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ORIGINAL ARTICLE

Subclinical liver traits are associated with structural and hemodynamic brain imaging markers

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Abstract

Background & Aims: Impaired liver function affects brain health and therefore understanding potential mechanisms for subclinical liver disease is essential. We assessed the liver–brain associations using liver measures with brain imaging markers, and cognitive measures in the general population.

Methods: Within the population-based Rotterdam Study, liver serum and imaging measures (ultrasound and transient elastography), metabolic dysfunction-associated fatty liver disease (MAFLD), non-alcoholic fatty liver disease (NAFLD) and fibrosis phenotypes, and brain structure were determined in 3493 non-demented and stroke-free participants in 2009–2014. This resulted in subgroups of $n = 3493$ for MAFLD (mean age 69 ± 9 years, 56% ♀), $n = 2938$ for NAFLD (mean age 70 ± 9 years, 56% ♀) and $n = 2252$ for fibrosis (mean age 65 ± 7 years, 54% ♀). Imaging markers of small vessel disease and neurodegeneration, cerebral blood flow (CBF) and brain perfusion (BP) were acquired from brain MRI (1.5-tesla). General cognitive function was assessed by Mini-Mental State Examination and the g-factor. Multiple linear and logistic regression models were used for liver-brain associations and adjusted for age, sex, intracranial volume, cardiovascular risk factors and alcohol use.

Results: Higher gamma-glutamyltransferase (GGT) levels were significantly associated with smaller total brain volume (TBV, standardized mean difference (SMD), −0.02, 95% confidence interval (CI) (−0.03 to −0.01); $p = 8.4{\cdot}10^{-4}$), grey matter volumes, and lower CBF and BP. Liver serum measures were not related to small vessel disease markers, nor to white matter microstructural integrity or general cognition. Participants with ultrasound-based liver steatosis had a higher fractional anisotropy (FA, SMD 0.11, 95% CI (0.04 to 0.17), $p = 1.5{\cdot}10^{-3}$) and lower CBF and BP. MAFLD and NAFLD phenotypes were associated with alterations in white matter microstructural integrity (NAFLD ~ FA, SMD 0.14, 95% CI (0.07 to 0.22), $p = 1.6{\cdot}10^{-4}$; NAFLD ~ mean

Abbreviations: 15-WLT, 15-word verbal learning test; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, brain perfusion; CARDIA, coronary artery risk development in young adults; CBF, cerebral blood flow; CSF, cerebrospinal fluid; CSVD, cerebral small vessel disease; FA, fractional anisotropy; GGT, gamma-glutamyltransferase; GM, grey matter; ICV, intracranial volume; LDST, letter-digit-substitution task; LSM, liver stiffness measurements; MAFLD, metabolic dysfunction-associated fatty liver disease; MD, mean diffusivity; MMSE, mini-mental state examination; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; RS, Rotterdam Study; TBV, total brain volume; TE, transient elastography; WFT, word fluency test; WM, white matter; WMH, white matter hyperintensities.

diffusivity, SMD −0.12, 95% CI (−0.18 to −0.05), $p = 4.7{\cdot}10^{-4}$) and also with lower CBF and BP (MAFLD ~ CBF, SMD −0.13, 95% CI (−0.20 to −0.06), $p = 3.1{\cdot}10^{-4}$; MAFLD ~ BP, SMD −0.12, 95% CI (−0.20 to −0.05), $p = 1.6{\cdot}10^{-3}$). Furthermore, fibrosis phenotypes were related to TBV, grey and white matter volumes.

Conclusions: Presence of liver steatosis, fibrosis and elevated serum GGT are associated with structural and hemodynamic brain markers in a population-based cross-sectional setting. Understanding the hepatic role in brain changes can target modifiable factors and prevent brain dysfunction.

KEYWORDS
brain, fibrosis, liver, MRI, non-alcoholic fatty liver disease

1 | INTRODUCTION

Liver disease is increasing worldwide and metabolic dysfunction-associated fatty liver disease (MAFLD), formerly named non-alcoholic fatty liver disease (NAFLD), is a growing cause contributing to chronic liver disease globally.[1–3] Increasing evidence suggests that liver disease may affect brain health and that several mechanisms are considered and constantly evolving.[4,5] While impaired liver function leads to insufficient detoxification and allows neurotoxins to the cerebral circulation, shared risk factors of cardiovascular health and metabolic comorbidities can play a particular role in the relation of NAFLD and brain function.[2,4–6] Other possible mechanisms of chronic liver disease and brain dysfunction are increase of several neurotoxins, increased permeability of blood–brain barrier, microglial activation and neuroinflammation.[4,7] Since liver-brain relations are mainly studied in disease-affected populations, it is important to elucidate underlying mechanisms in the general population to target modifiable factors and prevent brain dysfunction.

Global cognitive deficits like hepatic encephalopathy and Morbus Korsakoff are known to occur in advanced (alcoholic) liver disease.[4] Yet, much less is known on the effects of subclinical liver traits on cognitive functioning. Previously, elevated gamma-glutamyltransferase (GGT) was described as a potential risk factor for cognitive decline and dementia by its pro-oxidant and pro-inflammatory nature which may implicate oxidative stress in the vascular pathway.[8–10] Likewise, decreased alanine aminotransferase (ALT) serum levels and increased aspartate aminotransferase (AST) to ALT ratio values were observed in patient with Alzheimer's disease and those with lower scores of memory and executive function.[11] Within the general population, two recent studies described that having NAFLD was associated with lower cognitive performance and participants with NAFLD and a high risk for liver fibrosis had a poorer cognitive performance than those with low risk.[12,13]

Other studies have argued that NAFLD is associated with structural brain changes on non-invasive imaging, in particular smaller total brain volume (TBV), lower brain perfusion, and white matter hyperintensities (WMH).[14–18] More manifestations of cerebral small vessel disease (CSVD) besides WMH, like lacunes are linked to increased serum GGT and having NAFLD.[19,20] In fulminant hepatic failure and in some patients with cirrhosis, cerebral blood flow (CBF) autoregulation is impaired, but

Key points

- In a population-based cross-sectional setting, higher gamma-glutamyltransferase serum levels were associated with lower total brain and grey matter volumes and with lower cerebral blood flow and perfusion.
- Participants with liver steatosis had altered microstructural white matter integrity and lower cerebral perfusion. Higher liver stiffness, as a proxy for fibrosis, was related to lower total brain, grey and white matter volumes.
- Subclinical liver traits are associated with neurodegenerative and vascular brain impairment.

