THE ASSOCIATION OF LONG-TERM TRAJECTORIES OF ETHANOL INTAKE AND CHANGE IN COGNITIVE PERFORMANCE, AND EFFECT MODIFICATION BY ETHANOL INTAKE-ASSOCIATED GENETIC VARIANTS

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ABSTRACT

Shelly-Ann Mellissa Love: The Association of Long-term Trajectories of Ethanol Intake and Change in Cognitive Performance, and Effect Modification by Ethanol Intake-Associated Genetic Variants
(Under the direction of Kari E. North)

Faster rates of age-related cognitive decline may result in early onset of cognitive impairment and dementia. Ethanol use is highly prevalent(~70%) in the US. The relationship between ethanol intake and cognitive decline has been extensively studied. However, findings have inconsistent, which may be attributed to the use of single use of ethanol intake, short follow-up times (<5 years), and not taking genetic predisposition to ethanol drinking into account.

There is substantial genetic variability in ethanol consumption, and *in vitro* studies report differences in ethanol metabolism kinetic properties associated with genetic variants of different prevalence in diverse populations. The objective of this study was to assess the association of long-term trajectories of ethanol intake in mid-life with 15-year cognitive change from mid-to-late life among African-American and European-American adults, and of effect modification by ethanol drinking-associated genetic variants. We utilized data from a large biracial community cohort of men and women, who completed four assessments of ethanol intake and repeated assessments of cognitive function in three cognitive domains: processing speed, executive function, and language. Ethanol intake trajectories were defined using four measures during a 9-year interval (1987-1998) as i) stable never drinkers, ii) stable low-to-moderate drinkers, iii) stable heavy drinkers, iv) stable former drinkers, v) mostly low-to-moderate drinkers, vi) mostly heavy drinkers, and vii) mostly former drinkers.

The results from this study suggest that stable low-to-moderate drinking and stable heavy drinking in mid-life are not associated with 15-year cognitive decline from mid-to late-life among African-American and European-American adults. In addition, no effect modification was observed by an

unweighted genetic risk score comprised of ethanol intake-associated genetic variants (20 SNPs, African-Americans; 11 SNPs, European-Americans). Our findings of no association between stable low-to-moderate drinking during mid-life and 15- year cognitive decline from mid-life to late-life are consistent with previous studies finding demonstrating that moderate ethanol intake may not be protective of cognitive decline and suggests that low-to-moderate drinking should not be recommended to influence cognitive aging. Furthermore, the lack of evidence for effect modification suggests that the genetic variants tested do not influence the lack of ethanol intake-cognitive decline association observed in these data.

I dedicate this work to the wind beneath my wings: my husband, Jerry Lee Love, Jr.; son, Jerry Lee Love, III; daughter, Talia Rose Love; and twin sister, Stacey-Ann Celesia Meade
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"I can do all things through Christ which strengthens me."
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LIST OF ABBREVIATIONS

α	Alpha
β	Beta
γ	Gamma
π	Pie
σ	Sigma
χ	Chi
2SLS	Two stages least squares
3MS	Modified Mini-Mental State Examination
AB	Applied Biosystems
AC	Adenyl Cyclase
AD	Alzheimer's disease
ALSPAC	Avon Longitudinal Study of Parents and Children
AMPAR	α-Amino-3-Hydroxy-5-Methylisoxazole-4-Proprionic Acid
Arg48	Arginine 48
Arg272	Arginine 272
Arg370	Arginine 370
ARIC	Atherosclerosis Risk in Communities
ARIC-NCS	Atherosclerosis Risk in Communities Neurocognitive Study
AVENGEME	Additive Variance Explained and Number of Genetic Effects
BLSA	Baltimore Longitudinal Study of Aging
BMI	Body Mass Index
BNT	Boston Naming Test

BVRT	Benton Visual Retention Test
CAD	Coronary Artery Disease
CASI	Cognitive Abilities Screening Instrument
CDR	Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CHD	Coronary Heart Disease
CI	Confidence Intervals
COWA	Controlled Oral Word Association Test
cSNP	Coding SNPs
CVD	Cardiovascular Disease
CVLT	California Verbal Learning Test
Cys	Cysteine
Cys370	Cysteine 370
DAG	Directed Acyclic Graph
DALYs	Disability-Adjusted Life-Years
Del	Deletion
DNA	Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
DS-B	Digits Span-Backward
DS-F	Digits Span-Forward
DSST	Digit Symbol Substitution Test
DWRT	Delayed Word Recall Test
EPESE	Established Populations for Epidemiologic Studies of the Elderly
EVA	Epidemiology of Vascular Aging Study

FFQ	Food Frequency Questionnaire
FTD	Frontotemporal Dementia
FTLD	Frontotemporal Lobar Degeneration
GED	General Educational Development
GEE	Generalized Estimate Equations
GEE	Generalized Estimate Equations
GF	Graduate Frequency
Gln	Glutamine
Gln272	Glutamine 272
GSCAN	GWAS & Sequencing Consortium of Alcohol and Nicotine Use
H2O2	Hydrogen Peroxide
Hcγ	Homocysteine
HDL2	High Density Lipoprotein Cholesterol
HDL-C	High Density Lipoprotein Cholesterol
His	Histidine
His48	Histidine 48
HRT	Hormone Replacement Therapy
HWE	Hardy–Weinberg Equilibrium
Ile	Isoleucine
lle350	Isoleucine 350
In	Inversion
ISEL-SF	Interpersonal Support Evaluation List
IV	Instrumental Variable
K_m	Michaelis constant

LBD	Lewy bodies Dementia
LD	Linkage Disequilibrium
LDL-C	Low Density Lipoprotein Cholesterol
LM	Low-to-Moderate
LMEI	Low-to-Moderate Ethanol Intake
MAE	Multilingual Aphasia Examination
MAF	Minor Allele Frequency
MAR	Missing at Random
MCAR	Missing Completely at Random
MCI	Mild Cognitive Impairment
MD	Maryland
MEOS	Microsomal Ethanol Oxidizing System
MET	Metabolic Equivalent
mGluR	Metabotropic Glutamate Receptor
mGluRs	metabotropic glutamate receptors
MICE	Multivariate Imputation by Chained Equations
Min	Minute
mM	Millimeter
MMSE	Mini-Mental State Examination
MN	Minnesota
MoVIES	Monongahela Valley Independent Elders Survey
MR	Mendelian Randomization
MRI	Magnetic Resonance Image

MRC	Medical Research Council
MS	Mississippi
N	Number of participants
NAD+	Nicotinamide Adenine Dinucleotide
NAS	Nutrient Adequacy Score
NC	North Carolina
NHS	Nurses' Health Study
NIA-AA	National Institute on Aging-Alzheimer’s Association
NM	Nucleotide M
NMDAR	N-methyl-D-Aspartate Receptor
NOMAS	Northern Manhattan Study
PDD	Parkinson Disease Dementia
%	Percent
PKC	Protein Kinase C
PROSPER	The Pravastatin in the Elderly at Risk
QF	Quantity/Frequency
QIMR	Queensland Institute of Medical Research
RS	Reference SNP
RCT	Randomized Controlled Trials
RFLP	Restriction Fragment Length Polymorphism
ROS	Reactive Oxygen Species
SD	Standard Deviation
SE	Standard Error
SES	Social Economic Status

