Review

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Review

Understanding Proton Magnetic Resonance Spectroscopy Neurochemical Changes Using Alzheimer's Disease Biofluid, PET, Postmortem Pathology Biomarkers and APOE Genotype

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Abstract: In vivo proton (^{1}H) magnetic resonance spectroscopy (MRS) is a powerful noninvasive method which can measure Alzheimer's disease (AD) related neuropathological alterations at the molecular level. AD biomarkers include amyloid-beta (Aβ) plaques and hyperphosphorylated tau neurofibrillary tangles. These biomarkers can be detected via postmortem analysis, but also in living individuals through positron emission tomography (PET) or biofluid biomarkers of Aβ and tau. This review offers an overview of biochemical abnormalities detected by ^{1}H MRS within the biologically defined AD spectrum. It includes a summary of earlier studies that explored the association of ^{1}H MRS metabolites with biofluid, PET, and postmortem AD biomarkers, and examined how *apolipoprotein e4* allele carrier status influences brain biochemistry. Studying these associations is crucial for understanding how AD pathology affects brain homeostasis throughout the AD continuum and may eventually facilitate to develop potential novel therapeutic approaches.

Keywords: Alzheimer's disease; mild cognitive impairment; magnetic resonance spectroscopy; amyloid; tau; biofluid biomarkers; apolipoprotein *ε4*

1. Introduction

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder accounting for 60-80 of all dementia cases. More than 55 million people live with AD and other dementias, and this number is expected to rise [1]. AD dementia imposes a significant burden on the healthcare system and primary caregivers. Patients with AD dementia experience a progressive decline in both cognitive and physical abilities, leading to substantial challenges in daily functioning and quality of life. Recent progress in disease-modifying therapies, such as monoclonal antibodies, has emphasized the need for early and precise diagnosis of AD's underlying pathology through surrogate biomarkers [2]. Detecting AD pathology at the earliest stage, before significant brain damage occurs, is essential for timely intervention, which can improve outcomes. Proton (^{1}H) magnetic resonance spectroscopy (MRS) is a non-invasive method to detect changes in brain biochemistry linked to molecular-level pathological processes related to AD. The integration of ^{1}H MRS with other biomarkers acquired through positron emission tomography (PET) and biofluid analysis enhances the ability to diagnose AD at an early stage and monitor the impact of interventions, potentially leading to improved patient outcomes.

^{1}H MRS offers the advantage of monitoring biochemical alterations over time, making it a valuable tool for tracking disease progression as a prognostic tool and the efficacy of therapeutic interventions. Monitoring metabolite changes during the AD continuum provide a more detailed picture of the disease process, allowing for the identification of specific biochemical pathways affected by AD pathology. A variety of metabolites, which reflects different physiological functions, can be measured in the brain using ^{1}H MRS. For example, alterations in total N-acetylaspartate (tNAA: N-acetyl aspartate, NAA plus N-acetyl aspartylglutamate, NAAG) signal is associated with

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neuronal integrity, myo-inositol (mIns) reflects glial activity, glutamate (Glu) and gamma-aminobutyric acid (GABA) represent excitatory and inhibitory neurotransmission, respectively, while total choline (choline, Cho; phosphocholine, PCho; glycerophosphocholine, GPC) is associated with cell membrane turnover, and total creatine (tCr = creatine, Cr plus phosphocreatine, PCr) is linked to energy metabolism. Given the varying precision in naming some of the ^{1}H MRS metabolites NAA, Cr and Cho may sometimes refer the total sum as explained in the "total" definitions in this review.

The accumulation of amyloid-beta (Aβ) plaques outside the neurons and the accumulation of neurofibrillary tangles composed of hyperphosphorylated tau inside the neurons start more than two decades before the clinical symptoms of AD dementia manifest [3–5]. AD neuropathology can be measured through postmortem examination or using biomarkers in living people. The clinical diagnostic criteria for AD dementia have evolved over time. In 2018, the US National Institute on Aging and the Alzheimer's Association (NIA-AA) committee proposed the biological definition and diagnosis of AD as a research framework by using biomarkers acquired from living people during the AD continuum [6]. The NIA-AA framework has enabled a purely biological definition of AD by categorizing individuals based on biomarker evidence of AD pathology using amyloid-tau-neurodegeneration (ATN) [6]. In ATN classification, 'A' refers to Aβ proteinopathy pathway, 'T' to tau proteinopathy pathway, and 'N' to neurodegeneration. This biomarker classification scheme has recently been revised [7]. While the new revised biological disease staging using PET and biofluid markers will continue to increase our understanding about the disease progression, ^{1}H MRS biomarkers has potential to add a crucial neurochemical dimension to increase our understanding regarding neuropathological alterations in AD continuum and enhance the accuracy of early AD diagnosis. Unlike biofluid biomarkers, which reflect the global pathology, ^{1}H MRS data can provide regionally specific biochemical changes in the brain.

The biologically defined AD continuum that begins with the appearance of brain pathology in asymptomatic individuals and progresses through stages of increasing pathological burden, eventually leading to clinical symptoms [6,8]. The AD continuum stages includes preclinical stage, where the participants are cognitively unimpaired (with or without amyloid or tau biomarkers); the prodromal stage includes the early symptomatic phenotype (e.g. MCI) and AD dementia stage refers to the phase where severe cognitive symptoms are present which effects social and daily activities [9]. With recent advances in in vivo biomarker fields (i.e., neuroimaging and biofluid markers), progression of AD pathology in vivo is now possible starting from preclinical stages long before the irreversible brain damage occurs.

The focus of this review was to summarize the relationship between ^{1}H MRS metabolites and biomarkers acquired through PET (tau, amyloid), biofluid and post-mortem AD pathology analysis across the AD continuum in the brain (**Table 1**). There are several excellent older reviews on changes of ^{1}H MRS metabolites in AD continuum for further reading [10–18].

2. Commonly Studied ^{1}H MRS metabolites in AD

2.1. NAA

NAA is a small molecule which is synthetized from aspartate and acetyl-coenzyme A in the brain. It is a marker of neural health, viability, and synaptic integrity [19]. NAA has a high amplitude signal at 2.01 part per million (ppm), relative to the standard tetramethysilane. The peak also includes minor contributions from other metabolites such NAAG at 2.04 ppm. These overlapping signals from acetyl moieties of both molecule at 2.01-2.04 ppm range (plus lower amplitude signals from other ppm ranges, see de Graaf, 2007) is ascribed as NAA or total NAA (tNAA: NAA + NAAG) [20–25]. NAA is found primarily in neural cells and synthesized in mitochondria [21–23]. A lower NAA/Cr ratio has been associated with loss of synaptic integrity [19]. A reduction of NAA levels (using water as an internal reference or Cr) in patients with AD dementia compared to cognitively unimpaired participants is one of the most frequent findings of ^{1}H MRS studies [26–40].

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2.2. mIns

The well resolved spectral peak of mIns is assigned to 3.56 ppm [41,42]. mIns is considered as a glial marker and/or an osmolyte [43]. An increase in mIns was linked with elevated glial marker on PET (18kDa TSPO). TSPO PET uptake has been associated with neuroinflammation and glial cell activation [44] or density of inflammatory cells [45]. This association support the notion that mIns can be a marker of neuroinflammation or density of inflammatory cells. Many studies consistently demonstrated an increase in mIns (or mIns/Cr) in several gray and white matter brain regions in people with AD dementia compared to controls [12,26–29,31,36–38,40,43,46,47]. It has been proposed that an increase in the mIns/Cr ratio occurs during the early stages of the disease progression, which is then followed by a decrease in the NAA/Cr ratio and an increase in the Cho/Cr ratio at later stages of the disease [40].

