ARIC Manuscript Proposal #2564

PC Reviewed: 6/9/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Variation in ethanol-metabolizing genes modifies the relationship between ethanol intake and cognitive decline: The ARIC Neurocognitive Study.

b. Abbreviated Title (Length 26 characters): Ethanol intake, ethanol-metabolizing genes, and cognitive decline

2. Writing Group:
Writing group members: Shelly-Ann M. Love, Alanna Morrison, Kari E. North, Jim Pankow (invited), Jan Bressler, Sarah B. Jones, Lisa Wruck (invited), Tom Mosley

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

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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).

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3. Timeline:

Analysis and manuscript will be completed within a year of receiving approval.

4. Rationale:

The societal burden of dementia in the US is high due to its high prevalence, associated disability and health costs. Dementia has an estimated prevalence of 5.2 million in the US with an associated annual economic cost of $203 billion. Because of the increasing number of people aged 65 years and older in the US, the prevalence of dementia will quadruple to 16 million by 2050, creating an expected increase of healthcare cost to $1.2 trillion. [1]. To reduce the incidence of dementia it is important to identity modifiable factors that prevent, or delay, the progression of cognitive decline and dementia.

The relationship of ethanol intake with cognitive decline and dementia has been examined. These relationships, however, remain poorly understood. Previous studies have reported that heavy ethanol intake is associated with increased risk of cognitive decline and dementia; however results are conflicting with regard to the effect of low-to-moderate intake [2-14]. Several review studies have concluded that low-to-moderate ethanol is linked to protective effects on late-life cognitive decline and dementia [2, 5, 15]. On the contrary, some studies linked low-to-moderate ethanol intake to greater risk of late-life cognitive decline and dementia [16]. Several of these studies relied on single measures of ethanol intake [3, 7-9, 11, 12, 14], non-standardized definition of cognitive decline, outdated classification of dementia, short follow-up times (<5 years) [7, 8, 10, 12], lack of analytic control of confounders and effect measure modifiers [3, 7, 11, 12, 14]. A limited number of studies investigated the effects of ethanol in black populations despite the fact that the prevalence, incidence, and cumulative risk of Alzheimer's disease, the most common form of dementia, appears to be much higher in blacks than in whites [17-19]. Furthermore, few studies investigated the effects of mid-life ethanol intake with late-life cognition [20, 21].

The neurotoxic effects of ethanol that cause cognitive deficits may be mediated directly through damage to brain structures or indirectly through malnutrition, metabolite toxicity, electrolyte imbalance, or accompanying physical illnesses including liver disease and infection [22]. Korsakoff syndrome, a thiamine deficiency characterized by cognitive and memory deficits, which results from chronic ethanol abuse is an example of indirect ethanol neurotoxicity [23].

Positive effects of low-to-moderate ethanol on cognitive function have been attributed to flavonoids or other antioxidants, which may reduce the risk for dementia directly or indirectly by protecting against cardiovascular disease. Ethanol intake has been associated with fewer brain infarcts and was shown to have a U-shape relationship with the prevalence of white matter lesions [24]. Finally, low-to-moderate doses of ethanol might alter blood-clotting mechanisms through increase of prostacyclin concentrations and reduction of thromboxane A2 generation and thus may inhibit platelet function [24].

In vitro studies suggest that single nucleotide polymorphisms (SNPs) within ethanol-metabolizing genes (i.e., alcohol dehydrogenase, aldehyde dehydrogenase, and cytochrome P4502E1) alter the rate of ethanol oxidation (conversion of ethanol to acetaldehyde), thereby influencing the likelihood of excessive drinking. Yet, few studies have evaluated effect measure modification of the relationship of ethanol intake with cognitive decline by SNPs in the ethanol-metabolizing genes in ancestrally diverse populations. However, these studies were underpowered [25]; suffered from selection bias [25], residual confounding [25], and severe attrition bias [26]. As a result, there remains the need to study the effect measure modification of ethanol-metabolizing SNPs on the ethanol intake-cognitive decline relationship using large prospective studies with repeated measurements of ethanol intake and standardized measures of cognitive function to determine if effect measure modification exists.

The rich data source of the ARIC study which includes repeated measurements of ethanol intake of cognitive function collected on black and white men and women from mid- to-late life that were genotyped for ethanol-metabolism SNPS is well-suited to address this current research need. Evidence of effect measure modification of the ethanol intake-cognitive decline relationship by SNPs within ethanol-metabolizing genes will suggest possible mechanisms by which ethanol affects cognition, in ways that are relevant to understanding the potential public health risk and benefit of ethanol.