SLS	Seattle Longitudinal Study
SNP	Single nucleotide polymorphism
TIA	Transient Ischemic Attack
TIC-S	Telephone Interview for Cognitive Status
TICS-m	Modified Telephone Interview for Cognitive Status
TG	Triglycerides
TNFα	Tumor Necrosis Factor Alpha
μM	Micrometer
U.S.	United States
USA	United States of America
VaD	Vascular Dementia
Val	Valine
Val350	Valine 350
VFT-C	Verbal Fluency Test-Categorical
VFT-L	Verbal Fluency Test - Letter
VFT-L	Verbal Fluency Test-Letter
Vmax	Maximal Velocity
WFT	Word Fluency Test
WHIMS	Women's Health Initiative Memory Study
YLL	Life Years Lost
ω-3 FA	Omega-3 Fatty Acids

LIST OF GENES NAMES

ADH	Alcohol Dehydrogenases Class 1A
ADH1A	Alcohol Dehydrogenases Class 1A
ADH1B	Alcohol Dehydrogenases Class 1B
ADH1C	Alcohol Dehydrogenases Class 1C
ADH4	Alcohol Dehydrogenases Class 4
ADH5	Alcohol Dehydrogenases Class 5
ADH6	Alcohol Dehydrogenases Class 6
ADH7	Alcohol Dehydrogenases Class 7
ALDH	Aldehyde Dehydrogenase
ALDH1	Aldehyde Dehydrogenase 1
ALDH1A1	Aldehyde Dehydrogenase 1 Family Member A1
ALDH2	Aldehyde Dehydrogenase 2
APOB	Apolipoprotein B
APOE	Apolipoprotein E
APOE ε2	Apolipoprotein E ε2 allele
APOE ε4	Apolipoprotein E ε4 allele
Aβ	Amyloid β
CYP2E1	Cytochrome P450
RALDH1	Retinaldehyde dehydrogenase 1
sdLDL-c	Small Dense Low-Density Lipoprotein Cholesterol

CHAPTER I: INTRODUCTION

Cognitive impairment is a growing public health problem in the U.S. due to a rapidly aging and increasingly diverse population [1]. To reduce associated disability and morbidity [2], caretakers' burden [3-7], and high health care costs [8], it is important to identify and intervene upon modifiable factors that may prevent or reduce the risk of cognitive impairment. One such factor is ethanol use, which has a high prevalence of use (70%) and misuse (14%) in the U.S. [9]. Studies of the association of ethanol intake with cognitive decline have yielded inconsistent findings, likely attributable to a reliance on a single measurement of ethanol intake, non-standardized definitions of cognitive decline, short follow-up times, and or lack of analytic adjustment of confounders and effect measure modifiers. Few studies investigated the effects of ethanol intake in African Americans despite the disproportionate burden of cognitive impairment in this population.

Importantly, no study has investigated the effects of ethanol intake on cognition from mid-life to older adulthood. Further, ethanol-metabolizing gene variants alter the rate of ethanol oxidation, yet few studies have evaluated a possible effect measure modification of the ethanol intake-cognitive decline relationship by genetic variation in ancestrally diverse populations. Thus, studies based on diverse populations with repeated measurements of ethanol intake and cognitive function that have been genotyped for ethanol-metabolism SNPs are needed to better understand the relationship of ethanol intake with cognitive decline.

This doctoral research assessed the role of ethanol intake in cognitive impairment, as modified by genetic susceptibility in the bi-racial, population-based ARIC cohort of African-American and European-American adults. Analyses will include repeated measurements on participants who attended 5

examinations over 22 years of follow-up. Multiple imputation by chained equations will be used to account for attrition of the ARIC cohort during the years of follow-up.

CHAPTER II: SPECIFIC AIMS

2.1. Rationale

The proposed study seeks to estimate the relationship of ethanol intake and cognitive decline and evaluate possible modification of the ethanol intake-cognitive decline association by genetic susceptibility in a population-based sample of African-American and European-American participants aged 45-64 at baseline in the Atherosclerosis Risk in Communities (ARIC) study. This study will utilize data from ARIC visits 1-5. Manuscript 1 will address Specific Aim 1 and manuscript 2 will address Specific Aim 2.

2.2. Specific Aims

Specific Aim 1: Characterize 9-year trajectories of ethanol intake during mid-life in African-American and European-American adults and examine whether long-term trajectories of ethanol intake in mid-life are associated with 15-year rate of decline in cognition from mid-to-late life among African-American and European-American adults.

Hypothesis: a) Stable heavy drinking, mostly heavy drinking, stable former drinking, and mostly former drinking in mid-life is associated with greater 15-year cognitive decline compared to stable never drinking. b) Stable low-to-moderate drinking and mostly low-to-moderate drinking in mid-life is associated with lesser 15-year cognitive decline compared to stable never drinking.

Specific Aim 2: Assess effect modification of the ethanol intake-cognitive decline relationship by ethanol-intake associated SNPs in African-American and European-American men and women from mid-to-late life.

Hypothesis: Greater genetic ability to process ethanol (lower SNP set scores) is inversely related to 15-year cognitive decline per unit increase in ethanol intake.

2.3. Public Health Implications

Ethanol intake in mid-life is hypothetically associated with rate of cognitive decline from mid-to-late life through cerebrovascular and cardiovascular pathways. However, findings of a relationship between ethanol intake and cognitive decline have been inconsistent and limited by their use of single measurements of ethanol intake, assessment of ethanol intake in late life, and by not taking potential confounders and effect modifiers into account. Given the high prevalence of ethanol intake among adults in the U.S. and the dramatic increase in the prevalence of cognitive decline due to the projected aging and diversification of the population, understanding the relationship between ethanol intake in mid-life and rate of cognitive decline from mid-to-late life is important in reducing the burdens associated with cognitive decline.

This doctoral research work, by utilizing data from a large, racially-diverse population-based cohort with repeated measurements of ethanol intake and well-characterized cognitive function, quality controlled genetic data, and rich covariate data, has overcome some of the limitations of previous studies and aims to provide clear antecedent-consequent estimates of the ethanol intake-cognitive decline association. By exploring possible effect modification of this association by ethanol intake-associated genetic, this study aimed to inform mechanisms by which ethanol affects cognition. This study has added to the knowledge base by providing data on the effects of ethanol intake for future meta-analyses. In addition, study results may inform clinicians as they assess the risks and benefits of ethanol intake for their patients and public health practitioners in the areas of lifestyle modifications and policy.