Table 1. Summary of association studies between ^{1}H MRS and other biomarkers including biofluid, PET and postmortem pathology.

Authors	Cohort	Magnet field strength and acquisition parameters	Voxel locations and size	Key findings
[48]	CU (n=30)	7T, TR=644, MRSI, FIDLOVS	Posterior cingulate gyrus and precuneus	↑ GABA and ↑ Glu were associated ↑ Aβ burden on PET (PiB) with a positive effect modification by *APOE e4* allele.
[49]	AD (11), MCI (8), CU (n=26)	3T, TR/TE=2000/30 ms, MRSI, PRESS	Posterior cingulate gyrus, dorsolateral prefrontal cortex	↓Glu/tCr was associated with ↑ tau load on PET with florzolatau in the posterior cingulate gyrus of AD dementia patients. ↑ plasma NfL was associated with MRS metabolites (↓ tNAA/tCr and ↓ Glu/tCr) in the right dorsolateral prefrontal cortex of patients with AD dementia.
[50]	CU (Aβ – and Aβ+) (n=338), MCI (Aβ+)(n=90)	3T, TR/TE=2000/30 ms, single voxel, PRESS	Posterior cingulate cortex /precuneus region	↑ mIns/tCr ratio in the posterior cingulate gyrus was associated with ↑ posterior cingulate gyrus and neuocortical meta-ROI Aβ (flutemetamol) and tau (RO948) load on PET only in *APOE e4* allele carriers. ↑ plasma GFAP was associated with ↑ mIns/tCr (posterior cingulate gyrus) only in *APOE e4* allele carriers.
[51]	CU women: CSF Aβ negative (n=71); CU Aβ positive women (n=37); MCI (CSF Aβ positive) women (n=12)	3T, TR/TE=2000/20 ms; TR/TE=2000/68 ms/; single voxel; PRESS and MEGA-PRESS	Medial frontal cortex	↑ Glx, ↓ GABA, and ↑ mIns/tCr ratio in MCI compared to CU CSF Aβ42 negative and positive participants. ↑ Age was associated with ↓ levels of GABA in CU and MCI groups.

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[52]	CU (A−T−N−) (37); early AD (A+T+N−) (n=16); late AD (A+T+N+)(n=15)[a]	3T, TR/TE=2000/32 ms; single voxel; PRESS	Posterior cingulate cortex /precuneus region	↓ NAA/Cr in early AD (A+T+N−) and late AD (A+T+N+) compared to controls (A-T-N-; A+T-N-). ↑ mIns/Cr in late AD compared to controls. ↓NAA/Cr correlated with ↑ global Aβ load (PIB) and tau load (flortaucipir) on PET in whole cohort.
[53]	CU (n=40)	3T, TR/TE= 3000/30 ms, single voxel; sLASER	Posterior cingulate gyrus (automated VOI prescription)	↑Tau PET (flortaucipir) in posterior cingulate gyrus correlated with ↓NAA/tCr and ↓Glu/tCr.
[54]	CSF Aβ42 positive (n=111); CSF Aβ42 negative (n=174);	3T, TR/TE= 3000/30 ms, single voxel; PRESS	Posterior cingulate cortex /precuneus region	Visit 2 (~2.3 years after baseline): ↑ Cho/Cr, ↑mIns/Cr, ↓NAA/Cr, and ↓NAA/mI in CSF Aβ positive compared to CSF Aβ negative cases. Visit 3 (~4 years after baseline): ↑mIns/Cr, ↓NAA/Cr, and ↓NAA/mI in CSF Aβ positive compared to CSF Aβ negative cases. CSF Aβ positivity at baseline was associated with ↑mIns/Cr and ↓NAA/mIns ↑ Rate of change in the MCI Aβ positive for mIns/Cr and NAA/mIns compared to MCI Aβ negative.
[55]	CU younger controls (<60 years) (n=27); CU older controls (>60 years) (n=27); AD (>60 years) (n=25)	3T, TR/TE= 1600/(31-229) ms ms, single voxel, 2D J-PRESS	Posterior cingulate cortex /precuneus region	↑ mIns associated with ↑CSF tau, and ↑CSF p-Tau 181; ↑ GABA associated with ↑CSF p-Tau 181p in AD dementia group
[56]	Two cohorts: younger age (n=30) (20–40 years); CU (n=151): older individuals (60–85 years).	3T, TR/TE=4000/8.5 ms, single voxel, SPECIAL	Posterior cingulate cortex /precuneus region	↑ mIns, ↑ Cr, ↑mIns/NAA, ↓ GSH, ↓ Glu in older participants compared to younger participants.
[57]	CU (n=289)	1.5T, TR/TE=2000/25 ms, single voxel, PRESS	Posterior cingulate gyrus	↑ mIns/Cr ratio in participants with two copies of *APOE e4* allele compared with participants with non-carriers. ↓The NAA/mIns ratio in participants (*APOE e4*/*e4*) compared with those who were heterozygous for the *APOE e4* allele and non-carriers.
[58].	CU (n=15)	3T, TR/TE=1500/68 ms, single voxel, J-edited	Posterior cingulate cortex /precuneus region	↓GSH was associated with↑the temporal and parietal Aβ load on PET with PiB.

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		spin echo difference method		
[59]	aMCI (n=14); CU (n=32)	3T, TR/TE= 3000/30 ms, single voxel, sLASER	Posterior cingulate gyrus	↑ Global cortical Aβ load (PiB) on PET correlated with ↓Glu/mIns ratio in the entire cohort.
[60]	CU older adults (n=594) [c]	3T, TR/TE= 2000/30 ms, single voxel, PRESS	Posterior cingulate gyrus	↓ NAA/mIns and ↑ mIns/Cr at baseline were associated with ↑rate of Aβ deposition on serial PIB PET.
[61]	CU CSF Aβ42 negative (n=156); CU CSF Aβ42 positive (n=49), MCI CSF Aβ42 positive (n=88)	3T, TR/TE= 2000/30 ms, single voxel, PRESS	Posterior cingulate/precuneus	↑ mIns/Cr, ↑ Cho/Cr, ↓ NAA/Cr in MCI (CSF Aβ42 positive) compared to CU (CSF Aβ42 negative). ↑mIns/Cr in CU (CSF Aβ42 positive) compared to CU (CSF Aβ42 negative). ↑ mIns/Cr in *APOE e4* allele carrier CU (CSF Aβ42 negative) compared to non *e4* carrier CU (CSF Aβ42 negative). ↑ mIns/Cr and ↑ Cho/Cr were associated with ↑ Aβ deposition on PET (flutemetamol) in amyloid positive (on PET) cognitively unimpaired participants. ↑ mIns/Cr was associated with ↑ Aβ deposition on PET (flutemetamol) and in CSF Aβ42 positive cognitively unimpaired participants.
[62]	CU (n=16), aMCI (n=11)	3T; TR/TE = 2000/32ms, single voxel, 2D-PRESS	Bilateral hippocampi	No difference in mIns/Cr between *APOE e4* allele carriers and non-carriers
[63]	CU (n=21); aMCI (n=15)	3T, TR/TE= 3000/68 ms, single voxel, MEGA-PRESS	Posterior cingulate gyrus	↓ NAA was lower in Aβ positive subjects compared to Aβ negative (PiB PET) subjects. ↓ NAA was in *APOE e4* allele carriers compared to non-carriers.
[64]	*APOE e4* allele non carriers (n=89); *APOE e4* allele carriers (n=23)	3T, TR/TE= 1600/30 ms, single voxel, PRESS	Posterior cingulate gyrus	↑ Cho/Cr and ↑ mIns/Cr increase with age in *APOE e4* allele carriers. ↑ Cho/Cr ratio *APOE e4* carriers compared to non-carriers.
[19]	No to low likelihood of AD (n=17); Intermediate to high likelihood of AD likelihood (n=24)	3T, TR/TE= 2000/30 ms, single voxel, PRESS	Posterior cingulate gyrus	↓ NAA/Cr and NAA/mIns were associated with ↓synaptic integrity and ↑higher p-tau pathology. ↑Aβ burden was associated with ↑ mIns/Cr and ↓ NAA/mIns. ↑GFAP-positive astrocytic burden showed a trend of association with decreased NAA/Cr and NAA/mIns.

