD [5]. Hypoperfusion as a physiologic aspect of atrophy and neurodegeneration is one of the primary imaging manifestations of AD [6], that can be monitored through cerebral blood flow detection with five principal imaging methods single-photon emission computed tomography (SPECT), PET, dynamic susceptibility contrasts magnetic resonance imaging (DSC-MRI), and ASL [7]. The utilization of radiotracers or contrast agents is indispensable in SPECT, PET, and DSC-MRI [8]. ASL-MRI represents a novel noninvasive approach for identifying regional CBF perfusion in patients with AD. This method utilizes the changes in magnetic properties of arterial blood as an internal contrast [9]. Moreover, ASL is comparatively cost-effective, can be conducted in under 5 min, and is eligible for joint certification with structural MRI [1]. Numerous studies have been undertaken to investigate the relationship between changes in CBF and dementia by using ASL-MRI [6]. They have identified cerebral hypoperfusion in AD patients, particularly in the posterior cingulate and temporoparietal regions, which exhibit similarities to the frontal regions [10–12].

Other studies have identified the occipital regions, basal ganglia, thalamus, and insula as regions with CBF alterations in patients with AD [13–15]. Conversely, certain studies have demonstrated hyperperfusion in various cognitive-related regions, which could be attributed to a compensatory mechanism [16]. The assessment of CSF biomarkers plays a crucial role in the confirmation of AD [17]. Six CSF proteins have been manifested at increased CSF levels in AD patients which are amphiphysin (AMPH), aquaporin 4 (AQP4), cAMP-regulated phosphoprotein 21 (ARPP21), synuclein beta (SNCB), neurofilament medium polypeptide (NEFM), and GAP-43 [18]. GAP-43 is a protein expressed during the neuronal development of neurons and synaptogenesis [17]. It is implicated in synapse formation, axonal outgrowth, as well as learning and memory [18]. This protein is found in multiple regions of the brain, including the hippocampus, entorhinal cortex, neocortex, and olfactory bulb [19]. GAP-43 exhibits greater expression in brain regions affected by AD pathology, suggesting a potential association between extensive GAP-43 expression and AD-related neurodegeneration [17–19]. Recent research indicates that increased levels of GAP-43 in CSF could be a potential biomarker for AD [17–19].

This study aims to explore the relationship between changes in CSF levels of GAP-43, a potential biomarker for cognitive decline and disease progression in AD. We focus on how these changes may predict alterations in CBF, which is a key diagnostic imaging marker of cognitive decline Previous research has indicated a potential association between GAP-43 and hypoperfusion as observed on PET scans. Nevertheless, our study holds particular significance due to the recent proposal of ASL as a noninvasive and cost-effective alternative to PET scans.

2 | Methods

2.1 | ADNI Database and Participants

The data for this study were sourced from the ADNI database, accessible at adni.loni.usc.edu. Established in 2003, ADNI is a collaborative effort involving multiple institutions, including the National Institute on Aging (NIA) and private organizations, with the goal of advancing research on AD. The initiative aims to track the progression of MCI and early AD through a combination of neuroimaging, biological markers, and clinical evaluations. Initially designed to enroll 800 subjects, ADNI has expanded through subsequent phases—ADNI-GO and ADNI-2—resulting in over 1500 participants aged 55–90. These include cognitively healthy individuals and those with varying stages of cognitive impairment. Participants have the opportunity to continue their involvement across different study phases, contributing valuable data to enhance understanding of AD progression. For up-to-date information, please visit www.adni-info.org.

The data utilized in this study were obtained from ADNI database, which has received comprehensive ethical approval from multiple institutional review boards (IRBs). ADNI adheres to strict ethical standards in the collection and use of data, ensuring that all participant consent procedures are properly

followed. Specifically, informed consent was obtained from all participants or their authorized representatives before enrollment in the study. The initiative has received approval from various ethics committees, including those from prominent institutions such as Albany Medical Center, Boston University, and Duke University, among others. This rigorous ethical oversight guarantees that the rights and welfare of participants are prioritized throughout the research process.

The study population consisted of 92 CN and subjects with MCI from the ADNI-1,2 ASL sub-study. The inclusion and exclusion criteria for identifying CN and MCI are described in detail at www.adni-info.org. In summary, the ADNI study enrolled individuals aged 55–90 with both normal cognition and mild cognitive impairment. Participants were required to have a study partner or caregiver present at all scheduled visits, stable medication usage for at least 4 weeks before screening, adequate visual and auditory acuity for neuropsychological testing, good overall health without any additional conditions expected to interfere with the study, women who were at least 2 years postmenopausal or surgically sterile, completion of at least 6 grades of education or sufficient work history to exclude mental retardation, a Modified Hachinski score of $<=4$, and a Geriatric Depression Scale score of <6. For MCI following criteria were also administered: a memory complaint by the patient or study partner, an abnormal memory function score on the Wechsler Memory Scale (adjusted for education), MMSE score ranging from 24 to 30, a Clinical Dementia Rating of 0.5, and a Memory Box score of at least 0.5 were included in the study. Exclusion criteria encompassed significant neurological conditions other than AD, abnormal baseline MRI findings, major depression, bipolar disorder, a history of schizophrenia, alcohol or substance abuse within the past 2 years, clinically significant laboratory abnormalities, and the use of specific psychoactive medications, including antidepressants, anti-anxiety drugs, and sleep aids. Thirty-six CN (mean age = 70.68, SD = 6.49) and 56 patients with MCI (mean age = 70.92, SD = 7.15) were included in the study. All subjects had available CSF biomarkers (e.g., Tau, Ptau, and GAP 43) and MMSE scores. We further selected 42 subjects comprising 16 CN (mean age = 72.34, SD = 5.77) and 26 MCI (mean age = 70.75, SD = 6.45), who also had complete data available for ADNI cognitive composite scores (ADNI-MEM, ADNI-EF, ADNI-LAN, ADNI-VSP).

2.2 | Assessing Cerebral Blood Flow via ASL MRI

In this study, we employed ASL MRI to measure CBF, utilizing specific sequence parameters to optimize data acquisition. The ASL sequence was configured with a labeling duration of 1.5 s, a postlabeling delay of 2 s, a slice thickness of 5 mm, a field of view of 240 mm *240 mm, and a matrix size of 64*64. The repetition time (TR) was set to 3000 ms, and the echo time (TE) was maintained at 15 ms to ensure high-quality images suitable for analysis. Partial volume effects (PVE) are a significant consideration in neuroimaging studies, particularly in the context of measuring CBF using Arterial Spin Labeling (ASL) MRI. PVE occur when a single voxel contains a mixture of different tissue types, such as gray matter, white matter, and CSF, leading to inaccurate estimations of CBF values. This can result in an underestimation or overestimation of the true perfusion levels in specific brain regions, which is particularly critical when assessing conditions like Alzheimer's disease where subtle changes in blood flow may correlate with cognitive decline. To mitigate these effects, we employed advanced correction techniques using software tools such as SPM12 and the N4ITK algorithm, which facilitate accurate modeling of tissue composition within each voxel. By applying these corrections, we aimed to enhance the reliability of our CBF measurements and ensure that our findings regarding biomarkers like GAP-43 are based on precise data. This approach not only improves the validity of our results but also contributes to a better understanding of the relationship between CBF and neurodegenerative processes in Alzheimer's disease.

Following image acquisition, we implemented a comprehensive imaging processing workflow. Initial preprocessing steps included motion correction and alignment of the ASL images to a standard anatomical template. Subsequently, we defined regions of interest (ROI) based on anatomical landmarks relevant to Alzheimer's disease.

