

## RESEARCH ARTICLE

# Gray matter gamma-hydroxy-butyric acid and glutamate reflect beta-amyloid burden at old age

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## Abstract

Gamma-hydroxy-butyric acid (GABA) and glutamate are neurotransmitters with essential importance for cognitive processing. Here, we investigate relationships between GABA, glutamate, and brain  $\beta$ -amyloid (A $\beta$ ) burden before clinical manifestation of Alzheimer's disease (AD). Thirty cognitively healthy adults (age  $69.9 \pm 6$  years) received high-resolution atlas-based <sup>1</sup>H-magnetic resonance spectroscopic imaging (MRSI) at ultra-high magnetic field strength of 7 Tesla for gray matter-specific assessment of GABA and glutamate. We assessed A $\beta$  burden with positron emission tomography and risk factors for AD. Higher gray matter GABA and glutamate related to higher A $\beta$ -burden ( $\beta = 0.60$ ,  $p < 0.05$ ;  $\beta = 0.64$ ,  $p < 0.02$ ), with positive effect modification by apolipoprotein-E-epsilon-4-allele (APOE4) ( $p = 0.01$ - $0.03$ ). GABA and glutamate negatively related to longitudinal change in verbal episodic memory performance ( $\beta = -0.48$ ;  $p = 0.02$ ;  $\beta = -0.50$ ;  $p = 0.01$ ). In vivo measures of GABA and glutamate reflect early AD pathology at old age, in an APOE4-dependent manner. GABA and glutamate may represent promising biomarkers and potential targets for early therapeutic intervention and prevention.

## KEYWORDS

7 Tesla, aging, Alzheimer, APOE4, beta-amyloid, biomarker, cognitive impairment, dementia, GABA, glutamate, magnetic resonance spectroscopic imaging, magnetic resonance spectroscopy, memory, positron emission tomography, prevention, synaptic dysfunction, synaptic metabolism

## Highlights

- Gray matter-specific metabolic imaging with high-resolution atlas-based MRSI at 7 Tesla.
- Higher GABA and glutamate relate to  $\beta$ -amyloid burden, in an APOE4-dependent manner.
- Gray matter GABA and glutamate identify older adults with high risk of future AD.
- GABA and glutamate might reflect altered synaptic and neuronal activity at early AD.

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## 1 | INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia.<sup>1</sup> Neuropathology of AD is complex and is characterized by progressive neuronal dysfunction associated with the accumulation of the proteins  $\beta$ -amyloid (A $\beta$ ) and tau in cerebral gray matter.<sup>2,3</sup> Positron emission tomography (PET), with tracers such as Pittsburgh compound-B, are established molecular neuroimaging method to assess individual regional A $\beta$  burden in clinical populations,<sup>4</sup> including preclinical stages of AD.<sup>5</sup> Sporadic AD has a significant genetic etiology,<sup>6</sup> and genetic risk in European populations is mainly driven by the  $\epsilon$ 4 allele of the apolipoprotein E gene (APOE4), which may accelerate A $\beta$  accumulation.<sup>7</sup> A decade-long preclinical phase characterizes AD, during which neuropathological brain changes such as A $\beta$  accumulation take place, but affected individuals remain cognitively unimpaired.<sup>2</sup> The first clinical manifestation of AD is typically represented by gradual decline of verbal episodic memory functioning,<sup>8</sup> and gradual decline of episodic memory has been shown to indicate increased risk for AD in cognitively normal old-aged adults.<sup>9</sup> However, the pathophysiology of progressive deterioration from preclinical AD to overt cognitive impairment remains only incompletely understood.

Synaptic dysfunction represents a critical step in the progression of neurodegenerative brain damage in AD.<sup>10,11</sup> Consistently, changes of neuronal activity involving the major central nervous system neurotransmitters gamma-hydroxy-butyric acid (GABA) and glutamate (Glu) have been reported earlier in AD.<sup>12–15</sup> A concatenation of data from basic and *post mortem* studies suggests significant interactions between GABA and Glu activity with AD pathology,<sup>13,14,16</sup> with neuronal hyperactivity, peri-synaptic accumulation of glutamate, and enhanced GABAergic sprouting related to A $\beta$ .<sup>17–19</sup> There is an association between synaptic dysfunction and accumulation of tau, at this point however, their chronological sequence is not yet well understood.<sup>3,11</sup> Magnetic resonance spectroscopy (MRS) is a non-invasive method that allows measuring brain levels of GABA and Glu in living humans. Previous MRS studies showed decreased levels of GABA and/or Glu in patients diagnosed with mild cognitive impairment<sup>20–23</sup> or dementia due to AD,<sup>24,25</sup> thereby confirming *post mortem* evidence on reduced GABA and Glu in symptomatic stages of AD.<sup>26,27</sup> However, conventional MRS studies are limited by low spatial resolution, which makes investigations of GABA and Glu in the context of AD pathology difficult.

Magnetic resonance spectroscopic imaging (MRSI) at ultra-high magnetic field strength of 7 Tesla is a promising method to investigate gray and white matter-specific variation of the neurotransmitters GABA and Glu.<sup>28,29</sup> Thus, to better understand AD pathology-related metabolic changes, the current study investigates gray matter levels of GABA and Glu quantified with 7 Tesla MRSI in relation to A $\beta$  burden measured with PET. To support relevance for early disease stages, we performed this study in cognitively unimpaired old-aged adults, and assessed APOE4 carrier-status and longitudinal verbal episodic memory decline, as indicators of individual risk for AD. We hypothesized that A $\beta$  burden and risk factors for AD would be associated with higher GABA and Glu levels, in analogy to studies on increased A $\beta$ -related synaptic and neuronal activity during preclinical AD.<sup>12,15</sup>