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[65]	CU (n=17); AD (n=19)	3T, TR/TE= 2000/30 ms, single voxel, PRESS	Hippocampus, posterior cingulate gyrus and right parietal gyrus	↓NAA/Cr (hippocampus) was correlated with ↓CSF Aβ42. ↓NAA/Cr (parietal gyrus) was correlated with ↑CSF p-tau. ↑mIns/Cr (posterior cingulate gyrus) was correlated with ↑t-tau;
[66]	All subjects (n=109); AD dementia (n=40); non-AD dementia, (n=14); MCI of AD type (n=29) MCI of non-AD type (n=26)	1.5T, TR/TE= 2000/272, single voxel, PRESS	Medial temporal lobe	↓ NAA was correlated with ↓CSF Aβ42 in patient with AD dementia.
[67]	CU (n=311)	1.5 T, 2000/30 ms, single voxel, PRESS	Posterior cingulate gyrus	↑mIns/Cr and ↑Cho/Cr was associated ↑ Aβ load on PET (PIB).
[68]	Low AD likelihood (n=11); intermediate AD likelihood (n=9); high AD likelihood (n=34)	1.5 T/ 2000/30 ms, single voxel, PRESS	Posterior cingulate gyrus	↓ NAA/Cr, ↑mIns/Cr, ↓ NAA/mIns in postmortem frequent neuritic plaque group compared to neuritic sparse plaque group ↓ NAA/Cr in frequent neuritic plaque group compared to neuritic moderate plaque group. ↑mIns/Cr and ↓ NAA/mIns in neuritic moderate plaque group compared to neuritic sparse plaque group. ↓ NAA/Cr, ↑mIns/Cr, ↓ NAA/mIns in high-likelihood AD group compared to low-likelihood AD group ↑mIns/Cr in high-likelihood AD group compared to intermediate-likelihood AD group. ↓NAA/Cr, ↑mI/Cr, and ↓NAA/mI ratios were associated with higher Braak NFT stage, higher neuritic plaque score, and greater likeli-hood of AD.
[69]	CU (n=61); patient group (MCI + AD dementia (n=46)	1.5 T/ 2000/30 ms, single voxel, PRESS	Posterior cingulate/precuneus	No differences were noted on ^{1}H-MRS metabolite ratios (NAA/Cr, mIns/Cr, NAA/mIns) across *APOE e4* carriers and non-carriers.
[40]	CU (63); MCI (21); AD dementia (21)	1.5 T/ 2000/30 or 135 ms, single voxel, PRESS	Posterior cingulate gyrus; medial occipital; left superior temporal lobe	↑ NAA/Cr ratios (medial occipital) in patients with AD dementia correlated with *APOE e4* carrier status
[70]	postmortem brain with AD pathology (49);	In vitro ,11.7 T, perchloric acid extracts	Autopsy brain samples from various brain regions	↑ mIns, ↑ GPC, ↓ Glu, in *APOE e3/e3* samples from AD dementia patients compared to samples from normal control brains samples

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non-demented control (5)	↓NAA in *APOE e3/e3* and *APOE e4/e4* AD samples from AD dementia patients compared to samples from normal control brains (*APOE e3/e3*).

Abbreviations: Aβ: Amyloid-beta; AD: Alzheimer's Disease; aMCI: Amnestic Mild Cognitive Impairment; APOE: Apolipoprotein E; A−T−N−: Negative for amyloid-beta, tau, and neurodegeneration markers; A+T+N−: Positive for amyloid-beta and tau, negative for neurodegeneration markers; A+T+N+: Positive for amyloid-beta, tau, and neurodegeneration markers; CU: Cognitively unimpaired; CSF: Cerebrospinal fluid; FIDLOVS: Free-Induction-Decay-Localized-by-Outer-Volume-Suppression; GFAP: Glial Fibrillary Acidic Protein; GPC: Glycerophosphocholine; MCI: Mild Cognitive Impairment; MEGA-PRESS: Mescher-Garwood Point-Resolved Spectroscopy; MRSI: Magnetic resonance spectroscopy imaging; mIns: myo-inositol; NAA: N-Acetylaspartate; NfL: Neurofilament light; NFT: Neurofibrillary tangles; PET: Positron emission tomography; PiB: Pittsburgh compound B; PRESS: Point-Resolved Spectroscopy Single-Voxel Sequence; p-tau: Phosphorylated tau; RO948: Tau PET ligand; tCr: sLASER: adiabatic selective refocusing sequence; Total creatine; TR/TE: Repetition time/echo time; VOI: Volume of interest; MMSE: Mini Mental State Examination; MRS: Magnetic resonance spectroscopy; GABA: γ-Aminobutyric Acid; Glx: Glu (glutamate) + Gln (glutamine).

2.3. Cho

Choline is considered a cell membrane (phospholipid) turnover, white matter integrity and cellular density biomarker [43,71,72]. The peak at 3.2 ppm is assigned to mobile choline-containing compounds including PCho and GPC ascribed as total Cho (tCho), which are found in the myelin and the cell membrane [20,71–73]. While some studies reported a change in Cho signal related with AD pathology, the direction of change is not always consistent. Some reported an increase [37,40,74–77], and others reported a decrease or no change [26,29,31,33,78,79] in patients with AD dementia compared to controls. Elevation of Cho in the AD dementia may be due to an increased membrane catabolism in response to an increased demand for acetylcholine synthesis, which leads an increase in PCho and GPC [18,80,81].

2.4. Glu, Gln, Glx

The spectral peaks of Glu (at 2.35 ppm) and Gln (at 2.45 ppm), measured through conventional ^{1}H MRS sequences, overlap at commonly used clinical MR field strengths (1.5 T and 3T) [46]. Therefore, these two peaks are generally assigned as Glx (Glu +Gln). Glu, a precursor of GABA, is an excitatory neurotransmitter, and mainly synthesized through Glu-Gln cycle [82,83]. Earlier studies reported a decrease in glutamate or Glx in patients with AD dementia and MCI compared to cognitively unimpaired participants [34,59,63,84,85].

2.5. GABA

GABA is a primary inhibitory neurotransmitter in the brain. Differentiation of GABA from other overlapping peaks of Glx (at 2.35 ppm), NAA, Cr and PCr (at 3.02 ppm) at lower field strengths (≤ 3T) is challenging. Spectral editing methods or employment of 2 dimensional spectroscopy protocols are needed to resolve overlapping signals [86]. Riese et al. reported that GABA levels were lower in patients with amnestic MCI compared to elderly controls [63]. In contrast, a study observed no significant change in GABA levels between normal elderly participants and those with AD dementia [87].

