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# Increased α-HBDH levels exacerbate the detrimental effects of Aβ and tau pathology on cognitive function in Alzheimer's disease

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## Abstract

# BACKGROUND

The role of cardiovascular markers in Alzheimer's disease (AD) pathology is incompletely understood. We investigated whether serum α-hydroxybutyrate dehydrogenase (α-HBDH) is associated with amyloid and tau pathology and influences cognition in AD.

# METHODS

In 245 participants categorized by amyloid-PET status, blood levels of α-HBDH and AD biomarkers (p-tau217, p-tau181, Aβ42/40) were measured. Cognitive function was assessed across multiple domains.

# RESULTS

Higher α-HBDH correlated with greater amyloid positivity. Moreover, α-HBDH levels negatively correlate with cognitive performance in the Aβ − group. α-HBDH levels positively correlate with tau pathology and amyloid deposition in all participants, and specifically with p-tau181 in the Aβ + group. Notably, α-HBDH interacts with p-tau217 to exacerbate cognitive decline in all participants and the Aβ + group.

# DISCUSSION

α-HBDH is linked to both amyloid and tau pathology and interacts with p-tau217 to worsen cognition, highlighting its potential as a cardiovascular modulator in AD and supporting multi-target therapeutic strategies.

## 1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that manifests as progressive cognitive decline, driven by the deposition of amyloid-beta (Aβ) plaques and neurofibrillary tangles formed by hyperphosphorylated tau protein [1]. Recent research has increasingly challenged the central role of Aβ in AD pathogenesis: although therapies targeting Aβ clearance, such as anti-Aβ monoclonal antibodies, have been developed, clinical trials have reported that reducing the plaque burden has limited success in halting cognitive decline [2][3][4]. Furthermore, individuals who are Aβ negative often exhibit neurodegenerative changes, such as tau pathology and progressive cognitive dysfunction [5][6], highlighting the complexity of AD pathogenesis and the need to explore other contributing factors.

Notably, cardiovascular factors have emerged as critical modulators of AD progression [7][8][9][10]. Postmortem studies have shown that 80% of dementia patients, including those with AD, exhibit signs of vascular disease [11]. Cardiovascular disease (CVD) exacerbates neurodegeneration associated with

mild cognitive impairment (MCI), significantly increasing the risk of progression to AD [12]. These studies suggest that cardiovascular health may play a key role in the development and progression of AD [10][13], possibly independent of well-established pathological features. The interaction between cardiovascular factors and neurodegenerative diseases is an area of growing interest in AD research. Further investigation into this connection, exemplified by biomarkers, could yield valuable insights for preventive and therapeutic strategies.

α-Hydroxybutyrate dehydrogenase (α-HBDH) is traditionally used as a marker for myocardial infarction and is associated with adverse outcomes in various critical illnesses [14][15]. Increased levels of α-HBDH are not only indicative of acute myocardial infarction but are also linked to other severe conditions, including cerebral hemorrhage and cerebral infarction [16][17]. Moreover, α-HBDH is closely associated with atherosclerotic thrombotic events and is more sensitive than traditional laboratory indicators for assessing the risk of such events [18]. Increases in α-HBDH levels have been linked to chronic or acute ischemic cardiovascular conditions, suggesting a broader potential for α-HBDH in monitoring long-term outcomes, including those associated with peripheral artery disease [19]. The growing evidence for α-HBDH as a biomarker of cardiovascular injury has prompted interest in its potential role as a marker for neurodegenerative diseases.

Recent studies have suggested that α-HBDH may serve as a marker of oxidative stress [15], potentially influencing the pathophysiology of neurodegenerative diseases. Increased levels of α-HBDH have been observed in conditions such as intracerebral hemorrhage, stroke, and other diseases that share pathological features with neurodegeneration [16][17][19][20]. While there is growing evidence that α-HBDH is involved in the above diseases, its specific role in AD remains underexplored. The interactions among α-HBDH, Aβ, and tau proteins, as well as their combined effects on neurodegeneration and cognitive impairment, have not been thoroughly examined. Thus, an investigation into the broader role of α-HBDH in the context of AD is needed.

This study aims to fill this research gap by investigating the synergistic effects of α-HBDH and AD pathology on cognitive impairment in cohorts of AD patients according to the presence of Aβ. Specifically, this study sought to examine the independent and combined effects of α-HBDH and Aβ or tau on cognitive impairment. It also aimed to identify potential differences in these associations between participants expressing and not expressing Aβ. This research provides new insights into the cardiovascular factors contributing to AD progression, particularly regarding both the Aβ-dependent and Aβ-independent mechanisms of neurodegeneration. By exploring the role of α-HBDH in AD, this study provides valuable information for the development of potential therapeutic strategies targeting cardiovascular contributions to neurodegeneration in AD.

# 2. Methods

# 2.1 Study cohorts and participants

This study enrolled 245 volunteers from the Southern China Aging Brain Initiative (SCABI) cohort. Each participant received an 18F-florbetapir PET scan between March 2021 and January 2024. The recruitment process is outlined in **Supplementary Figure S1**. Written informed consent was obtained from all subjects or their legal representatives. The study followed the principles of the Declaration of Helsinki and was authorized by the Ethics Committees of the Affiliated Brain Hospital of Guangzhou Medical University.

Detailed descriptions of the SCABI cohort have been published previously [21]. Eligible participants met the following inclusion criteria: 1) age 50 years or older, and 2) cognitive status classified as normal, mild cognitive impairment (MCI; based on Peterson criteria [22]), or dementia (diagnosed according to DSM-IV criteria for any dementia [23]). Exclusion criteria comprised: 1) malignant tumors or significant cerebrovascular disease (including ischemic stroke and intracerebral hemorrhage accompanied by neurological deficits); 2) major neurological conditions (e.g., metabolic encephalopathy, encephalitis, multiple sclerosis, epilepsy, traumatic brain injury, or normal pressure hydrocephalus); 3) severe psychiatric disorders (such as schizophrenia, bipolar disorder, schizoaffective disorder, paranoid psychosis, or intellectual disability); and 4) systemic illnesses known to affect cognition (e.g., hepatic or renal dysfunction, thyroid abnormalities, severe anemia, folate or vitamin B12 deficiency, syphilis, HIV infection, or substance abuse). The first three categories were verified through clinical neurological assessment, whereas the fourth category relied on patient or family-reported medical history.

AD diagnosis followed the National Institute on Aging–Alzheimer's Association criteria for probable AD dementia [24] and was further supported by positive Aβ-PET findings. The final cohort included 245 individuals with a mean age of 68.7 years (standard deviation, SD: 7.10). Among them, 30.6% were male, and 78.8% exhibited cognitive impairment due to either MCI (58.4%) or dementia (20.4%).