### RESEARCH IN CONTEXT

- **Systematic review:** To our knowledge, the present study is the first to investigate changes of gray matter gamma-hydroxy-butyric acid (GABA) and glutamate in a preclinical population at risk for Alzheimer's disease (AD). We used state-of-the-art neuroimaging with tissue-specific atlas-based magnetic resonance spectroscopic imaging (MRSI) at ultra-high field strength of 7 Tesla, to quantify gray matter GABA and glutamate, and positron emission tomography (PET) for  $\beta$ -amyloid (A $\beta$ ) burden.
- **Interpretation:** Our data suggest an association between gray matter levels of GABA and glutamate with A $\beta$  burden, episodic memory change, and genetic risk for AD reflected by apolipoprotein E4 (APOE4) carrier-status. As these findings were observable in old-aged persons without significant cognitive impairment, GABA and glutamate might reflect changes of synaptic metabolism or neuronal activity at early stages of AD.
- **Future directions:** Longitudinal studies are needed to better understand the role of GABAergic and glutamatergic metabolism in early AD, and its potential as biomarker and therapeutic target for early intervention and prevention.

## 2 | METHODS

### 2.1 | Participants and ethics

The study was approved by the local cantonal ethics committee of canton Zurich, Switzerland, and conducted in accordance with the declaration of Helsinki. The study sample included 30 cognitively healthy participants, who gave written informed consent and were recruited from ongoing cohort studies at the Hospital for Psychogeriatric Medicine, University of Zurich, Switzerland.<sup>30</sup> Participants underwent a clinical work-up including medical history, blood sampling, APOE4 genotyping, medical, psychiatric, and neurological examination. Inclusion criteria were age 55–80 years, Mini-Mental State Examination (MMSE) score  $\geq 27/30$ , absence of depression as indicated by the Geriatric Depression Scale,<sup>31</sup> available data for gray matter levels of GABA and Glu, and Verbal Learning and Memory Test (VLMT) performance at two time points. Exclusion criteria included contraindications to MRI, severe medical illness, or any conditions possibly affecting cognition, for example, drug abuse, neurological or mental conditions.

### 2.2 | Cognitive testing

After inclusion, we assured normal cognition by administering a neurocognitive test battery at baseline, which was repeated at follow-up. For the current study, the longest available follow-up interval was selected ( $3.15 \pm 2.16$  mean  $\pm$  SD years). We used the German version

of the VLMT,<sup>32</sup> because verbal episodic memory performance is typically most sensitive to AD-related cognitive decline.<sup>8</sup> To capture the earliest possible variations in verbal episodic memory performance among cognitively normal participants, we chose to specifically analyze immediate recall performance at the VLMT, as this task may deteriorate prior to other tasks of verbal episodic memory testing.<sup>33</sup> Annual change in VLMT performance was calculated as follows: [performance at follow-up – performance at baseline]/years between baseline and follow-up.

### 2.3 | PiB-PET acquisition and preprocessing

Our approach for analyzing PiB-PET has been previously described in detail.<sup>34</sup> Briefly, we intravenously injected 350 MBq of <sup>11</sup>C-PiB and collected dynamic PET data on a GE Discovery PET/CT scanner at the University Hospital of Zurich, Division of Nuclear Medicine. We semi-automatically calculated cortical PiB retention using PMOD PNEURO tool, version 3.4 (PMOD Ltd, Zurich, Switzerland). For each voxel, standardized uptake value ratios (SUVR) were computed (matrix dimensions: 128 × 128 × 47, voxel size: 2.34 × 2.34 × 3.27 mm) using late frame signals (50–70 min) of the time-activity-curve and the cerebellar gray matter as reference (Figure 1A). For the present study, we calculated regional mean PiB-SUVR in the PCP gray matter using in-house written code and SPM12, as previously described.<sup>35</sup> In brief, the preprocessed PET image was realigned to structural MRI and then multiplied with the customized binary ROIs of PCP gray matter. We inspected all images to assure accurate alignments. For purposes of illustration, we divided our sample in participants with relatively high versus low local Aβ burden using the median PiB-SUVR in the PCP as cutoff (PiB-SUVR = 1.26), which corresponds with our previously derived cutoff for Aβ-positivity.<sup>30</sup>