2.6. GSH

Glutathione (GSH) is considered as an important antioxidant in the brain [88]. A decrease in GSH levels in a variety of brain regions, including the hippocampus, frontal cortex, posterior cingulate cortex, and anterior cingulate cortex, has been demonstrated in patients with AD dementia as compared with age-matched cognitively unimpaired participants [89–91]. However, a recent meta-

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analysis reported that there was no change in GSH peroxidase and GSH reductase activities and GSH levels in human specimens [92].

2.7. Cr

The Cr and PCr system play role in energy metabolism for adenosine triphosphate regeneration and act as an energy buffer [93]. Since there are overlapping singlets of Cr and PCr peaks at 3.03 ppm and 3.9 ppm at 3T and lower fields, their sum ascribed as Cr or total Cr (tCr) is used in many studies, generally as an internal reference metabolite to calculate relative metabolite levels (metabolite/tCr) [17,94]. While tCr remains constant in various diseases such as AD dementia [28,29,31,94], it has been shown that total Cr levels may change with age [56,95–97] and white matter hyperintensity volume [98]. It is highly recommended that tCr levels should be used as an internal reference after confirming that its concentration (relative to water) is not changed [99].

3. Association of ^{1}H MRS Metabolites with Postmortem Neuropathology

Correlation studies between antemortem ^{1}H MRS metabolite alterations and postmortem neuropathology are limited. Histopathological findings serve as a gold standard to validate ^{1}H MRS findings to monitor AD continuum and better understand how metabolite changes are associated with topographical neuropathological alterations [19,68].

We reported that a decrease in NAA/Cr and an increase in mIns/Cr (posteriorcingulate gyrus) correlates with postmortem Alzheimer-type pathology including the postmortem Braak neurofibrillary tangle stage, higher neuritic plaque score, and greater likelihood of AD (**Figure 1**) [68]. The study suggested that the mIns/Cr ratio may be more sensitive to early pathologic changes than the NAA/Cr ratio. Melissa et al. showed that antemortem ^{1}H MRS metabolites (e.g., NAA/Cr and NAA/mIns) linked to postmortem AD neuropathology, including the amyloid burden, synaptic integrity, and tau pathology [19]. In particular, the study identified a correlation between increased mIns/Cr and decreased NAA/mIns in the posterior cingulate gyrus with postmortem amyloid burden. Additionally, the study found an association between NAA/Cr and synaptic vesicle immunoreactivity but not neural density in the posterior cingulate gyrus across the entire cohort, including AD patients and control subjects. No such association was observed between Cho/Cr and mIns/Cr ratios and synaptic vesicle immunoreactivity which.

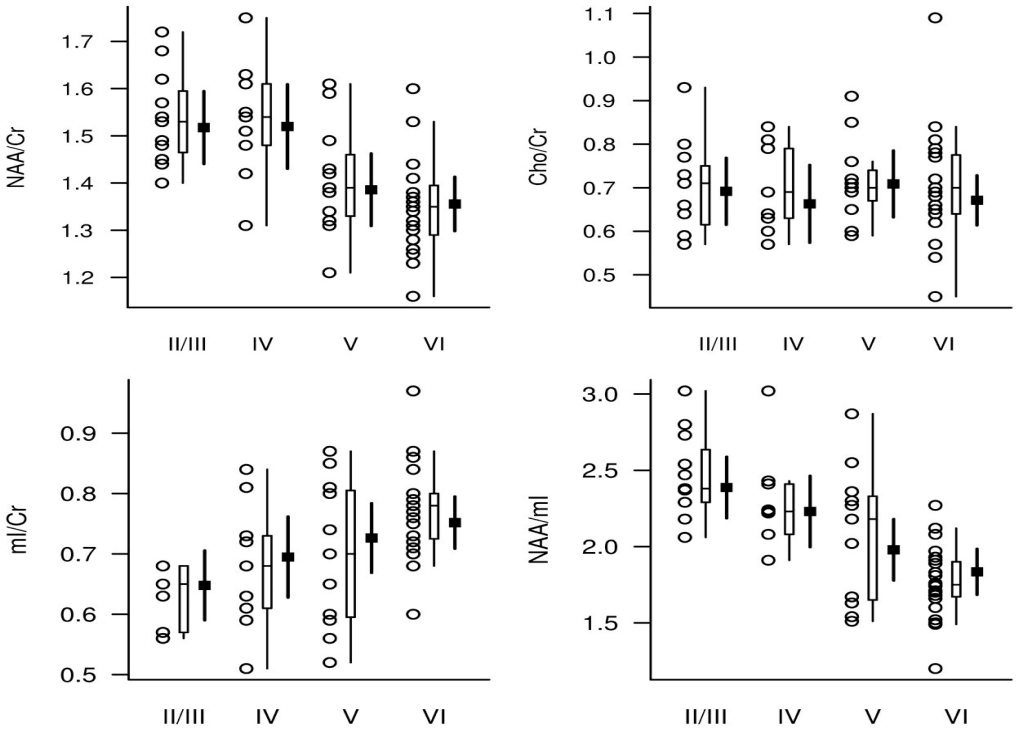

Figure 1. ^{1}H MR spectroscopic metabolite ratios plotted according to Braak NFT stage (horizontal

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axis). For each Braak NFT stage diagnosis, individual values, a box plot of the distribution, and the estimated mean and 95% CI (darker lines) for the mean are shown. The mean and CI were derived from ANCOVA models and are assumed for a 78-year-old woman in whom the interval from ^{1}H MR spectroscopy to death is 2 years. With permission from Radiology [68].serves as synaptic integrity marker. Furthermore, a higher postmortem pTau burden was associated with lower NAA/Cr and NAA/mIns ratios, while Cho/Cr was not associated with postmortem pTau.

The study reported that postmortem late extracellular neurofibrillary tangle pathology was not correlated with NAA/Cr, mIns/Cr, Cho/Cr, and NAA/mIns. There was also no association between CD68 (a marker for activated phagocytic microglia)-positive microglia and any of the metabolite ratios studied in the study (i.e., NAA/Cr, mIns/Cr, Cho/Cr, and NAA/mIns) [19]. One of the important findings of this study was the association between NAA/Cr with synaptic integrity marker and pTau, but not with neural loss or late extracellular neurofibrillary tangle pathology. Supporting evidence of these findings reported later in an in vivo study using tau (Flortaucipir) PET with [53].

4. Association of ^{1}H MRS Metabolites with Tau and Amyloid PET

4.1. NAA

In cognitively unimpaired cohort, there was no statistically significant association between NAA/Cr (posterior cingulate gyrus) and Aβ deposition on PET imaging (11 C Pittsburgh compound B, henceforth PiB) after adjusting for sex and age [67]. In line with this study, it was reported that neither global cortical Aβ nor local (posterior cingulate gyrus) Aβ load on PiB PET were correlated with NAA (posterior cingulate gyrus) in a cohort consist of cognitively unimpaired participants and those with amnestic MCI [63]. However, the study reported that NAA was lower in Aβ positive participants compared to Aβ negative participants. Zeydan et al. examined ^{1}H MRS metabolite profile in the posterior cingulate gyrus in two groups (i.e. cognitively unimpaired participants and participants with amnestic MCI) using advanced sLASER MRS protocol [59]. A study reported that the level of NAA, mIns, Cr, and Cho between amnestic MCI participants, who were Aβ-positive on PiB PET, and cognitively unimpaired participants, who were Aβ-negative on PiB PET were not statistically significant [59]. We recently reported that Aβ deposition on PET was not associated with NAA/tCr ratios in the posterior cingulate gyrus of cognitively unimpaired participants, while higher in tau PET load was associated with a lower NAA/tCr ratio [53]. Extending these findings, a current study reported no significant association between tNAA/tCr with Aβ-load on PIB PET within the gray matter (posterior cingulate gyrus or dorsolateral prefrontal cortex) [49]; however this study reported a decrease in tNAA/tCr ratio in patients with patients with AD dementia in the gray matter. These studies suggest that an increase in Aβ-load may not be directly associated with NAA in preclinical and prodromal stages of AD pathology.

