

ARIC Manuscript Proposal #3487

PC Reviewed: 10/8/19
SC Reviewed: _____

Status: _____
Status: _____

Priority: 2
Priority: _____

1.a. Full Title: Liver enzymes, cognitive function and impairment. The ARIC-Neurocognitive Study.

b. Abbreviated Title (Length 26 characters): Liver enzymes and brain health

2. Writing Group members: Yifei Lu, Alvin Thomas, Liz Selvin, Priya Palta, Richey Sharrett, Thomas Mosley, David Knopman, Lynne Wagenknecht, James Pike, Laura Loehr, others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __YL__

First author: Yifei Lu
Address: Department of Epidemiology
123 W. Franklin Street, Suite 410
Chapel Hill, NC 27516-8030
E-mail: yifeilu@live.unc.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: Gerardo Heiss
Address: Department of Epidemiology
123 W. Franklin Street, Suite 450
Chapel Hill, NC 27516-8030
Phone: (919) 962-3253
E-mail: gerardo_heiss@unc.edu

3. Timeline: Anticipated completion of a manuscript within 8 mos.

4. Rationale:

A recent report by Nho and collaborators (Nho, 2019) documents associations between biomarkers of liver function and impaired memory, reduced executive function, mild cognitive impairment (MCI), dementia diagnosis, amyloid- β deposition, reduced cerebral glucose metabolism, and A/T/N neurodegenerative biomarkers in cerebrospinal fluid (CSF) relative to individuals whose cognitive performance was defined as normal. Decreased levels of serum alanine aminotransferase (ALT) and an elevated ratio of aspartate aminotransferase (AST) to ALT were higher in patients with Alzheimer's disease diagnosis than cognitively intact individuals, as was the case for reduced brain glucose metabolism, amyloid β 1-42 levels in CSF, and higher CSF levels of p-tau and t-tau levels. Lower levels of ALT were also associated with structural cerebral atrophy and amyloid- β deposition on PET.

The study was conducted in 1,581 members of the ADNI cohort. The comparison groups included 407 cognitively normal individuals, cohort members identified as having significant memory concerns (N = 20), early MCI (n=298), late MCI (n= 544), or mild forms of dementia (n=312). Although markers of liver function were measured in what is referred to as baseline (September 1,

2005, to August 31, 2013; n=800) the study participants seem to have been drawn from ADNI 1, ADNI-GO, or ADNI 2 in ways that are not explained in detail. The criteria by which the control group of cognitively normal individuals were selected are not mentioned.

All analyses were cross-sectional and reverse causation cannot be ruled out. Alcohol consumption (which is well documented in its association with altered liver enzymes) was not available as a covariate. In its place the investigators used levels of gamma-glutamine transferase, an indicator of long-term, heavy alcohol consumption as a covariate.

Based on the extensive characterization of functional and morphologic neurodegenerative processes and their association with serum of ALT and the AST to ALT ratio the authors propose that liver function may play a role in the pathogenesis of dementia. Although elevated levels of alkaline phosphatase were found to be associated with lower cognitive function and Alzheimer's (KelleTT, 2001), the report by Nho and colleagues appears to be the first to suggest a role of liver function in the pathophysiology of neurodegeneration, cerebral metabolism, cognitive performance and dementia.

The causal pathways underlying the associations reported by Nho et al are not known, although the authors review several metabolic pathways that can speak to the plausibility of their results. Abnormal levels of ALT and the AST to ALT ratio are indicative of dysregulation of liver-associated metabolites such as branched-chain amino acids, phosphatidylcholines, and several lipid moieties. Each of these was reported to be associated with Alzheimer's disease. (Toledo, 2017; Pietzner, 2018; Kaddurah-Daouk, 2013)

ALT as well as AST are related to hepatic gluconeogenesis (Qian, 2015) as well as the production of neurotransmitters required for the maintenance of synapses. (Rui, 2014) Reduced ALT levels are known to be associated with lower availability of pyruvate and may be associated with reduced gluconeogenesis in the liver (Reis, 2009) (and thus lower levels of glucose available as energy source to various tissues), as well as a reduction in the levels of the excitatory neurotransmitter glutamate, related to memory performance. It is postulated by Nho and collaborators that disturbed energy metabolism, indexed by lower levels of ALT and increases in the AST to ALT enzyme ratio, may contribute to the reduced brain glucose metabolism observed to affect primarily the orbitofrontal cortex and temporal lobes (related to memory and executive function).

The aim of this manuscript proposal is to attempt a replication of the association between altered liver function enzymes and indicators of cognitive performance, MCI, and dementia in the ARIC-NCS study. Measurements of ALT, AST and serum gammaglutamyltransferase (GGT) were performed at ARIC-NCS examination visits six (2016-2017) and seven (2018-2019). The analyses proposed are cross-sectional and longitudinal.