For ROI value extraction, we calculated mean CBF values by averaging the measurements across all voxels within each defined region. This approach allowed us to quantify CBF in specific brain areas and correlate these values with GAP-43 levels in CSF. By focusing on these methodological details, we aimed to provide a clear understanding of how ASL MRI was utilized in our study to assess cerebral blood flow in the context of Alzheimer's disease.

The Clinical Neurochemistry Lab at the University of Gothenburg in Sweden has developed an internal enzyme-linked immunosorbent assay (ELISA) method for quantifying CSF GAP-43 levels. The research introduces a cutting-edge cell-ELISA method to measure the levels of GAP-43 protein in multiple microcultures of mature dorsal root ganglion neurons. The analysis shows that the number of GAP-43 ranges from 1 to 10 ng, suggesting its presence in less than 500 DRG neurons. The ELISA was established through the utilization of a monoclonal GAP-43 antibody NM4 (as the coating antibody) in combination with the polyclonal GAP-43 antibody ABB-135 (as the detector antibody) for the specific recognition of the C-terminal region of GAP-43. The CSF GAP-43 demonstrated a measurable range of 312–20,000 pg/mL in the ELISA assay. CSF samples for quality control were acquired from the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital in Molndal, Sweden. The ELISA assay for CSF GAP-43 has been previously documented [17].

2.3 | ADNI Cognitive Composite Scores

A subpopulation of subjects that had all ADNI cognitive composite scores was selected for this study. Full details on the creation of each cognitive composite score are available at www.adni-info.org. Briefly, ADNI-MEM (i.e., memory composite score) contains The Rey Auditory Verbal Learning Test (RAVLT), ADAS-Cog, logical memory, and some MMSE subscores. The ADNI-EF (i.e., executive function composite score) was created by combining category fluency animals, category fluency vegetables, trails A and B, digit span backward, WAIS-R

digit symbol substitution, and five clock drawing items (circle, symbol, numbers, hands, time) scores. ADNI- LAN (i.e., language composite score) contains neuropsychological battery language-related tests (e.g., 1. category fluency–animals, 2. category fluency–vegetables, 3. Boston naming (total), and MMSE language tasks (including 1. naming an object–watch, 2. naming an object–pencil; 3. repeating a sentence; 4. reading a sentence; 5. writing a sentence), and ADAS-Cog language tasks (including 1. following commands, 2. object naming, 3. ideational practice), and some Montreal Cognitive Assessment MoCA (e.g., letter F fluency, three animal naming items, and two sentence repetition tasks). Finally, the ADNI-VSP (i.e., visuospatial composite score) was created using the clock-copy based Neuropsychological Battery test (1. copy circle; 2. copy symmetry; 3. copy number; 4. copy hand; 5. copy time), ADAS-Cog's constructional praxis score, and MMSE's copy design score.

2.4 | Statistical Analysis

Descriptive statistics are reported utilizing the measures of means and standard deviations. For variables that fall into specific categories, we utilize numerical values and percentages. Independent samples *t*-tests and Mann-Whitney tests were used to compare continuous variables between groups, whereas a chi-square test was performed to compare the categorical variables across the groups. Multiple linear regression was employed to investigate whether GAP 43 measures can predict the CSF values of any of the ROIs in each group. The association between GAP-43 and the CBF measure of each ROI with the subject's MMSE score or ADNI cognitive composite scores was also investigated using the same analysis method. We further performed the analyses after dividing each group based on APOE 4 or Tau/ABETA status to address the potential role of genetics in our findings (i.e., APOE 4 - CN, APOE4 + CN, APOE 4 - MCI, APOE 4 + MCI, Tau/ABETA - CN, Tau/ABETA + CN, Tau/ABETA - MCI, Tau/ABETA + MCI). For the latter, the Tau level was divided by the ABETA level for each subject, and the result was considered positive if it was above 0.48 [20]. Statistical significance was set at the $p < 0.05$ level. The patient's socioeconomic status, including age, sex, and years of education, as well as AD-related accumulations of tau levels and the genetic factor of APOE 4 status, were considered as covariates. The statistical analyses were conducted utilizing the SPSS 26 software.

3 | Result

This study involved the analysis of 92 participants from the ADNI database, comprising 36 CN and 56 MCI subjects (Table 1). Additionally, a subgroup of 42 participants 16 CN and 26 with MCI was selected based on their cognitive status assessed using ADNI composite scores (Table 2). There were no significant differences in baseline characteristics, such as age, gender, and education, among the subgroups. This aligns with our hypothesis that alterations in synaptic integrity, reflected by GAP-43 levels, are linked to cognitive decline in Alzheimer's disease. Although GAP-43 levels did not differ significantly between CN and MCI groups, the cognitive decline observed in MCI suggests that GAP-43 may serve as a potential biomarker for synaptic dysfunction, warranting further investigation into its longitudinal changes and their relationship with cognitive progression and CBF alterations in the Alzheimer's dementia continuum. CSF biomarkers and ASL ROI did not exhibit a statistically significant difference between diagnostic groups. However, MCI subjects had significantly lower MMSE scores than CN (Table 1).

The APOE 4 gene expression was notably elevated, while the ADNI-MEM score was significantly reduced among participants with MCI with $p < 0.01$(Table 2). Significant inverse correlations were found between ASL perfusion values in various brain regions and GAP-43 levels, particularly in the left and right cuneus cortex, lateral occipital cortex, and right superior parietal cortex for the control group, as well as the left pallidum in MCI subjects. These negative associations suggest that lower cerebral blood flow corresponds with higher GAP-43 levels, which supports our hypothesis that increased GAP-43 in CSF may indicate synaptic dysfunction associated with cognitive decline in AD. The visual representation of these associations (Figure 1) underscores the potential role of GAP-43 as a biomarker for monitoring synaptic integrity and its relationship to cerebral blood flow alterations within the Alzheimer's dementia continuum (Figure 1).

In APOE4-negative individuals within the MCI group, significant negative correlations were observed between perfusion values in the right lateral occipital cortex (A) and both the left (B) and right (C) frontal pole cortex (Figure 2). This suggests that alterations in blood flow may be particularly relevant for understanding cognitive deficits in this subgroup Significant positive correlations were noted between the GAP 43 value and (A) ADNI_EF in CN individuals with $p < 0.01$, and (B) ADNI_LAN in individuals with MCI (Figure 3). These results support our hypothesis that increased GAP-43 levels in CSF reflect synaptic alterations that correlate with cognitive function and cerebral blood flow changes, highlighting GAP-43's potential as a biomarker for monitoring disease progression and synaptic integrity within the Alzheimer's dementia continuum.

The relationships between ROI CBF and MMSE are shown in Table 3. Participants are grouped into distinct subcategories according to their APOE-4 and Tau/ABETA status. Multiple linear regression was conducted, controlling for age, gender, education, mean precentral cortex perfusion, and Tau level. This adjustment was made when comparing groups based on APOE4 status, Tau/ABETA status, or both Tau level and APOE4 status about the diagnosis. In the CN group, negative correlations between CBF in the left and right regions and MMSE scores were observed specifically within the APOE ε4-positive subgroup ($p < 0.001$; $p = 0.01$). The right rostral anterior cingulate CBF of the CN group exhibited a significant positive correlation with MMSE in the Tau/ABETA- subgroup ($p = 0.01$). These findings suggest that CBF alterations may reflect underlying synaptic dysfunction linked to cognitive decline, supporting our hypothesis that elevated GAP-43 levels in CSF correspond to changes in cerebral blood flow and cognitive status within the Alzheimer's dementia continuum. This reinforces the potential of GAP-43 as a biomarker for assessing synaptic integrity and cognitive function in AD.

TABLE 1 | Baseline characteristic and priori ROI CBF of all subjects.