Current studies have investigated the association between ^{1}H MRS and both tau and Aβ loads on PET in cognitively unimpaired individuals. Our group recently investigated the association between brain metabolites with tau and Aβ load on PET [53]. An increase in the posterior cingulate gyrus tau load on Flortaucipir PET was associated with lower NAA/tCr in cognitively unimpaired older adults [53] (**Figure 2**). Extending these findings, a decreased NAA/Cr ratio in the posterior cingulate gyrus was associated with elevated tau and Aβ load on PET in a cohort consisting of participants with non-AD and AD dementia who were categorized based on their A/T/N status based on PET and MRI [52]. The study reported that NAA/Cr ratio in early AD (A+T+N−) and late AD (A+T+N+) was lower compared to controls (A-T-N− and A+T-N-). An association between elevated NAA/Cr and an increase in global Aβ load on PET and tau load on PET was present in the whole cohort. Furthermore, the study reported that NAA/Cr ratio

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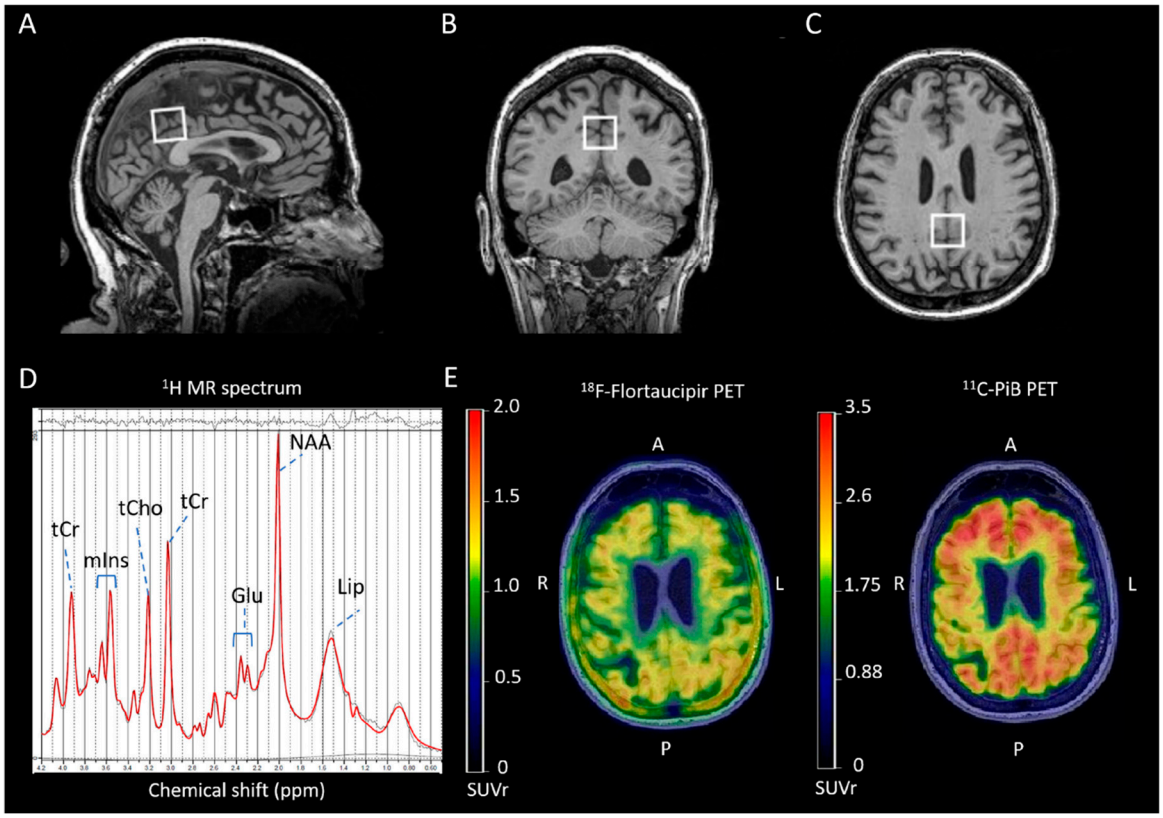

Figure 2. Sagittal (A), coronal (B), and transverse (C) T1– weighted magnetic resonance images with superimposed posterior cingulate gyrus magnetic resonance spectroscopy volume of interest (20×20×20 mm3). Representative 1H MRS spectra, AV-1451-PET and PiB-PET of a clinically normal individual. The thick red curve on the representative MRS spectra is the LCModel fit to the data. The thin curve under the spectra is the baseline. The residual (data minus the fit to the data) is shown on the top of the spectra. (D) Single voxel proton (1H) magnetic resonance (MR) spectrum acquired from the posterior cingulate gyrus of a cognitively unimpaired participant (age = 81) at 3 T with sLASER sequence. The thick red curve on the representative MR spectrum is the LCModel fit to the data. The thin curve under the spectrum is the fitted baseline. The residual (data minus the fit to the data) is shown at the top of the spectrum. The chemical shift axis is labeled in parts per million (ppm) unit. The Y axis is an intensity scale of each spectral line with no unit. (E) The representative cortical flortaucipir PET and PiB PET scans were acquired from the same participant. The participant had low NAA/tCr = 1.20 and Glu/tCr = 0.99, high PCG flortaucipir standard value uptake unit ratio of 1.22, and high PCG PiB standard value uptake unit ratio of 3.06. The PET scans were registered to the T1-weighted MR image and displayed together. We observed flortaucipir uptake in the skull of this participant. The meningeal and bone uptake of flortaucipir is known manifestation of off-target binding. The cause is unknown. In contrast such off-target uptake in PiB is not seen except with rare cases of bone uptake in diseases with high rates of bone remodeling (e.g., hyperostosis frontalis interna). The representative color scale shows the standardized uptake value ratios. Abbreviations: Glu, glutamate; Lip, lipid signal; NAA, N-acetylaspartate; PET, positron emission tomography; PiB, Pittsburgh compound-B; tCr, phosphocreatine + creatine; tCho, phosphocholine + glycerophosphocholine; mIns, myo-inositol. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.). This figure, originally appearing as Figure 1 and Figure 2; is reprinted from "1H MR spectroscopy biomarkers of neuronal and synaptic function are associated with tau deposition in cognitively unimpaired older adults", Neurobiology of Aging, Volume 112, April 2022, Pages 16-26, with permission from Elsevier.could be used to discriminate A-T-N− and A+T-N-) from participants with early AD (A+T+N-).