## 2.2 Cognitive evaluation

A detailed neuropsychological assessment was performed on all participants, as outlined in prior work [25]. The evaluation incorporated a series of validated tests designed to examine multiple cognitive domains. These instruments included the Mini-Mental State Examination (MMSE), the Clinical Dementia Rating (CDR) scale, the Activities of Daily Living (ADL) scale, and the Hachinski Ischemic Score (HIS). Furthermore, specific functions were measured using the Auditory Verbal Learning Task (AVLT), the Trail-Making Test (TMT), and the Symbol-Digit Modality Test (SDMT). Language abilities were assessed via the Boston Naming Test (BNT), while visuospatial and executive functions were evaluated with the Rey-Osterrieth Complex Figure (ROCF) test, the Stroop Color and Word Test (STROOP), the Digit Span Test (DST), and the Clock Drawing Test (CDT).

## 2.3 Blood sample acquisition

Venous blood was drawn from all 245 participants into 5 mL polypropylene tubes. Samples were delivered to the laboratory without delay and processed within a four-hour window post-collection. After centrifugation at 2000 × g for 10 minutes under 4°C conditions, 0.5 mL plasma aliquots were pipetted

into individual polypropylene tubes and subsequently preserved at − 80°C until biomarker assessment. Blood acquisition was completed within a three-month period relative to each participant's PET scan.

## 2.4 Measurement of serum α-HBDH concentration

Serum α-HBDH levels were determined via an enzyme cycling method implemented on an automated clinical analyzer (AU5800, Beckman Coulter, Brea, CA). All assays were performed by a research assistant who remained blinded to the clinical diagnoses and group assignments of the participants.

## 2.5 Quantification of plasma tau and Aβ biomarker concentrations

On the day of analysis, plasma samples were thawed under ambient conditions. To minimize pre-analytical variability, only aliquots without prior thawing cycles were utilized. Concentrations of Aβ1–42, Aβ1–40, p-tau181, and p-tau217 were measured directly from the original 0.5 mL storage tubes. Analyses were conducted on a Lumipulse G 1200 automated immunoassay system (Fujirebio) using the corresponding Lumipulse G chemiluminescent assays for each biomarker. All procedures adhered strictly to the manufacturer's protocols, which included vortex mixing and brief centrifugation after thawing to eliminate potential interference from fibrin clots.

## 2.6 ApoE status assessment

Apolipoprotein E (ApoE) status was evaluated using the Lumipulse G ApoE4 and Pan-ApoE chemiluminescent assays (Fujirebio). Measurements of ApoE4 and total ApoE were performed sequentially, and the ApoE4/Pan-ApoE ratio was calculated to determine the proteotype. Based on established thresholds [26], samples were categorized as follows: “null” (absence of ApoE4) if the ratio was below 5%, “heterozygous” (presence of ApoE4 alongside ApoE2 or ApoE3) if the ratio was between 5% and 75%, and “homozygous” (exclusively ApoE4) if the ratio reached or exceeded 75%.

## 2.7 Imaging acquisition, visual rating, and quantitative analysis of Amyloid-PET

A total of 236 participants underwent 18F-florbetapir amyloid PET imaging. As reported previously [21], scans were acquired on either a SIGNA PET magnetic resonance (MR) or a Siemens PET computed tomography (CT) system, starting 50 minutes after intravenous administration of 10 ± 1 mCi of 18F-florbetapir.

For PET/MR acquisitions, data were collected over 15 minutes. Image reconstruction employed an ordered-subset expectation maximization algorithm with time-of-flight and point-spread-function corrections, using 28 subsets and six iterations. Attenuation correction was applied via zero-echo-time sequences. The resulting images had a matrix size of 256 × 256, a display field of view of 46.2 × 30 cm, a slice thickness of 2.78 mm, and a pixel size of 2.8 × 2.8 mm.

PET/CT scans were obtained over 15–20 minutes using a three-dimensional iterative reconstruction algorithm (four iterations, 21 subsets). The reconstruction incorporated time-of-flight and point-spread-function modeling with five iterations and 16 subsets. The final images featured a matrix size of 336 × 336, a zoom factor of 2.0, a slice thickness of 2.0 mm, and a Gaussian filter with a full-width-at-half-maximum of 5.0 mm.

All scans were visually assessed by experienced readers who were blinded to clinical and biomarker data. Following FDA guidance, scans were rated as “positive” if one or more cortical regions exhibited increased gray-matter signal with loss of gray-white matter contrast, and as “negative” if gray-white matter contrast remained clearly distinguishable.

For quantitative analysis, each PET image was spatially normalized to a Montreal Neurological Institute (MNI) 152 18F-florbetapir template via linear and nonlinear transformations. Mean tracer uptake was computed within a composite region comprising the frontal, lateral parietal, and anterior/posterior cingulate cortices. An 18F-florbetapir standardized uptake value ratio (SUVR) map was then generated using the whole cerebellum as the reference region.

## 2.8 Statistical analysis

Data preprocessing: Missing data points, with their frequencies detailed in **Supplementary Tables S1–S2**, were handled through multiple imputation via chained equations. This procedure was implemented using the “mice” package in R (v4.3.2), a well-established method for managing incomplete datasets in clinical and epidemiological studies. To mitigate the influence of extreme values, potential outliers were identified via the Tukey method, defined as observations exceeding 1.5 times the interquartile range (IQR). These outliers were subsequently replaced with the corresponding first (Q1) or third (Q3) quartile values utilizing the “outliers” package (v3) in R, thereby reducing their impact on subsequent correlation and regression analyses.

Data analysis: Group comparisons between Aβ-positive and Aβ-negative participants were performed using independent samples t-tests for continuous variables and chi-square tests for categorical variables. For the Trail Making Test part B (TMT-B), scores were reversed so that lower values reflected worse executive function, aligning its interpretation with other cognitive scales. For cognitive domain assessments, memory function was evaluated using the sum of AVLT trials N4 and N5, while other domains were quantified by averaging their two respective scales (BNT and VFT for language; TMT-B and STROOP for executive function; SDMT and DST for attention; and CDT and ROCF for visuospatial skills). Multivariable linear regression analyses (adjusted for years of education and APOE status) examined plasma α-HBDH interactions with Aβ status, sex, and age across six cognitive domains. Exploratory partial correlation analyses, controlling for sex, age, education, and APOE status, were conducted to evaluate associations between α-HBDH levels and (1) performance in each cognitive domain, and (2) established AD biomarkers, including plasma protein concentrations and PET-derived parameters. Confirmatory linear regression models were constructed to assess the respective contributions of blood α-HBDH, p-tau217, and global amyloid deposition (quantified by SUVR) to cognitive performance. For

each domain, three nested models were tested: one with α-HBDH alone, one with α-HBDH and p-tau217, and one with α-HBDH and SUVR. To account for multiple testing, all regression p-values were adjusted using the false discovery rate (FDR) correction. Finally, a LASSO regression model, optimized with λ.min, was employed to identify seven blood-based biomarkers and MMSE contributing to brain Aβ status classification. All analyses were performed in R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed α level of 0.05 defined statistical significance. To verify the robustness of the primary results, two sensitivity analyses were conducted. First, a complete case analysis was performed by excluding all participants with any missing data on AD biomarkers (Aβ40, Aβ42, p-tau181, and p-tau217), rather than using imputation. Second, all primary regression models were rerun separately in male and female subgroups to explore potential sex differences.