Variable			CN ($N = 36$)	MCI ($N = 56$)	p value
Age			70.68 ± 6.49	70.92 ± 7.15	0.78[a]
Gender (F/M)			21 (58%)/15 (42%)	25 (44%)/31 (56%)	0.2
Education			16.25 ± 2.61	16.71 ± 2.65	0.32
APOE4 (−/+)			23 (63%)/13 (37%)	36 (64%)/20 (36%)	0.96
Tau			242.91 ± 109.73	260.40 ± 112.79	0.46[a]
P-Tau			22.23 ± 11.16	24.59 ± 11.97	0.34[a]
Tau/ABETA (−/+)			32 (88%)/4 (12%)	46 (82%)/10 (18%)	0.37
MMSE			29.14 ± 1.26	28.36 ± 1.66	**0.01**[a]
GAP 43			5098.76 ± 2697.36	4987.71 ± 2448.49	0.98[a]
ASL perfusion metric	**Caudal anterior cingulate**	Left	225633.94 ± 16377.00	241639.80 ± 11180.83	0.40
		Right	226905.07 ± 105990.98	241998.27 ± 92120.76	0.47
	Entorhinal	Left	243379.16 ± 85940.59	229153.85 ± 81372.56	0.42
		Right	233693.75 ± 90937.23	234219.39 ± 98220.59	0.83[a]
	Hippocampus	Left	293374.72 ± 88061.42	292823.33 ± 69007.56	0.97
		Right	283969.47 ± 89607.52	291031.71 ± 68124.19	0.66
	Inferior parietal	Left	201025.08 ± 110748.65	31294080 ± 104698.10	0.60
		Right	325615.13 ± 118997.49	318052.51 ± 88607.73	0.99[a]
	Lateral occipital	Left	288178.63 ± 117178.80	283161.75 ± 93497.14	0.82
		Right	285317.05 ± 110479.02	287325.52 ± 94136.83	0.92
	Middle temporal	Left	318084.42 ± 92210.17	303562.73 ± 88101.79	0.70[a]
		Right	323269.47 ± 109915.35	310537.55 ± 87388.88	0.68[a]
	Parahippocampus	Left	310086.75 ± 96025.25	326409.14 ± 90648.66	0.41
		Right	313208.75 ± 98110.40	314748.10 ± 82310.32	0.93
	Pericalcarine	Left	402771.14 ± 121926.43	433021.68 ± 118222.21	0.12[a]
		Right	416405.44 ± 127083.43	443084.55 ± 117743.93	0.30
	Posterior cingulate	Left	292432.00 ± 141807.22	292589.54 ± 111504.34	0.99
		Right	299721.28 ± 140646.80	303763.35 ± 109447.00	0.88
	Precentral	Left	239482.28 ± 96802.02	232140.86 ± 74918.64	0.68
		Right	237104.28 ± 96361.70	236415.83 ± 70404.90	0.66
	Precuneus	Left	300270.02 ± 108653.68	307885.19 ± 93735.05	0.72
		Right	304780.38 ± 108901.52	322652.31 ± 89468.48	0.39
	Rostral anterior cingulate	Left	240361.25 ± 86685.80	229490.50 ± 87163.39	0.57[a]
		Right	227118.07 ± 80716.79	214242.30 ± 82732.08	0.37[a]
	Rostral middle frontal	Left	259615.91 ± 101052.41	241238.28 ± 83194.81	0.34
		Right	249380.59 ± 105363.59	237775.39 ± 83527.71	0.55
	Caudal middle frontal	Left	248537.14 ± 112170.67	230726.07 ± 87030.03	0.39
		Right	239784.94 ± 127873.41	241026.14 ± 87481.48	0.95
	Supramarginal	Left	283356.38 ± 114344.71	291099.20 ± 117322.07	0.51[a]
		Right	293418.22 ± 119174.00	285576.62 ± 95834.58	0.83[a]
	Inferior temporal	Left	258441.66 ± 92793.83	244270.91 ± 75482.03	0.42
		Right	252032.52 ± 86073.75	241728.30 ± 75034.21	0.74[a]
	Insula	Left	265402.00 ± 15189.18	282271.44 ± 11491.56	0.37
		Right	266412.76 ± 16035.36	277724.49 ± 9454.13	0.51

(Continues)

TABLE 1 | (Continued)

Variable			CN ($N = 36$)	MCI ($N = 56$)	p value
	Caudate	Left	168247.88 ± 70117.95	163853.74 ± 47508.18	0.74
		Right	165765.74 ± 81344.57	156968.43 ± 44655.63	0.56
	Cuneus	Left	387695.60 ± 116971.77	398713.63 ± 111312.13	0.65
		Right	379699.09 ± 129816.14	406100.66 ± 111912.57	0.30
	Fusiform	Left	312576.40 ± 93835.78	333759.80 ± 85267.76	0.27
		Right	310731.57 ± 87923.88	335538.57 ± 81401.47	0.17
	Inferior lateral ventricle	Left	223571.06 ± 74338.68	227300.17 ± 70720.24	0.81
		Right	194943.78 ± 88029.11	213568.35 ± 66835.39	0.26
	Lateral occipital	Left	28811.88 ± 118827.02	283161.75 ± 93497.14	0.80
		Right	289500.94 ± 109159.94	287325.52 ± 94136.83	0.92
	Cerebral white matter	Left	75255.85 ± 27797.60	74696.46 ± 19896.40	0.91
		Right	73283.71 ± 27653.31	74659.01 ± 17697.52	0.79
	Choroid plexus	Left	230247.36 ± 92472.98	248539.94 ± 71728.34	0.30
		Right	215771.72 ± 90964.24	241643.29 ± 66723.33	0.12
	Brain stem	N/A	89789.26 ± 40225.72	89713.77 ± 32764.53	0.99
	Cerebellum cortex	Left	214473.00 ± 89228.85	201909.31 ± 74821.03	0.60
		Right	204794.97 ± 85721.14	194137.87 ± 84805.00	0.65
	Isthmus cingulate	Left	319394.24 ± 114465.48	343222.19 ± 99063.45	0.29
		Right	325537.80 ± 121195.91	352145.51 ± 102394.69	0.26
	Lateral orbitofrontal	Left	248059.94 ± 81058.81	251387.42 ± 65422.53	0.83
		Right	259087.65 ± 91848.07	250759.70 ± 63169.70	0.93
	Lingual	Left	382176.91 ± 114895.13	408547.75 ± 99597.00	0.25
		Right	374664.09 ± 105809.8	405787.88 ± 89009.94	0.13
	Medial orbitofrontal	Left	245823.28 ± 82080.63	236409.63 ± 70395.19	0.56
		Right	249363.11 ± 88125.09	238146.52 ± 87597.52	0.52
	Parsopercularis	Left	279031.73 ± 102354.98	278295.55 ± 83640.79	0.97
		Right	287936.48 ± 116734.95	296256.19 ± 83325.14	0.71
	Parsorbitalis	Left	277152.83 ± 100547.27	271845.51 ± 94184.22	0.80
		Right	285444.23 ± 105849.27	278350.88 ± 94655.77	0.74
	Parstriangularis	Left	280256.57 ± 98303.20	272479.16 ± 88201.36	0.69
		Right	280876.92 ± 105626.67	279387.33 ± 88757.50	0.94
	Postcentral	Left	230663.66 ± 95014.20	238581.75 ± 84827.21	0.68
		Right	233332.83 ± 95842.43	233707.07 ± 70842.33	0.98
	Transverse Temporal	Left	38904468 ± 128170.44	410568.17 ± 136091.93	0.45
		Right	373472.09 ± 133864.32	394703.32 ± 138389.78	0.89[a]
	CSF	N/A	292544.04 ± 128761.55	298310.47 ± 122094.25	0.70[a]
	Accumbens	Left	210560.74 ± 88311.83	222183.89 ± 69715.12	0.66[a]
		Right	208467.87 ± 85642.41	216361.20 ± 71148.67	0.63
	Amygdala	Left	235804.89 ± 90652.84	223627.04 ± 77685.89	0.49
		Right	232015.60 ± 96853.97	232080.11 ± 66533.85	0.99
	Frontal pole	Left	227013.49 ± 104914.08	228680.03 ± 97351.73	0.93
		Right	232955.76 ± 108180.90	211013.25 ± 91069.04	0.30