Lay summary

Within the population-based Rotterdam study, we found that having a fatty liver or liver stiffness measured on liver images and elevated liver enzymes in the blood are related to structural and blood flow markers in the brain. These findings were independent of various cardiovascular factors, lifestyle factors, metabolic syndrome and alcohol consumption in 3493 participants. Impaired liver function may affect brain dysfunction in the general population, to identify this in an early stage could target modifiable factors and contribute to prevent brain dysfunction.

underlying mechanisms are largely unknown.[21] Recently, within healthy young adults higher serum ALT was related to perfusion alterations in certain brain regions using multimodal magnetic resonance imaging (MRI).[22] Nevertheless, little is known on how liver traits relate to microstructural and hemodynamic markers in the brain in community-dwelling individuals. Moreover, this is the first study to assess MAFLD and these brain measures. We performed a comprehensive assessment of liver and brain traits in a cross-sectional setting within the Rotterdam Study, we assessed the associations of serum liver enzymes, hepatic steatosis detected by ultrasound and transient elastography (TE) measures of the liver with imaging markers of CSVD and neurodegeneration, CBF and brain perfusion and cognitive measures. Additionally, we defined phenotypes of MAFLD, NAFLD and elevated liver stiffness (fibrosis) in our population-based setting to examine these phenotypes with structural and hemodynamic brain MRI measures, and cognitive status.

2 | MATERIALS AND METHODS

2.1 | Setting and study population

The Rotterdam Study (RS) is a population-based cohort study, originated in 1990 and including 14 926 participants aged ≥45 years who are examined every 3–4 years and followed up for causes and determinants of diseases in the Netherlands.[23] Brain MRI was incorporated in the RS from August 2005 onwards.[24]

For the purpose of our cross-sectional study, we included hepatic examination with blood sampling, abdominal ultrasound and TE, brain MRI and neuropsychological tests recruited during the period of 2009–2014. The average time interval between liver assessments/ neuropsychological tests and brain MRI assessments was 1.4 months. Blood samples were available for 5967 out of 14 926 participants, and 5764 had complete measurements for liver blood samples and steatosis on ultrasound (Figure S1). Participants without informed consent (n = 39) and diagnosed with stroke (n = 171) or dementia (n = 40) at time of MRI scan were excluded. Furthermore, we excluded participants without MRI invitation, incomplete or insufficient quality scans for rating (n = 1932), and those with MRI-defined cortical infarcts (n = 89). The total study population resulted in 3493 stroke-free and non-demented participants with complete liver blood samples, liver ultrasonography and TE data, and structural brain imaging available for analyses. Additionally, we defined three subgroups, according to definitions as described in the next paragraph (see also flowchart, Figure S1): MAFLD (N = 3493), NAFLD (N = 2938) and fibrosis (N = 2252).

The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) according to the Population Study Act, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.

2.2 | Liver function measures and diagnostic phenotypes

Fasting blood samples were collected, including AST, ALT, GGT using automatic enzyme procedures (Roche Diagnostic GmbH). The trained nurse ultrasonographer carried out abdominal ultrasound (Hitachi HI VISION 900) and liver stiffness measurements (LSM) using TE (Fibroscan®, EchoSens). Images derived from abdominal ultrasound were stored digitally and reviewed by two expert hepatologists.

Hepatic steatosis was diagnosed according to the protocol of Hamaguchi et al,[25] and dichotomized as either presence or absence of hyper-echogenic liver parenchyma as compared to right kidney parenchyma. LSM were performed on the right lobe of the liver in between costae, while participant was lying flat on the back with the right arm in maximal abduction. We excluded participants with intracardiac devices and physical disability hampering the examination. If there was no valid LSM record after 10 attempts, then LSM were regarded to have failed and excluded. Reliability criteria of Boursier et al. were applied; hence, LSM with an interquartile range/ median LSM > 0.3 kPa with median LSM ≥ 7.1 kPa were considered poorly reliable and excluded.[26]

For the subgroup MAFLD, we included participants with the presence of hepatic steatosis on ultrasound and having a body mass index (BMI) ≥25 kg/m^2, diabetes mellitus or ≥2 of metabolic abnormalities.[3] Metabolic abnormalities included (1) waist circumference ≥88 cm (♀) or ≥102 cm (♂); (2) blood pressure ≥130/85 mm Hg or blood pressure–lowering medication; (3) serum triglycerides ≥1.70 mmol/L or lipid-lowering medication; (4) high-density lipoprotein (HDL) cholesterol <1.3 mmol/L (♀) or <1.0 mmol/L (♂) or lipid-lowering medication; (5) prediabetes defined as fasting serum glucose of 5.6–6.9 mmol/L; (6) homeostatic model assessment of insulin resistance (HOMA) of ≥ 2.5. Unfortunately, the minor MAFLD criteria on C-reactive protein levels were unavailable. Presence of NAFLD was defined as having hepatic steatosis on ultrasound in the subgroup analysis after excluding participants with excessive alcohol use (n = 440), viral hepatitis (n = 27) and use of steatogenic medication (n = 88). For the fibrosis assessment, we excluded participants without LSM (n = 1006) and poorly reliable or failed transient elastography measures (n = 235), we used a cut-off of LSM ≥ 8 kPa as a proxy for clinically significant liver fibrosis.[27]

2.3 | Brain MRI protocol and focal, volumetric and microstructural markers

Participants were scanned on a 1.5-tesla MRI scanner (GE-Healthcare). We performed four high-resolution sequences: T1-weighted sequence, proton density-weighted sequence, fluid-attenuated inversion recovery sequence and T2*-weighted gradient-recalled-echo sequence. More details of the imaging protocol have been described elsewhere.[24] Automated tissue segmentation was used to quantify supratentorial grey matter (GM), white matter (WM), WMH and cerebrospinal fluid (CSF).[28] Tissue segmentations were visually inspected

and manually corrected if needed. Intracranial volume (ICV) was calculated as the sum of GM, WM, and CSF. TBV was the sum of GM, normal-appearing WM, and WMH volumes. Hippocampal volume was automatically segmented using FreeSurfer software (version 6.0).[29] We summed up right and left hippocampal volumes for total hippocampal volume. Presence and number of cortical infarcts, lacunes and microbleeds were rated by trained research physicians.[24] Focal lesions with tissue loss involving cortical GM were defined as cortical infarcts. Subcortical lesions between ≥3 mm and <15 mm were rated as lacunes. Microbleeds were determined as focal round to ovoid areas <10 mm of low signal intensity on T2*-weighted imaging. Diffusion-weighted echo-planar imaging sequence was embedded in the RS protocol from March 2006 onwards. For the 2D diffusion tensor imaging (DTI) scan, we used a *b*-value of 1000 s/mm^2 applied in 25 directions, and voxel size of 3.3×2.2×3.5 mm^3.[24] All DTI data were pre-processed using a standardized pipeline which includes correction for subject motion and eddy currents.[30] Afterwards, diffusion tensors were estimated from which fractional anisotropy (FA) and mean diffusivity (MD) were determined. By registering the diffusion data to the tissue segmentation global measures of FA and MD in normal-appearing WM were obtained. Lower FA and higher MD are regarded indicative of lower white matter microstructural integrity.