## 2.9 Data Availability Statement

The original neuroimaging and clinical data from the SCABI cohort supporting the findings of this study are not publicly available due to patient privacy and ethical restrictions. De-identified data can be made available from the corresponding author or the Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University upon reasonable request, subject to approval and a data use agreement.

# 3. Results

## 3.1 Characterization of the study population

Table 1 presents the clinical, demographic, and plasma biomarker data of all participants grouped on the basis of α-HBDH levels (low vs. high groups, dichotomized at the median due to nonnormal distribution). Brain Aβ status differed significantly between the groups, with the high α-HBDH group having a significantly higher prevalence of Aβ positivity than the low α-HBDH group did (50.4% vs. 32.5%, $p = 0.007$). The high α-HBDH group had significantly lower education levels (9.22 vs. 11.0 years, $p = 0.001$) and a higher proportion of females (77.6% vs. 60.8%, $p = 0.007$), while age and APOEε4 carrier status showed no significant group differences (both $p > 0.05$).

Compared to the low group, the high α-HBDH group exhibited significantly elevated phosphorylated tau biomarkers (p-tau181: 3.16 vs. 2.76 pg/mL, $p = 0.020$; p-tau217: 0.45 vs. 0.32 pg/mL, $p = 0.020$) and higher SUVRs (1.21 vs. 1.13, $p = 0.001$), although no significant differences were observed in Aβ40, Aβ42 or the Aβ42/40 ratio (all $p > 0.7$). With respect to cardiovascular biomarkers, the high α-HBDH group had higher total cholesterol (TC: 5.52 vs. 5.13 mmol/L, $p = 0.006$) and high-density lipoprotein cholesterol levels (HDL-C: 1.67 vs. 1.49 mmol/L, $p < 0.001$) but lower triglycerides (TG: 1.26 vs. 1.47 mmol/L, $p = 0.042$). No significant differences were found in low-density lipoprotein cholesterol (LDL-C) or glucose levels (both $p > 0.05$).

There was no significant difference between the high and low α-HBDH groups in either activities of daily living (ADL: 12.7 vs. 13.1, $p = 0.184$) or Hachinski Ischemic Scores (HIS: 1.84 vs. 1.78, $p = 0.661$). However, the high α-HBDH group demonstrated worse cognitive performance across multiple domains.

Global cognition, as measured by the MMSE, was significantly poorer in the high group (19.0 vs. 21.6, $p = 0.002$), and Clinical Dementia Rating (CDR) scores were higher (0.74 vs. 0.60, $p = 0.036$). Memory function tests (AVLT N1-N3: 12.4 vs. 14.7; N4-N5: 5.84 vs. 8.51; N6: 2.23 vs. 3.48; all $p \leq 0.006$) and language ability (BNT: 17.2 vs. 19.0, $p = 0.002$) were significantly impaired, although verbal fluency (VFT) showed no difference ($p = 0.303$). Attention (DST: 13.8 vs. 14.9, $p = 0.017$) and visuospatial skills (ROCF: 20.9 vs. 23.2, $p = 0.041$) were also worse in the high group compared with the low group.

Initial interaction analyses were performed to evaluate the associations between plasma α-HBDH levels and Aβ status, sex, and age across six cognitive domains (**Supplementary Table S3**). The analyses revealed significant interactions between α-HBDH and Aβ status that were consistent across all cognitive domains (all β coefficients negative, range: -0.014 to -0.035; all $p_{FDRs} < 0.01$), indicating amplified cognitive impairment from α-HBDH in Aβ + individuals compared to Aβ − counterparts. More limited interactions were observed for sex (Memory: β = 0.021, $p_{FDR}=0.006$; Language: β = 0.01, $p_{FDR}=0.038$; Attention: β = 0.026, $p_{FDR}=0.003$) and age (Memory: β=-0.001, $p_{FDR}=0.024$; Attention: β=-0.002, $p_{FDR}=0.003$). Given the robust amyloid-dependent pattern, we adopted Aβ-stratified analyses for all subsequent investigations.

Table 1
Demographic and clinical characteristics of all participants based on α-HBDH group.

| | ALL | Low αHBDH | High αHBDH | *p.overall* |
|---|---|---|---|---|
| | *N = 245* | *N = 120* | *N = 125* | |
| **Brain Aβ status**: | | | | 0.007** |
| Negative | 143 (58.4%) | 81 (67.5%) | 62 (49.6%) | |
| Positive | 102 (41.6%) | 39 (32.5%) | 63 (50.4%) | |
| Sex: | | | | 0.007** |
| Male | 75 (30.6%) | 47 (39.2%) | 28 (22.4%) | |
| Female | 170 (69.4%) | 73 (60.8%) | 97 (77.6%) | |
| Age (years) | 68.7 (7.10) | 68.1 (7.24) | 69.3 (6.94) | 0.174 |
| Education (years) | 10.1 (4.14) | 11.0 (4.00) | 9.22 (4.11) | 0.001** |
| APOEe4: | | | | 0.507 |
| Non-Carrier | 178 (72.7%) | 90 (75.0%) | 88 (70.4%) | |
| Carrier | 67 (27.3%) | 30 (25.0%) | 37 (29.6%) | |
| **AD biomarkers** | | | | |
| Aβ40 (pg/mL) | 269 (41.9) | 269 (41.5) | 268 (42.4) | 0.974 |
| Aβ42 (pg/mL) | 24.6 (4.93) | 24.7 (4.96) | 24.5 (4.92) | 0.749 |
| Aβ42/40 | 0.09 (0.01) | 0.09 (0.01) | 0.09 (0.02) | 0.772 |
| Ptau181 (pg/mL) | 2.96 (1.32) | 2.76 (1.20) | 3.16 (1.41) | 0.020* |
| Ptau217 (pg/mL) | 0.39 (0.42) | 0.32 (0.38) | 0.45 (0.44) | 0.020* |
| **SUVRs** | 1.17 (0.20) | 1.13 (0.17) | 1.21 (0.22) | 0.001** |
| **Cardiovascular biomarkers** | | | | |
| α-HBDH (U/L) | 121 (19.3) | 105 (8.58) | 136 (13.1) | < 0.001*** |
| TC (mmol/L) | 5.33 (1.11) | 5.13 (1.07) | 5.52 (1.13) | 0.006** |
| HDLC (mmol/L) | 1.58 (0.40) | 1.49 (0.36) | 1.67 (0.41) | < 0.001*** |
| LDLC (mmol/L) | 3.17 (0.94) | 3.07 (0.97) | 3.26 (0.90) | 0.103 |
| TG (mmol/L) | 1.36 (0.78) | 1.47 (0.89) | 1.26 (0.66) | 0.042* |