(Continues)

TABLE 1 | (Continued)

Variable			CN ($N = 36$)	MCI ($N = 56$)	p value
	Pallidum	Left	29582.60 ± 13460.53	28479.68 ± 10840.61	0.94[a]
		Right	26907.36 ± 1902.64	25379.48 ± 11449.72	0.56[a]
	Paracentral	Left	213617.65 ± 105621.09	216946.67 ± 97385.36	0.87
		Right	223211.56 ± 107311.30	220769.50 ± 87220.69	0.90
	Putamen	Left	197703.41 ± 68020.97	196212.29 ± 54175.55	0.90
		Right	194034.75 ± 78340.45	192490.11 ± 46559.78	0.91
	Superior frontal	Left	203564.06 ± 93170.52	200420.57 ± 88156.64	0.76[a]
		Right	199729.24 ± 99315.20	201960.15 ± 88493.35	0.84[a]
	Superior parietal	Left	223517.25 ± 93441.86	219170.63 ± 83006.92	0.81
		Right	220985.22 ± 90467.97	220292.29 ± 71469.38	0.96
	Superior temporal	Left	307334.03 ± 101366.80	317543.13 ± 96282.92	0.63
		Right	328648.26 ± 110561.58	327919.45 ± 100374.98	0.88[a]
	Temporal pole	Left	257974.82 ± 105635.16	237031.11 ± 85356.08	0.30
		Right	255816.27 ± 109848.39	253776.39 ± 96503.56	0.92
	Thalamus	Left	190934.10 ± 70621.64	196858.32 ± 62001.36	0.67
		Right	179009.87 ± 69393.22	184975.96 ± 61680.62	0.67
	Optic chiasm	N/A	137293.89 ± 89808.38	132198.85 ± 78526.73	0.83[a]
	Cerebellum white matter	Left	77068.26 ± 39310.25	71372.70 ± 26742.04	0.76[a]
		Right	72851.49 ± 38107.67	66127.27 ± 24282.83	0.30
	Lateral Ventricle	Left	101184.86 ± 46641.41	108222.19 ± 42134.15	0.36[a]
		Right	105533.36 ± 55502.00	110580.49 ± 42433.31	0.47[a]
	Fifth ventricle	N/A	30103.17 ± 28241.41	26191.15 ± 41872.88	0.13[a]
	Fourth ventricle	N/A	267380.53 ± 107622.48	252386.07 ± 87489.08	0.47
	Third ventricle	N/A	226644.75 ± 81398.01	216548.74 ± 78098.73	0.52[a]

Note: Continuous and categorical variables are presented as mean ± standard and number(percentage) respectively. Chi-square test used for dichotomous variables. Bold format represents significant value.
Abbreviations: ASL, arterial spin label; MMSE, mini mental state exam.
[a]Indicates Mann-Whitney test; otherwise, Independent T-test p value presented for continuous variables.

In the MCI Tau-ABETA negative group, the right accumbens area and the fifth ventricle were found to be significantly associated with MMSE score with $p < 0.01$, showing a positive and negative correlation, respectively. In the Tau-ABETA positive MCI patients, there was a positive relation between left middle temporal cortex cerebral perfusion and MMSE score. The left superior parietal cortex and 5th ventricle exhibited a negative correlation with the MMSE score in MCI patients who tested negative for the APOE4 gene variant. These findings suggest that specific brain regions are differentially impacted by cognitive decline in relation to GAP-43 levels. The positive correlation between left middle temporal cortex perfusion and cognitive function aligns with our hypothesis that elevated GAP-43 levels in CSF reflect synaptic dysfunction associated with cognitive decline. Conversely, the negative correlations observed in other regions may indicate that reduced cerebral blood flow is linked to worsening cognitive performance, further supporting the notion that GAP-43 serves as a potential biomarker for synaptic integrity and cognitive status within the Alzheimer's dementia continuum.

When comparing individuals with the MCI to the CN group, and controlling for standard covariates along with the presence of APOE4 and Tau-ABETA, it was observed that the 5th ventricle in the MCI group retained its inverse relationship with MMSE scores, while the bilateral accumbens area exhibited a positive correlation. Table 4 illustrates the notable correlations between ADNI composite scores and ROI CBF in various groups and subgroups. These findings support our hypothesis that elevated GAP-43 levels in CSF correspond to alterations in cerebral blood flow, reflecting underlying synaptic dysfunction associated with cognitive decline in AD. The distinct relationships observed in different brain regions emphasize the complexity of how synaptic integrity and cerebral perfusion interact within the Alzheimer's dementia continuum, potentially providing insights into early diagnostic markers for cognitive impairment.

Parameters with nonsignificant results are presented in the supplementary materials. In terms of the ADNI-MEM score, a significant negative correlation was observed in MCI APOE4- patients between the left pallidum. Conversely, in APOE4-

TABLE 2 | Baseline characteristic of subgroup analyses.

Variables	CN ($N = 16$)	MCI ($N = 26$)	p value
Age	72.34 ± 5.77	70.75 ± 6.45	0.42
Gender (F/M)	11 (68%)/5 (32%)	10 (38%)/16 (62%)	0.05
Education	16.56 ± 2.50	17.04 ± 2.40	0.51[a]
APOE 4 (−/+)	13 (81%)/3 (19%)	13 (50%)/13 (50%)	**0.04**
Tau	235.35 ± 96.85	264.73 ± 110.67	0.42[a]
P-Tau	21.14 ± 9.27	25.87 ± 12.03	0.23[a]
Tau/ABETA (−/+)	15 (93%)/1 (7%)	20 (76%)/6 (24%)	0.22
MMSE	29.13 ± 1.20	28.27 ± 1.88	0.17[a]
ADNI-MEM	0.88 ± 0.63	0.30 ± 0.73	**0.01**
ADNI-EF	0.54 ± 0.84	0.88 ± 0.93	0.25
ADNI-LAN	0.36 ± 0.79	0.48 ± 0.88	0.40[a]
ADNI-VSP	0.25 ± 0.58	0.09 ± 0.71	0.49[a]
GAP 43	5077.22 ± 2786.64	4705.25 ± 1944.41	0.89[a]

Note: Continuous and categorical variables are presented as mean ± standard and number(percentage) respectively. Chi-square test used for dichotomous variables. Bold formats represent significant values.
Abbreviations: ADNI-EF, ADNI executive function composite score; ADNI-LAN, ADNI language composite score; ADNI-MEM, ADNI memory composite score; ADNI-VSP, ADNI visuospatial composite score; MMSE, mini mental state exam.
[a]Indicates Mann-Whitney test; otherwise, independent T-test p value presented for continuous variables.