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4.2. mIns

In a cognitively unimpaired cohort, elevated mIns/Cr in the posterior cingulate gyrus was associated with an increased Aβ load on PiB PET [67]. Voevodska et al. reported an association between higher Aβ load on PET with higher mIns/Cr and mIns/NAA (posterior cingulate gyrus) ratios in cognitively unimpaired participants who were classified as Aβ positive based on Aβ CSF levels [61]. The study also reported that when Aβ positivity was based on PET with Flutemetamol, the association between elevated mIns/Cr and increased Aβ load in cognitively unimpaired controls was sustained [61]. However, these associations were not present in the CSF Aβ42 negative cognitively unimpaired controls, suggesting that a certain level of neuropathological accumulation driven by amyloid load may be required to observe these associations [61]. Nedelska et al. reported that elevated mIns/Cr and lower NAA/mIns (posterior cingulate gyrus) in cognitively unimpaired participants at baseline were associated with baseline Aβ load and an increased rate of Aβ deposition on PiB PET over time [60]. Extending these findings, a higher level of mIns/Cr (posterior cingulate gyrus) was reported in participants with biologically defined late AD dementia (A+ T+ N+) with cognitive impairment compared with cognitively unimpaired participants (A-T-N- and A+ T-N-)[52] , but no statistically significant difference in mIns/Cr ratio was observed between cognitively unimpaired and biologically defined early AD participants (A+T+N-). The study also demonstrated an association between higher mIns/Cr and higherglobal Aβ load on PET (PiB) and tau load on PET in the entire cohort.

4.3. Cho

The Cho/Cr ratio in the posterior cingulate gyrus was associated with an increased Aβ load on PET (PIB) in a relatively large cognitively unimpaired cohort (n = 311) [67]. Partially in line with this study, Voevodskaya et al. reported that elevated Cho/Cr in the posterior cingulate gyrus region in cognitively unimpaired participants was associated with an increased Aβ load on PET with flutemetamol, but only in amyloid PET positive cognitively unimpaired participants [61]. Interestingly, when the CU group classified as amyloid positive based on CSF Aβ42 levels instead of PET, Cho/Cr was no longer associated with Aβ load on PET or CSF Aβ load [61]. This suggest that Aβ load in CSF and PET might be capturing distinct aspect of amyloid pathology. In addition, the variability in CSF Aβ42 level in these participants might have influenced the relationship between Cho/Cr and amyloid load, making the association less detectable in the Aβ positive group based on CSF analysis.

Spotorno et al. reported no correlation between tCho/tCr and Aβ and tau load on PET, and no moderation effect of *APOE* ε4 genotype on these associations in a cohort consisting of CU (Aβ negative and Aβ positive on PET), MCI (Aβ positive on PET) [50]. Most recently, Chen et al. reported no change in Cho/Cr between normal (A−T−N− and A+T-N-), biologically defined early AD (A+T+N−) and late AD (A+T+N+) groups [52]. The results of Sportorno et al. are not fully in line with result from those of Kantarci et al. (2011) which might be attributed to differences in the characteristics of participants among the studies. The participants in the study by Kantarci et al. (2011) were relatively older than those enrolled in the study by Sportorno et al. (2022). This suggests that the association between Cho and Aβ load on PET might be more detectable when neuropathological alterations have progressed further in older participants.

4.4. Glx and Glu

Rieze et al. reported no association between Glx and Aβ deposition on PiB PET (global and local [posterior cingulate gyrus]) in a cohort consist of participants with amnestic MCI and cognitively unimpaired participants [63]. Zeydan et al. reported a decrease in Glu and Glu/mIns ratio in the amnestic MCI group (Aβ positive on PET with PiB) compared to the cognitively unimpaired group (Aβ negative on PET with PiB) in the posterior cingulate gyrus [59]. A decrease in Glu/mIns ratio was associated with a higher global cortical Aβ deposition in whole cohort consist of amnestic MCI and cognitively unimpaired participants [59]. In addition, the study reported that this correlation was not

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present when groups (participants with amnestic MCI and cognitively unimpaired participants) were analyzed independently. Only few studies have investigated the association between ^{1}H MRS metabolites and both tau and amyloid loads on PET in cognitively unimpaired individuals and patients with AD. An increase in the posterior cingulate gyrus tau deposition on PET with 18F-flortaucipir was associated with lower Glu/tCr ratios in cognitively unimpaired older adults [53] (**Figure 2**) and biological sex modified this association. However, association between Glu/tCr and Aβ deposition on PET with PIB was not statistically significant. Chen et al. categorized their cohort as cognitively unimpaired controls (A−T−N− and A+T-N-), early AD (A+T+N−), and late AD (A+T+N+) dementia using PET and MRI data. The study reported no difference in Glu/tCr across groups (controls, biologically defined early AD and late AD). Matsuaoka et al. reported that a decrease in Glu/tCr in posterior cingulate was associated with an increase in tau load on PET with florzolatau in participants AD dementia [49]. Riese et al. (2015) studied a cohort of MCI and healthy controls and found no difference in Glx between groups categorized as Aβ negative and Aβ positive on PET [63].

4.5. GABA

GABAergic dysfunction has been reported in the AD continuum [100]. Some studies reported lower GABA/Cr in patients with AD dementia [100], others found no change in GABA levels compared to controls [87]. Rieze et al. reported that similar GABA levels in posterior cingulate gyrus among groups classified as Aβ positive and negative on PET [63]. The study also reported no correlation between GABA and Aβ deposition on PiB PET (global and local [posterior cingulate gyrus]) in a cohort consist of participants with amnestic MCI and no cognitive impairment.

4.6. GSH

In cognitively unimpaired participants, a negative correlation between GSH levels (posterior cingulate gyrus) and brain amyloid load on PET (PiB) in the temporal and parietal regions was reported suggesting preclinical changes in GSH level might be early biomarker of AD pathology [58]. In a recent study, no difference in GSH/tCr (posterior cingulate gyrus) across groups (controls: A-T-N- and A+T-N-;biologically defined early AD: A+T+N- and late AD: A+T+N+) was found [52]. Further research is needed to explore the relationship between GSH levels and Aβ and tau pathology [92].

5. Association of ^{1}H MRS Metabolites with Biofluid Biomarkers

The emergence of blood-based plasma biomarkers represents a major recent breakthrough in identifying biological indicators of AD. These biofluid biomarkers are non-invasive, readily accessible, and cost-effective, making them crucial for detecting AD throughout its preclinical, prodromal and dementia stages. The most extensively evaluated AD-related plasma biomarkers include Aβ, especially $A\beta_{40}$, $A\beta_{42}$, and their ratio $A\beta_{42}/A\beta_{40}$, phosphorylated tau (p-tau) protein at epitopes 181, 217, and 231 (p-tau181, p-tau217, and p-tau231) which reflect neuritic plaques and neurofibrillary tangle pathologies [101–103]. It has been demonstrated that $A\beta_{42}/A\beta_{40}$ ratio in plasma correlates with CSF AD biomarkers, and amyloid PET [104–106]. Similarly, the level of p-tau proteoforms were associated with CSF, PET, and post-mortem AD neuropathological markers [107–110].

Plasma neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) have been commonly studied in AD research [101–103]. The NfL is a marker of neuroaxonal damage, and elevated NfL is associated with progression of neurodegenerative disorders such as AD, Huntington's disease, Multiple Sclerosis [111,112]. Interestingly, it has been reported that plasma NfL but not CSF NfL was significantly associated with cognition [113] suggesting CSF and plasma biomarkers might provide complementary information rather than being directly interchangeable. Plasma GFAP, a marker of astrocytic activation, is associated with the elevated risk and severity of AD type and non-AD type dementia [114,115]. While biofluid AD markers in plasma can also be

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measured in CSF, lumbar punctures, however, are burdensome and costly, and requires specialized training, limiting their use, if serial assessment is required.

