ARIC Manuscript Proposal #2761

PC Reviewed: 6/7/16 **Status: A** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: **Associations between glutamate-regulated enzymes (AST, ALT and GGT) levels and stroke and migraine in ARIC**

b. Abbreviated Title (Length 26 characters): Glutamate-regulated enzymes, stroke and migraine in ARIC

2. Writing Group:
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ALCS **[please confirm with your initials electronically or in writing]**

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3. Timeline:

Manuscript preparation will be complete in 12 months.

4. Rationale:

During brain damage like stroke or traumatic brain injury, glutamate acts as an important mediator of neuronal death.[1, 2] The concentration of extracellular glutamate is tightly regulated to maintain physiological concentrations through sodium dependent transporters. [3, 4] When glutamate accumulates in the endothelial cells to a concentration that exceeds plasma levels, it is moved via facilitated diffusion through the luminal side into the blood stream. It has been reported that in pathologic conditions associated with a large increase of glutamate in brain tissue (which has been shown to occur in pathologies such as ischemia, and migraine), glutamate diffuses from the extracellular space to the blood following a gradient of concentration, which could explain the increase of systemic glutamate levels observed in these diseases. [5-8]

Blood glutamate levels are mainly regulated by two enzymes, glutamate-oxaloacetate transaminase (GOT1 or AST [aspartate aminotransferase]) and glutamate-pyruvate transaminase (GPT or ALT [alanine aminotransferase]); both are able to metabolize blood glutamate and facilitate the lowering of extracellular levels of glutamate. Low levels of AST and ALT may be a significant factor for the brain-to-blood glutamate efflux, and possibly contribute to the stroke patient's clinical outcome. In two independent studies, patients with poor neurological outcome after ischemic stroke showed higher glutamate and lower blood AST and ALT levels on admission, compared to those with better outcome. [5, 6] This association was stronger for AST than ALT levels. This favorable effect was also supported by positive effects on the reduction of lesion volume. In addition, a few studies have indicated that higher levels of serum gamma-glutamyl transpeptidase (GGT) are associated with stroke incidence. [9-11] GGT is a well-known enzyme marker for alcohol consumption as well as liver disease, and a potential marker for oxidative stress.[12] However, in the last decade there has been growing evidence of a linear association between GGT activity and risk of cardiovascular disease, independent from alcohol intake.[13] GGT is a cell-surface enzyme that hydrolyzes the gamma-glutamyl bond of extracellular reduced and oxidized glutathione, initiating their cleavage into glutamate, cysteine (cystine) and glycine.[12] The fact that high AST and ALT levels are associated with lower neurological damage, while GGT is associated with incidence of stroke, strongly supports the hypothesis that glutamate-regulated enzymes play an important role in the pathology of stroke.

In analogy with stroke, the role of glutamate in migraine has resumed importance, mainly focused on the activity of glutamate as well as its receptors and transporters. [14-16] The association between the dysregulation of glutamate and migraine attacks is broadly described in the literature. Previous studies have observed that migraine patients show higher glutamate levels than controls in plasma, platelets, saliva or cerebrospinal fluid (CSF). [17, 18] Moreover, systemic increase in glutamate levels seems to be associated with the occurrence of migraine attacks. In fact, it has been reported that diets rich in monosodium glutamate act as a potential trigger for migraine headaches. [10] In addition, migraine patients show increased glutamate levels and lower AST activity compared to control healthy subjects. Furthermore, AST activity has been inversely associated with glutamate levels during inter-ictal periods, while increased glutamate levels have been associated with duration of pain during ictal periods. [19] As a result of these studies, some authors suggest that systemic modifications of blood glutamate levels could reduce the excessive glutamatergic signal in the brain and therefore be used as a potential prophylactic treatment against migraine attacks. [9]

5. Main Hypothesis/Study Questions:

Specific Aims:

1. To assess the association between AST, ALT and GGT blood levels and stroke risk.
2. To study the association between AST, ALT and GGT blood levels and stroke outcome (death).
3. To assess the association between AST and ALT blood levels and migraine.