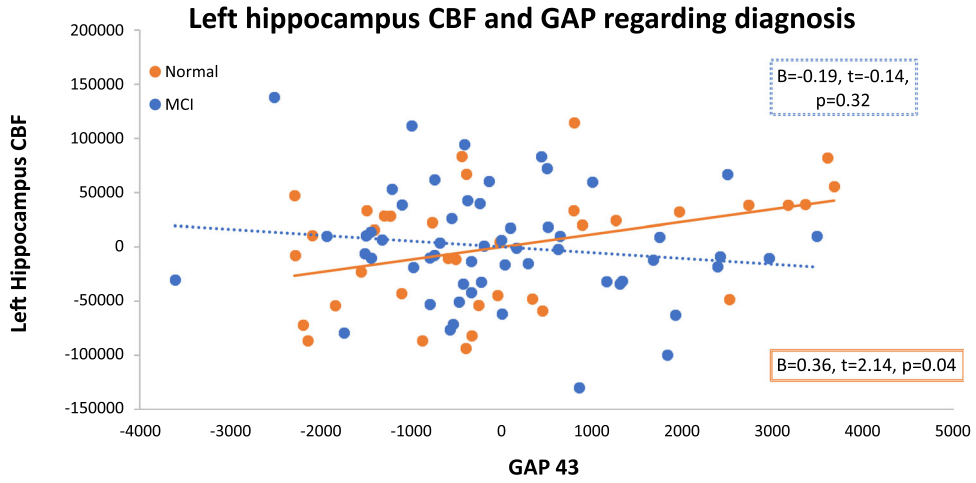

FIGURE 1 | Effect of GAP 43 and subject's diagnosis on Left Hippocampus CBF.

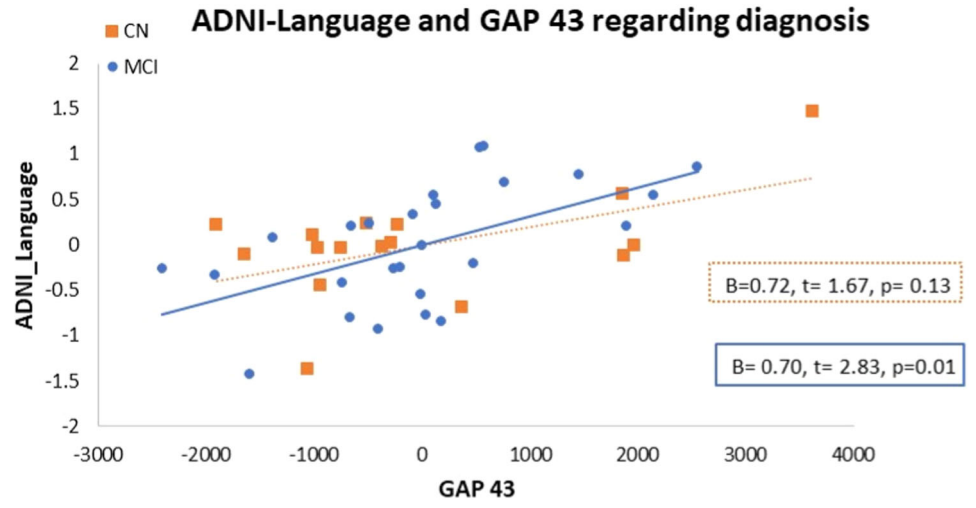

FIGURE 2 | Association of Gap 43 and subject's diagnosis on ADNI language composite score (ADNI-LAN).

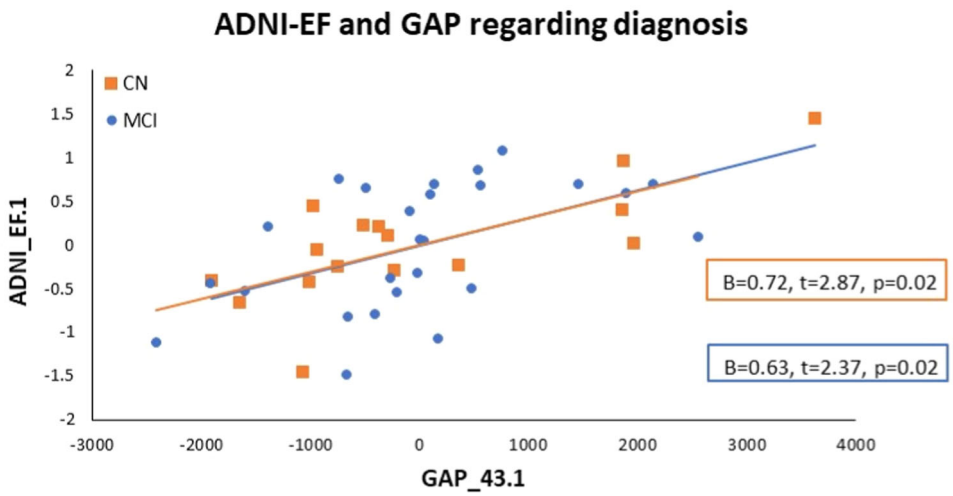

FIGURE 3 | Association of Gap 43 and subject's diagnosis on ADNI executive function composite score (ADNI-EF).

TABLE 3 | Significant associations of ROI CBF with MMSE.

Group	ROI	Subgroup	β	*t*	*p* value
CN	**Left pericalcarine**	APOE4 +	−0.89	−6.90	**< 0.001**[a]
	Right pericalcarine	APOE4 +	−0.95	−4.02	**0.01**[a]
	Right rostral anterior cingulate	Tau/ABETA −	0.48	2.55	**0.01**[b]
MCI	**Fifth ventricle**	APOE4 −	−0.75	−3.16	**< 0.01**[a]
	Left superior parietal cortex	APOE4 −	−0.67	−1.16	**0.01**[a]
	Fifth ventricle	Tau/ABETA −	−0.73	−3.86	**< 0.001**[b]
	Right accumbens area	Tau/ABETA −	0.42	2.20	**0.03**[b]
	Left middle temporal cortex	Tau/ABETA +	0.82	22.26	**< 0.01**[b]
CN versus MCI	**Right rostral anterior cingulate**	CN	0.42	2.65	**0.01**[c]
	Fifth ventricle	MCI	−0.44	−2.72	**0.01**[c]
	Left accumbens area	MCI	0.45	2.88	**< 0.01**[c]
	Right accumbens area	MCI	0.33	2.15	**0.03**[c]

Note: Significant associations using multiple linear regression with age, gender, education, mean precentral cortex perfusion and [a]Tau level (when comparing based on APOE4 status or [b]APOE4 status (when comparing based on Tau/ABETA status or [c]Tau level and APOE4 status (when comparing regarding diagnosis).
Abbreviations: CBF, cerebral blood flow; CN, cognitively normal; MCI, mild cognitive impairment; MMSE, mini mental state exam; ROI, region of interest.

positive patients, the bilateral hippocampus, bilateral anterior cingulate cortex, right peri calcarine, the 3rd ventricle, right cuneus cortex, bilateral inferolateral ventricle, right lingual cortex, bilateral transverse temporal cortex, bilateral Amygdala, bilateral Pallidum, and left putamen exhibited a positive correlation with the score. In the tau/ABETA negative patients with MCI, the only positive correlation of ADNI-MEM score was with the cerebral blood flow in the left pallidum. These findings imply that specific brain regions have differential impacts on cognitive performance based on genetic and biomarker profiles. The observed correlations align with our hypothesis that elevated GAP-43 levels in CSF correspond to alterations in cerebral blood flow and cognitive function within the Alzheimer's dementia continuum. This underscores the potential of GAP-43 as a biomarker for synaptic integrity and cognitive status, emphasizing its role in understanding the neurobiological underpinnings of AD progression.

Accounting for both APOE4 and tau/ABETA, it was observed that the right hippocampus, right cerebral white matter, left cuneus cortex, left inferolateral ventricle, right amygdala, bilateral pallidum, right putamen, and right thalamus exhibited a negative correlation, while the right cerebellum cortex showed a positive correlation with ADNI-MEM score. The ADNI-EF score exhibited positive correlations with the CBF of the right caudal anterior cingulate cortex and the left temporal pole cortex in the APOE4 positive group. When considering both APOE-4 and TAU/ABETA levels CBF of bilateral pallidum negatively correlated with ADNI-EF scores. Positive correlations were observed between the left superior frontal and temporal cortex and the right caudal anterior cingulate cortex. In our study, individuals who tested negative for APOE4 for TAU-ABETA did not exhibit any notable associations between CBF in various regions and the ADNI-EF. These findings suggest that specific brain regions are differentially affected by cognitive impairment based on genetic and biomarker profiles.