5. Main Hypothesis/Study Questions:

Aim 1: Estimate the relationship between ethanol intake and 20-year cognitive decline in black and white men and women from mid-to-late life.

Hypothesis 1: Heavy drinking will be associated with greater 20-year cognitive decline compared to never drinking

Hypothesis 2: Low-to-moderate drinking will be associated with lesser 20-year cognitive decline compared to never drinking.

Aim 2: Estimate the relationship between SNPs within ethanol-metabolizing genes and ethanol intake in black and white ARIC men and women from mid- to-late life.
Hypothesis 3: SNPs within ethanol-metabolizing genes will be associated with ethanol intake.

Aim 3: Evaluate the effect modification of the ethanol intake-cognitive decline relationship by SNPs within ethanol-metabolizing genes in each of the two ancestral populations.
Hypothesis 4: Individuals with greater genetic ability to process ethanol will experience lesser 20-year cognitive decline per unit increase in ethanol intake.
Hypothesis 5: Individuals with lower genetic ability to process ethanol will experience greater 20-year cognitive decline per unit increase in ethanol intake.

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 population and design: The primary analysis will include all participants in the ARIC-Neurocognitive Study (ARIC-NCS): 1) with at least two measurements of cognitive function across the 5 ARIC visits, 2) with at least one measurement of ethanol intake from visits 1-4, and 3) were genotyped for SNPs within ethanol-metabolizing genes. Ethanol intake was collected over a 22-year period among all ARIC participants prior to measurement of cognitive impairment and dementia in ARIC-NCS, which began in 2010. Excluded from the study population will be participants who did not self-identify as black or white, blacks residing in Washington County or Minneapolis (due to low numbers), and participants who had missing cognitive data at visit 2.

Exposures: The primary exposure of interest is ethanol intake. Ethanol intake was assessed at all visits by trained interviewers using an interview-administered ethanol intake questionnaire. Ethanol intake was collected in terms of its frequency (days per week), usual quantity (glasses of a specified volume), and type (wine, beer, liquor) at all 5 ARIC cohort study visits. Questions were identical in all surveys and also included items on drinking history to assess never and past drinking status as well as the time since cessation of drinking among former drinkers. Current drinkers were asked how often they usually drank wine, beer, or hard liquor. The amount of ethanol consumed (in grams per week) was calculated assuming the following ethanol content: 4oz of wine = 10.8 grams; 12 oz. of beer = 13.2 grams; and 1.5 oz. of distilled spirits = 15.1 grams. For a drinker who reported less than one drink per week, the ethanol intake was recorded as 0 g per week.

The secondary exposure is SNPs within Ethanol-Metabolizing Genes. ARIC cohort participants who consented to the use of their DNA for research purposes was genotyped for previously studied functional / non-synonymous variants in ethanol-metabolizing genes. Genotyping of the SNPs of interest were performed using the TaqMan Assay ® between visits 4 and 5 as part of an ancillary study.

Outcome: Cognitive function was assessed at visit 2 (1990-1992), visit 4 (1996-1998), and the ARIC-NCS at visit 5 (2011-2013) using 3 standard cognitive tests that assessed different domains of cognition: 1) the Delayed Word Recall Test (DWRT)- a test of memory [27], 2) the Digit Symbol Substitution Test (DSST) - a test of language [28], and 3) the Word Fluency Test (WFT)- a test of executive function [29]. All three tests were administered by trained examiners using standardize protocols in a quiet room. Recordings were reviewed for quality control.

Statistical analysis: We will use linear mixed models to estimate the relationship between ethanol intake and cognitive decline. The models will be adjusted for covariates, age, sex, education, center, BMI, smoking status, and the Apolipoprotein E ε4 polymorphism. Linear mix model utilizes all available data over follow-up, handle differences in length of follow-up, and account for the fact that repeated measures on the same individual are correlated. Both the intercept and slope will be fitted as random effects, allowing for differences in cognitive function at baseline and rate of cognitive decline. We will use unstructured correlation matrices and robust variance estimates. Time since baseline (visit 2) will be modeled using a linear spline with a knot at 6 years (the mean duration between visits 2 and 4). The spline term will allow for a nonlinear association between time and cognitive decline, more appropriately fit the study design than a quadratic term, and is supported by diagnostics. The primary coefficients of interest will be the interactions between ethanol intake and the time spline terms, which will address the hypothesis of greater decline among participants with heavy ethanol intake after adjustment of age and other covariates.