2.4 | Cerebral blood flow and brain perfusion

For CBF measurement, 2D phase-contrast imaging was performed with MRI and calculated using interactive data language-based custom software (Cinetool version 4, GE-Healthcare).[31] No contrast agents were administered. Total CBF (in mL/min) was computed by adding up flow rates for carotid arteries and basilar artery and multiplying by 60 s/min. Total brain perfusion (in mL/min per 100 mL) was calculated by dividing CBF (mL/min) by each individual's brain volume (mL) and multiplying by 100.

2.5 | Cognitive Functioning

Mini-Mental State Examination (MMSE), letter-digit-substitution task (LDST), word fluency test (WFT), Stroop test, 15-word verbal learning test (15-WLT) and Perdue Pegboard test were assessed during each research visit.[32] For global cognition, we computed a standardized composite score (g-factor) with principal component analysis on the error-adjusted Stroop interference subtask, LDST, WFT, delayed recall of the 15-WLT, and Perdue Pegboard. The g-factor explained 50.2% of the total variance in cognitive test scores in our population.

2.6 | Covariates

During center visits participants were interviewed and underwent laboratory and physical examinations for information on demographic, genetic and cardiovascular risk factors.

Hypertension, diabetes mellitus, blood pressure- and lipid-lowering medications, body mass index, alcohol, smoking, education, serum total cholesterol, and serum high-density lipoprotein cholesterol, were of interest for this study. Definitions of (additional) covariates and other characteristics are presented in the Appendix S1.

2.7 | Statistical analysis

Descriptive statistics were stratified by presence of steatosis on ultrasound in the total population. LSM and WMH were natural log-transformed because of their skewed distribution. Measures for liver enzymes, log-transformed LSM, brain volumes, DTI, CBF and brain perfusion were standardized to *Z*-scores. Presence of steatosis, MAFLD, NAFLD, fibrosis, lacunes and microbleeds were investigated dichotomously. MMSE and g-factor were investigated continuously. Missing covariate data were imputed using 10-fold multiple imputation based on determinants, outcomes, and covariates included in the models. Distribution of covariates in imputed and non-imputed datasets showed no differences. We used multivariable linear regression to determine the association between liver enzymes, liver ultrasound measures and diagnostic phenotypes (MAFLD, NAFLD and fibrosis) with structural brain measures, CBF and brain perfusion and cognitive functioning. Logistic regression was used to study relations of liver measures and diagnostic phenotypes with presence of lacunes and microbleeds. Pooled regression coefficients and 95% CIs were obtained with Rubin's method.[33]

All models were corrected for age, sex and ICV (model 1). Additionally, we took into account hypertension, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, use of lipid lowering medication, body mass index, smoking status and alcohol consumption (model 2). All analyses involving GM and WM volumes were adjusted for each other. For DTI measures we also corrected for normal-appearing WM volume and log-transformed WMH. Analyses involving cognitive functioning were further adjusted for education level. Assumptions for linear and logistic regression were met. We explored non-linear effects of age in our models by adding age-squared and 3 splines. Furthermore, we studied above-mentioned associations between liver and brain measures with additional adjustments for lifestyle factors (dietary quality, energy intake, smoking, alcohol consumption, and physical activity) and metabolic syndrome (see Appendix S1).[34]

Sensitivity analyses (see Appendix S1) were performed by repeating descriptive statistics stratified by presence of MAFLD, presence of NAFLD and presence of fibrosis in each subgroup. We categorized liver enzymes in normal and abnormal values, namely <within normal limits and ≥1× upper limits of normal and used a more strict definition of ALT. In addition, subgroup analysis was performed among persons with presumably alcoholic fatty liver disease, defined as ≥3 units/day for men and ≥2 units/day for women (N = 395). Also, we analyzed if excluding alcohol consumption as a

potential confounder influenced the primary analysis between liver and brain measures.

Since several outcomes may be correlated, we used permutation testing to assess independence of outcomes for the brain imaging markers and cognitive function. Multiple testing thresholds have been calculated through 10 000 permutations, namely $p < 4.58 \times 10^{-3}$ for independent brain markers and cognitive function and $p < 7.70 \times 10^{-4}$ based on independent liver and brain markers and cognitive function.

All analyses were conducted using statistical software packages SPSS (version 24.0) and R (version 3.5.2).

3 | RESULTS

Population characteristics are presented in Table 1. Of the 3493 participants, 56.3% were women, mean age was 68.9 years (SD 8.8) and 34.3% had steatosis on ultrasound. Compared to participants with no steatosis, participants with steatosis had significantly higher presence of diabetes mellitus, hypertension and metabolic syndrome, used more blood pressure-lowering and lipid-lowering medications, had excessive alcohol use and lower education, and presented with elevated serum triglycerides, glucose, HOMA, liver enzymes, liver stiffness and fibrosis, adjusted for age and sex.

The following sections provide results grouped by liver traits and phenotypes in relation to structural and hemodynamic brain MRI measures, and cognitive status.

3.1 | Liver serum markers

Serum GGT was associated with all measured brain volumes in model 1 and overall associations attenuated after adjustments in model 2. The associations between serum GGT and TBV and GM volumes survived multiple testing and showed mean differences per SD incremental of GGT of −0.02, 95% CI (−0.03 to −0.01) and −0.03, 95% CI (−0.04 to −0.01), respectively (Figure 1 and Table S3A). No associations were found between liver serum markers and CSVD and microstructural brain markers (Figure 1, Table 2 and Table S3B).

All liver enzymes were associated to lower CBF and brain perfusion (Table 3). Only the associations with GGT remained significant in model 2 after correction for multiple testing.

Overall, the liver enzymes showed decreases in MMSE and g-factor, yet none survived the multiple testing thresholds (Figure 2).

3.2 | Liver ultrasound measures

We observed significant lower TBV, GM and WM volumes with the presence of steatosis and increase in LSM in model 1 which attenuated in model 2 (Figure 1 and Table S3A). Presence of liver steatosis was associated with higher FA (Figure 1 and Table S3B). Steatosis on ultrasound was also related to lower cerebral hemodynamics (Table 3). No associations were seen for LSM and microstructural markers, and brain blood flow and perfusion (Figure 1 and Table 3). Steatosis and LSM were not associated with CSVD markers and cognitive function (Table 2 and Figure 2).