TABLE 4 | Significant associations of ROI CBF with ADNI composite scores.

Composite score	Group	ROI	Subgroup	Beta	*t*	*p* value
ADNI-MEM	**MCI**	**Left pallidum**	APOE4 −	−1.73	−7.18	**< 0.001**[a]
		Left hippocampus	APOE4 +	−0.69	−4.50	**< 0.01**[a]
		Right hippocampus	APOE4 +	−0.83	−6.55	**< 0.001**[a]
		Left rostral anterior cingulate	APOE4 +	−0.97	−5.05	**< 0.01**[a]
		Right rostral anterior cingulate	APOE4 +	−1.04	−6.16	**< 0.01**[a]
		Right pericalcarine	APOE4 +	−0.73	−4.06	**< 0.01**[a]
		Third ventricle	APOE4 +	−0.47	−2.77	**< 0.05**[a]
		Right cuneus cortex	APOE4 +	−0.62	−2.62	**< 0.05**[a]
		Left inferior lateral ventricle	APOE4 +	−0.67	−5.35	**< 0.01**[a]
		Right inferior lateral bentricle	APOE4 +	−0.55	−3.01	**< 0.05**[a]
		Right lingual cortex	APOE4 +	−0.99	−6.31	**< 0.001**[a]
		Left transverse temporal cortex	APOE4 +	−0.94	−2.59	**< 0.05**[a]
		Right transverse temporal cortex	APOE4 +	−0.99	−2.51	**< 0.05**[a]
		Left amygdala	APOE4 +	−0.63	−3.39	**< 0.05**[a]
		Right amygdala	APOE4 +	−0.68	−2.70	**< 0.05**[a]
		Left pallidum	APOE4 +	−0.59	−3.82	**< 0.01**[a]
		Left pallidum	APOE4 +	−0.78	−3.36	**< 0.05**[a]
		Left putamen	APOE4 +	−0.49	−3.03	**< 0.05**[a]
		Left pallidum	Tau/ABETA -	−0.57	−3.14	**< 0.01**[b]
	CN versus MCI	**Right hippocampus**	MCI	−0.43	−2.24	**< 0.05**[c]
		Right cerebral white matter	MCI	−0.72	−2.48	**< 0.05**[c]
		Left cuneus cortex	MCI	−0.55	−2.12	**< 0.05**[c]
		Left inferior lateral ventricle	MCI	−0.43	−2.41	**< 0.05**[c]
		Right cerebellum cortex	MCI	0.44	2.42	**< 0.05**[c]
		Right amygdala	MCI	−0.46	−2.32	**< 0.05**[c]
		Left pallidum	MCI	−0.71	−6.29	**< 0.001**[c]
		Right pallidum	MCI	−0.78	−4.35	**< 0.001**[c]
		Right putamen	MCI	−0.60	−2.91	**< 0.01**[c]
		Right thalamus	MCI	−0.40	−2.23	**< 0.05**[c]
		Left lingual cortex	CN	1.46	2.46	**< 0.05**[c]
		Left parsorbitalis cortex	CN	−1.06	−3.13	**< 0.05**[c]
		Right paracentral cortex	CN	1.05	2.35	**< 0.05**[c]
ADNI-EF	**MCI**	**Right caudal anterior Cingulate**	APOE4 +	1.27	3.29	**< 0.05**[a]
		Left temporal pole cortex	APOE4 +	0.77	4.25	**< 0.01**[a]
	CN versus MCI	**Right caudal anterior cingulate**	CN	1.99	3.54	**< 0.01**[c]
		Left parsorbitalis cortex	CN	−1.14	−2.54	**< 0.05**[c]
		Right paracentral cortex	CN	1.27	2.42	**< 0.05**[c]
		Left pallidum	MCI	−0.53	−2.84	**< 0.05**[c]
		Right pallidum	MCI	−0.66	−2.76	**< 0.05**[c]
		Left superior frontal cortex	MCI	0.61	2.35	**< 0.05**[c]
		Left superior temporal cortex	MCI	0.60	2.40	**< 0.05**[c]
		Left temporal pole cortex	MCI	0.51	2.38	**< 0.05**[c]
		Right caudal anterior cingulate	MCI	0.78	2.27	**< 0.05**[c]

(Continues)

TABLE 4 | (Continued)

Composite score	Group	ROI	Subgroup	Beta	*t*	*p* value
ADNI-LAN	**MCI**	**Left pallidum**	Tau/ABETA -	−0.68	−3.32	**< 0.01[b]**
		Left pallidum	APOE4 +	−0.57	−2.76	**< 0.05[a]**
		Right pallidum	APOE4 +	−0.89	−3.98	**< 0.01[a]**
		Left amygdala	APOE4 +	−0.69	−3.42	**< 0.05[a]**
	CN versus MCI	**Right choroid plexus**	CN	−0.94	−2.56	**< 0.05[c]**
		Left parsorbitalis cortex	CN	−1.13	−2.69	**< 0.05[c]**
		Right parsorbitalis cortex	CN	−0.89	−2.39	**< 0.05[c]**
		Left pallidum	MCI	−0.65	−4.42	**< 0.001[c]**
		Right pallidum	MCI	−0.75	−3.72	**< 0.01[c]**
		Left temporal pole cortex	MCI	0.45	2.16	**< 0.05[c]**
		Left thalamus	MCI	−0.46	−2.57	**< 0.05[c]**
		Right thalamus	MCI	−0.47	−2.58	**< 0.05[c]**
ADNI-VSP	**MCI**	**Left cerebellum white matter**	APOE4 +	−1.16	−2.24	**< 0.05[a]**
		Right paracentral cortex	Tau/ABETA -	0.67	2.36	**< 0.05[b]**
	CN versus MCI	**Right lateral ventricle**	CN	−1	−2.78	**< 0.05[c]**
		Right choroid plexus	CN	−1.01	−2.49	**< 0.05[c]**
		Left temporal pole cortex	CN	−1.3	−3	**< 0.05[c]**
		Right insula cortex	MCI	−0.69	−2.15	**< 0.05[c]**
		Third ventricle	MCI	−0.44	−2.27	**< 0.05[c]**
		Right pallidum	MCI	−0.61	−2.29	**< 0.05[c]**

Note: Significant associations using multiple linear regression with age, gender, education, mean precentral cortex perfusion and [a]Tau level (when comparing based on APOE4 status or [b]APOE4 status (when comparing based on Tau/ABETA status or [c]Tau level and APOE4 status (when comparing regarding diagnosis).
Abbreviations: ADNI-EF, ADNI executive function composite score; ADNI-LAN, ADNI language composite score; ADNI-MEM, ADNI memory composite score; ADNI-VSP, ADNI visuospatial composite score; CBF, cerebral blood flow; CN, cognitively normal; MCI, mild cognitive impairment; ROI, region of interest.

The negative correlations in areas such as the bilateral pallidum may indicate that reduced perfusion in these regions is linked to poorer cognitive performance. Conversely, positive correlations in regions like the right cerebellum and caudal anterior cingulate cortex imply that enhanced blood flow may support better cognitive function. These results align with our hypothesis that elevated GAP-43 levels in CSF correspond to alterations in cerebral blood flow and cognitive status within the Alzheimer's dementia continuum. The distinct patterns of correlation reinforce the potential of GAP-43 as a biomarker for synaptic integrity, highlighting its role in understanding the neurobiological mechanisms underlying cognitive decline in AD.