5.1. NAA

Lower medial temporal lobe NAA was correlated with lower CSF Aβ42 within patients with AD dementia [66]. Neither CSF tau nor CSF pTau181 were correlated with NAA within all dementia group (i.e. AD dementia, non-AD dementia, MCI of AD type and MCI of non-AD type) or any individual dementia groups. Bittner et al. studied the correlation of hippocampal, posterior cingulate gyrus and right parietal gyrus NAA with CSF Aβ42, CSF p- and t-tau in cognitively unimpaired and AD dementia patients [65]. Lower hippocampal NAA/Cr ratio in patients with AD dementia was associated with lower CSF Aβ42 levels, but not with CSF p-tau or t-tau, whereas lower parietal NAA/Cr was associated with higher CSF p-tau, but not with CSF Aβ42, or t-tau [65]. Voevodska et al. (2016) showed that NAA/Cr ratio in posterior cingulate gyrus was lower in MCI (CSF Aβ42 positive) compared to cognitively unimpaired individuals (CSF Aβ42 negative) [61]. Voevodskaya et al. (2019) reported that estimated rate of change in NAA/Cr in the posterior cingulate gyrus was -2.0%/year in a cohort consist of cognitively normal controls, mild cognitive impairment, and subject with cognitive decline who were classified based on their CSF Aβ load as CSF Aβ positive or CSF Aβ negative at baseline [54]. However, the estimated rate of change of NAA/Cr was not significant in CSF Aβ negative group. The metabolite ratios were also compared based within CSF Aβ positive and negative groups at baseline (visit 1), visit 2 and visit 3 with a gap of approximately 2 years between visits. It was shown that NAA/Cr ratio was lower in CSF Aβ negative group compared to CSF Aβ positive group at visit 2 and visit 3 [54].

Hone-Blanchet reported that tNAA in the medial frontal cortex did not change among older women categorized as cognitively unimpaired Aβ positive, cognitively unimpaired Aβ negative and MCI (Aβ positive) groups [51]. Furthermore, CSF Aβ 42 levels were not associated with level of tNAA and other metabolites (e.g., tCho, tNAA/mIns, Glx, mIns, GABA, GABA/tCr) [51]. Matsuoka et al. showed that there was a significant association between increased plasma NfL and a decreased tNAA/tCr in the right dorsolateral prefrontal cortex of participants with AD dementia; however, there was only a trend of association between elevated plasma NfL and decreased tNAA/tCr from the posterior cingulate gyrus, but this correlation did not reach statistically significance.

These studies highlight the value of integrating data from AD-specific CSF and plasma markers. Combination of both fluid and MRS biomarkers can track the progression of cognitive decline during the preclinical and prodromal phases of AD [54]. These studies also show that there are regional differences regarding how MRS metabolites correlated with AD CSF biomarkers which might be related with regional progression of NFT and amyloid pathology.

5.2. mIns

A serial MRI/MRS study was conducted in cognitively unimpaired individuals for 7 years [57]. Seven years after the baseline measurements, CSF and ^{1}H MRS data were collected in subjects who were converted to MCI/AD, Parkinson's disease, and dementia with Lewy bodies. The study demonstrated that CSF Aβ42, CSF p-tau were not correlated with NAA/mIns ratio in this cohort. Voevodska et al. (2019) showed that there were no differences between NAA/mIns in posterior cingulate gyrus/precuneus region between CSF Aβ42 positive and Aβ42 negative participants (60 years or older) at baseline (visit 1) in a longitudinal design. However approximately 2.3 years (visit 2), and approximately 4 years (visit 3) after the baseline visit, a decrease in NAA/mIns in CSF Aβ42 positive compared to CSF Aβ42 negative participants was observed. The study reported that being CSF Aβ42 positive at visit 1 was associated with a decrease in NAA/mIns over time in all cohort (CSF Aβ42 positive and negative cases) (the model was adjusted for baseline age, sex, and *APOE* ε4 carriership). Furthermore, the study reported that a higher rate of change in the MCI CSF Aβ42 positive participants compared to MCI CSF Aβ42 negative participants [54]. Hone-Blanchet reported that mIns/tCr ratio in the medial frontal cortex was elevated in MCI (CSF Aβ positive) compared to cognitively unimpaired CSF Aβ negative and Aβ positive women [51]. In another study, an increase

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in plasma GFAP associated with elevated mIns/tCr in posterior cingulate gyrus/precuneus region in a cohort consist of cognitively unimpaired (Aβ negative and positive on PET with flutemetamol) and MCI (Aβ positive on PET) participants who were *APOE* ε4 carriers [50].

5.3. Cho

Voevodska et al. (2016) reported that Cho/Cr in participants with MCI (all CSF Aβ42 positive) was higher compared to cognitively unimpaired participants who were CSF Aβ42 negative. Voevodska et al. (2019) showed that there were no differences between Cho/Cr in posterior cingulate gyrus/precuneus region between CSF Aβ42 positive and CSF Aβ42 negative participants (60 years or older participants) at baseline (visit 1)[54]. However approximately 2.3 years (visit 2) after the baseline visit, an increase in Cho/tCr ratio in CSF Aβ42 positive compared to CSF Aβ42 negative participants was observed when the groups compared with each other at the same visit. There was no difference in Cho/Cr ratios among CSF Aβ42 positive and negative groups at visit 3 (approximately 4 years after the baseline)[54].

5.4. Glu

Matsuoka et al showed that there was a significant association between increased plasma NfL and a decreased Glu/tCr in the right dorsolateral prefrontal cortex of participants with AD dementia, and there was only a trend of association between elevated plasma NfL and decreased Glu/tCr in the posterior cingulate gyrus, but this correlation did not reach statistical significance [49].

5.5. GABA

Hone-Blanchet et al. demonstrated that GABA levels in the medial frontal cortex of participants with MCI were lower compared to cognitively unimpaired participants (CSF Aβ42 positive and Aβ42 negative) Blanchet et al., 2022). While older age was correlated with lower GABA levels in both cognitively unimpaired participants CSF Aβ42 positive and Aβ42 negative participants, CSF biomarkers (Aβ42, t-tau and p-tau) were not associated with GABA, and GABA/Cr levels.

6. Influence *APOE* ε4 Allele on ^{1}H MRS Metabolites

Carrying one or two copies of *APOE* ε4 allele elevates the risk factor for late-onset AD dementia. A recent study showed that almost all participants who were homozygotes for *e4* allele (ε4/ε4) exhibited AD pathology (A+T+N+)[48]. However, only few studies investigated whether *APOE* ε4 carrier status effect the metabolite levels or *e*4 carrier status modify the relationship between ^{1}H MRS metabolites and AD biomarkers. Some studies reported no effect of *APOE* ε4 carrier status on metabolite levels, and their association of with AD biomarkers (Aβ and tau load), but others reported that *APOE* ε4 allele carrier status effects the metabolite levels and/or the relationship between the metabolites and AD biomarkers [40,48,50,56,57,60–63,69,70].