3.3 | Diagnostic phenotypes

Overall, MAFLD phenotypes were associated with lower brain volumes and only TBV, GM and WM volumes remained significant in model 2 (Figure 1 and Table S3A). Except for GM volume, NAFLD phenotypes were associated with all other volumetric measures in model 1 which attenuated in model 2 (Figure 1 and Table S3A). The fibrosis phenotypes were related to all volumes besides hippocampal volume and remained significant surviving multiple testing thresholds for TBV, GM and WM in model 2. The presence of MAFLD and NAFLD resulted in significant higher FA and lower MD in model 2 (Figure 1 and Table S3B). MAFLD and NAFLD phenotypes were also associated with lower CBF and brain perfusion in model 1 (Table 3). The presence of fibrosis was related to a lower g-factor in model 1 and attenuated in model 2, yet neither of the phenotypes were related to MMSE (Figure 2).

None of the phenotypes were associated with CSVD markers (Table 2). We also observed no associations for those with fibrosis and DTI measures, and CBF and perfusion (Figure 1, Table 3 and Table S3B).

3.4 | Sensitivity and subgroup analyses

After adjusting for lifestyle factors, we only found a stronger association of AST with WMH ($p = 0.003$). Other results did not alter after adjustments for lifestyle factors. The liver–brain associations remained similar to the primary analysis when adjusted for metabolic syndrome.

Similar associations as above were found after categorizing liver enzymes in normal (<within normal limits) and abnormal (≥1 x upper limits of normal) values with volumetric measures (Table S4). The associations between abnormal levels of serum GGT and volumes of total brain, GM and WM even strengthened. For DTI measures, we found that abnormal values of serum AST and the strict cut-off of ALT were associated with higher fractional anisotropy, but none survived the multiple testing thresholds (Table S5). In contrary to the primary analysis, though not significant, abnormal values of serum GGT showed lower FA and higher MD, reflecting worse global microstructural integrity. Within abnormal values of all liver enzymes, comparable results with lower total cerebral blood flow and brain perfusion were seen (Table S6).

For the analysis of alcoholic fatty liver disease, none of the associations were significant in 395 participants. Finally, excluding adjustments for alcohol consumption did not influence the primary analysis between liver and brain measures (data not shown).

TABLE 1 Characteristics of the study population by steatosis status.

Characteristic	Total N = 3493	No steatosis n = 2294	Steatosis n = 1199
Age at blood sample, years	68.8 (8.8)	68.6 (9.0)	69.1 (8.2)
Age at MRI scan, years	68.9 (8.8)	68.7 (9.0)	69.2 (8.2)
Female sex, *n*	1965 (56.3)	1330 (58.0)*	635 (53.0)*
History of diabetes mellitus, *n*	411 (11.8)	147 (6.4)*	264 (22.0)*
History of viral hepatitis, *n*	27 (0.8)	14 (0.6)	13 (1.1)
Hypertension[a], *n*	2488 (71.3)	1506 (65.6)*	982 (82.0)*
Blood pressure-lowering medication, *n*	1493 (42.7)	863 (37.6)*	630 (52.5)*
Serum lipid-lowering medication, *n*	999 (28.6)	579 (25.2)*	420 (35.0)*
Steatogenic medication use, *n*	88 (2.5)	57 (2.5)	31 (2.6)
Body mass index[a], kg/m^2	27.3 (4.0)	26.0 (3.4)*	29.6 (4.0)*
Alcohol[a], units/week	0.6 (0.09–1.6)	0.6 (0.08–1.6)*	0.5 (0.08–1.8)*
Excessive alcohol ue[a], *n*	395 (12.6)	232 (11.3)*	163 (15.0)*
Smoking status[a], *n*			
Never	1176 (33.7)	813 (35.5)*	363 (30.3)*
Current	505 (14.5)	346 (15.1)	159 (13.3)
Former	1805 (51.8)	1131 (49.4)*	674 (56.4)*
Education[a]			
Low	946 (27.4)	597 (26.3)*	349 (29.6)*
Intermediate	1681 (48.8)	1080 (47.7)	601 (50.9)
High	820 (23.8)	589 (26.0)*	231 (19.6)*
Biochemistry and liver imaging			
Aspartate transaminase, U/L	24.0 (21.0–28.0)	24.0 (21.0–28.0)*	25.0 (21.0–29.0)*
Alanine aminotransferase, U/L	19.0 (15.0–24.0)	17.0 (14.0–22.0)*	22.0 (17.0–29.0)*
Gamma-glutamyltransferase, U/L	24.0 (17.0–35.0)	21.0 (16.0–30.0)*	29.0 (21.0–42.0)*
Total cholesterol[a], mmol/L	5.5 (1.1)	5.5 (1.1)*	5.4 (1.1)*
HDL cholesterol[a], mmol/L	1.5 (0.4)	1.6 (0.4)*	1.3 (0.4)*
Steatosis on ultrasound, *n*	1199 (34.3)	–	–
Liver stiffness measurements[b], kPa	4.8 (3.9–6.1)	4.7 (3.8–5.8)*	5.2 (4.2–6.8)*
Liver stiffness measurements ≥8 kPa[b], *n*	153 (5.6)	64 (3.5)*	89 (10.1)*
Cerebral small vessel disease markers			
WMH volume, mL	3.6 (1.9–7.5)	3.5 (1.8–7.4)	3.8 (2.0–8.2)
Lacunes, *n*	296 (8.5)	184 (8.0)	112 (9.3)
Cerebral microbleeds, *n*	791 (22.6)	544 (23.7)*	247 (20.6)*
Volumetric markers			
Intracranial volume, mL	1136.6 (114.4)	1140.3 (114.4)*	1129.6 (114.1)*
Total brain volume, mL	925.6 (96.5)	930.8 (96.9)*	915.7 (95.0)*
Grey matter volume, mL	525.9 (54.0)	527.4 (54.4)*	523.2 (53.0)*
NAWM volume, mL	392.6 (58.8)	396.4 (58.7)*	385.3 (58.3)*
Hippocampal volume[c], mL	7.7 (0.8)	7.8 (0.8)*	7.7 (0.9)*
Microstructural markers			
Fractional anisotropy[c]	0.34 (0.02)	0.34 (0.02)	0.34 (0.02)
Mean diffusivity[c], ($\times 10^{-3}$ mm^2/s)	0.75 (0.03)	0.75 (0.03)	0.75 (0.03)
Cerebral blood flow and perfusion			
Total cerebral blood flow[c], (mL/min)	512.6 (95.3)	520.4 (96.2)*	497.5 (91.8)*
Total brain perfusion[c], (mL/min per 100 mL)	55.5 (9.4)	56.1 (9.6)*	54.4 (9.0)*

TABLE 1 (Continued)

Characteristic	Total N = 3493	No steatosis n = 2294	Steatosis n = 1199
Cognitive measures			
Mini Mental State Exam[c]	29.0 (27.0–29.0)	29.0 (27.0–29.0)	28.0 (27.0–29.0)
G-factor[c], standardized	0.0 (1.0)	0.04 (1.0)*	−0.08 (1.0)*

Note: Non-imputed values are means (SD), median (25th–75th percentiles) or numbers (valid percentages).