CHAPTER III: BACKGROUND AND SIGNIFICANCE

This chapter reviews the epidemiology of cognitive decline and how cognitive decline is related to cognitive impairments such as mild cognitive impairment (MCI) and dementia. In addition, we will review literature on the effects of ethanol intake on cognitive decline, evidence of and reasons for inconsistent studies finding, and proposed mechanisms that may underlie both the neurotoxic effects and neuroprotective effects of ethanol on cognition. Furthermore, we acquired knowledge from *in vitro* and genetic association studies that polymorphisms within ethanol-metabolizing genes affects ethanol metabolism and may modify the ethanol intake-cognitive decline relationship.

First, in Section 3.1, we will discuss the epidemiology and public health burden of cognitive decline, and risk factors of cognitive decline that were identified in observational studies. Second, in Section 3.2,we will discuss the most widely used questionnaire measures of ethanol intake in epidemiological studies. Third, in Section 3.3,we will discuss the six primary neurocognitive domains, and in Section 3.4, we will discuss the neuropsychological tests and test batteries commonly used to assess decline in single or multiple neurocognitive domains. Fourth, in Section 3.5, we will discuss the absorption and elimination of ethanol intake in the human body, the different pathways of ethanol metabolism and polymorphic enzymes affecting the rate of ethanol metabolism. Fifth, in Section 3.6,we will discuss the proposed mechanisms that underlie the ethanol intake – cognitive decline relationship . Sixth, in Section 3.7, we will review prospective studies of ethanol intake and cognitive decline. We will conclude this chapter with a review of genetic association studies of *ADH* and *ALDH* polymorphisms with ethanol dependence and ethanol consumption (Section 3.8) and of gene-environment studies of ADH and ALDH polymorphisms and ethanol intake on cognitive decline (Section 3.9).

3.1. Epidemiology of Cognitive Decline

3.1.1. Cognitive Decline

Cognitive decline refers to the decline in mental processes, such as attention, short-term and long-term memory, reasoning, movement coordination, and planning of tasks, which are important for the conduct of daily living activities (Table 1) [10, 11]. Neurobiological and cognitive performance studies suggest that declines in cognitive function are gradual and develop from early adulthood, mid-20s or early 30s. The extent of decline depends on the type of cognitive domain [12-14]. Crystallized cognitive abilities (i.e., language and visuospatial), which refers to the skills, ability, and knowledge that is overlearned, well-practiced and familiar, remain stable or gradually improve at a rate of 0.02 to 0.003 standard deviations per year through the sixth and seventh decades of life [15, 16]. Fluid cognitive abilities (i.e., processing speed, attention, memory, language, visuospatial, and executive function), which refer to abilities to problem-solve and reason, independent of one's past knowledge, peak in the third decade of life and then decline at an estimated rate of -0.02 standard deviations per year [15, 16]. By age 70, most individuals have a significantly lower cognitive performance compared to their mid-life cognition levels [17, 18]. Although cognitive decline with age is normal, decline is not inevitable. Studies indicate that older adults retain exceptional cognitive function until their 70s and 80s and have performance that is comparable or better than younger adults [19-22].

The rate of cognitive decline varies among individuals [23-27]. Studies have shown that rate of cognitive decline in older adults is associated with lifetime differences in experiences, health status, lifestyles, education, attitudinal and emotional factors, socioeconomic status, and genetics [11]. It is well documented that there exist racial and ethnic differences in cognitive function at older ages [28-30]. However, it is unclear whether racial and ethnic differences exist for rates of cognitive decline. Findings are inconsistent on the effect of race on cognitive decline, with some studies reporting African-Americans having higher rates of cognitive decline compared to European-Americans [31-33], while other studies report no difference in cognitive decline by race [34, 35], and others report that African-Americans have slower rates of cognitive decline than European-Americans [29, 36-38].

Faster rates of cognitive decline may lead to earlier onset of cognitive impairment and dementia, which may result in significant burden in those experiencing decline and their caregivers [39]. Age-related cognitive decline occurs on a continuum, and there is not yet a consensus of a boundary that distinguishes physiological and pathological changes [40]. Consequently, there is no standardization in the methods of research to define age-related cognitive decline, making comparisons of study results difficult. As a result, reliable epidemiological evidence on risk of cognitive decline are difficult to collect, and are therefore lacking in literature [41].

By 2050, it has been projected that the number of Americans over the age of 65 will double to 83.7 million, from 43.1 million in 2010 [1]. In addition to an increase in the number of individuals aged 65 years and older, it expected that the U.S. will become more racially and ethnically diverse [1]. Consequently, the number of Americans at risk for cognitive impairment and dementia will increase dramatically as the population ages. Furthermore, studies indicate that African American and other racial minority groups are disproportionately burdened with Alzheimer's disease (AD), the most common form of dementia, and other forms of cognitive impairment [37-40]. In an attempt to reduce the incidence of cognitive impairment and dementia, current research has focused on identifying modifiable risk factors that can prevent or delay the progression of cognitive decline in diverse populations.

3.1.2. Mild Cognitive Impairment and Dementia

3.1.2.1. Definition, Prevalence, and Incidence of MCI

MCI is a syndrome defined as cognitive decline greater than expected for an individual's age and education level, but that does not interfere notably with activities of daily life [42]. The National Institute on Aging-Alzheimer's Association (NIA-AA) core clinical criteria for MCI include 1) evidence of concern about a change in cognition, in comparison with the individual's previous cognitive level, 2) impairment in one or more cognitive domains that is greater than would be expected for the patient's age and educational background, 3) mild problems performing complex functional tasks which they used to perform previously (e.g., paying bills, preparing a meal, shopping, etc.) while able to maintain independence of function in daily life, with minimal aids or assistance, 4) no evidence of a significant

impairment in social or occupational functioning, or 5) a score of 1 to 1.5 standard deviations below the mean for their age and education-matched peers on culturally appropriate normative data on cognitive tests [43, 44]. Cognitive and functional severity within the MCI definition varies widely, thereby the MCI syndrome is heterogenous. The heterogeneity of MCI explains the variability in prevalence rates, incidence rates, and rates of progression to dementia.

Subtypes of MCI are characterized clinically by the presence of memory impairment (amnestic MCI) or the absence of memory impairment with presence of impairment in one or more non-memory cognitive domain (non-amnestic) [45]. Classification of MCI subtypes relates to the underlying etiology, pathology, clinical presentation, and outcomes (Table 2). The etiology of MCI includes neurogenerative disease, apolipoprotein E ε4 allele (*APOE* ε4), spontaneous features of parkinsonism damage, and cerebrovascular disease. Pathology of MCI can include neurodegenerative, amyloid β (Aβ) plaques, neurofibrillary tangles, hippocampal atrophy, reduced brain volume, cerebrovascular, cortical infarctions, subcortical infarctions, and white matter hyperintensities. MCI may consist of impairment in a single or multiple cognitive domains [45]. The number of impaired domains determines disease severity and the likelihood of progression to dementia. Multiple-domain MCI represents greater disease severity compared to single domain MCI, which in turn implies a higher rate of progression from MCI to dementia. Single or multiple domain amnesia MCI may progress to AD if there is an underlying degenerative or vascular pathology. Typically, single or multiple domain non-amnestic MCI as a manifestation of degenerative etiology progresses to non-AD dementias (e.g., frontotemporal dementia) or dementia with Lew Bodies, [46].