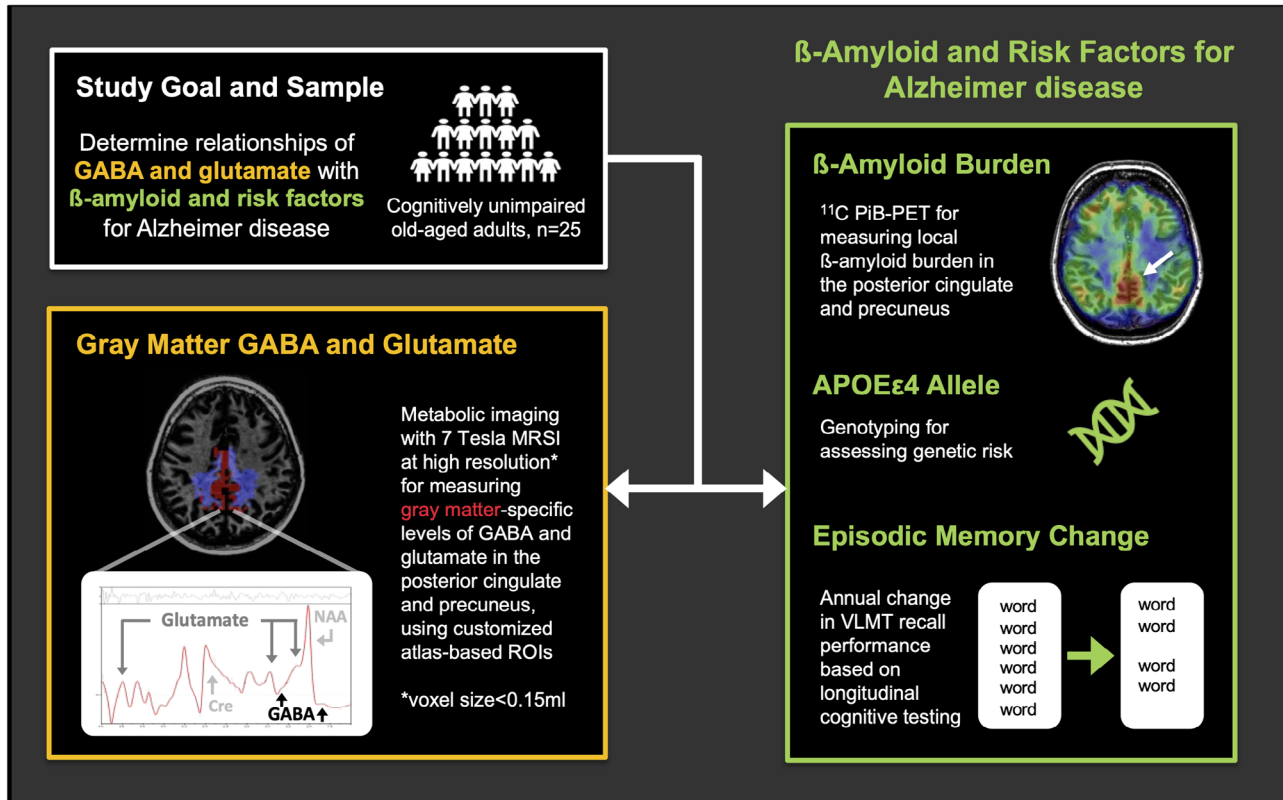
### 2.4 | MRI and MRSI scanning

Detailed descriptions of MRI and MRSI acquisition have been previously published.<sup>35–37</sup> In brief, all participants were scanned on a Philips Achieva 7T scanner (Philips Healthcare, Best, The Netherlands) equipped with a quadrature transmit head coil and a 32-channel receive coil (Nova Medical, Wilmington, MA), located at the Institute for Biomedical Engineering, ETH and University of Zurich, Switzerland. We collected two structural images including high-resolution T1-weighted three-dimensional magnetization prepared rapid gradient echo (MPRAGE) (TE = 3.74 ms; TR = 8.22 ms; scan mode = 3D; resolution [x, y, z] = 0.8 × 0.8 × 0.9 mm; scan time = 10:54 min), which we used for further post-processing (tissue segmentation; definition of ROIs) and localization of the MRSI volume-of-interest, and a three-dimensional FLAIR sequence (TE = 310.74 ms; TR = 8000 ms; resolution [x, y, z] = 0.43 × 0.43 × 2 mm; scan time = 7:00 min), which we added for multi-contrast segmentation. We also acquired MRSI (acquisition delay = 2.5 ms; TR = 644 ms; scan time = 29:22 min), comparable to “free-induction-decay-localized-by-outer-volume-suppression”

(FIDLOVS) MRSI<sup>28</sup> but without suppression of outer volume or fat,<sup>38</sup> using water suppression similar to VAPOR,<sup>39</sup> and dedicated active B0 shimming.<sup>40</sup> To allow selecting voxels in gray or white matter subregions of the PCP after post-processing, a very high spatial resolution was chosen (voxel size < 0.15 mL; in-plane resolution = 3.5 × 3.5 mm<sup>2</sup>, field of view = 200 × 168 mm<sup>2</sup> with 57 × 48 grid; slice thickness = 12 mm). The MRSI slab was manually placed above the lateral ventricles and tilted to cover the PCP (see Figure 1A). Prior to MRSI acquisition, we also collected a structural MRI (T1-weighted turbo spin-echo image; TE = 2.9 ms; TR = 5.7 ms; resolution [x, y, z] = 0.9 × 0.9 × 1.5 mm; scan time = 28.2 seconds) with the identical field of view as the MRSI slab, which we used for exact dimensional reference of the MRSI during post-processing. All MR images were visually inspected by a trained neurologist experienced in neuroimaging (P.G.U.) for presence ischemic lesions, tumors, or hemorrhage.

### 2.5 | Post-processing and analysis of MRSI data

The MRSI data were processed as described in earlier publications.<sup>35,36</sup> MRSI raw data were read into MATLAB using MRRecon (Gyrotools Ltd, Winterthur, Switzerland). Before summing up spectra from individual MRSI voxels across the anatomical gray and white matter ROIs, the following processing steps have been performed: (1) over-discrete MRSI reconstruction including frequency offset correction based on B0 maps as well as channel combination and phase correction based on complex sensitivity maps acquired with the vendor provided SENSE reference scan protocol<sup>41</sup>; (2) Hamming filtering in both spatial directions; (3) off-center alignment between MRSI and MRI data caused by different spatial resolutions, which lead to slightly different half-voxel-shifts; (4) HLSVD water filtering; and (5) truncation of the FIDs after 512 of 2048 data points. We then calculated customized tissue-specific atlas-based ROIs of the PCP within the MRSI slab by intersecting the MRSI slab, tissue probability maps derived from SPM12,<sup>42</sup> and an anatomical template of the PCP defined by a reference atlas<sup>43</sup> (Figure 1A). Next, we averaged MRSI spectra from voxels within PCP gray or white matter, and then estimated tissue-specific levels of brain metabolites, including GABA and glutamate using LC model.<sup>44</sup> The LC Model basis set was simulated using the GAMMA library<sup>45</sup> and comprised the following 18 metabolites: NAA, ml, GABA, creatine (Cre), glycerophosphoryl-choline (GPC), phosphoryl-choline (PCh), glutamate (Glu), glutamine (Gln), glycine (Glc), ascorbate (Asc), ml, aspartate, N-acetylaspartateglutamate (NAAG), glutathione (GSH), scyllo-inositol (Scyllo), and taurin (Tau). Phosphorylethanolamine (PE) was omitted from the basis set used for spectral fitting. Simulated macromolecules were considered during the fitting<sup>28,46</sup> and the default setting for the stiffness of the spline baseline was chosen. tCho and Glx represent compound measures of GPC and PCh, and Glu and Gln, respectively. As demonstrated in the original FIDLOVS <sup>1</sup>H-MRSI investigation, Glu and GABA fit results do not show a strong correlation at 7T, and the respective spectral signatures are generally well separated from one another.<sup>28</sup> Cre-levels were used as an internal reference by normalizing all other metabolites to Cr as suggested earlier.<sup>47,48</sup> The resulting