Abbreviations: HDL, high-density lipoprotein; MRI, magnetic resonance imaging; NAWM, normal-appearing white matter; WMH, white matter hyperintensities.

[a]Missing values in the total population ≤11%.

[b]Missing values for LSM are 19.8%.

[c]Missing values for hippocampal volume ($n = 56$), fractional anisotropy and mean diffusivity ($n = 63$), cerebral blood flow and brain perfusion ($n = 17$), Mini Mental State Exam ($n = 12$) and g-factor ($n = 658$).

*Significant difference ($p < 0.05$) between persons with and without steatosis adjusted for age and sex.

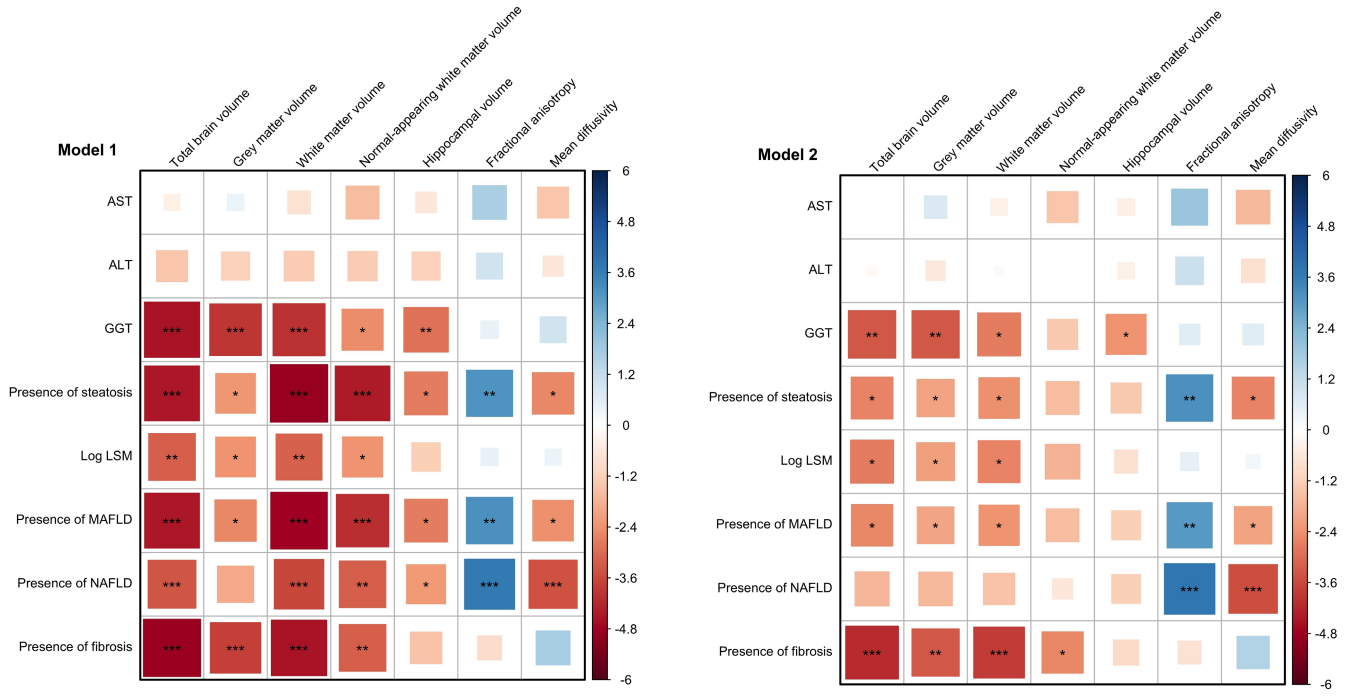


FIGURE 1 Heat maps of associations between liver measures and diagnostic phenotypes and volumetric and microstructural brain markers. ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease. Model 1: adjusted for age, sex and intracranial volume (ICV). Model 2: adjusted for age, sex, ICV, hypertension, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, body mass index, smoking status and alcohol intake. Missing data for hippocampal volume and fractional anisotropy/mean diffusivity in total study population and subgroup for MAFLD[⁋] assessment, subgroup for NAFLD[‡] assessment and subgroup for LSM and fibrosis[†] assessments: $n = 56$ and $n = 63$; $n = 49$[‡] and $n = 55$[‡]; $n = 35$[†] and $n = 34$[†], respectively. [⁋]MAFLD derived from the subgroup of $N = 3437$ participants for hippocampal volume in which $n = 1145$ had MAFLD and $N = 3430$ for fractional anisotropy and mean diffusivity in which $n = 1146$ had MAFLD. [‡]NAFLD derived from the subgroup of $N = 2889$ participants for hippocampal volume and $N = 2883$ for fractional anisotropy and mean diffusivity in which $n = 960$ had steatosis on ultrasound. [†]For hippocampal volume $N = 2217$ had LSM with $n = 110$ participants having LSM ≥ 8 kPa and for fractional anisotropy and mean diffusivity $N = 2218$ participants had LSM and $n = 104$ had LSM ≥8 kPa. Colours and sizes of squares correspond to t values, blue indicates positive and red indicates negative associations. Larger squares indicate stronger associations. Significance levels: *$p < 0.05$; **$p < 4.58$ e-3; ***$p < 7.70$ e-4.

4 | DISCUSSION

We found that subclinical liver traits are associated with structural and hemodynamic imaging markers in the brain. Higher gamma-glutamyltransferase levels, were related to smaller total and grey matter volumes, lower cerebral blood flow and brain perfusion. Ultrasound-based liver steatosis was associated with an increased fractional anisotropy, and lower cerebral blood flow and

TABLE 2 Associations of liver enzymes, liver ultrasound measures and diagnostic phenotypes with small vessel disease brain markers.