5. Main Hypothesis/Study Questions:

- a. Lower levels of ALT and GGT and a higher AST to ALT ratio are associated with lower performance on measures of memory and executive function, a higher prevalence of MCI, and a higher prevalence of dementia.
- b. Lower levels of ALT and GGT and a higher AST to ALT ratio are associated with lower (cortical) volume in cerebral regions of interest.
- c. MRI measures of brain morphologic abnormalities of vascular origin are not associated with levels of ALT, AST or their ratio.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Measurements

Exposures: Serum ALT, AST, AST to ALT ratio, and GGT measured at visits 6 and 7. Serum ALT and AST were measured by two ancillary studies, namely AS2009.16 (PI: Selvin) at visits 2 and 4, and AS2014.13 (PI: Selvin) on visit 5 specimens.

Dependent variables:

Cognitive function was examined in 3 domains using a comprehensive neuropsychological battery at ARIC-NCS/visit 6 and 7: (1) memory (delayed word recall, logical memory, and incidental learning); (2) executive function (trail making tests parts A and B, and digit symbol substitution); and (3) language (semantic and phonemic fluency, and Boston naming test). A longitudinal categorical confirmatory factor analysis model was utilized to generate factor scores for each domain. In addition, a global cognition factor score was computed utilizing the nine tests from the three domains and the digit span backwards. The recently developed V2_V6_CNF factor scores will be used.

Cognitive status was classified as normal cognition, MCI, and dementia based on syndromic diagnosis. Specifically, MCI was assigned to participants with FAQ ≤ 5 /CDR sum of boxes ≤ 3 , at least one cognitive domain Z score ≤ -1.5 , and a general cognitive performance score decline rate >0.055 /year. Diagnosis of dementia was made if satisfying either low mini-mental state examination score (<21 for whites or <19 for blacks), or FAQ >5 /DR sum of boxes >3 , at least two cognitive domain Z score ≤ -1.5 , and a decline rate in general cognitive performance score >0.055 /year. Participants neither in MCI nor dementia were classified as cognitively normal (Manual 17, ARIC visit 6).

In the absence of neuroimaging at visit 6 and 7, brain volume and structural abnormalities will be assessed by using brain MRI scans performed at ARIC-NCS/visit 5 (2011-2013) instead. White matter hyperintensity volume, regional cortical volume, and Alzheimer disease signature region volume (including hippocampus, parahippocampal, entorhinal, inferior parietal lobules, and precuneus) were quantified in cm^3 . Brain infarcts (cortical, subcortical, and lacunar infarcts), and cerebral microbleeds were identified as presence/absence or counted in frequency.

Covariates

- Demographics: age, sex, race-center, education;
- Physical information: body mass index;
- Lifestyle: smoking, alcohol consumption, physical activity;
- Comorbidity: diabetes, hypertension;
- Genotype: apolipoprotein E $\epsilon 4$ (0 or ≥ 1 allele)

Exclusion criteria:

- Self-reported as non-white/black participants and black participants from Minneapolis and Washington County due to the small number;
- Liver disease mentioned in hospital discharge diagnoses (ICD-10 CM codes K70-K77);
- ALT, AST, and GGT values greater than 4 standard deviations above or below the mean;
- Missing information on ALT, AST, and GGT;

- Multiple imputation will be used to address potential bias due to informatively missing cognitive outcomes. Among the variables to be considered in the imputation are Mini-Mental State Exam (MMSE) score; Telephone Interview for Cognitive Status (TICS); Six-Item Screener (SIS), Clinical Dementia Rating (CDR), and the Eight-item Interview to Differentiate Aging and Dementia (AD8)

Sensitivity analysis

Missing covariates of interest

Statistical evaluation

Participant characteristics and liver function measurements (ALT, AST, AST to ALT ratio) at Visit 2 will be compared across outcome categories at Visit 6 (cognitively normal, MCI, and dementia), summarized as means (standard deviation) or counts (proportions), as appropriate. Descriptive statistics will be used to examine the distributional properties of the liver enzymes and their age-adjusted association with the dependent variables.

At each examination visit the (cross-sectional) association of liver enzymes with cognitive function, and with MCI and dementia where available, will be modelled continuously, and their cross-sectional associations with domain-specific and the global cognition factor scores will be assessed using multivariable linear regression. Multinomial logistic regression will be used to model cognitive status (normal cognition, MCI, and dementia) as the dependent variable. Models will be adjusted for age, sex, race-center, education level, abdominal girth, smoking, habitual alcohol intake, physical activity, diabetes, hypertension, and apolipoprotein E ϵ 4 genotype.