The ADNI-LAN score exhibited negative correlations with CBF in the left pallidum among Tau/ABETA-negative patients but showed positive associations with bilateral pallidum perfusion among APOE ε4-positive individuals Upon considering all dementia biomarkers, it was observed that the bilateral pallidum regions and, notably, the bilateral thalami maintained their negative correlations. Only a positive correlation was observed in the Left temporal pole cortex for this score. These findings align with our hypothesis that elevated GAP-43 levels in CSF correspond to alterations in cerebral blood flow and cognitive performance within the Alzheimer's dementia continuum. The distinct patterns of correlation suggest that specific brain regions are critical for language function and may reflect underlying synaptic integrity as indicated by GAP-43 levels. This emphasizes the potential of GAP-43 as a biomarker for assessing cognitive function and understanding neurobiological changes associated with AD progression.

The ADNI-VSP score exhibited positive correlations with CBF of left cerebellum white matter in the APOE4 positive group and a positive correlation with the right paracentral cortex in the TAU-ABETA negative subgroup. These findings imply that enhanced perfusion in these brain regions may support better visuospatial processing abilities, which are critical for cognitive function. Accounting for APOE, Tau, and ABETA status, negative correlations of the right insula cortex, 3rd ventricle and right pallidum were found. This suggests that reduced blood flow in these areas may be associated with cognitive decline, particularly in visuospatial tasks.

These results align with our hypothesis that elevated levels of GAP-43 in CSF correspond to alterations in cerebral blood flow and cognitive performance within the Alzheimer's dementia continuum. The distinct patterns of correlation highlight the role of specific brain regions in cognitive functioning and underscore the potential of GAP-43 as a biomarker for synaptic integrity. By linking CBF alterations to cognitive performance, our findings contribute to a deeper understanding of the neurobiological mechanisms underlying AD progression.

4 | Discussion

Recent research suggests a correlation between cerebral blood flow (ASL perfusion) and GAP43 in individuals diagnosed with AD. The study unveiled noteworthy correlations between the average perfusion of the precentral cortex and Tau levels when stratified by APOE4 status, Tau/ABETA status, or Tau levels and APOE4 status, as well as when comparing based on diagnosis. These findings also support the results of previous studies using ASL MRI, which identified a link between lower perfusion in AD patients and poorer overall instrumental activities of daily living (IADL) abilities [21, 22]. This correlation highlights the potential role of cerebrovascular health in the progression of neurodegenerative changes associated with AD. The precentral cortex is crucial for motor function and coordination, suggesting that impaired perfusion may not only reflect underlying pathological processes but also contribute to functional decline in daily activities. Furthermore, the stratification by APOE4 status underscores the importance of genetic factors in influencing both vascular health and neurodegeneration, potentially guiding personalized therapeutic approaches. The relationship between Tau levels and perfusion reinforces the hypothesis that neuroinflammation and vascular dysfunction may co-occur in AD, which could lead to a vicious cycle exacerbating cognitive decline. Therefore, these findings could inform future research aimed at exploring interventions targeting both vascular health and Tau pathology, ultimately enhancing our understanding of AD's multifaceted nature and improving patient outcomes.

The results of our study are consistent with previous research that has found higher levels of CSF GAP-43 in patients with AD and those with MCI caused by AD. This suggests that CSF GAP-43 may be a valuable biomarker for detecting and monitoring the progression of AD and related conditions. These results have important implications for the advancement of novel diagnostic and therapeutic strategies for AD. Further research is needed to better understand the underlying mechanisms of CSF GAP-43 in AD and to explore its potential as a target for intervention. In conclusion, our study provides additional evidence of the potential usefulness of CSF GAP-43 as a biomarker for AD and related conditions. This emphasizes the importance of ongoing research in this field [23, 24]. The previous studies have reported a more significant increase in GAP-43 compared to the current study. This discrepancy in findings could be attributed to variations in the assay methods employed or the heterogeneous patient populations included in the previous studies. However, the current study has demonstrated that GAP-43 can be accurately measured in CSF using a novel in-house sandwich ELISA. To further expand on this, future studies could explore the potential clinical implications of measuring GAP-43 levels in CSF, such as its potential use as a biomarker for neurological disorders.

Additionally, further research could explore the underlying mechanisms of GAP-43 in the central nervous system and its role in neuronal plasticity [18, 24]. The significant association between GAP-43 and the biomarkers T-tau and P-tau, along with the inverse correlation with Aβ42, aligns with prior studies indicating that elevated levels of GAP-43 are linked to elevated tau and amyloid deposition in the brain [18]. The correlation among synaptic, tau, and amyloid pathology in AD has been the focus of scientific investigation. Studies have shown that synaptic damage occurs during the early stages of AD and is associated with amyloid pathology and memory impairment. Furthermore, findings from in vivo studies suggest a strong correlation between tau pathology and a decrease in synaptic density, as well as changes in synaptic function in AD. Nevertheless, the biological relationship between synaptic, tau, and amyloid pathology has not been fully clarified. Moreover, the association between a low MMSE score and a decline in MMSE score over time in relation to a higher GAP-43 concentration has been poorly established when analyzed in all groups. This gap in knowledge suggests that longitudinal studies are needed to determine whether elevated GAP-43 levels predict cognitive decline more accurately than traditional biomarkers. Investigating these dynamics could yield critical insights into the temporal progression of AD and enhance our understanding of how synaptic dysfunction contributes to cognitive impairment. Ultimately, elucidating these complex relationships may pave the way for novel interventions aimed at preserving synaptic function and mitigating cognitive decline in AD patients.

Previous MRI studies have commonly utilized conventional structural MRI to detect structural alterations, such as hippocampal atrophy or white matter lesions, that underlie cognitive decline in AD. The article “Synaptic Tau: A Pathological or Physiological Phenomenon?” explores the synaptic implications of tau pathology in AD and its potential correlation with memory deficits, a prominent feature of the condition. The presence of neurofibrillary tangles containing hyperphosphorylated tau and amyloid-beta plaques is emphasized as the histological diagnosis of AD. It also suggests that AD may involve a transition from physiological to pathological synaptic tau function. This review also underscores the importance of establishing the direct correlation between neuronal activity and tau pathology [18]. The utilization of noninvasive ASL MRI for quantifying CBF could potentially elucidate the mechanisms that occur before the onset of irreversible parenchymal damage.

Significant positive associations between CBF and cognitive scores were observed in various brain regions for different subgroups. The findings of this study indicate a notable correlation between AD-related CSF pathology and cognitive composite scores. This indicates a strong connection between cognitive outcomes and AD pathology. These results offer empirical evidence to support the use of cognitive composite scores as the primary endpoint for clinical trials aimed at preventing AD. They suggest that CSF-directed treatments have the potential to correlate well with AD lesions. This section summarizes the key findings and their implications discussed earlier. The text emphasizes the utilization of ASL MRI for assessing CBF and its correlation with cognitive outcomes in the context of AD pathology [25]. The results are noteworthy, particularly in the context of the revised guidelines from the Food and Drug Administration (FDA) for the development of medications for early-stage AD. The revised guidance permits increased flexibility in the use of cognitive measures to assess the effectiveness of treatments. Nevertheless, it is essential to establish a connection between the observed cognitive changes and the underlying disease pathology, as suggested by the findings of this study. This underscores the significance of verifying that any enhancements in cognitive function due to

drug therapies truly reflect favorable alterations in the disease process. This is consistent with the overarching goal of creating more efficient interventions for AD, which necessitates a thorough comprehension of the connection between cognitive outcomes and the fundamental pathology. Consequently, additional research and clinical evidence are necessary to establish this critical link and further the advancement of treatments for early AD [26].