	Log WMH standardized mean difference (95% CI)	Lacunes odds ratio (95% CI)	Cerebral microbleeds odds ratio (95% CI)
Liver enzymes, per SD increase			
AST			
Model 1	0.04 (0.01 to 0.06)*	1.03 (0.92 to 1.14)	1.07 (0.99 to 1.15)
Model 2	0.03 (0.01 to 0.06)*	1.02 (0.91 to 1.14)	1.06 (0.99 to 1.15)
ALT			
Model 1	0.03 (0.01 to 0.06)*	1.01 (0.90 to 1.15)	0.99 (0.90 to 1.07)
Model 2	0.03 (−0.00 to 0.05)	1.00 (0.88 to 1.14)	0.99 (0.90 to 1.07)
GGT			
Model 1	0.02 (−0.00 to 0.05)	1.02 (0.91 to 1.14)	1.03 (0.96 to 1.12)
Model 2	0.01 (−0.01 to 0.04)	1.01 (0.90 to 1.14)	1.04 (0.96 to 1.13)
Liver ultrasound measures			
Steatosis (yes/no)			
Model 1	0.08 (0.02 to 0.14)*	1.19 (0.92 to 1.52)	0.82 (0.68 to 0.97)*
Model 2	0.04 (−0.02 to 0.10)	1.16 (0.87 to 1.54)	0.81 (0.66 to 0.99)*
Log LSM[a], per SD increase			
Model 1	0.01 (−0.02 to 0.05)	0.98 (0.82 to 1.19)	0.96 (0.85 to 1.08)
Model 2	−0.00 (−0.04 to 0.03)	0.96 (0.80 to 1.16)	0.93 (0.83 to 1.06)
Diagnostic phenotypes (yes/no)			
MAFLD[b], $n = 1169$			
Model 1	0.09 (0.03 to 0.14)**	1.19 (0.93 to 1.53)	0.81 (0.68 to 0.96)*
Model 2	0.04 (−0.02 to 0.11)	1.15 (0.86 to 1.54)	0.79 (0.65 to 0.97)*
NAFLD[c], $n = 981$			
Model 1	0.07 (0.01 to 0.14)*	1.19 (0.90 to 1.57)	0.82 (0.68 to 0.99)*
Model 2	0.03 (−0.04 to 0.10)	1.15 (0.84 to 1.58)	0.83 (0.66 to 1.03)
Fibrosis[a], $n = 110$			
Model 1	0.04 (−0.10 to 0.19)	1.72 (0.93 to 3.13)	0.89 (0.54 to 1.45)
Model 2	−0.03 (−0.18 to 0.11)	1.67 (0.90 to 3.06)	0.79 (0.48 to 1.31)

Note: Model 1: adjusted for age, sex and intracranial volume (ICV). Model 2: adjusted for age, sex, ICV, hypertension, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, body mass index, smoking status and alcohol intake.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; SD, standard deviation; WMH, white matter hyperintensities.

[a]LSM and fibrosis (LSM ≥ 8 kPa) derived from the subgroup of 2252 participants after excluding unavailable, poorly reliable and failed measures.

[b]MAFLD defined as the presence of hepatic steatosis on ultrasound and having a body mass index ≥25 kg/m^2, diabetes mellitus or ≥2 of metabolic abnormalities.

[c]NAFLD derived from the subgroup of 2938 participants after excluding excessive alcohol use, viral hepatitis and steatogenic medication use.

*$p < .05$; **$p < 4.58$ e-3.

perfusion. Participants with MAFLD and NAFLD also showed alterations in microstructural white matter integrity and those with fibrosis had lower volumes of total brain, grey matter and white matter.

Of all liver serum measures we studied, GGT was most evidently related to brain measures. In a recent population-based study, the highest GGT tertile was independently related to presence of silent brain infarcts.[19] Contrariwise, we did not find associations between serum liver markers and CSVD markers, compared to the previous mentioned study we only adjust for more cardiovascular factors. Three previous studies have described an increased risk of dementia with higher GGT levels in patient and cohort settings.[9,35,36] Mechanisms for these findings include inflammation and oxidative stress, common pathways for cardiovascular and cerebrovascular diseases.[36] GGT has been noted an independent marker of cardiovascular risk involving these mechanisms and along ALT

TABLE 3 Liver enzymes, liver ultrasound measures and diagnostic phenotypes with total cerebral blood flow and total brain perfusion.

	Total cerebral blood flow, mL/min	Total brain perfusion, mL/min per 100 mL
Standardized mean difference (95% CI)		
Liver enzymes, per SD increase		
AST		
Model 1	−0.04 (−0.07 to −0.01)**	−0.05 (−0.08 to −0.02)**
Model 2	−0.04 (−0.07 to −0.01)*	−0.04 (−0.08 to −0.01)*
ALT		
Model 1	−0.06 (−0.09 to −0.03)***	−0.06 (−0.10 to −0.03)***
Model 2	−0.04 (−0.07 to −0.01)*	−0.05 (−0.08 to −0.01)*
GGT		
Model 1	−0.09 (−0.12 to −0.06)***	−0.08 (−0.11 to −0.05)***
Model 2	−0.07 (−0.10 to −0.04)***	−0.06 (−0.10 to −0.03)***
Liver ultrasound measures		
Steatosis (yes/no)		
Model 1	−0.18 (−0.24 to −0.12)***	−0.17 (−0.24 to −0.10)***
Model 2	−0.12 (−0.19 to −0.05)**	−0.11 (−0.19 to −0.04)**
Log LSM[a], per SD increase		
Model 1	−0.04 (−0.09 to −0.00)*	−0.03 (−0.08 to 0.01)
Model 2	−0.03 (−0.07 to 0.01)	−0.02 (−0.07 to 0.02)
Diagnostic phenotypes (yes/no)		
MAFLD[b], $n = 1162$		
Model 1	−0.19 (−0.25 to −0.13)***	−0.18 (−0.25 to −0.11)***
Model 2	−0.13 (−0.20 to −0.06)***	−0.12 (−0.20 to −0.05)**
NAFLD[c], $n = 977$		
Model 1	−0.17 (−0.24 to −0.10)***	−0.16 (−0.24 to −0.09)***
Model 2	−0.11 (−0.19 to −0.03)*	−0.11 (−0.19 to −0.02)*
Fibrosis[a], $n = 109$		
Model 1	−0.19 (−0.36 to −0.01)*	−0.10 (−0.28 to 0.08)
Model 2	−0.12 (−0.29 to 0.05)	−0.04 (−0.22 to 0.15)

Note: Model 1: adjusted for age, sex and intracranial volume (ICV). Model 2: adjusted for age, sex, ICV, hypertension, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, body mass index, smoking status and alcohol intake.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease.

[a]LSM and fibrosis (LSM ≥ 8 kPa) derived from the subgroup of 2252 participants after excluding unavailable, poorly reliable and failed measures.

[b]MAFLD defined as the presence of hepatic steatosis on ultrasound and having a body mass index ≥25 kg/m^2, diabetes mellitus or ≥2 of metabolic abnormalities.