**FIGURE 1** Overview of study procedures. This study investigated relationships of gray matter gamma-hydroxy-butyric acid (GABA) and glutamate (Glu) with pathological, genetic, and cognitive markers of early Alzheimer's disease (AD) in cognitively normal old-aged adults. Left lower box: Gray matter levels of GABA and Glu were measured with magnetic resonance spectroscopic imaging (MRSI) at 7 Tesla, which enables tissue-specific atlas-based metabolic mapping of the posterior cingulate and precuneus (PCP) as previously described<sup>31</sup>: Customized regions of interest (ROIs) cover the gray and white matter of the PCP region; these ROIs are generated by intersecting (1) tissue probability maps, (2) a template of the PCP region defined by an anatomical reference atlas, and (3) the MRSI slab. The axial brain image shows exemplary customized ROIs with voxels in PCP gray and white matter (red = gray matter; blue = white matter). The image below displays an exemplary spectrum, averaged across all voxels in the PCP gray matter (NAA = N-acetylaspartate, Cre = creatine). Right box: We measured local  $\beta$ -amyloid ( $A\beta$ ) burden in vivo with  $^{11}\text{C}$ -PiB-PET. Note the high  $A\beta$  burden in the PCP region on the exemplary  $^{11}\text{C}$ -PiB-PET image. Further, we assessed established risk factors for AD, such as the apolipoprotein E4 (APOE4) allele, and longitudinal change in verbal episodic memory performance.

normalized metabolite-to-creatine ratios were used for subsequent statistical analyses for group differences.

Spectra were visually inspected for major artifacts including lipid contamination and insufficient water suppression leading to gradient modulation sidebands, ghosting artifacts or substantial baseline distortions, and excluded if these artifacts hampered the quantification of the metabolites of interest. In addition, spectra with broad linewidth (full width at half-maximum, FWHM)  $> 1.31$  ppm, signal-to-noise ratio (SNR)  $< 4$ , or Cramér-Rao lower bounds (CRLB)  $> \text{sample mean} \pm 1\text{SD}$  as calculated by LC Model, and substantial structured residuals were excluded. We used a sample-specific CRLB cutoff derived by using the sample mean  $\pm 1\text{SD}$  CRLB calculated after excluding those with CRLB of 999%. These criteria have been chosen in alignment with recent recommendations of the MRS expert's consensus initiative, to avoid bias by underestimating changes in metabolites with low concentrations.<sup>49</sup> We only included participants with sufficient quality for both GABA and Glu. Structured information according to the Minimum Reporting Standards for in vivo MRS are attached as supplement.<sup>50</sup> Given our experience from earlier studies showing that gray matter metabolites

are more informative in a context of aging and AD, we a priori focused on gray matter levels of GABA and Glu.<sup>35-37</sup>

## 2.6 | Statistical analysis

We used multivariate linear regression to investigate whether gray matter GABA and Glu were associated with  $A\beta$  burden, APOE4, the interaction of APOE4 and  $A\beta$  burden, or changes in VLMT performance. To test our main hypothesis, we used gray matter GABA or Glu as dependent variable, and first entered  $A\beta$  burden and APOE4 as independent variables, followed by their interaction term ( $A\beta \times \text{APOE4}$ ). In an exploratory analysis, we tested whether gray matter GABA and Glu were associated with changes in VLMT performance, and whether  $A\beta$  burden or APOE4 influenced the relationships of GABA and Glu with VLMT performance: We first entered GABA or Glu, followed by  $A\beta$  burden and APOE4 (independent variables), to predict longitudinal change in VLMT performance (dependent variable).

We conducted a control analysis, to infer about specificity of GABA and Glu, and exclude potential bias due to alterations of Cre levels that are used as a reference (GABA/Cre, Glu/Cre). In this control analysis, we repeated the above-mentioned linear regression models with another metabolite (NAA/Cre) instead of GABA and Glu. All models were adjusted for age, sex, and education. To avoid collinearity, independent variables were mean-centered for the model that included the interaction term. Plotting and inspecting of residuals assured that residuals were normally distributed, and data were log-transformed if necessary. We corrected for multiple testing using the false-discovery-rate method at  $\alpha < 0.05$ . We report FDR-corrected  $p$ -values, adjusted coefficients of determination ( $R^2$ ), standardized beta coefficients ( $\beta$ ), and F change, which indicates if adding variables to the model improves its predictive power.

Given the interest in biomarker for early detection of subjects at risk for future cognitive decline due to AD, we also explored if gray matter GABA or Glu could classify subjects at risk, using the percurve function in MATLAB. For this classification, we used a risk profile of future AD. We defined this risk profile by A $\beta$ -related memory decline indicated by (1) absent improvement, that is, no or negative annual change between the first and second VLMT assessment, and (2) relatively high A $\beta$  burden of PiB-SUVR  $> 1.26$  in the PCP (see the PET imaging methods section). We chose to use this rather liberal definition of A $\beta$ -related cognitive decline because of the relatively high cognitive performance level, with many participants showing improvement in cognitive test performance at follow-up due to learning effects, and overall minor A $\beta$  burden in our sample.