No differences in metabolite ratios (NAA/Cr, mIns/Cr, NAA/mIns) were found across *APOE* genotype (i.e., *e4* carriers and non-carriers) within cognitively unimpaired control and patient (MCI+AD dementia) group [69]. Riese et al. reported no difference in GABA and Glx levels between *APOE* ε4 carriers and non-carriers in a cohort consisting of cognitively unimpaired individuals and those with amnestic MCI [63]. In another study, no difference in mIns/Cr between *APOE* ε4 carriers and non-carriers was found in a cohort which included both cognitively unimpaired participants and subjects with amnestic MCI [62]. In line with this study, Voevodska et al. reported that *APOE* ε4 allele carrier status did not affect the mIns/Cr levels (posterior cingulate gyrus) across cognitively unimpaired CSF Aβ42 positive, and CSF Aβ42 negative, MCI groups (CSF Aβ42 positive) [61]. Nedeslska et al. reported that *APOE* ε4 allele carrier status did not modify the relationship between MRS metabolites (NAA/mIns, mIns/Cr) and rate of Aβ deposition on serial PET [60]. A serial MRI/MRS study was conducted in cognitively unimpaired individuals for 7 years [57]. At baseline, the mIns/Cr ratio was elevated in subjects with two copies of the *APOE* ε4 allele compared to non-carriers. Additionally, the NAA/mIns ratio was significantly decreased in subjects who were

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homozygous for the *APOE* ε4 allele compared to those who were heterozygous for the *APOE* ε4 allele and non-carriers. However, the NAA/Cr ratio showed no significant difference between subjects with and without the *APOE* ε4 allele [57]. Suri et al. showed that there was no significant effect of three *APOE* groups (*e*3 carrier, *e*3 homozygotes, *e*4 carriers) or an interaction between *APOE* groups and age on the metabolite profile in the posterior cingulate gyrus in individuals who were younger (between 20-40 years old) and cognitively unimpaired older age cohort (between 60 and 85 years old) [56]. In a cohort composed of subjects without cognitive impairment and with MCI who were *APOE* ε4 allele carriers, no association between tau load on PET with mIns/tCr (posterior cingulate gyrus) were observed [50].

In postmortem perchloric acid brain extracts, an increase in mIns and GPC, a decrease in Glu and NAA was observed in AD brains with *APOE e3/e3* allele carriers status compared to normal control brains with APOE e3/e3 allele carrier status [70]. The study also reported differences between e3/e3 AD and e4/e4 AD brains. For example, NAA was lower, and GPC was higher in e4/e4 AD brains compared to e3/e3 AD brains. We reported NAA/Cr ratio of patients with AD dementia significantly correlated with *APOE* ε4 carrier status [40]. Riese et al. reported that NAA levels were lower in cohort of participants who were cognitively unimpaired participants and those with amnestic MCI, who had *APOE* ε4 allele compared to those without it [63]. A recent study compared metabolite ratios of cognitively unimpaired group who were carrying two copies of *APOE* ε4 allele (i.e. *APOE* ε4 homozygotes) with non-carriers [57]. The study reported a higher mIns/Cr in *APOE* ε4/ ε4 homozygotes compared to non ε4 carriers. Furthermore, a decrease in NAA/mIns ratio was reported in those with ε4/ ε4carriers compared with subjects with only one copy of ε4 allele. A recent study using voxel wise analysis demonstrated an association between elevated Aβ load on PET with increased mIns/tCr ratio (posterior cingulate gyrus) only in *APOE ε*4 allele carrier group (cognitively unimpaired + MCI) [50]. A recent study investigated the influence of *APOE ε*4 carrier status on the relationship between GABA and Glu (posterior cingulate gyrus) and Aβ load on PET. The study reported that elevated gray matter GABA and Glu was associated with higher Aβ load on PET with positive effect modification by *APOE ε*4 *allele* carrier status [48].

More research is needed to understand the impact of *APOE ε*4 on ^{1}H MRS metabolites. While some findings suggest significant alterations in certain metabolite ratios among *APOE ε*4 carriers, particularly homozygotes, further research is needed to clarify these relationships and their implications for understanding and diagnosing AD.

7. Future Directions

AD biomarker (PET, CSF, plasma) and MRS studies suggest that the association of ^{1}H MRS with Aβ and/or tau pathology may vary based on the AD stage and the topographical heterogeneity of the disease, with these associations being region-specific. For example, some studies observed an association between ^{1}H MRS metabolites, such as NAA, and Aβ load on PET in a cohort involving participants with prodromal and AD dementia stages of the disease. In contrast, the association between ^{1}H MRS metabolites (NAA/Cr and Glu/Cr) and tau load on PET was detected even at the preclinical stage in cognitively unimpaired participants. These findings indicate that correlations between AD biomarkers and ^{1}H MRS metabolites vary by region, which may be related to the spatial progressions of amyloid and tau pathologies. For example, amyloid pathology progresses from the neocortical regions to the limbic and subcortical regions, while tau pathology begins in transentorhinal cortex and spreads to the paralimbic and neocortical areas [116,117]. While considering the association between metabolic changes in the various brain regions with AD biomarkers (PET, CSF, plasma), it is crucial to consider the spatial and temporal dynamics of amyloid and tau pathologies to understand the underlying mechanisms of AD progression.

Various studies reported conflicting findings regarding the association of brain metabolites with Aβ/tau pathology. These differences can be partially attributed to variations in cohort characteristics, acquisition and quantification methodologies, disease stage and progression, and genetic factors. Variability in the characteristics populations studied, including differences in age, cognitive status (cognitively unimpaired, MCI, AD dementia), and genetic factors (e.g., *APOE* status and carrying one

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of two copies of ε4 allele), and race, can influence the outcomes. Some studies focused on cognitively unimpaired individuals, while others included participants with amnestic MCI or MCI and AD dementia or mix groups. There are also methodological variations between studies. Some studies used higher field MRS and other used relatively lower field MRS with varying acquisition protocols and regions of interest. Advanced techniques such as the sLASER protocol coupled with automated volume of interest prescription may provide increased sensitivity and specificity compared to other methods [118]. Variation in the disease staging might be a source of contrasting findings. The relationship between metabolites and AD biomarkers may not be as pronounced as in later stages, where significant neuronal loss and metabolic changes are more evident. Understanding these differences may help in interpreting the results and drawing more comprehensive conclusions about the underlying biochemical processes in AD.

Ultra-high field MR clinical systems (7T and higher) offer promising opportunities, including enhanced spectral resolution, improved signal-to-noise ratio, and reliable quantification of low-concentration metabolites like Glu, Glc, Gln, GSH, and GABA. Further research is required to fully understand and harness the clinical potential of ultra-high-field MRS in the AD continuum.

^{1}H MRS holds significant promise for monitoring disease progression, especially in clinical trials targeting early predementia pathology. Future studies should focus on evaluating the potential of ^{1}H MRS alongside plasma biomarkers in this setting, with careful consideration given to underrepresented racial and ethnic groups, as well as the role of biological sex as a variable [119].

There are ongoing affords to harmonize, standardize and optimize ^{1}H MRS methods for both single-center and multicenter studies [120–123]. Future MRS studies should consider the consensus recommendations from experts to facilitate multicenter studies and ensure reproducibility of results [121]. Recent advancements in ^{1}H MRS including the automated volume of interest prescription pipeline [118] which enables fast and automated voxel placement and eliminates the requirement of manual voxel placement, and enables higher inter- and intra-subject consistency of voxel placement, would enhance the clinical integration of MRS and enhance its use in clinical trials as an outcome measure [118]. Furthermore, future MRS studies can incorporate the advanced MRS protocols such as modified sLASER to overcome limitations of conventional MRS sequences such as chemical shift displacement errors at 3T and 7T [121].

Overall, integrating advancements in ^{1}H MRS with recent developments in AD biomarkers field offer a comprehensive approach to understand disease progression, and evaluating treatment strategies.

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