[c]NAFLD derived from the subgroup of 2938 participants after excluding excessive alcohol use, viral hepatitis and steatogenic medication use. Missing data for CBF and brain perfusion in total study population and MAFLD assessment ($n = 17$)[b], subgroup for NAFLD assessment ($n = 14$)[c], and subgroup for LSM and fibrosis assessments ($n = 9$)[a].

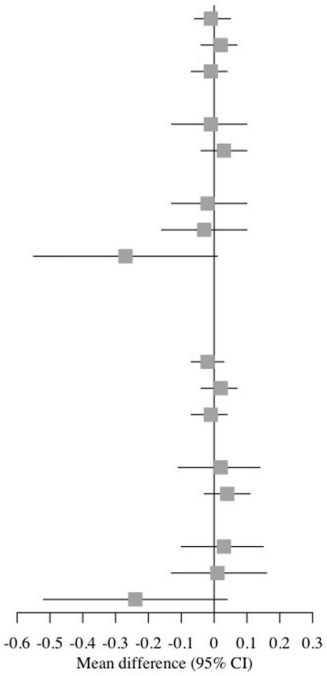
*$p < 0.05$; **$p < 4.58$ e-3; ***$p < 7.70$ e-4.

was described as another independent marker.[37] Lower ALT levels were found in participants with Alzheimer's disease compared with normal controls in the Alzheimer's Disease Neuroimaging Initiative Study.[11] We only found a stronger association of AST with WMH after adjusting for lifestyle factors compared to our primary analysis. Additional sensitivity analyses with a more strict cut-off for ALT and categorizing liver enzymes, showed strengthened associations between GGT and TBV, GM and WM volumes.

In this study, we show that associations of serum GGT, ultrasound-based steatosis and MAFLD phenotypes with CBF remained significant after further adjustments. Similar findings were seen for brain perfusion. To our knowledge only two other

Panel (A) Mini Mental State Examination

	Number of participants N	Mean differences (95% CI)
Model 1		
Liver enzymes, per SD increase		
AST	3481	-0.01 (-0.06; 0.05)
ALT	3481	0.02 (-0.04; 0.07)
GGT	3481	-0.01 (-0.07; 0.04)
Liver ultrasound measures		
Presence of steatosis (yes/no), n=1195	3481	-0.01 (-0.13; 0.10)
Log LSM, per SD increase	2244	0.03 (-0.04; 0.10)
Diagnostic phenotypes (yes/no)		
Presence of MAFLD, n=1165	3481	-0.02 (-0.13; 0.10)
Presence of NAFLD, n=977	2926	-0.03 (-0.16; 0.10)
Presence of fibrosis, n=110	2244	-0.27 (-0.55; 0.01)
Model 2		
Liver enzymes, per SD increase		
AST	3481	-0.02 (-0.07; 0.03)
ALT	3481	0.02 (-0.04; 0.07)
GGT	3481	-0.01 (-0.07; 0.04)
Liver ultrasound measures		
Presence of steatosis (yes/no), n=1195	3481	0.02 (-0.11; 0.14)
Log LSM, per SD increase	2244	0.04 (-0.03; 0.11)
Diagnostic phenotypes (yes/no)		
Presence of MAFLD, n=1165	3481	0.03 (-0.10; 0.15)
Presence of NAFLD, n=977	2926	0.01 (-0.13; 0.16)
Presence of fibrosis, n=110	2244	-0.24 (-0.52; 0.04)



Panel (B) G-factor

	Number of participants N	Mean difference (95% CI)
Model 1		
Liver enzymes, per SD increase		
AST	2835	-0.02 (-0.06; 0.01)
ALT	2835	0.00 (-0.03; 0.03)
GGT	2835	-0.01 (-0.04; 0.02)
Liver ultrasound measures		
Presence of steatosis (yes/no), n=961	2835	-0.05 (-0.12; 0.01)
Log LSM, per SD increase	1888	-0.02 (-0.06; 0.02)
Diagnostic phenotypes (yes/no)		
Presence of MAFLD, n=937	2835	-0.06 (-0.12; 0.00)
Presence of NAFLD, n=777	2361	-0.05 (-0.12; 0.02)
Presence of fibrosis, n=95	1888	-0.26 (-0.43; -0.10)**
Model 2		
Liver enzymes, per SD increase		
AST	2835	-0.03 (-0.07; 0.00)
ALT	2835	0.01 (-0.02; 0.03)
GGT	2835	0.00 (-0.03; 0.03)
Liver ultrasound measures		
Presence of steatosis(yes/no), n=961	2835	0.02 (-0.04; 0.09
Log LSM, per SD increase	1888	-0.01 (-0.04; 0.03)
Diagnostic phenotypes (yes/no)		
Presence of MAFLD, n=937	2835	0.03 (-0.04; 0.10)
Presence of NAFLD, n=777	2361	0.03 (-0.05; 0.10)
Presence of fibrosis, n=95	1888	-0.19 (-0.35; -0.04)*

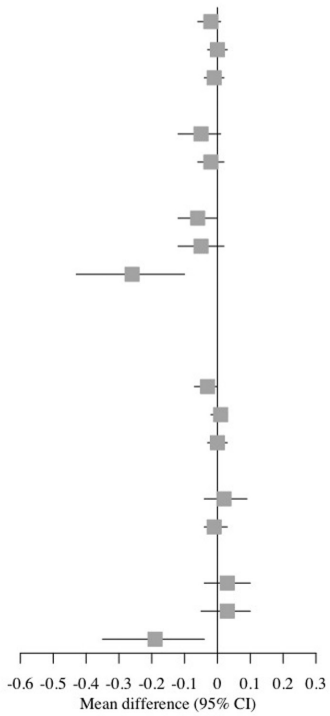


FIGURE 2 Forest plots of associations between liver markers and cognitive measures. ALT, alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease. Model 1: adjusted for age, sex and intracranial volume (ICV). Model 2: adjusted for age, sex, ICV, hypertension, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, body mass index, smoking status, alcohol intake and education. Missing data for Mini Mental State Examination and g-factor in total study population and subgroup for MAFLD assessment, subgroup for NAFLD[‡] assessment and subgroup for LSM and fibrosis[†] assessments; $n = 12$ and $n = 658$; $n = 12^{‡}$ and $n = 577^{‡}$; $n = 8^{†}$ and $n = 364^{†}$, respectively. Significance levels: $^{*}p < 0.05$; $^{**}p < 4.58$ e-3 .