### 3 | RESULTS

Five participants were excluded from the analysis owing to incomplete neuropsychological data ( $n = 1$ ) or insufficient MRSI quality ( $n = 4$ ). After excluding two subjects with CRLB of 999% for GABA, relative CRLB was  $16.4 \pm 13.5$  (range 5-70) % for GABA and  $4.4 \pm 2.7$  (range 2-15) % for Glu, resulting in a study-specific cutoff of  $\text{CRLB} \geq 29.52\%$  (mean  $\pm 1\text{SD}$  CRLB for GABA); linewidth was  $0.45 \pm 1.87$  FWHM and SNR  $14.41 \pm 4.88$ . After exclusion of two more subjects due to insufficient quality, remaining MRSI spectra showed relative CRLB of  $12.8 \pm 6.5$  (range 5-27) % for GABA and of  $4.3 \pm 2.9$  (range 2-15) % for Glu; linewidth was  $0.48 \pm 1.94$  FWHM and SNR  $14.48 \pm 4.82$ , in 25 finally included participants (Table 1).

#### 3.1 | Gray matter GABA and Glu relate to A $\beta$ burden, in an APOE4-dependent manner

In the main analysis, we investigated relationships of gray matter GABA and Glu with A $\beta$  burden. We found that higher gray matter levels of GABA were associated with higher A $\beta$  burden ( $\beta = 0.53$ ;  $p = 0.032$ ;  $R^2 = 0.27$ ). This association was driven by the presence of APOE4. That is, entering the interaction term A $\beta$ \*APOE4 significantly improved the predictive power of the model (F change,  $p = 0.03$ ) and showed a

**TABLE 1** Subject characteristics, neuroimaging, and cognitive features.

Parameter	Value
Sample size $n$ (females)	25 (14)
Age (years)	$69.92 \pm 5.95$
Education (years)	$15.92 \pm 2.52$
APOE4 carrier $n$ (%)	8 (27)
Cortical PiB-SUVR	$1.26 \pm 0.37$
Local PiB-SUVR in PCP	$1.36 \pm 0.31$
Follow-up time (years)	$3.15 \pm 2.16$
<b>7T MRSI metabolites</b>	
Gray matter GABA/Cre	$0.53 \pm 0.61$
Gray matter GABA CRB (%)	$12.80 \pm 6.48$
Gray matter Glu/Cre	$1.38 \pm 0.65$
Gray matter Glu CRB (%)	$4.25 \pm 2.88$
<b>Cognition at baseline</b>	
Mini-Mental State Examination	$29.40 \pm 1.00$
Boston Naming Test	$14.60 \pm 0.71$
Digit Span Forward	$6.60 \pm 1.41$
Digit Span Backward	$6.20 \pm 1.55$
Trail Making Test A/B	$2.41 \pm 0.65$
WMS visual pair learning	$15.76 \pm 2.39$
WMS visual pairs recall	$5.56 \pm 1.26$
VLMT immediate recall	$10.20 \pm 2.63$
VLMT delayed recall	$9.72 \pm 3.12$
VLMT supported recall	$11.52 \pm 3.27$
<b>Cognition at follow-up</b>	
Mini Mental State Examination	$29.28 \pm 1.02$
Boston Naming Test	$14.76 \pm 0.44$
Digit Span Forward	$7.08 \pm 1.63$
Digit Span Backward	$6.72 \pm 1.72$
Trail Making Test A/B	$2.40 \pm 0.68$
WMS visual pair learning	$15.60 \pm 2.99$
WMS visual pairs recall	$5.44 \pm 1.26$
VLMT immediate recall	$11.20 \pm 3.30$
VLMT delayed recall	$10.96 \pm 3.92$
VLMT supported recall	$11.76 \pm 4.15$
<b>Annual cognitive change</b>	
Mini Mental State Examination change/y	$0.01 \pm 0.54$
Boston Naming Test change/y	$0.05 \pm 0.33$
Digit Span Forward change/y	$0.12 \pm 0.82$
Digit Span Backward change/y	$0.32 \pm 0.79$
Trail Making Test A/B change/y	$-0.03 \pm 0.33$
WMS visual pair learning change/y	$0.00 \pm 1.16$
WMS visual pairs recall change/y	$-0.02 \pm 0.58$
VLMT immediate recall change/y	$0.32 \pm 1.04$

(Continues)



**TABLE 1** (Continued)

Parameter	Value
VLMT delayed recall change/y	0.53 ± 1.69
VLMT supported recall change/y	0.09 ± 1.33

Note: Table shows mean ± SD if not indicated otherwise.

Abbreviations: GABA, gamma-hydroxy-butyric acid; PiB-SUVR, <sup>11</sup>C-Pittsburgh compound-B standard uptake value ratio; VLMT, Verbal Learning and Memory Test; WMS, Wechsler Memory Scale.

synergistic association of APOE4 and Aβ burden with gray matter GABA (Aβ\*APOE4 vs. GABA:  $\beta = 0.60$ ;  $p = 0.045$ ; adjusted  $R^2 = 0.41$ ; Figure 2A). Similarly, we observed that higher levels of gray matter Glu were associated with higher Aβ burden ( $\beta = 0.63$ ;  $p = 0.018$ ; adjusted  $R^2 = 0.41$ ), even more when APOE4 was present, which interacted with Aβ burden on Glu (Aβ\*APOE4 vs. Glu:  $\beta = 0.59$ ;  $p = 0.024$ ;  $R^2 = 0.59$ ; F change,  $p = 0.01$ ; Figure 2B).