Study Hypotheses:

1. Higher blood levels of AST and ALT, and lower blood levels of GGT will be associated with decreased risk of stroke and death after stroke.
2. Higher AST and ALT blood levels will be associated with a lower prevalence of migraine.

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).

Study Design:

- ***Stroke analysis:*** Prospective cohort study: Baseline Visit 2 (1990-1992, measures of ALT, AST, and GGT) with follow-up through 31 December 2013 for stroke events.
- ***Migraine analysis:*** Non-concurrent cross-sectional study: Visit 2 (1990-1992) measures of ALT, AST, and GGT and Visit 3 (1993-1995) and migraine.

Inclusion/Exclusion Criteria:

Stroke analysis*:*

- Inclusion Criteria: ARIC participants with data on liver enzymes at Visit 2
- Exclusion Criteria:
    - Participants with stroke occurring prior to baseline (Visit 2) (prevalent stroke)
    - Non-white race, non-black race, or black race at the Minnesota or Maryland field centers
    - Abnormal liver function (AST> 60U/L or ALT> 60U/L or GGT> 50U/L) at baseline (Visit 2)
    - Missing data on covariates included in statistical models

Migraine analysis*:*

- Inclusion Criteria: ARIC participants with data on liver enzymes at Visit 2 and data on migraine at Visit 3
- Exclusion Criteria:
    - Non-white race, non-black race, or black race at the Minnesota or Maryland field centers
    - Abnormal liver function (AST> 60U/L or ALT> 60U/L or GGT> 50U/L) at baseline (Visit 2)

Exposure: AST, ALT and GGT levels measured at Visit 2.

- Alanine aminotransferase (ALT) was measured in serum using Roche ALT reagent on the Roche Modular P Chemistry analyzer. In this reaction, ALT catalyzes the reaction of alpha-ketoglutarate with L-alanine to form L-glutamate and pyruvate. Under the action of LDH, pyruvate converts to lactate, and NADH is converted to NAD. The activator pyridoxal phosphate is not included in this reagent system. The decrease in absorbance of NADH, measured at 340 nm (secondary wavelength is 700 nm), is directly proportional to the serum activity of ALT. It is a kinetic rate reaction. The laboratory inter-assay CV is 5.6% at a value of 40 U/L and 3.5% at a value of 135 U/L.
- Aspartate aminotransferase (AST) was measured in serum using Roche AST reagent on the Roche Modular P Chemistry analyzer. In this reaction, AST activity is determined by a modification of the method recommended by the International Federation of Clinical Chemistry (IFCC). AST catalyzes the reaction of alpha-ketoglutarate with L-aspartate to form L-glutamate and oxaloacetate. Under the action of malate dehydrogenase (MDH), oxaloacetate converts to malate, and NADH is oxidized to NAD. The decrease in absorbance of NADH, measured at 340 nm (secondary wavelength = 700 nm), is directly proportional to the serum activity of AST. It is a kinetic rate reaction. The laboratory inter-assay CV is 6.5% at a value of 29 U/L and 1.6% at a value of 137 U/L.
- Gamma-glutamyl transferase (GGT) was measured in serum using Roche GGT reagent on the Roche Modular P Chemistry analyzer. In the presence of glycyl glycine, L-gamma-glutamyl-3-carboxy-4-nitroanilide is converted by GGT to 5-amino-2-nitrobenzoate and L-gamma-glutamyl-glycylglycine. The rate of colored product formation is directly related to the amount of GGT in the specimen, and the rate of its appearance is measured at 415 nm (secondary wavelength 700 nm). This is a kinetic (Rate-A) reaction. The laboratory inter-assay CV is 5.1% at a value of 39 U/L and 2.9% at a value of 171 U/L.