Population based cohort studies of MCI estimated the prevalence of MCI in the US to be 16-20% in individuals aged 65 and older [45]. The few studies on MCI incidence rates observed estimates ranging from 5.1 to 168 per 1000 years [45, 47].

Relatively few studies examined the mortality of MCI cases. Study findings suggest increased mortality among MCI cases compared to cognitively normal individuals over a median follow-up time of 5.7 years [48-50].

An important MCI outcome is the increased risk of progression to dementia [51], with most studies reporting rates of progression from MCI to dementia from 10-15% per year [45, 51]. Risk factors for progression to dementia include the degree of functional impairment, severity of neuropsychological test scores [52], and presence of neuropsychiatric behavior [53] at the time of MCI diagnosis.

MCI is an important public health concern due to the increased risk of progression to dementia and increased mortality.

3.1.2.2. Definition, Prevalence, and Incidence of Dementia

Dementia is a disorder characterized by a decline in cognition involving one or more cognitive domains that affects an individual's ability to perform everyday activities [54], unlike MCI where the ability to function in daily life is preserved . The NIA-AA defines dementia when there are cognitive or behavioral (neuropsychiatric) symptoms that 1) interfere with the function of usual daily activities, 2) represent a significant decline in from previous level of functioning, and 3) are not explained by delirium or major psychiatric disorder; 4) cognitive impairment detected and diagnosed through a combination of medical history and mental status examination or neuropsychological tests; cognitive or behavioral impairment that involves a minimum of two of the following domains: recent memory, executive function, visuospatial abilities, and language [55]. AD is the most common form of dementia. Other, less common forms of dementia include Vascular (multi-infarct) dementia (VaD), Lewy bodies Dementia (LBD), Parkinson Disease Dementia (PDD), and Frontotemporal Dementia (FTD).

AD is irreversible and progressive, it slowly destroys memory and thinking skills and eventually the ability to carry out simple tasks. AD accounts for an estimated 60 to 80 percent of dementia cases. For a diagnosis of probable Alzheimer's, the criteria adapted from the NIA-AA include dementia established by examination and objective testing, progressive worsening of memory and other cognitive functions, and deficits in two or more cognitive areas (executive function, visuospatial abilities, and language). Absence of systemic disorders or other brain diseases, which could account for the deficits in memory and cognition, should also be established [55].

VaD may arise as a sequel to any form of cerebrovascular disease. VaD accounts for about 20 percent of dementia cases. A diagnosis of probable vascular dementia VaD is based on the following information: history of stroke, evidence of relevant cardiovascular disease (CVD) by brain imaging including multiple large-vessel infarcts or a single strategically placed infarct, any combination of onset of dementia within three months following a recognized stroke; abrupt deterioration in cognitive functions; or fluctuating, stepwise progression of cognitive deficits [56].

LBD is caused by abnormal deposits of a protein called alpha-synuclein in the brain. These deposits, called Lewy bodies, can lead to impairment in thinking, movement, behavior, and mood. The LBD Consortium core clinical revised criteria for the diagnosis of probable LBD include at least two of the following features: fluctuating cognition with pronounced variations in attention and alertness, recurrent visual hallucinations that are typically well formed and detailed, and spontaneous features of parkinsonism [57].

PDD should be used to describe dementia that occurs in the context of well-established Parkinson disease. The diagnosis is PDD when an individual is originally diagnosed with Parkinson's based on movement symptoms and dementia symptoms don't appear until a year or more later [57].

FTD is caused by a family of brain diseases known as frontotemporal lobar degeneration (FTLD). These disorders are the result of damage to neurons in parts of the brain called the frontal and temporal lobes. The diagnosis of FTD requires a thorough history, verified by a caregiver, and a neurological examination [58].

In 2015, the prevalence of AD in the US was estimated at 5.2 million among individuals aged 65 years and older. In the US, an individual develops AD every 67 seconds. By 2050, a new case of AD is expected to develop every 33 seconds, resulting in approximately 1 million new cases per year, and the expected prevalence to triple to 16 million due to the aging population [59, 60].

AD is the sixth-leading cause of death in the US and the fifth-leading cause of death in those aged 65 years and older [61]. Between 2000 and 2013, the mortality rate from AD increased by 71% while

deaths from heart disease, stroke, and prostate cancer decreased by 14%, 23%, and 23%, respectively [61]. In 2015, it was estimated that 700,000 individuals aged 65 years and older will die from AD.

AD is also a leading cause of disability and morbidity. From 1990 to 2010, AD's rank in disability-adjusted life-years (DALYs) rose from 25th to 12th, and from 32nd to 9th in life year lost (YLL), the largest increase for any disease [2].

AD is burdensome on patients, care givers, and society. In 2014, an estimated 17.9 billion hours of unpaid care was provided by 15.7 million caregivers of individuals with AD [59]. The economic value of care provided by unpaid caregivers in 2014 was $217.7 billion [59]. Family members who care for AD patients experience distress including emotional stress and depression, deteriorated health, and depleted income and finance due to disruptions in employment [3-7]. AD is one of the most expensive chronic diseases. In 2015, the cost of health care, long-term care and hospice for individuals with AD was $226 billion [8].

3.1.3. Risk Factors for Cognitive Decline

Given the public health importance of preventing cognitive impairment in individuals and promoting cognitive health, achieving a better understanding of the various beneficial and deleterious risk factors that influence cognitive decline is important so that prevention and remediation efforts can be developed [11]. Over the past several decades many modifiable risk and protective factors have been studied in relation to cognitive decline [10, 62, 63]. Presented in Table 3 is a summary of findings on potential risk factors for cognitive decline from observational studies. In this section, risk factors for cognitive decline will be discussed.

Risk factors for cognitive decline include demographic factors (age, sex, race, educational attainment, and social support), genetic factors (*APOE* ε4 allele), lifestyle factors (smoking, physical activity, omega-3 fatty acids (ω-3 FAs)), and medical factors (diabetes, hypertension, hyperlipidemia, obesity, stroke, and depression).

3.1.3.1. Demographic Factors

Demographic risk factors of cognitive decline include age, sex, race, educational attainment, and social support.

A3.1.3.1. Age

As discussed in Section A.1., cognitive abilities tend to decline with age [64]. “Fluid abilities” are most affected by age, while “crystallized” abilities are more resistant.