### 3.2 | Gray matter GABA and Glu relate to change in VLMT performance

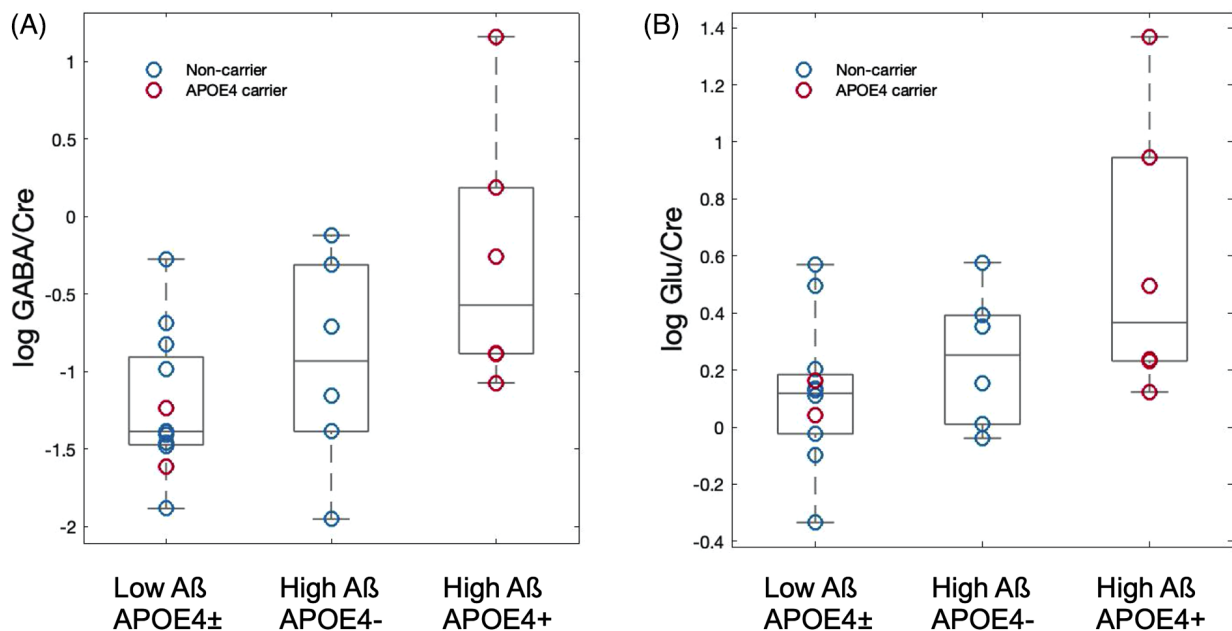
In an exploratory analysis, we tested whether GABA or Glu were associated with longitudinal change in VLMT performance, and whether Aβ burden or APOE4 influenced the relationships of GABA and Glu with VLMT change. We found that higher gray matter GABA and Glu were

negatively associated with VLMT change (GABA:  $\beta = -0.48$ ;  $p = 0.023$ ;  $R^2 = 0.20$ , Figure 2; Glu:  $\beta = -0.50$ ;  $p = 0.020$ ;  $R^2 = 0.22$ ), suggesting that higher levels of GABA and Glu relate to subtle decline in verbal episodic memory performance (Figure 3). Iteratively entering Aβ burden and APOE4 into the model did not attenuate the associations of GABA and Glu with VLMT change. After adjusting for age, sex, and education, however, the associations of GABA and Glu with VLMT change remained at statistical trend level only (GABA:  $p = 0.068$ ; Glu:  $p = 0.058$ ), although none of these covariates was associated with VLMT change (results not shown).

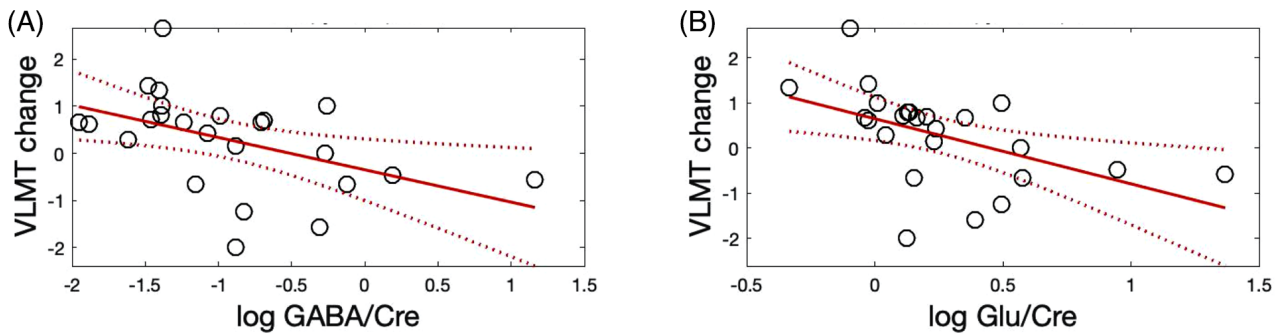
In a control experiment, we repeated the above-mentioned linear regression analyses using gray matter levels of NAA instead of GABA and Glu, to exclude potential bias due to the common reference Cre used for GABA, Glu (GABA/Cre and Glu/Cre), and other metabolites (e.g., NAA/Cre). Gray matter NAA was not associated with Aβ burden or APOE4 (results not shown), suggesting that the associations of GABA and Glu with Aβ burden observed in the main analysis were specific for GABA and Glu and were not confounded by potential changes in Cre.

### 3.3 | Gray matter GABA and Glu identify subjects at increased risk of incipient AD

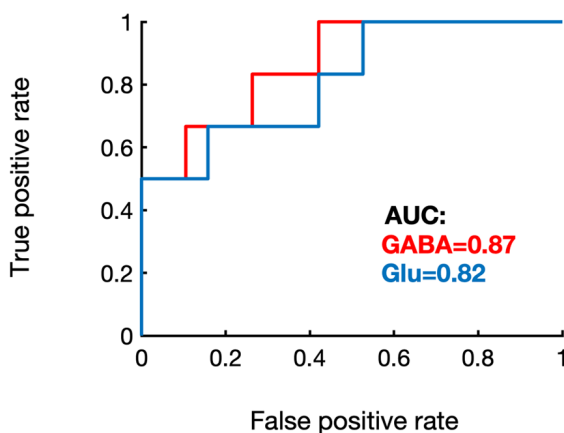
Given the observed associations of GABA and Glu with Aβ burden and APOE4, we investigated properties of GABA and Glu for early risk stratification of AD among old-aged individuals. Specifically, we tested



**FIGURE 2** Associations of gray matter gamma-hydroxy-butyric acid (GABA) and glutamate (Glu) with  $\beta$ -amyloid (Aβ) burden and apolipoprotein E4 (APOE4). Gray matter levels of (A) GABA and (B) Glu in the posterior cingulate and precuneus (PCP) are plotted as a function of high/low Aβ burden and presence/absence ( $\pm$ ) of the APOE4 allele. Higher GABA and Glu were associated with higher Aβ burden (GABA:  $\beta = 0.60$ ,  $p < 0.05$ ; Glu:  $\beta = 0.64$ ,  $p < 0.02$ ), especially when APOE4 was present. For illustration purpose, high Aβ burden was defined using the median <sup>11</sup>C-Pittsburgh compound-B standard uptake value ratio (PiB-SUVR) in the PCP gray matter as cutoff. Note that there are two subjects overlaid at the second lowest data point in the group on the right side in (B).