Poisson or negative binomial regression with a log-transformed outcome will be used to estimate the association of liver enzymes (ALT, AST, AST to ALT ratio) over examination visits 2 – 6 with the measures of cognitive performance at visit 6, as well as cognitive status (normal cognition, MCI, and dementia). Patterns of attrition associated with levels liver enzymes will be examined and addressed analytically as needed to control for selection bias.

Subsequently, we will restrict our analyses to the sample selected for a brain MRI scan at ARIC-NCS/visit 5. Associations of liver function measurements with brain MRI markers will be quantified using linear regression models for continuous dependent variables of log-transformed volumes of white matter hyperintensity, regional cortical volume, Alzheimer disease signature region volume, and frequency of brain infarcts and cerebral microbleeds, and using logistic regression model for binary dependent variable of presence/absence of brain infarcts, and cerebral microbleeds adjusting for the same set of covariates as mentioned above. Analyses for brain volumes will be additionally adjusted for total intracranial volume as the indicator of head size.

Finally, we will conduct subgroup analysis stratified by age, sex, race, drinking, diabetes, hypertension, and apolipoprotein E ϵ 4 genotype. Although all of aforementioned analyses will be conducted at visit 6, to confirm the consistency of our results, we will explore data from visit 7 as well. Bonferroni adjustment for multiple testing will apply to the study questions formulated a priori. All analyses will be performed with Stata version 14.0

References

Nho K, Kueider-Paisley A, Ahmad S, et al. Alzheimer's Disease Neuroimaging Initiative and the Alzheimer Disease Metabolomics Consortium. Association of Altered Liver Enzymes With

Alzheimer Disease Diagnosis, Cognition, Neuroimaging Measures, and Cerebrospinal Fluid Biomarkers. JAMA Netw Open. 2019 Jul 3;2(7):e197978. doi: 0.1001/jamanetworkopen.2019.7978

Kellett KAB, Williams J, Vardy ER, et al. Plasma alkaline phosphatase is elevated in Alzheimer's disease and inversely correlates with cognitive function. Int J Mol Epidemiol Genet. 2011;2(2):114-121.

Toledo JB, Arnold M, Kastenmüller G, et al. Alzheimer's Disease Neuroimaging Initiative and the Alzheimer Disease Metabolomics Consortium . Metabolic network failures in Alzheimer's disease: a biochemical road map. Alzheimers Dement. 2017;13(9):- . doi:10.1016/j.jalz.2017.01.020

Pietzner M, Budde K, Homuth G, et al. Hepatic steatosis is associated with adverse molecular signatures in subjects without diabetes. J Clin Endocrinol Metab. 2018;103(10):3856-3868. doi:10.1210/jc.2018-00999

Kaddurah-Daouk R, Doraiswamy PM, Zhu H, et al. Alterations in metabolic pathways and networks in mild cognitive impairment and early Alzheimer's disease. Alzheimers Dement. 2013;9(4)(suppl):P571. doi:10.1016/j.jalz.2013.05.1126

Qian K, Zhong S, Xie K, Yu D, Yang R, Gong D-W. Hepatic ALT isoenzymes are elevated in gluconeogenic conditions including diabetes and suppressed by insulin at the protein level. Diabetes Metab Res Rev. 2015;31(6):562-571. doi:10.1002/dmrr.2655

Rui L. Energy metabolism in the liver. Compr Physiol. 2014;4(1):177-197. doi:10.1002/cphy.c130024

Reis HJ, Guatimosim C, Paquet M, et al. Neuro-transmitters in the central nervous system & their implication in learning and memory processes. Curr Med Chem. 2009;16(7):796-840. doi:10.2174/09298670978754927

Deal, J. A., Gottesman, R. F., Griswold, M., et al. (2015). Application of Latent Variable Methods to the Study of Cognitive Decline When Tests Change over Time. Epidemiology, 26(6), 878–887. doi: 10.1097/EDE.0000000000000379

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes ___X___ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No

(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes ___X___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC

Investigators have access to the publications lists under the Study Members Area of the web site at: <http://www.csc.unc.edu/atic/mantrack/maintain/search/dtSearch.html>

Yes No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

None found

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes No

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (list number*

B. primarily based on ARIC data with ancillary data playing a minor role

(usually control variables; list number(s)* AS2009.16 (PI: Selvin) and AS2014.13 (PI: Selvin)

*ancillary studies are listed by number at <https://www2.csc.unc.edu/atic/approved-ancillary-studies>

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from <http://publicaccess.nih.gov/> are posted in <http://www.csc.unc.edu/atic/index.php>, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.