A.3.1.3.2. Sex

Reports on sex differences in cognitive function have been inconsistent [36, 65-69]. Some studies reported lesser age-related cognitive decline in women [65-67], while others reported greater age-related cognitive decline in women [36], and no sex differences in age-related cognitive decline [68, 69]. Sex differences in age-related cognitive decline have been attributable to improved living conditions and less gender-restricted educational opportunities favoring women in some cognitive functions (episodic memory) and decreasing or eliminating differences in other cognitive abilities [70].

A.3.1.3.3. Race

As discussed in Section A.1., studies finding on the effect of race on cognitive decline are inconsistent, with some studies reporting African-Americans having higher rates of cognitive decline compared to European-Americans [31-33], while other studies report no difference in cognitive decline by race [34, 35], and others report that African-Americans have slower rates of cognitive decline than European-Americans [29, 36-38]. An explanation for racial differences in cognitive impairment is that elderly African-Americans have fewer years of education than European-Americans [31, 71, 72] and such differences may contribute to racial differences in cognitive decline [31].

A.3.1.3.4. Educational Attainment

Low educational attainment has been associated with poor cognitive function and faster age-related cognitive decline [73-75] . The concept of cognitive reserve has been proposed as an explanation for why less education is associated with greater cognitive decline. Education may directly modify brain structure by increasing synapse number or vascularization and creating a cognitive reserve. The

"cognitive reserve capacity" hypothesis postulates that conditions in early-life affect the rate of cognitive decline in later-life [76]. Early-life education may have effects in late-life if individuals with more education continue to engage in mental stimulation, which may result in beneficial neurochemical or structural alterations in the brain [77].

A.3.1.3.5. Social Support

Some studies demonstrated that social activities, larger social networks, and a history of social contact are associated with cognitive function [78-90]. In the study by Yeh et al., marriage and perceived positive support from friends were significantly and positively associated with cognitive function, while loneliness and living alone were not significantly associated with cognitive function [88]. Because social activities provide the challenge of effective communication and participation in complex interpersonal exchanges, social support has been thought to inhibit cognitive decline in the elderly [89]. However, an independent coordinated analysis of four longitudinal studies found no effect social activity on cognitive function [91]. Most studies of social engagement and cognitive function are small, are combined with cognitive training and/or physical activities, and/or dissimilar in types of social engagement, making it difficult to draw any conclusions [63].

A.3.1.3.2. Lifestyle Factors

Lifestyle risk factors of cognitive decline include smoking status, physical activity, and ω-3 FAs.

A.3.1.3.2.1. Smoking

Prospective studies of smoking exposure on age-related cognitive decline has been inconclusive [92-100], with some studies reporting null associations [92-94], and others reporting a positive association [96-101].

The British 1946 birth cohort study considered the difficulty of finding an association between smoking and cognitive impairment provided the differential high morality of smokers especially among the elderly population [101]. This study, after controlling for socioeconomic and health status variables, found that smokers who survive into later life maybe at risk of clinically significant cognitive decline. These effects were observed mostly among heavy smokers (i.e., individuals smoked 20 cigarettes per day

or more). Early studies in middle-aged adults indicated that current smoking and number of pack-years of smoking are related with reduced performance on tests of psychomotor speed assessed 5 years later [99]. Similar results were observed for cognitive decline in a large cohort study (Rotterdam Study) conducted in multiple European countries [96] and in a recent study that was conducted in the US [102].

The mechanisms by which smoking affects cognitive decline remain unclear. However, it has been shown that smoking exposure is associated with periventricular and subcortical white matter lesion progression, which themselves are associated with greater cognitive decline independent of known cardiovascular risk factors [95].

A.3.1.3.2.2. Physical Activity

Physical activity is postulated to have potential protective effects on cognitive function by reducing the risk of related comorbidities (coronary heart disease (CHD), stroke, and diabetes mellitus), sustaining cerebral blood flow [103], improving aerobic capacity and cerebral nutrient supply [104, 105] as well as growth factors (i.e., brain-derived neurotropic factor) [106, 107]. In their system review, Beydoun et al. suggested that physical activity could represent an important and potent protective factor for cognitive decline in elderly persons, with 21 of 24 prospective studies reporting an association between physical activity and cognitive outcomes [10].

Several randomized trials and a Cochrane review of such trials reported significant improvement in cognitive function among previously inactive, but healthy, seniors who started an exercise program [108, 109] . Studies consistently demonstrated that exercise must be regular and vigorous [110-113]. However, studies have been unable to determine the optimal duration, type and intensity of physical activity, and time period in an individual's lifespan that physical activity should occur so that its protective effect on cognitive decline is maximized [63].

A.3.1.3.2.3. ω-3 FAs

Epidemiological studies suggest that ω-3 FAs are protective of cognitive decline [114]. In animal and *in vitro* studies, ω-3 FAs have been shown to have a wide variety of beneficial effects on neuronal functioning, inflammation, oxidation and cell death [114].

A.3.1.3.3. Genetic Factor

A genetic risk factor for cognitive decline is the *APOE ε4* allele.

A.3.1.3.3.1. Apolipoprotein E ε4 genotype

The *APOE ε4* allele is a well-established risk factor for AD [115], and has been implicated in an earlier age of onset of AD [116] compared to non-carriers. *APOE*, a lipid transport protein that is encoded by the polymorphic *APOE* gene, has three functional main isoforms that are encoded by three common polymorphisms, ε2 (cys112, cys158), ε3 (cys112, arg158), and ε4 (arg112, arg158). Although these allelic forms differ from each other by only one or two amino acids at positions 112 and 158, these differences alter *APOE* structure and function. While the *APOE ε2* allele may be protective of AD [117], the *APOE ε4* allele has been associated with reduced neuronal survival and cognitive impairment [117, 118]. Prospective studies indicate that carriers of the *APOE ε4* allele are at increased risk of cognitive decline [119-125].

A.3.1.3.4. Cardiovascular Disease Risk Factors

Cardiovascular disease risk factors of cognitive decline include diabetes, hypertension, hyperlipidemia, obesity, stroke, and depression.

A.3.1.3.4.1. Diabetes

Diabetes has been consistently associated with an increased risk of cognitive decline [126-132]. The ARIC study of 13,351 African-American and European-American adults ages 48 to 67 at visit 2 reported mid-life diabetes is associated with greater cognitive decline over 20-years compared to diabetes [62, 131]. The mechanisms that underlie the associations of diabetes with cognitive decline remain unclear, but glycemic control may play a crucial role in this association and could contribute to both neurodegenerative and vascular damage [133].