*Note*: The continuous variables in the table are expressed as the mean and standard deviation.

| | ALL | Low αHBDH | High αHBDH | *p.overall* |
|---|---|---|---|---|
| | *N = 245* | *N = 120* | *N = 125* | |
| GLU (mmol/L) | 5.51 (1.70) | 5.62 (1.71) | 5.40 (1.68) | 0.321 |
| ADL | 12.9 (2.37) | 13.1 (2.24) | 12.7 (2.49) | 0.184 |
| HIS | 1.81 (1.01) | 1.78 (0.90) | 1.84 (1.11) | 0.661 |
| CDR | 0.67 (0.51) | 0.60 (0.45) | 0.74 (0.56) | 0.036* |
| **Global cognition** | | | | |
| MMSE | 20.3 (6.43) | 21.6 (6.21) | 19.0 (6.42) | 0.002** |
| **Memory** | | | | |
| AVLT N1-N3 | 13.5 (6.44) | 14.7 (6.27) | 12.4 (6.44) | 0.006** |
| AVLT N4-N5 | 7.15 (6.38) | 8.51 (6.48) | 5.84 (6.02) | 0.001** |
| AVLT N6 | 2.84 (3.18) | 3.48 (3.20) | 2.23 (3.05) | 0.002** |
| **Language** | | | | |
| BNT | 18.1 (4.70) | 19.0 (4.62) | 17.2 (4.63) | 0.002** |
| VFT | 11.5 (4.12) | 11.8 (4.16) | 11.3 (4.08) | 0.303 |
| **Executive function** | | | | |
| TMT-B time | 99.2 (55.7) | 94.6 (57.7) | 104 (53.5) | 0.201 |
| STROOP | 41.3 (8.42) | 41.9 (8.31) | 40.8 (8.52) | 0.329 |
| **Attention** | | | | |
| SDMT | 25.3 (14.3) | 26.7 (14.6) | 24.0 (13.9) | 0.142 |
| DST | 14.3 (3.73) | 14.9 (3.77) | 13.8 (3.62) | 0.017* |
| **Visuospatial skill** | | | | |
| CDT | 2.90 (1.08) | 3.02 (1.05) | 2.78 (1.10) | 0.081 |
| ROCF | 22.0 (8.61) | 23.2 (8.74) | 20.9 (8.38) | 0.041* |
| Cognitive Status: | | | | 0.136 |
| Cognitively Unimpaired | 52 (21.2%) | 31 (25.8%) | 21 (16.8%) | |
| MCI | 143 (58.4%) | 69 (57.5%) | 74 (59.2%) | |
| Dementia | 50 (20.4%) | 20 (16.7%) | 30 (24.0%) | |

*Note*: The continuous variables in the table are expressed as the mean and standard deviation.

Abbreviations: Aβ, amyloid β; pTau, phosphorylated tau; tTau, total tau; NFL, Neurofilamentlightchain; SUVRs, Standardized Uptake Value Ratios; TC, Total Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; TG, Triglycerides; GLU, Glucose; ADL, activities of daily living; CDR, clinical dementia rating; HIS, Hachinski Ischemic Score; MMSE, Mini-Mental State Examination; AVLT N1-3, Auditory Verbal Learning Test Immediate recall; AVLT N4, Auditory Verbal Learning Test Short-term delayed recall; AVLT N5, Auditory Verbal Learning Test Long-term delayed recall; AVLT N6, Auditory Verbal Learning Test Recognition; BNT, Boston Naming Test; VFT, Verbal fluency test; TMT, Trail-Making Test; STROOP, Stroop Color and Word Test; SDMT, Symbol-Digit Modality Test; DST, Digit Span Test; CDT, Clock Drawing Test; ROCF, Rey-Osterrieth Complex; MCI, Mild Cognitive Impairment. *** ($p < 0.001$), ** ($p < 0.01$), and * ($p < 0.05$). Adjusted $R^2$ values are reported for each group.

### *3.2 Blood α-HBDH levels are associated with cognitive impairment in an amyloid-dependent manner*

Heatmaps revealed distinct patterns of correlation between blood α-HBDH levels and cognitive performance on the basis of amyloid status. In all participants, α-HBDH levels were associated with MMSE scores, language function, and executive function (**Figure 1A**) (all $p_{FDRs} < 0.05$). In the Aβ+ group, no significant correlations were found (**Figure 1B**) (all $p_{FDRs} > 0.05$). Conversely, in the Aβ− group, α-HBDH levels were associated with MMSE scores, language function and attention (**Figure 1C**) (all $p_{FDRs} < 0.05$).

Scatter plots were used to confirm the findings of the heatmap analysis. In the overall cohort, higher α-HBDH levels were significantly associated with poorer global cognition (MMSE: $R^2 = 0.286$, $p = 0.005$, $p_{FDR} = 0.015$), language function ($R^2 = 0.194$, $p = 0.016$, $p_{FDR} = 0.037$), and executive function ($R^2 = 0.117$, $p = 0.014$, $p_{FDR} = 0.043$) (**Figure 1D, F, and G**). When the patients were stratified by amyloid status, these associations were found to be driven primarily by the Aβ− group, in which higher α-HBDH levels were associated with worse global cognition (MMSE: $R^2 = 0.275$, $p = 0.04$), language function ($R^2 = 0.208$, $p = 0.025$, $p_{FDR} = 0.037$), and attention ($R^2 = 0.439$, $p = 0.01$, $p_{FDR} = 0.031$) (**Figure 1 D, F, and H**). In contrast, no significant associations were observed in the Aβ+ group (all $p > 0.05$, $p_{FDRs} > 0.05$).

These findings demonstrate clear negative correlations between α-HBDH levels and cognitive performance in Aβ− participants, whereas Aβ+ individuals present no discernible patterns of correlation. The consistent patterns across different cognitive domains in Aβ− participants highlight the potential role of α-HBDH in cognitive decline-related pathways independent of amyloid.

### *3.3 Blood α-HBDH levels are associated with AD biomarker levels in an amyloid-dependent manner*

Heatmap analysis revealed significant associations of blood α-HBDH levels with p-tau181 levels, p-tau217 levels, and SUVRs across all participants (**Figure 2A**) (all $p < 0.05$). In the Aβ+ group, the α-HBDH level was associated with the p-tau181 level (**Figure 2B**) ($p < 0.05$). Conversely, no significant correlations were observed in the Aβ− group (**Figure 2C**) (all $p > 0.05$).