studies examined the relation between liver measures and CBF.[14,22] NAFLD participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study, had a decreased total CBF than those without NAFLD.[14] Another population-based study showed that higher serum ALT was related to increased CBF in the superior frontal gyrus and decreased CBF in the middle occipital gyrus, angular gyrus, precuneus, and middle temporal gyrus.[22] AST, ALT and NAFLD phenotypes were only nominally significant within our study. Several differences in image acquisition may play a role in these latter studies. The CARDIA study used non-contrast computed tomography to define NAFLD and both studies used a 3-tesla MRI with an arterial spin labeling protocol to measure CBF which differed from our method to obtain CBF and brain perfusion measurements using 2D phase-contrast 1.5-tesla MRI without injecting any contrast agents. The importance of CBF on the progression of brain atrophy and dementia has been studied in several studies and investigation of longitudinal assessments of liver measures on CBF would be valuable.[38–40]

In Korean individuals with fatty liver disease, a higher risk for lacunar infarcts and no associations with WMH were observed.[20] Conversely, another Korean study showed significant higher burden of WMH and no associations for lacunes and microbleeds.[16] One case–control study investigated the relation of NAFLD with WMH and revealed that fibrosis severity of NAFLD was associated with WMH.[18] Also, within a healthy Korean study, the presence and severity of NAFLD were associated with WMH.[17] In our population, we obtained nominal significant results for the relation between NAFLD and WMH burden. Similar to our study, NAFLD was associated with smaller TBV in the Framingham Heart Study.[15] Moreover, they also reported non-significant associations with WMH, lacunar infarcts and hippocampal volume, which could relate to differences in study size and frequency of NAFLD in their population, as well as a CT-based measurement of NAFLD they used. The Coronary-Artery-Risk-Development in Young-Adults study also quantified NAFLD with CT and noted smaller TBV and decreased CBF, after adjusting for cardiovascular factors only CBF remained significant.[14] Interestingly, they found that liver fat and NAFLD decreased TBV after controlling for BMI. We explored the relations of liver and brain function controlling for BMI and metabolic syndrome which did not change our initial findings (see Appendix S1). Consensus is still needed within this area of adiposity and brain changes.[41,42] NAFLD could have a mediating role in this pathway and other potential mediators could be cardiovascular risk factors, inflammation and adipose tissue-derived hormones.

Lower cognitive performance was described in a smaller and older population with higher levels of GGT compared to our population.[35] Nonetheless, we only found a nominal significant association with fibrosis and declined global cognition.

This is the first study describing the relation between liver markers and brain microstructural integrity. Additionally, there are no other studies of MAFLD and the brain-axis yet. Categorization of liver enzymes in normal and abnormal values, showed that serum AST and the strict cut-off of ALT were nominally associated with higher fractional anisotropy and only higher values of serum GGT, even though not significant, with lower fractional anisotropy and higher mean diffusivity. Our results between MAFLD and NAFLD phenotypes and microstructural integrity were unexpected and the reversed association of (non-alcoholic) steatosis with higher FA and lower MD is complex, because this pathophysiological reflection is unknown. We know that a lower FA and higher MD reflect worse global WM microstructural integrity, and is related to aging and neurodegeneration.[43] In acute settings lower MD can be related to ischemia, but this is unlikely in our population. Other speculative mechanisms could be inflammation or cholesterol-mediated processes in which liver steatosis or fat accumulation could decelerate the age-related degeneration of microstructural integrity which can result in higher FA and lower MD values, because of preserved microstructural integrity. Still speculatively, higher lipid levels and lipid metabolism could play a role within this mechanism as lipids are an important content of myelin. Yet, within a healthy Korean population, the triglycerides/high-density lipoprotein ratio was positively associated with silent brain infarcts and subcutaneous adipose tissue negatively with WMH.[44,45] Other hypotheses are the disorganization or reorganization theory on the bi-directional changes in FA, that could indicate structural remodelling after (traumatic) brain injury or brain plasticity as a reflection of axonal regeneration, plasticity or gliosis.[46,47] Potential other contributions could be artifacts to confound the magnetic resonance signal and the interpretation of DTI like partial volume effects, iron/hemosiderin-related products, gliosis and degeneration. Along with unforeseen confounding bias, although we controlled for well-known factors like cardiovascular factors and alcohol in this relatively healthy population. These findings remain however controversial and hypothetical, as contrasting findings in the majority of previous published demonstrate, and thus confirmations in other studies and exploration of possible mechanisms longitudinally in diverse populations are needed.

Furthermore, all our analyses were corrected for alcohol consumption and sensitivity analyses showed no significant differences when no adjustments were performed for alcohol (data not shown).

4.1 | Strengths and limitations

The large population-based setting and extensive data on liver and brain markers and potential confounders are strengths of this study.

However, several limitations need to be addressed. The cross-sectional design of our study restrained us from conclusions regarding causality. Hence, associations between trajectories of liver markers and brain aging would be interesting to investigate. Ultrasonography has an important clinical role for screening for liver steatosis and though sensitivity for mild steatosis is poor, for moderate steatosis it is considered good.[48] We used presence of liver steatosis and elevated liver stiffness measures as a proxy to define MAFLD, NAFLD and liver fibrosis phenotypes, in our population-based setting. Nonetheless, liver biopsy remains the gold standard for the diagnosis of both, which would be unethical to perform in our setting. Moreover, underlying mechanisms are still unclear and therefore not all possible confounders could be adjusted for, residual confounding could be a reason for conflicting results in studies so far. Likewise, data on homocysteine and C-reactive protein were not available to explore potential mechanisms of vascular and immunity pathways. Lastly, the distribution of liver enzyme values were within normal limits for most participants. Although, we were interested in these values in relation to brain outcomes, there is little clinical evidence of the height of liver serum levels within a normal range and their meaning in clinical practice.

5 | CONCLUSIONS

Presence of liver steatosis, fibrosis and GGT levels are associated with structural and hemodynamic brain markers. These associations indicate that there could be a hepatic role leading to accelerated brain aging and pathologies, such as alterations in cerebral hemodynamics and microstructural integrity that could lead to vascular damage and brain atrophy. Nevertheless, liver dysfunction itself could also induce cardiovascular disease and metabolic disorders, which are known factors that could cause brain disorders. Therefore, to explore these findings and the causal pathways of the liver-brain axis further, longitudinal and pathway analysis studies are needed. Understanding the hepatic role in brain changes can target modifiable factors and prevent brain dysfunction.

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CONFLICT OF INTEREST STATEMENT

None.

DISCLOSURES

P. Yilmaz, L. J. M. Alferink, L. G. M. Cremers, S. Darwish Murad, M. A. Ikram and M. W. Vernooij report no disclosures relevant to the manuscript. W. J. Niessen, reports Quantib B.V. as a relevant financial activity outside the submitted work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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