A.3.1.3.4.2. Hypertension

Longitudinal studies have provided strong evidence of a high blood pressure (hypertension) and cognitive decline relationship [134]. Mid-life hypertension is consistently reported as a risk factor for cognitive decline [135-141]. The ARIC study of 13,467 African-Americans and European-American

adults ages 48-67 at study baseline reported that hypertension in mid-life was independently associated with a steeper decline in cognitive performance over 20 years [135]. By contrast, studies indicate that late-life hypertension may not be a critical risk factor in cognitive aging [142].The effect of hypertension on cognitive function is likely mediated by several mechanisms, which includes small and large vessel disease, microinfarcts, leukoaraiosis, and changes in cerebral metabolism [143].

A.3.1.3.4.3. Hyperlipidemia

Hyperlipidemia, especially hypercholesterolemia, is associated with cognitive decline [144]. Lipid regulation plays a crucial role in neuroplasticity and survival [145]. Like hypertension, hyperlipidemia may be a stronger risk factor in mid-life cognitive decline than in late life [146, 147].

A.3.1.3.4.4. Obesity

Although additional longitudinal studies are needed [62], emerging evidence support the existence of obesity-related brain changes and dysfunction [148]. The mechanisms by which obesity contributes to cognitive decline remains unclear. The effects of obesity on cognition are likely mediated through pathways such as the effects of diabetes or metabolic syndrome (i.e., inflammation, insulin resistance, endothelial dysfunction, and microvascular disease). Obesity may also increase the risk of cognitive aging directly through the presence of excessive adipose tissue and the secretion of inflammatory proteins (i.e., leptin), which is associated with cognitive impairment and decline [149-151]. A meta-analysis conducted by Anstey et al. of 15 prospective studies with follow-up times ranging from 3.2 to 36.0 years suggests the effects of obesity on cognition may differ between mid-life and late-life [152].

A.3.1.3.4.5. Stroke

Stroke is associated with cognitive decline [153, 154]. Stroke may cause long-term cognitive decline by inducing or exacerbating neurodegenerative disease [155, 156], or neurodegenerative disease may amplify brain injury and cognitive deficits after stroke [157].

A.3.1.3.4.6. Depression

Studies have found an association between depressive symptoms and rate of cognitive decline that is independent of the neuropathologic conditions most strongly linked to late-life cognitive decline [158-160]. However, it remains unknown if depression increases an individual's risk of cognitive decline or serves as an early marker of brain changes [63].

3.1.4. Summary

In summary, the public health burden of cognitive decline is increasing due to an aging population. Therefore, it is of public health significance to identify and intervene upon modifiable factors that may prevent or reduce the risk cognitive decline. Few prospective studies have examined the relationship between modifiable factors and cognitive decline. These studies provide evidence from which we can conclude that regular physical activity, a healthy diet, smoking cessation, and reduction and management of obesity, diabetes, hypertension, and stroke may reduce the risk of cognitive decline [63]. However, much remains to be known about the relationship between other modifiable factors such as ethanol intake and cognitive decline, and how this relationship maybe modified by single polymorphisms in ethanol-metabolizing genes. As a result, the focus of this work will be on the association between ethanol intake and cognitive decline, and the possible modification of this relation by single nucleotide polymorphisms (SNPs) within ethanol-metabolizing genes.

In Section 3.2, we will discuss the proposed mechanisms that underlie the ethanol intake-cognitive decline relationship.

3.2. Measurements of Ethanol Intake

The most commonly used methods in epidemiological studies to measure self-reported ethanol intake include the food frequency questionnaire (FFQ), the quantity/frequency (QF), and the graduated frequency (GF). Epidemiological studies of ethanol intake largely use self-reports [161-163]. Self-reports of ethanol intake are mostly reliable [164-166]. Regarding validity, in the absence of a "gold standard", assessing the validity of self-report is difficult [162, 163]. Systematic variations of ethanol intake

measurements may be attributable to the construct validity of the assessment tool (questions and response categories) and the assessment mode (e.g., self-report, interviewer administered, observer rating, or computer assisted) [167, 168].

The FFQ [169] is the most common method of dietary assessment that is used by large epidemiological studies of diet and health [170]. The FFQ asks participants to report the frequency and quantify of food items and food groups over a specified period. More specifically, in epidemiological studies of ethanol intake, participants are asked how often on average over a defined time (e.g., day, week, months and year), they consumed each type of alcoholic beverage (i.e., beer, wine, and spirits/hard liquor, separately). Advantages of the FFQ include a low burden on the respondent, a low administrative cost, and the ease at which the test can be administered compared to other methods of dietary assessments (~30 minutes) [170, 171]. Disadvantages of the FFQ include a heavy reliance on participant's long-term memory, and with an FFQ the precision in quantifying intake is not possible [172, 173].

The QF [174] is among the earliest measures of ethanol intake. QF measures are known as estimation formulae because they query participants about their "average" ethanol intake patterns with two questions that inquire about: 1) the overall frequency of ethanol intake within a specified period of time (F) ("How often do you drink?"), and 2) the number of drinks consumed on days that the participant drank (Q) ("How many per occasion?") [175]. Total volume of ethanol intake is derived by multiplying frequency (F) and quantity (Q). QF measures generally provide reliable information on quantity and frequency of drinking day and are most useful when time is limited and information about atypical drinking is not required. One advantage of the QF is that it is simple and relatively easy to complete. The QF has numerous disadvantages [176, 177]. Researchers have suggested that responses to QF tend to 1) describe 'modal' rather than 'average' behavior [162, 175, 178], 2) misclassify drinkers and under-report occasions of heaving drinking [179, 180], and 3) underestimate volume of ethanol intake [181-183] .

The GF [176] measure was developed to overcome some of the limitations of QF and allows pattern information as well as generate volume of ethanol intake directly. GF comprises of a series of questions that asks about ethanol intake in terms of graded amount (e.g., one to two drinks, three to four

drinks, etc.) or thresholds [184]. Compared to QF, GF estimates for volume consumer is higher [182]. There is also evidence to suggest that GF overestimate ethanol intake [185]. Some of this overestimate may have been partly due to the algorithm for calculating total frequency which used the middle range for each frequency category [186]. Nonetheless, the GF measure is widely used in survey research involving ethanol intake.

3.3. Neurocognitive Domains

Cognition is important for functional independence (i.e., an individual's ability to perform activities of daily living) such as living independently, managing finances, adhering to medication, and driving safety. Intact cognition is critical for effective communication, which includes processing and integrating sensory information and appropriately responding to others. Cognitive abilities often decline with age. Because cognitive abilities generally decline with age, it is of public health significance to comprehend the types of cognition changes that occurs with normal aging [187].

In this section, we will describe the primary neurocognitive domains, as well as decline in each domain.

3.3.1. Memory

Memory is the capacity to process, maintain, and to immediately manipulate available information [188]. The two main types of memory are working memory (short-term memory) and long-term memory (Table 4) [189]. Both types of memory are important to everyday functioning [11].