**FIGURE 3** Gray matter gamma-hydroxy-butyric acid (GABA) and glutamate (Glu) relate to longitudinal episodic memory performance. Higher levels of gray matter (A) GABA and (B) Glu in the posterior cingulate and precuneus (PCP) were negatively associated with longitudinal dynamics of episodic memory performance, defined by annual changes in performance at the Verbal Learning and Memory Test (VLMT), in cognitively normal old-aged humans (GABA:  $\beta = -0.48$ ,  $p = 0.023$ , adjusted  $R^2 = 0.20$ ; Glu:  $\beta = -0.50$ ,  $p = 0.020$ ,  $R^2 = 0.22$ ).



**FIGURE 4** Gray matter gamma-hydroxy-butyric acid (GABA) and glutamate (Glu) identify old-aged adults at high risk of Alzheimer's disease (AD). Gray matter GABA and Glu classified old-aged adults with a high risk of incipient AD, determined by A $\beta$ -related episodic memory decline, with good accuracy. AUC = area under the curve.

if GABA and Glu identified individuals with increased risk of AD as indicated by A $\beta$ -related memory decline. Gray matter GABA classified individuals with increased risk of AD with good accuracy (AUC = 0.87; Figure 4). The classification performance of Glu was less accurate but still good (AUC = 0.82; Figure 4). Classification performances improved after adding age, sex, and education as additional predictors (AUC for GABA = 0.97; AUC for Glu = 0.94).

## 4 | DISCUSSION

We tested the hypothesis that gray matter levels of GABA and Glu, measured in vivo with 7T MRSI, relate to imaging-defined AD pathology and established risk factors of AD at old age. Indeed, higher gray matter GABA and Glu were associated with higher A $\beta$  burden and conformingly APOE4 and subtle episodic memory decline. Relevance for early therapeutic intervention and prevention is supported by the

fact that these associations were observed in cognitively unimpaired old-aged adults.

In contrast, previous studies using 3T MRS could not detect associations of GABA or Glu (or Glx, a compound of Glu and glutamine), measured in the PCP or frontal cortex, with A $\beta$  burden<sup>20,23,51</sup> or APOE4<sup>20,52,53</sup> in old-aged adults with unimpaired or mildly impaired cognition. There are several possible methodological explanations for why our current approach using MRSI at ultra-high field strength of 7T was able to detect these associations of GABA and Glu with A $\beta$  load and risk factors for AD. First, we considered effects of both A $\beta$  burden and APOE4, which may facilitate the detection of more subtle effects that could be less evident when either A $\beta$  burden or APOE4 are considered alone. The previous studies, however, either focused on APOE4 but did not measure A $\beta$  burden,<sup>52,53</sup> or did not explore possible synergistic effects of A $\beta$  burden and APOE4.<sup>20,23,51</sup> Moreover, Hone-Blanchet et al. (2022) used cerebrospinal fluid biomarkers of A $\beta$  pathology,<sup>23</sup> whereas we and the studies by Riese et al. (2015) and by Kara et al. (2022) exploited the potential of PET to assess local A $\beta$  burden in critical brain regions.<sup>20,35,37,51</sup> This latter approach of combining PET with MRS/MRSI may optimize sensitivity to detect regional effects, by acquiring neuropathological (i.e., A $\beta$ , tau) and metabolic (e.g., GABA, Glu) readouts from the same volume-of-interest, ideally regions with early AD-related brain changes, such as the PCP.<sup>12</sup> Second, we used atlas-based tissue-specific high-resolution MRSI at ultra-high magnetic field strength of 7T, whereas the previous studies used MRS at 3T with manual placement of a single-voxel in the PCP region.<sup>20,51-53</sup> Since AD pathology-related changes of synaptic metabolism and neuronal activity primarily pertain to the gray matter, the ability to differentiate between signals from gray and white matter (as possible with 7T MRSI) is crucial in order to be able to detect subtle variation.<sup>54,55</sup> Moreover, MRS at ultra-high field strength of 7T yields more precise metabolite measures compared to 3T owing to higher signal-to-noise ratio and increased spectral and spatial resolution.<sup>56</sup> Accordingly, our approach leverages these benefits of MRSI at 7T to measure GABA and Glu in PCP gray and white matter separately,<sup>36</sup> a major advantage over conventional single-voxel MRS. Specifically, our previously developed approach collects multi-voxel

spectral information from PCP gray or white matter at very high spatial resolution (voxel size < 0.15 mL), whereas MRS assesses a single-voxel at lower spatial resolution (typical voxel size = 8 mL), which contains gray matter, white matter, and cerebrospinal fluid. Importantly, however, levels of GABA and Glu show tissue-specific variations, with higher levels in gray compared to white matter, across cortical regions,<sup>28,29,57,58</sup> including the PCP.<sup>37</sup> These tissue-specific differences are unlikely to be captured by single-voxel MRS but are relevant, as we found that metabolite levels relate to cognition and other imaging markers in a tissue-specific manner, with gray matter levels being more informative than white matter levels in a context of aging and neurodegeneration.<sup>35–37</sup> Moreover, we have implemented an atlas-based approach that enables metabolic mapping of a distinct brain region precisely defined by an anatomical reference atlas.<sup>35–37</sup> Atlas-based MRSI approaches may yield optimized measures of GABA compared to manual single-voxel approaches.<sup>59</sup> Therefore, our study and the previous studies do not sample the same anatomical region, and the sensitivity to detect AD-related metabolic alterations could be higher in our study owing to higher anatomical precision. Nevertheless, measurements of GABA in our study were based on improved spectral resolution at 7T and not spectral editing, a commonly used method. The lack of spectral editing is a limitation of our study, as it introduces the possibility of some overlap between spectral peaks of GABA with macromolecules and other metabolites. Our finding of increased GABA should therefore be interpreted with caution and needs replication.