General linear regression analyses revealed distinct patterns of associations between blood α-HBDH levels and AD biomarker levels on the basis of amyloid status. In the overall cohort, α-HBDH levels were significantly positively associated with p-tau181 levels ($R^2$=0.143, $p$ <0.001, $p_{FDR}$=0.003), p-tau217 levels ($R^2$=0.134, $p$ =0.011, $p_{FDR}$=0.032), and SUVRs ($R^2$=0.125, $p$ =0.002, $p_{FDR}$=0.007) (**Figure 2 D, E, and F**). Notably, these associations were driven primarily by Aβ+ individuals, with significantly positive correlations observed between α-HBDH levels and p-tau181 levels ($R^2$=0.146, $p$ =0.012, $p_{FDR}$=0.019) (**Figure 2 D**) in this group. In contrast, no significant associations were found in the Aβ− group (all $p$ > 0.05, $p_{FDRs}$ > 0.05).

### *3.4 Interaction effect of α-HBDH and p-tau217 on cognitive impairment in Aβ+ participants*

In Aβ+ individuals, significant interaction effects of α-HBDH levels and p-tau217 levels were observed for specific cognitive domains. The interaction of α-HBDH and p-tau217 showed significant negative effects on global cognition (MMSE: β=-0.052, 95% CI=-0.073 to -0.031), memory (β=-0.02, 95% CI=-0.038 to -0.002), language function (β=-0.014, 95% CI=-0.026 to -0.002), executive function (β=-0.024, 95% CI=-0.041 to -0.007), and attention (β=-0.029, 95% CI=-0.053 to -0.004), indicating that higher levels of both biomarkers synergistically contribute to cognitive impairment in these domains. No significant interactions were found for visuospatial function (β=-0.005, 95% CI=-0.0230.013) or between α-HBDH levels and SUVRs for any cognitive domain (all 95% CIs spanning zero) (**Figure 3A and Supplementary Table S4**). These findings demonstrate that elevated α-HBDH levels and elevated p-tau217 levels exert combinatory effects on specific cognitive domains in Aβ+ individuals, with a consistent pattern of impairment across all affected domains. Similar results were observed among all participants (**Supplementary Figure S2**). However, among Aβ− participants, the interaction between α-HBDH levels and p-tau217 levels was observed only for memory impairment (β=-0.264, 95% CI=-0.423-0.105) (**Figure 3B**).

Patients were divided into high and low α-HBDH groups on the basis of median values, and the predicted scores for specific cognitive domains were plotted to illustrate the interaction (**Figure 3C-H**). Across all participants, at higher α-HBDH levels, the decline in cognitive function was more pronounced with increasing p-tau217 levels, indicating that elevated α-HBDH levels exacerbate the negative impact of tau pathology on cognitive impairment. In contrast, at lower α-HBDH levels, the effect of p-tau217 on cognitive function was less pronounced, suggesting a diminished influence of tau pathology. These findings highlight that increased α-HBDH levels may amplify the adverse effects of p-tau217 on cognitive impairment.

### *3.5 Machine Learning-Derived Predictive Hierarchy of Blood Biomarkers for Brain Aβ Status Classification*

The LASSO regression model optimized with λ.min identified six blood-based biomarkers predictive of brain Aβ status classification (**Figure 4, Supplementary Table S8**). P-tau217 emerged as the strongest predictor of brain Aβ positivity with the largest positive coefficient (β = 5.68), whereas the Aβ1-42/Aβ1-

40 ratio showed the most pronounced negative coefficient (β = -0.90). Additional predictors included α-HBDH (β = 0.32), SUVRs (β = 0.67), and P-tau181 (β = 0.31), which demonstrated moderate positive coefficients, while Aβ1-42 alone (β = -0.55) had a moderate negative importance.

### *3.6 Sensitivity analyses*

Sensitivity analyses supported the robustness of the primary findings as follows: 1) A reanalysis excluding missing AD biomarker data (**Supplementary Table S5**) confirmed the key interaction effect of α-HBDH and p-tau217 on global cognition (MMSE) in the overall cohort, despite some domain-specific variations. 2) Separate analyses of males and females (**Supplementary Tables S6–S7**) revealed that these interaction effects were largely consistent across both males and females, with minor differences in statistical significance for certain cognitive domains within amyloid-positive subgroups.

## 4. Discussion

This study provides new insights into the amyloid status-dependent associations among a cardiovascular biomarker (α-HBDH), AD biomarkers and cognitive function. The key findings are as follows: First, blood α-HBDH levels were lower in the Aβ− group than in the Aβ+ group and were negatively associated with cognitive performance in all participants (MMSE scores, language function, and executive function) and in the Aβ− group (MMSE scores, language function, and attention). Second, blood α-HBDH levels were positively correlated with tau pathology (p-tau181 and p-tau217) and amyloid deposition (SUVR) in the full cohort. Within the Aβ+ group, this correlation with tau pathology was specific to p-tau181. Third, and most notably, an interactive effect between α-HBDH and p-tau217 on cognitive function was identified. Elevated α-HBDH levels amplified the detrimental influence of p-tau217 on cognitive decline in all participants and in the Aβ+ group. These findings highlight that α-HBDH, as a representative cardiovascular factor, may be a modifiable risk factor for AD, suggesting that interventions targeting cardiovascular factors could strongly complement anti-amyloid therapies.

### *4.1 α-HBDH as a cardiovascular biomarker in cognitive impairment*

The present study revealed that α-HBDH is a biomarker that is significantly associated with cognitive impairment. Increased levels of α-HBDH were observed in Aβ+ participants and were negatively correlated with cognitive performance across various domains, specifically with MMSE scores, language function, and executive function, in all participants. These findings suggest that α-HBDH, a cardiovascular marker typically used to assess myocardial infarction and other critical illnesses, may play a significant role in cognitive impairment. Prior studies have demonstrated that α-HBDH serves as an index for evaluating the severity of COVID-19 [27] and that the α-HBDH level is elevated in conditions such as cerebral hemorrhage, cerebral infarction, and acute ischemic stroke [17][19][20][27]. This finding not only elucidates the relationship between cardiovascular dysfunction and neurodegeneration but also paves the way for the use of cardiovascular biomarkers, such as α-HBDH, as valuable diagnostic indicators in AD. Future research should aim to elucidate the mechanisms by which α-HBDH influences

AD pathology, potentially leading to the development of innovative therapeutic approaches targeting both the cardiovascular system and neurodegeneration.

### *4.2 Interaction between α-HBDH and tau pathology: A new dimension in AD mechanisms*

The key finding of this study is the observed interaction effect between α-HBDH and p-tau217, as higher levels of α-HBDH were found to be associated with the exacerbating effects of p-tau217 on cognitive impairment in AD. This finding fills a gap in previous research by establishing a link between vascular factors, specifically α-HBDH, and tau-related neurodegeneration in AD. Elevated α-HBDH levels may indicate underlying cardiovascular dysfunction that exacerbates tau-mediated brain damage, thereby accelerating cognitive decline. This interaction suggests that α-HBDH not only may serve as a marker of cardiovascular damage but also could contribute to the pathophysiology of tau-related neurodegeneration. These findings underscore the need for further research into the intersection of cardiovascular health and tau pathology in AD, as this relationship may provide new insights into the mechanisms underlying AD progression and potential therapeutic targets.