Outcome(s):

Stroke analysis*:*

- Definite/probable incident stroke (as defined by ARIC adjudication) overall and stratified by stroke type (ischemic and hemorrhagic)
- Stroke outcome (death)

Migraine analysis*:*

- Definitive Migraine Definition: 1) headache lasting at least 4 hours, 2) 2/3 of throbbing/pulsing/pounding OR need to lie down OR unilateral, 3) (nausea AND vomiting) OR (photophobia AND phonophobia)
- Probable Migraine Definition: 2/3 of the above three definitive migraine criteria
- Primary and secondary outcome variables will be 1.) Definitive migraine and 2.) Definitive + probable migraine
- Above definitions used in: Dearborn JL, Schneider AL, Gottesman RF, Kurth T, Pankow JS, Couper DJ, Rose KM, Williams MA, Peterlin BL. Adiponectin and leptin levels in migraineurs in the Atherosclerosis Risk in Communities Study. Neurology. 2014; 83:2211-8.
- Will also look at migraines with and without aura.

Covariates: Age at baseline (continuous; years), sex (male; female), race/center (Minnesota whites; Maryland whites; North Carolina whites; North Carolina blacks; Mississippi blacks),

diabetes (defined as self-reported diagnosis by physician, diabetes medication use, fasting glucose ≥126 mg/dl, or HbA1c ≥6.5%, yes; no); hypertension medication use (yes; no), estrogen use (among women only, yes; no), coronary heart disease (adjudicated events, yes; no), alcohol use (never; former; current), and body mass index (continuous).

Summary of Data Analysis:

Stroke analysis*:* Adjusted Cox Proportional Hazards models will be used to explore the associations between ALT, AST, and GGT with stroke risk (baseline Visit 2 [1990-1992] with follow-up through 31 December 2012). Linear spline models will be used to explore the continuous associations of ALT, AST, and GGT with stroke risk (overall and stratified by ischemic/hemorrhagic subtype) and death after stroke. We will also explore the relationships with stroke risk by tertiles of ALT, AST, and GGT. In a sensitivity analysis, we will assess the association between changes in levels of ALT, AST, and GGT from Visit 2 (1990-1992) to Visit 4 (1996-1998) and stroke outcome (overall and stratified by ischemic/hemorrhagic subtype) with follow-up time defined from Visit 4 through 31 December 2012. Due to the strong association of alcohol and obesity with liver enzyme levels, we will additionally perform stratified analyses by alcohol and obesity status.

Migraine analysis*:* Adjusted logistic regression models will be used to explore the associations between ALT, AST, and GGT measured at visit 2 (1990-92) with migraine measured at Visit 3 (1993-1995). Linear spline models will be used to explore the continuous associations of ALT, AST, and GGT with migraine. We will also explore the relationships with migraine by tertiles of ALT, AST, and GGT. Due to the strong association of alcohol and obesity with liver enzyme levels, we will additionally perform stratified analyses by alcohol and obesity status.

Limitations:

- Data on liver enzymes and migraine are available at different ARIC visits
- There might not be enough power for the assessment of associations between liver enzymes levels and hemorrhagic stroke
- Residual confounding

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.cscc.unc.edu/ARIC/search.php

   __**X**__ 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)?**

PRIOR ARIC LIVER ENZYMES PUBLICATIONS:

- Schneider AL, Lazo M, Ndumele CE, Pankow JS, Coresh J, Clark JM, Selvin E. Liver enzymes, race, gender and diabetes risk: the Atherosclerosis Risk in Communities (ARIC) Study. Diabet Med. 2013; 30:926-33.
- Lazo M, Rubin J, Clark JM, Coresh J, Schneider AL, Ndumele C, Hoogeveen RC, Ballantyne CM, Selvin E. The association of liver enzymes with biomarkers of subclinical myocardial damage and structural heart disease. J Hepatol. 2015; 62:841-7.
- Folsom AR, Lutsey PL, Roetker NS, Rosamond WD, Lazo M, Heckbert SR, Basu S, Cushman M, Selvin E. Elevated hepatic enzymes and incidence of venous thromboembolism: a prospective study. Ann Epidemiol. 2014 Nov;24(11):817-821
- Alonso A, Misialek JR, Amiin MA, Hoogeveen RC, Chen LY, Agarwal SK, Loehr LR, Soliman EZ, Selvin E. Circulating levels of liver enzymes and incidence of atrial fibrillation: the Atherosclerosis Risk in Communities cohort. Heart. 2014 Oct;100(19):1511-6.