Ethanol intake will be modeled both as a continuous variable and as a categorical variable (never drinker, low-to-moderate drinker, heavy drinker). For the categorical ethanol intake measure, the referent group will only include never drinkers, thus avoiding the potential problem of including past drinkers which may include persons who have abstained from alcohol due to poor health (the "sick quitter effect") [30]. For the continuous ethanol intake measure, ethanol intake will be quantified as the average daily intake (grams/day) across all study visits. The categorical ethanol intake variable will be evaluated as an indicator variable in the analysis models. The DWRT, DSST, WFT, and global cognition z-scores will be assessed as continuous measures of cognitive function.

The Mendelian randomization (MR) study design will be used to verify the causal role of ethanol intake on cognitive decline. MR is a technique that uses, as instrumental variables, genetic variants that are not associated with the outcome variable, but instead alter the action of a putative risk factor. If the genetic variants are distributed at random with regard to confounders such as social economic status (SES), an interaction of genotype with the exposure variable of interest (in the present context, ethanol intake) on the outcome (in the present context, cognitive performance) indicates causal effects of the exposure variable. We will use linear regression models to determine which SNPs within the ethanol-metabolizing genes correlate with ethanol intake in ARIC black and white participants. These SNPs will be used as instrumental variants in the race-stratified MR analysis. Using linear regression models, we will test the assumptions of the MR design, namely that the SNPs are unrelated to the potential confounders (sex, age, education, center, BMI, smoking status, and the Apolipoprotein E ε4 polymorphism) and to the outcome (cognitive function from mid-to-late life).

If the assumptions of the MR design are met, we will proceed with evaluating if the ethanol intake-cognitive decline relationship is modified by SNPs in the ethanol-metabolizing genes in each of the two ARIC ancestral populations (i.e., blacks and whites). An additive SNP set score of minor alleles across the SNPs in the ethanol-metabolizing genes will be created. Scores of the SNP set will range from a possible minimum value of zero to a maximum value of 2 times the total number of ethanol-metabolizing SNPs, theoretically corresponding to highest to lowest ability to metabolize alcohol [31]. Gene x environment interaction between the SNP set and ethanol intake will be evaluated for in linear regression models with cognitive function as the dependent variable. The DWRT, DSST, WFT, and global cognition z-scores will be assessed as continuous measures of cognitive function. The models will include SNP set score, log-ethanol intake, and SNP set score x log-ethanol intake as predictors and covariates: age, sex, education, center, BMI, smoking status, and the Apolipoprotein E ε4 polymorphism. Models will also be adjusted for principal components to account for population stratification. If the beta estimate for the interaction between log-ethanol intake and SNPs within the ethanol-metabolizing genes has a p-value<0.05, this will indicate that ethanol intake interacted with the SNP set score to significantly predict cognitive decline.

Twenty-five percent of ARIC participants died over the course of 22 years of follow-up, and 45% of those alive at the time of the exam 5 visit did not attend. As a result, attrition is a methodological concern. Multivariate imputation by chained equations (MICE) will be used to evaluate and correct for attrition due to missing cognitive data. MICE involves filling in the missing cognitive data values multiple times, creating multiple "complete" datasets. The missing values are imputed based on the observed values for a given individual and the relations observed in the data for other participants, assuming the observed variables are included in the imputation model [32]. Because multiple imputations involves creating multiple predictions for each missing value, the analyses of multiple imputed data take into account the uncertainty in the imputations and yield accurate standard errors [33].

Covariates: Potential confounder were identified through directed acyclic graphs and a priori knowledge from existing literature and include age, sex, education level, study recruitment center, body mass index, smoking status, and the Apolipoprotein E ε4 polymorphism [34-40].

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? __X__ Yes ____ No (We will use ethanol-metabolizing and ApoE genotype data)

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"? __X__ 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)?

MS410: Longitudinal association of alcohol consumption and cognition (lead: M. Eigenbrodt)

MS1635: Variation in alcohol-metabolizing genes modifies the relationship between steady alcohol consumption and incidence of CVD (lead: K. Volcik)

MS1636: Variation in alcohol-metabolizing genes modifies the relationship between steady alcohol consumption and plasma lipid levels (lead: K. Volcik)

MS1651: Genome-Wide Association Study of Alcohol Consumption in the CHARGE consortium (lead: D. Levy)

MS 2195: Association between alcohol consumption and cognitive impairment: The ARIC Neurocognitive Study (lead: Sara B. Jones)

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

11.b. If yes, is the proposal

__X__ A. primarily the result of an ancillary study (ARIC-NCS 2008.06)

__X__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* (alcGene 2006.09)

*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.

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