This study offers additional evidence that reinforces the necessity for prospective participants to exhibit positive amyloid scans as a prerequisite for meeting the eligibility criteria for AD prevention trials. These experiments necessitate the participation of individuals who are clinically asymptomatic but exhibit substantial AD pathology and are at an elevated risk of experiencing cognitive symptoms. These individuals stand to gain the most from preclinical interventions that have the potential to delay or prevent the onset of cognitive decline. The study also revealed that an increased presence of gap43 in cortical regions associated with AD is correlated with diminished performance across a range of cognitive domains. Therefore, pinpointing and focusing on these areas could serve as a promising approach to combat cognitive decline in people at risk of developing AD [26, 27]. Previous research has indicated that they exclusively examined the relationship between amyloid-PET and CBF [28]. The findings of our investigation into the association between CSF GAP-43 and ASL perfusion align with prior studies that have examined the link between increased levels of CSF biomarkers and perfusion in cognitively normal APOE-ε4 carriers. Furthermore, our findings are consistent with post-mortem evidence indicating that elevated Braak stages of tau pathology are linked to heightened expression of the vascular endothelial growth factor. Our study also uncovers connections between specific brain regions, including the hippocampus, rostral anterior cingulate, caudal anterior cingulate, and CSF GAP-43 in ASL perfusion. The results indicate that CSF GAP-43 may function as a distinctive biomarker for AD, given its association with amyloid and tau accumulation in the brain, and its lack of significant elevation in other neurological disorders.

Additionally, our findings suggest that there is a significant increase in GAP-43 levels among individuals with AD and MCI who carry the APOE-ε4 gene. This finding implies a potential association between this phenotype and the early susceptibility of synapses, which in turn contributes to cognitive deterioration. Our study also illustrates a notable correlation between CBF and GAP-43 in distinct brain regions. The mentioned studies discuss the association of CSF GAP-43 with cognitive decline and progression to dementia in individuals with AD. The research delves into the longitudinal and cross-sectional relationships between CSF GAP-43 and AD biomarkers, highlighting how high levels of GAP-43 in Aβ-positive participants are linked to worse brain metabolic decline, atrophy, and cognitive scores over time. This underscores the importance of monitoring GAP-43 levels as part of a comprehensive biomarker panel that could provide insights into disease progression and treatment efficacy. Ultimately, elucidating the precise role of GAP-43 in the context of AD could pave the way for novel therapeutic strategies aimed at enhancing synaptic resilience and preserving cognitive function in at-risk populations.

Additionally, the given studies emphasize the increased risk of conversion to AD dementia in individuals with high baseline GAP-43 levels. These findings underscore the potential of CSF GAP-43 as a biomarker for AD pathology and its implications for disease progression. The research revealed that MCI subjects had lower MMSE scores compared to CN individuals, and there were significant associations between brain regions and cognitive status. Notably, there were inverse correlations in various brain regions like the Cuneus cortex, Occipital cortex, and superior parietal cortex in the control group. Additionally, adverse connections were observed in specific brain regions among APOE4-negative individuals with MCI. The study also highlighted significant correlations between perfusion values and cognitive scores, such as ADNI-EF and ADNI-LAN scores. Furthermore, distinct associations were found between ROI CBF and MMSE scores in different subgroups based on APOE4 and Tau/ABETA status. These findings emphasize the intricate relationships between brain regions, cognitive function, genetic factors like APOE4, and biomarkers in individuals with cognitive impairment. The study also highlighted significant correlations between perfusion values and cognitive scores, such as ADNI-EF and ADNI-LAN scores. This suggests that alterations in cerebral blood flow may not only reflect but also potentially contribute to the cognitive decline observed in these populations. Furthermore, distinct associations were found between ROI CBF and MMSE scores in different subgroups based on APOE4 and Tau/ABETA status. These findings emphasize the intricate relationships between brain regions, cognitive function, genetic factors like APOE4, and biomarkers in individuals with cognitive impairment. To provide deeper insight, it is crucial to consider the neurobiological implications of these findings. The elevated levels of GAP-43 may indicate an underlying neurodegenerative process characterized by synaptic dysfunction and plasticity changes, which are hallmarks of AD pathology. Understanding how GAP-43 interacts with other neurodegenerative markers could elucidate its role in the progression of cognitive decline. Moreover, the observed inverse correlations between brain regions suggest a potential compensatory mechanism where certain areas may attempt to mitigate dysfunction in others, highlighting the complexity of neural networks involved in cognition.

Moreover, the research explores the intricate relationships between brain regions, cognitive function, genetic factors like APOE4, and biomarkers in individuals with cognitive impairment. It reveals significant correlations between perfusion values, cognitive scores, and different subgroups based on APOE4 and Tau/ABETA status. These findings highlight the complex interplay between brain regions, cognitive function, and genetic factors in individuals with cognitive impairment. This association has the potential to function as a predictive factor for the onset of AD during the transition from normal cognition to MCI. The findings offer insights into the potential correlation between GAP-43 expression and distinct cognitive functions across different cognitive states. Furthermore, through the consideration of variables such as age, gender, education, and Tau level, our research provides a thorough comprehension of the circumstances under which these correlations manifest, thereby enhancing our insight into the cognitive and biological determinants that impact these connections. The results of our study carry substantial implications for elucidating the

pathophysiology of Alzheimer's disease and mild cognitive impairment. The correlations between CSF GAP-43 levels and ASL perfusion, along with the identification of the particular brain regions implicated, offer significant insights into the prospective utility of CSF GAP-43 as a biomarker for AD. These findings highlight the significance of additional research to clarify the potential of CSF GAP-43 as a diagnostic and prognostic tool for Alzheimer's disease and related cognitive disorders. In discussing our findings, we must acknowledge certain limitations. The sample size may restrict generalizability, and variations in participant characteristics could influence results. Moreover, while we highlight the utility of GAP-43 as a biomarker for AD pathology, it is essential to consider other potential confounding factors that may affect these relationships.

5 | Conclusion

In conclusion, our study offers evidence of a notable association between CSF GAP-43 and ASL perfusion in individuals without clinical dementia, as well as in those in advanced stages of AD. This correlation persists irrespective of Aβ pathology, APOE genotype, or tau markers. Therefore, ASL perfusion monitoring of CBF has the potential to identify alterations in GAP-43 associated CBF during the preclinical phase, offering a non-invasive biomarker for the early detection of AD. This indicates that ASL perfusion may have a significant impact on the early detection of AD, thereby contributing to the advancement of effective diagnostic approaches and interventions for the disease. The correlation between CSF GAP-43 and ASL perfusion, along with the brain regions impacted, offers valuable insights into the potential utility of CSF GAP-43 as a biomarker for AD. This implies that alterations in CSF GAP-43 levels could serve as an indicator of AD progression. Moreover, identifying the brain regions implicated in this correlation could facilitate the creation of precise diagnostic and therapeutic strategies for AD. This study has the potential to significantly contribute to the early detection and management of AD.

Author Contributions

Neda Songhori: writing – original draft, conceptualization, methodology, validation, writing – review and editing, investigation, visualization, project administration, supervision, resources, software, data curation. **Maryam Shamsi Goushky:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, supervision, resources. **Mohammad Mehdi Khaleghi:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, supervision. **Marzieh Mojerloo:** conceptualization, investigation, writing – original draft, methodology, validation. **Mohammad Sadeghi:** conceptualization, investigation, supervision, funding acquisition. **Mehrdad Mozafar:** formal analysis, data curation, methodology, software, validation. **Zahra Babaie Aghdam:** supervision, investigation, visualization, project administration. **Maryam Ghajar:** supervision, validation, conceptualization, writing – original draft. **Yalda Kianifar:** conceptualization, writing – original draft. **Farough Karimzadeh:** writing – original draft, investigation. **Farbod Khosravi:** supervision, writing – review and editing. **Mahsa Mayeli:** supervision.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Transparency Statement

The lead author Neda Songhori affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.