3.3.1.1. Working Memory - Short-term Memory - Recent Memory

Working memory is the ability to temporarily retain information while it is processed or being used. Working memory encompasses the active manipulation of information, the maintenance of some information, while concurrently processing incoming information [11]. Working memory plays an essential role in the execution of many activities, such as adherence to a medication schedule [11], and is a central component of other cognitive abilities such as language processing, problem solving, decision making, and new learning [11]. Studies of the effects of aging on working memory indicate that working

memory generally declines with age, largely for complex task [190-193]. In this work, the association between ethanol intake and decline in short-term (recent) memory will be assessed.

3.3.1.2. Long-Term Memory

Long-term memory is the system for storage of permanent knowledge and a repository of an individual's knowledge [11]. Long-term memory is divided in 2 types: explicit (or declarative) memory and implicit (or procedural) memory (Table 4). Declarative memory ("knowing what") is memory of facts and events and refers to those memories that can be consciously recalled (or "declared"). It is sometimes called explicit memory, since it consists of information that is explicitly stored and retrieved, although it is more properly a subset of explicit memory. Declarative memory can be further sub-divided into episodic memory and semantic memory [11]. Procedural memories also known as skill learning, refers to learning and remembering how to perform an activity such as driving a car, riding a bicycle, cooking a favorite recipe, or using a software program. These memories are typically acquired through repetition and practice [11]. Long-term memory was not assessed in the ARIC study. Therefore, we will not provide details on the different types of long-term memory and we will not describe decline in each long-term memory domain.

3.3.2. Speed of Information Processing

Speed of information processing reflects the efficiency of cognitive operations [11]. Declines in processing speed may affect an individual's ability to recall spoken instructions, address important information, or perform tasks that have pacing demands [11]. In this work, the association between ethanol intake and decline in processing speed will be assessed.

3.3.3. Executive Function

Executive function refers to cognitive skills used to regulate behavior and modify responses based on environmental cues [11]. These cognitive skills include the ability to plan actions, organize information, think abstractly, allocate mental resources, reason, problem solve, adjust to new situations, and appropriate behavior during social interactions [11]. Declines in executive function may affect an

individual's ability to make decisions, to respond, and to concurrently process relevant and irrelevant information. Declines in executive function have been linked to declines in the ability to perform important daily life activities such as medication management [194]. In this work, the association between ethanol intake and decline in executive function will be assessed.

3.3.4. Attention/Concentration

Attention is the capacity for processing information [11]. Humans are limited by the quantity of information they can process with a specified timeframe. For most individuals, especially for older adults, performing tasks at full capacity for long durations can be tiresome [195, 196]. Cognitive performance in attention tends to decline and be susceptible to error, when capacity limits have been exceeded [195]. Summarized in Table 5 are the different types of attention which includes selective attention, divided attention, and sustained attention. The attention neurocognitive domain was not assessed in the ARIC. Therefore, declines in attention will not be evaluated in this work.

3.3.5. Language

Language function refers to the ability to comprehend and formulate speech, read, write, and promptly name words by category or sound [11]. Language function is an important component of human behavior and a main mechanism for communication [11]. Language processing is essential for completing cognitive tasks and includes comprehending written and spoken instructions and social interactions. Individuals whose spoken language is impeded, for example, by hearing loss may withdraw from social interaction [197]. Reduced social interaction not only negatively impacts an individual's quality of life but may contribute to cognitive decline. In this work, we will assess decline in the language neurocognitive domain.

3.3.6. Sensory and Motor Function - Visuospatial Skills

Visuospatial skills refer to an individual's ability to identify visual and spatial relationships between objects [11]. Visuospatial skill is measured in terms of the ability to imagine objects, produce objects, or to comprehend the similarities and differences between objects [11]. Visuospatial skills are

pertinent for tasks such as learning environmental layouts and routes, map reading, and translating directions [11]. The visuospatial skills domain was not assessed in the ARIC. Therefore, decline in visuospatial skills will not be evaluated in this work.

3.4. Measurements of Cognitive Function

In the previous section, we discussed the different neurocognitive domains and described decline in each cognitive domain. In this section, we will discuss neuropsychological tests that are used in clinical settings and epidemiological studies to screen for dementia and measure cognitive changes over time.

Neuropsychological tests are specifically designed tasks used to measure a psychological function known to be linked to a brain structure or pathway. Neuropsychological tests are utilized in research of brain function and in clinical settings to diagnose cognitive deficits. Since neuropsychological tests are usually administered to an individual by a trained individual in a quiet room, these tests provide an estimate of an individual's peak level of cognitive performance [198].

Most neuropsychological tests that are in current use are based on psychometric theory. In this psychological model, an individual's raw score on a test is compared to a large general population ("normative sample") that is similar to the individual in one or more characteristics such as age, gender, level of education, and race. Research findings have found that these characteristics are associated with cognitive performance in cognitively healthy people. The comparison of an individual's raw score to a normative sample, therefore provide a fair assessment of their cognitive function [11].

Most forms of cognition involve multiple cognitive functions working in unison, however tests can be organized into broad categories based on the cognitive function which they predominantly assess [15]. Some tests appear under multiple headings as different versions and aspects of tests can be used to assess different functions [15].

In this work, we narrowed our discussion of psychological tests to the digit symbol substitution test (DSST), delayed word recall test (DWRT), word fluency test (WFT) that were employed in the ARIC Study to assess cognitive deficits in the attention and psychomotor speed, verbal learning and short-term memory, executive function and language domains, respectively. We will also discuss test batteries which

combine multiple tests to provide an overview of cognitive abilities. Test batteries are commonly used in prospective studies to assess the relationship between ethanol intake and cognitive decline. If implemented early, these tests may be used to rule out problems in certain cognitive functions and provide an indication of functions which may be tested specifically. Tests of mental status include the MMSE, the modified Mini-Mental State Examination (3MS), the Telephone Interview for Cognitive Status (TIC-S), and the Cognitive Abilities Screening Instrument (CASI).

3.4.1. Neuropsychological Tests

The DSST is a subset of the Revised Wechsler Adult Intelligence Scale. The DSST assesses the attention and psychomotor speed cognitive domains. DSST requires use of motor speed, sustained attention, and visual spatial skills. Participants are given 90 seconds to fill in blank squares with symbols corresponding to digits from 1 to 9 using a key that matches digits to symbols [199]. The DSST test scores ranges from 0 to 93 and has high reliability (0.82-0.88) in older adults [199]. The DSST test is more sensitive to brain damage, cognitive decline, and dementia than other Wechsler Adult Intelligence tests [199]. Unlike the MMSE, the DSST does not suffer from a ceiling effect and can identify changes at the highest levels of cognition [200].

The DWRT assesses verbal learning and short-term memory. Participants are asked to learn 10 items, and after a five-minute delay are given 60 seconds to recall the word. The DWRT score ranges from 0 to 10 and has a high test-retest reliability of 0.75 in older adults [201]. Of all the cognitive tests, the DWRT is the most discriminating test in identifying individuals with early AD. DWRT appears to be affected by age and sex, and there is evidence of practice effects [202].