### *4.3 Amyloid status and α-HBDH: Differentiating pathological profiles in AD*

The inclusion of both Aβ+ and Aβ− participants in this study makes it particularly valuable, as it allows for a more nuanced understanding of the role of α-HBDH depending on brain amyloid status. In the Aβ+ group, increased levels of α-HBDH were not only directly associated with p-tau181 but also synergistically worsened cognitive impairment in conjunction with p-tau217. However, in the Aβ− group, higher levels of α-HBDH were associated with cognitive impairment but not with the levels of plasma AD biomarkers, suggesting that cardiovascular dysfunction may influence cognitive decline even in the absence of amyloid plaques. The possible reasons for this are that cognitive impairment in Aβ− individuals could be driven by other mechanisms, such as cardiovascular dysfunction, impaired blood flow or other vascular pathologies [28]. These factors could contribute to cognitive decline through mechanisms such as a reduced oxygen supply [29] or increased inflammation [30], both of which could accelerate neurodegeneration in the absence of amyloid plaques.

## 5. Limitations and future research directions

Several constraints of this study should be acknowledged. First, the small sample size and low diversity may limit the generalizability of the results. Future investigations utilizing larger and more diverse cohorts are required to validate these findings across different populations and settings. Second, due to its cross-sectional design, this study provides only a snapshot of the association between α-HBDH levels and cognitive impairment, making it difficult to infer causality. Longitudinal studies are necessary to confirm whether increased α-HBDH levels predict cognitive decline over time, particularly in the context of AD. Third, although an association between α-HBDH and AD pathology has been established, the exact biological mechanisms linking cardiovascular dysfunction and neurodegeneration remain unclear. Further investigations are needed to explore how α-HBDH influences tau and amyloid pathology at the molecular level, which could provide deeper insights into the mechanisms driving cognitive decline in AD.

## 6. Conclusion

In conclusion, this study highlights the significant association of α-HBDH, a cardiovascular biomarker, with the pathogenesis of AD. Increased α-HBDH levels are linked to cognitive impairment, tau pathology, and amyloid deposition, suggesting that cardiovascular dysfunction may be related to the progression of cognitive impairment, even in the absence of substantial amyloid pathology. These findings provide a new perspective on AD treatment, underscoring the importance of considering treatments targeting cardiovascular factors alongside traditional amyloid-targeting therapies.

## Declarations

# Competing interests

The authors report no competing interests.

# Supplementary material

Supplementary material is available at Neurology online.

### Consent Statement

Written informed consent was obtained from all participants or their legally authorized representatives prior to inclusion in this study. The study protocol was approved by the Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University.

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# Author Contribution

H.R.Z., Q.X.C., and Q.W. contributed equally to this work and share first authorship. H.R.Z. was responsible for the collection and organization of clinical data. Q.X.C. and Q.W. performed the data analysis, statistical modeling, and visualization, including the preparation of figures and tables. Q.X.C. drafted the initial version of the manuscript. M.F.Y., Z.D.X., D.Y.X., H.Y.T., S.L., P.B.G., G.H.L., and H.M.L.

critically reviewed and revised the manuscript for important intellectual content. X.M.Z. and Y.P.N. conceptualized and supervised the study, secured funding, and provided overall guidance. All authors reviewed and approved the final version of the manuscript.


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# Data Availability

The original neuroimaging and clinical data from the SCABI cohort supporting the findings of this study are not publicly available due to patient privacy and ethical restrictions. De-identified data can be made available from the corresponding author or the Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University upon reasonable request, subject to approval and a data use agreement.