Heterogeneity in methods for MRS/MRSI processing should also be considered when discussing discrepant findings. While rather rigid measures for quality control of MRS/MRSI spectra, namely a CRLB cutoff < 20%, have been used over many years, recent consensus guidelines recommend against this practice and promote more liberal, tailored approaches, given that a strict CRLB cutoff may introduce bias for metabolites with low concentrations, such as GABA.<sup>49,60,61</sup> These differences in practice of quality control in recent years may affect the comparison of current and future studies with older studies. Moreover, there is variability in the usage of absolute metabolite concentrations or metabolite ratios. While normalizing metabolites of interest to creatine measured within the same voxel has been performed in most studies,<sup>21,24,25,51,52</sup> including our present and previous studies,<sup>35–37</sup> some studies normalize to water<sup>20,53</sup> or use absolute metabolite concentrations.<sup>23</sup> Future studies should assess if the choices of quality control, especially CRLB cutoffs, or reference metabolites may influence the relationship of AD biomarkers or clinical outcomes with MRS/MRSI metabolites, especially metabolites with low concentrations such as GABA.

Our findings add to a growing body of evidence, mainly from basic research, supporting a role of GABAergic and glutamatergic alterations as potential biomarkers, mechanisms, and therapeutic targets in early AD.<sup>13–18</sup> For example, A $\beta$ -related hyperactivity has been linked with peri-synaptic glutamate accumulation<sup>19</sup> and enhanced GABAergic sprouting.<sup>17</sup> Hence, our observations may support recent studies on synaptic dysfunction as an important pathophysiological event in the progression of AD, which may directly promote propa-

gation of pathological tau.<sup>10,11,62</sup> Further, our findings resonate with clinical neuroimaging studies linking neuronal hyperactivity at the network level to risk and biomarkers of AD in old-aged adults.<sup>12,15</sup> Interestingly, a combined functional MRI-MRS study showed that GABA and Glu in the PCP explained half of the variance of functional connectivity in networks strongly implicated with AD.<sup>63</sup> Noteworthy, we previously showed that gray matter GABA and Glu in the PCP moderate A $\beta$ -related alterations in functional connectivity at old age, suggesting a link between GABA and Glu with A $\beta$ -related network alterations prior to clinical onset of AD.<sup>37</sup> Thus, we speculate that the associations of higher GABA and Glu with A $\beta$  burden and risk factors for AD in the present study, could be features of increased brain activity at the borderland of aging and preclinical AD.

A key question is whether the A $\beta$ - and APOE4-related increases in GABA and Glu levels observed in our study reflect detrimental or compensatory metabolic changes related to brain reserve,<sup>64</sup> although these two are not mutually exclusive. It is impossible to answer this question based on our data, and the meaning of GABAergic and glutamatergic alterations could possibly change as a function of time and stage along the AD continuum. We found that higher gray matter levels of GABA and Glu were negatively associated with longitudinal changes in VLMT performance in formally cognitively unimpaired old-aged adults, but this association was independent of A $\beta$  burden and APOE4. Therefore, higher levels of GABA and Glu could reflect either compensation despite AD risk, or decompensation due to incipient AD, but also non-AD age-related cognitive decline. The fact that both glutamate (as an excitatory neurotransmitter) and GABA (as an inhibitory neurotransmitter) are upregulated, might seem counterintuitive at first. Here, we think further studies are needed to answer the question whether GABA upregulation may be a (futile) attempt to counteract neuronal hyperactivity – which is a key feature of early stages of AD.<sup>17,65</sup>

Non-invasive biomarkers for early detection of cognitive decline due to AD are paramount, given the recent approval of disease-modifying therapies for early AD. In our study, gray matter GABA, and to a lesser extent also Glu, showed good accuracy for detecting subjects with a high risk profile of AD, suggesting that gray matter GABA and Glu are potential biomarkers of incipient AD and may serve for risk stratification. Moreover, gray matter GABA and Glu could be used as outcome parameters in interventional trials modulating GABAergic or glutamatergic activity, especially in the context of neurodegenerative diseases, such as AD.

A major limitation of our study is the small sample size. We therefore consider our study as preliminary, and our findings should be interpreted with caution. Larger studies including more participants and longer follow-up periods that also include repeated measures of biomarkers are needed to clarify the trajectory and exact meaning of GABAergic and glutamatergic alterations measured with MRS/MRSI during aging and the early AD continuum. Our data encourage applications of MRI/MRSI at 7T in research and perspective clinical settings, to fully explore the potential of dedicated imaging methods at ultra-high magnetic field strength in aging and neurodegeneration.



## 5 | SUMMARY

We show that gray matter GABA and Glu, measured in vivo with advanced 7T MRSI, are associated with A $\beta$  burden and established risk factors for AD, namely APOE4 and subtle episodic memory decline, at old age. Our findings resonate with previous studies implicating GABAergic and glutamatergic alterations, synaptic dysfunction, and abnormal neuronal activity with early AD. Additional longitudinal studies are warranted to explore the potential of GABAergic and glutamatergic alterations as biomarkers, mechanisms, and therapeutic targets in neurodegenerative diseases such as AD.

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## CONFLICT OF INTEREST STATEMENT

See COI disclosure forms. Author disclosures are available in the [supporting information](#).

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## SUPPORTING INFORMATION

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