PRIOR ARIC MIGRAINE PUBLICATIONS:

- Hamedani AG, Rose KM, Peterlin BL, Mosley TH, Coker LH, Jack CR, Knopman DS, Alonso A, Gottesman RF. Migraine and white matter hyperintensities: the ARIC MRI study. Neurology. 2013; 81:1308-13.
- Carson AP, Rose KM, Sanford CP, Ephross SA, Stang PE, Hunt KJ, Brown CA, Szklo M. Lifetime prevalence of migraine and other headaches lasting 4 or more hours: the Atherosclerosis Risk in Communities (ARIC) study. Headache. 2004; 44:20-8.
- Dearborn JL, Schneider AL, Gottesman RF, Kurth T, Pankow JS, Couper DJ, Rose KM, Williams MA, Peterlin BL. Adiponectin and leptin levels in migraineurs in the Atherosclerosis Risk in Communities Study. Neurology. 2014; 83:2211-8.

PRIOR ARIC MIGRAINE PROPOSALS:

- Catherine Paton. MSP #363. A descriptive study of migraine headache prevalence in a biracial population: the ARIC Study, 1993-95.
- Sara Ephross. MSP #400. Migraine and Preclinical/Clinical Cardiovascular Disease and its Risk Factors: The Atherosclerosis Risk in Communities (ARIC) Study.
- Kathryn Rose #951. Migraine Headaches and Retinal Microvascular Abnormalities.
- Lee Peterlin. MSP #2015. An Evaluation of Obesity-related Proteins and Genes in Migraine Participants from the Atherosclerosis Risk in Communities Study.

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

11.b. If yes, is the proposal

__X__A. primarily the result of an ancillary study (list number* 2009.16)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

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.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __**X**__ No.

References

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2. Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: identifying novel targets for neuroprotection. Prog Neurobiol. 2014; 115: 157-88.
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6. Campos F, Rodríguez-Yáñez M, Castellanos M, Arias S, Pérez-Mato M, Sobrino T, Blanco M, Serena J, Castillo J. Blood levels of glutamate oxaloacetate transaminase are more strongly associated with good outcome in acute ischemic stroke than glutamate pyruvate transaminase levels. Clin Sci (Lond). 2011; 121(1): 11-7.
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11. Weikert C, Drogan D, di Giuseppe R, Fritsche A, Buijsse B, Nöthlings U, Willich SN, Berger K, Boeing H. Liver enzymes and stroke risk in middle-aged German adults. Atherosclerosis. 2013; 228(2): 508-14.
12. Hanigan MH. Gamma-glutamyl transpeptidase: redox regulation and drug resistance. Adv Cancer Res. 2014; 122:103-41.
13. Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: a meta-analysis of prospective cohort studies. Atherosclerosis. 2014; 236(1):7-17.
14. Ferrari A, Spaccapelo L, Pinetti D, Tacchi R, Bertolini A. Effective prophylactic treatments of migraine lower plasma glutamate levels. Cephalalgia 2009; 29: 423–429.
15. Leira R and Rodrıguez R. Diet and migraine. Rev Neurol 1996; 24: 534–538.
16. Castillo J, Martı´nez F, Corredera E, Aldrey JM, Noya M. Amino acid transmitters in patients with headache during the acute phase of cerebrovascular ischemic disease. Stroke 1995; 26: 2035–9.
17. Edvinsson L, Villalo´n CM and Maassen Van Den Brink A. Basic mechanisms of migraine and its acute treatment. Pharmacol Ther 2012; 136: 319–333.
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19. Campos F, Sobrino T, Pérez-Mato M, Rodríguez-Osorio X, Leira R, Blanco M, Mirelman D, Castillo J. Glutamate oxaloacetate transaminase: a new key in the dysregulation of glutamate in migraine patients. Cephalalgia. 2013; 33(14):1148-54.