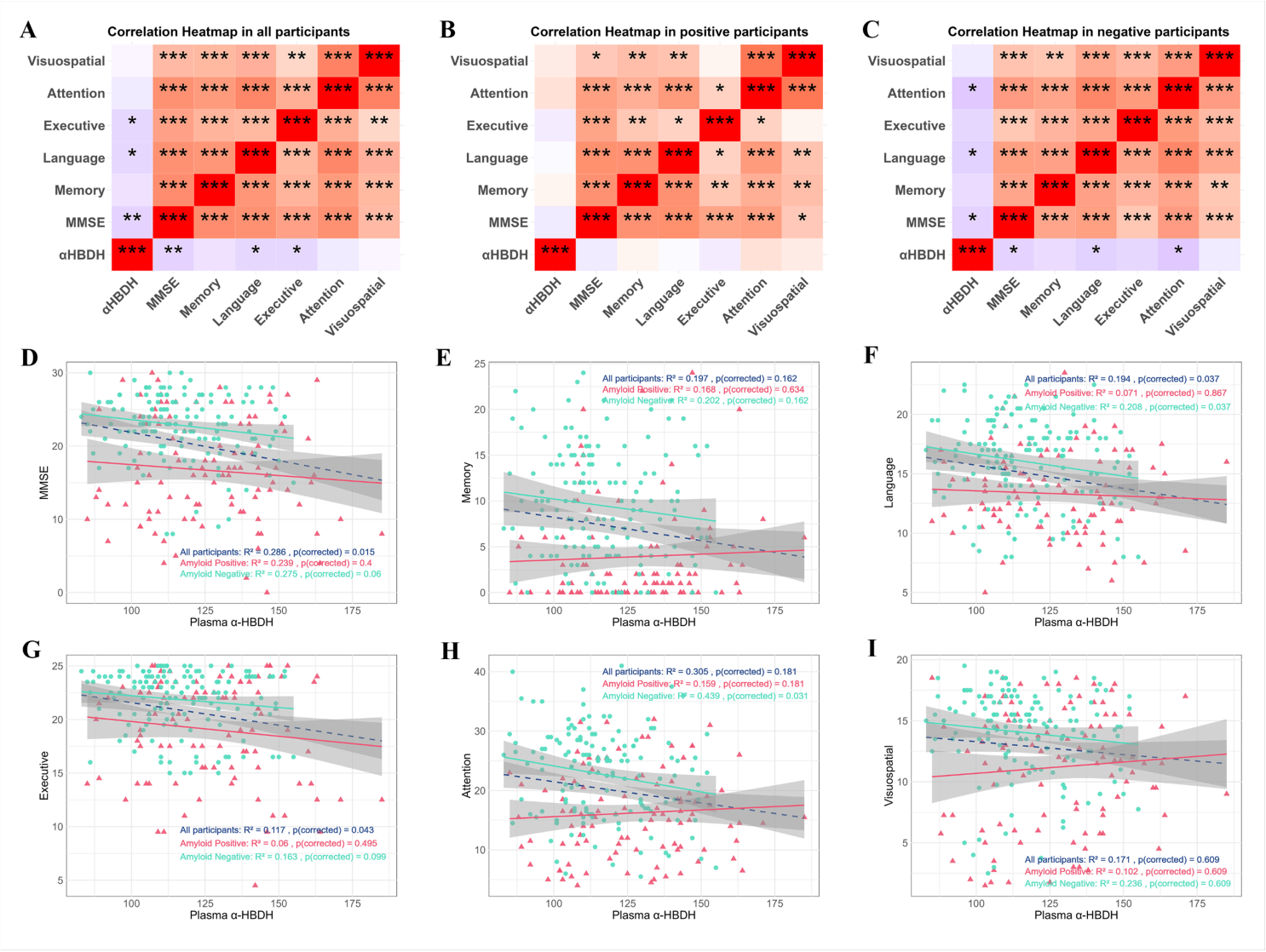
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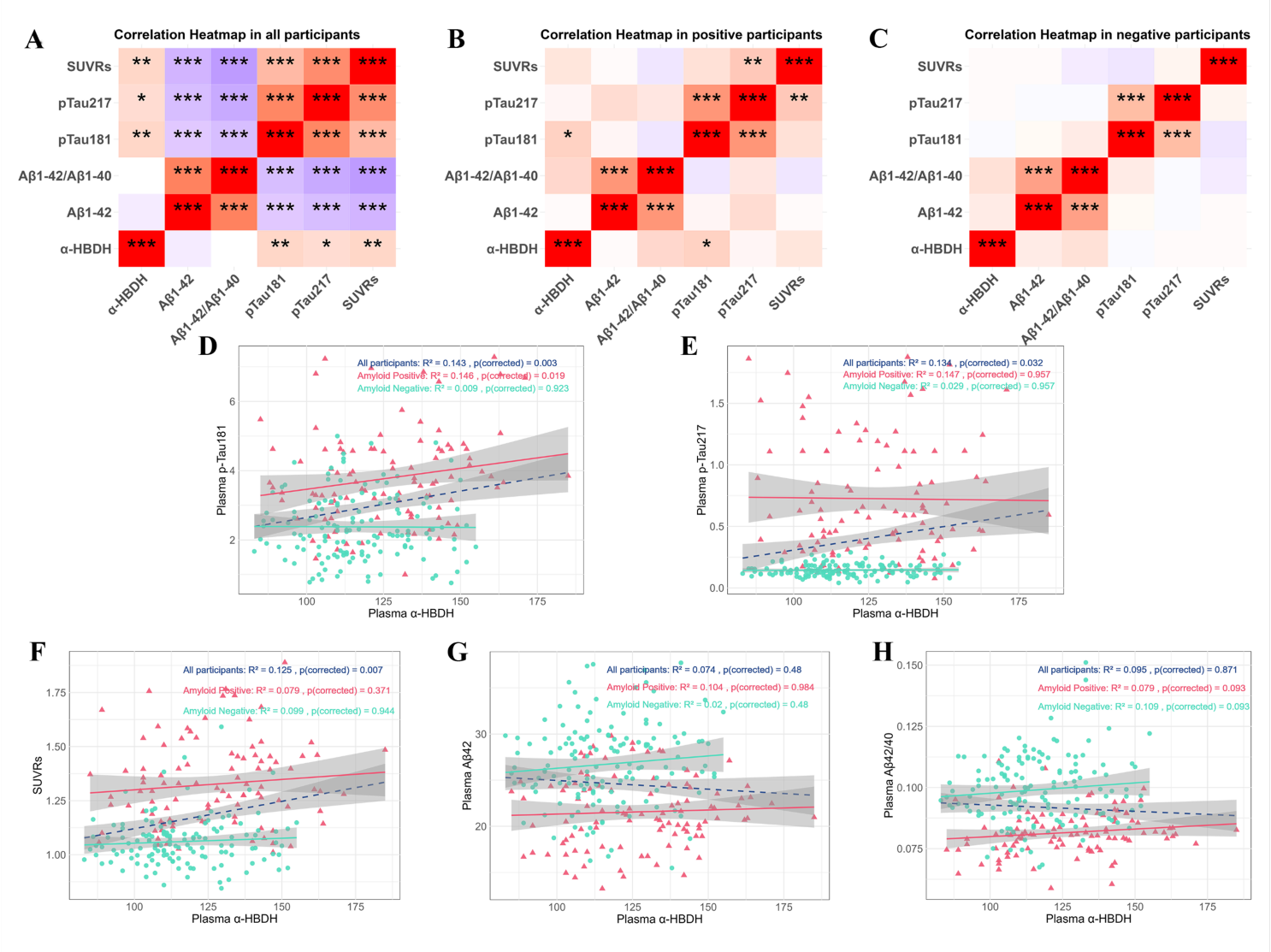
# Figures




## Figure 1

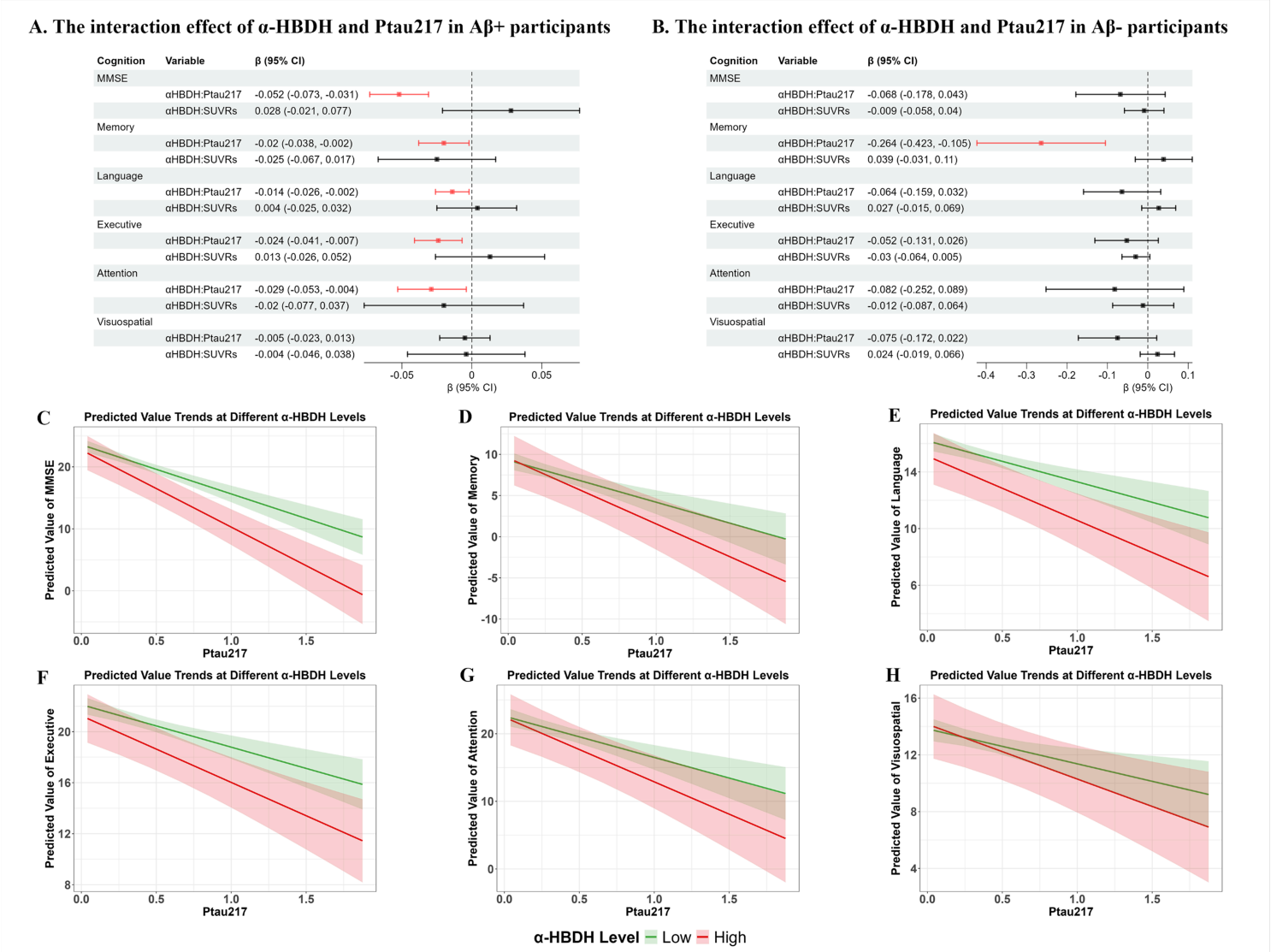
**Correlations between blood α-HBDH levels and cognitive function across groups stratified by Aβ positivity (FDR corrected; controlled for sex, age, years of education, and APOE status). (A-C)** Heatmaps

of associations between α-HBDH and cognitive domains across groups. **(D-I)** Scatterplots with regression lines between α-HBDH levels and specific cognitive scores.




## Figure 2

**Correlations between blood α-HBDH levels and AD biomarker levels across groups stratified by Aβ positivity (FDR corrected; controlled for sex, age, years of education, and APOE status). (A-C)**Heatmaps of associations between α-HBDH and AD biomarker levels across groups. **(D-H)**Scatterplots with regression lines between α-HBDH levels and individual AD biomarker levels.




## Figure 3

**Interaction effect of α-HBDH and p-tau217 on cognitive function across groups (adjusted for sex, APOE status, cognitive status, age, and years of education). (A-B)** Forest plots show the interaction effects of α-HBDH and p-tau217 on multiple cognitive domains in (A) Aβ+ participants and (B) Aβ− participants. **(C-H)** Interaction plots illustrate the relationship between p-tau217 levels and predicted cognitive scores in groups with high versus low α-HBDH levels.

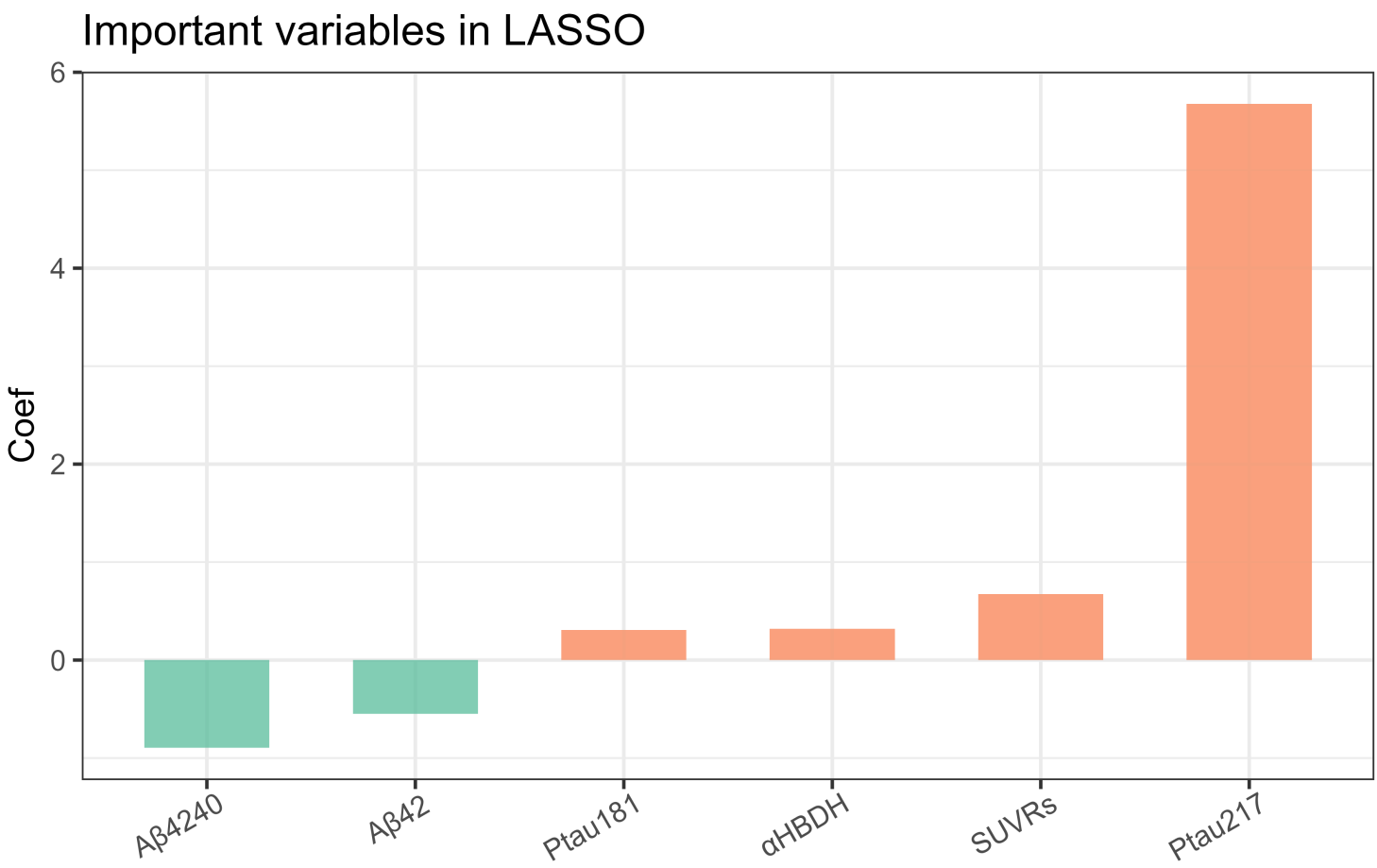



**Figure 4**

**Coefficients of blood biomarkers in the LASSO Model (λ.min).**

## Supplementary Files

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- 6August2025Supplementary.docx