The WFT , also known as the Controlled Oral Word Association Test (COWA) is a part of the Multilingual Aphasia Examination (MAE) [203]. The WFT assesses executive function and language [203, 204]. Participants are given 60 seconds to generate as many words as possible for the letters F, A and S, avoiding proper nouns. The WFT score is the total number of acceptable words generated for the three letters [205], and has a test-retest reliability of 0.88 in older adults [206]. The WFT is useful for

detecting frontal lobe damage and early mental decline in older persons [207]. Factors that may influence WFT include education, sex, and age [207].

3.4.2. Batteries Assessing Multiple Neuropsychological Functions

The MMSE is used extensively in clinical and research settings to measure global cognitive impairment in older adults [208, 209]. The MMSE is commonly used in medicine to screen for dementia, and in longitudinal studies to measure cognitive chance over time [210]. The MMSE is a quick (~5 to 10 minutes to administer) and short exam (consists of 11 questions, maximum total score of 30) that assesses seven areas of cognitive functioning (i.e., orientation to time, orientation to place, registration of three words, attention and calculation, recall of three words, language, and visual construction), and has a cut-off of 23/24 out of 30 to show significant cognitive impairment. The MMSE has been shown to have both good test-retest reliability (0.80–0.95) [208-211] and acceptable sensitivity and specificity to detect mild to moderate stages of dementia [208-212]. However, the MMSE is less sensitive in detecting MCI and fails to discriminate patients with early stages of AD from normal patients. It is also insensitive to impairments in executive functioning, abstract reasoning, and visual perception/construction [213-215]. Moreover, the MMSE is affected by demographic factors such as age, education and race/ethnicity, with age exerting the greatest effect [216]. This has limited its use for detecting change in clinical work and in research studies. MMSE has a “floor” effect in terms of its inability to detect changes in established advanced dementia in those with little formal education and those with severe language problems, and a ‘ceiling’ effect in that it may fail to detect very mild illness, and mild/moderate cognitive impairment in people at high educational level or premorbid intelligence [217].

Because of the shortcomings of the MMSE can be attributable to the narrow range of possible scores and ceiling effect, an expanded version of the MMSE, 3MS was developed to include four additional questions (date and place of birth, word fluency, similarities, and delayed recall of words), and to increase the maximum total score to 100 points [218]. Several studies have consistently shown the reliability of the 3MS to be higher than the MMSE in a variety of samples [219-223]. The 3MS is more in

detecting dementia in comparison to the MMSE [220, 222, 224]. Additionally, a reduced rate of false-negative classifications and an increased sensitivity of the 3MS over the MMSE [223].

The TICS [225], is a global mental status test that can be administered over the phone or face-to-face. The TICS are used in epidemiological studies and clinical trials to monitor changes in cognitive functioning over time, and is known to have high reliability and validity [225, 226]. The TICS is a quick (~10 minutes to administer) and easy measure (consists of 11 questions, maximum total score of 41) that assesses six cognitive domains (i.e., orientation, concentration, short-term memory, language, praxis, and mathematical skills), and has a cut-off of 24 out of 41 to show significant cognitive impairment. The TICS demonstrates a high correlation with the MMSE and has been found to have excellent sensitivity (94%) and specificity (100%) in differentiating participants with AD from those who have normal cognitive functioning [225]. Although the TICS was modeled after the MMSE, it has less ceiling effects than the MMSE, and can be reliably used even for persons with visual or physical deficits [226, 227]. A modified version of the TICS, the TICS-m [226, 228] was developed to include the delayed recall item, known as the most sensitive cognitive measure for MCI and AD detection. The addition of the delayed recall item of the TICS-m has resulted in enhanced sensitivity of the measure for detecting cognitive impairment and reduced ceiling effects relative to the MMSE [229, 230].The TICS-m has been found to have excellent sensitivity (>99%) and specificity (86%) in the screening and detection of AD [231, 232].

The CASI is an instrument designed for identifying cognitive changes in the elderly. The CASI has been used to screen for dementia in epidemiological studies [233-237]. The CASI consists of items either identical or similar to the ones used in the Hasegawa Dementia Rating Scale (Hasegawa DRS[238]), the MMSE [208], and the 3MS [218]. The CASI can be administered in approximately 15-20 minutes and consists of 25 questions that assesses nine cognitive domains of attention, concentration, orientation, short-term memory, long-term memory, language abilities, visual construction, category fluency and abstraction and judgment, which adds up to a total score of 100. The CASI has a maximum score of 100 and has a cut-off $\leq$ 65 points to show significant cognitive impairment [239].

3.5. Ethanol Intake Metabolism

3.5.1. Ethanol Absorption and Elimination

Ethanol intake is probably the most extensively investigated drug due to its widespread use and distinct pharmaceutical properties [240]. After oral intake, ethanol is transported from the stomach to the small intestine, where it is rapidly absorbed into the blood and distributed throughout the body (Figure 3) [241, 242]. Approximately 20% of ethanol is absorbed from the stomach while 80% is absorbed from the small intestine [240]. The rate of absorption of ethanol depends on a variety of factors that include ethanol volume and concentration [241, 243, 244], and fed or fasting state [245]. Ethanol is highly miscible to water and can also be found in body fluids and tissues [242]. Ethanol is primarily metabolized in the liver (~95% of ingested ethanol), but other metabolic pathways include breath (0.7%), sweat (0.1%) and urine (0.3%) [242].

Ethanol metabolism involves several enzymes. The primary enzymes involve in ethanol metabolism in the liver are ADH and ALDH (Figure 4). These enzymes help break apart the ethanol molecule, making it possible to eliminate it from the body. Upon ingestion and absorption into the blood stream, ADH metabolizes ethanol to acetaldehyde, a highly toxic substance and known carcinogen [246]. Then, in a second step, acetaldehyde is further metabolized to a less active byproduct, namely acetate [246], which then is broken down into water and carbon dioxide allowing for easy elimination [247]. This reaction is mediated by the mitochondrial enzyme ALDH (Figure 4). In this metabolic chain of events, two basic mechanisms result in the accumulation of acetaldehyde in the body: 1) faster metabolism of ethanol to acetaldehyde, which is related to increased ADH activity, and/or 2) slower metabolism of acetaldehyde to acetate, which is caused by decreased ALDH activity. The excessive production or accumulation of acetaldehyde results in the flushing response, which may be accompanied by lightheadedness, nausea, accelerated heart rate, and headaches [248]. Individuals experiencing flushing typically drink little or no ethanol due to the unpleasantness of this reaction [248].

The enzymes cytochrome CYP2E1 and catalase also break down ethanol to acetaldehyde thus contributing to ethanol metabolism (Figure 4) [249]. However, CYP2E1 only is active after an individual

has consumed large amounts of ethanol, and catalase metabolizes only a small fraction of ethanol in the body [246].

There exists a substantial degree of inter-individual and ethnic variability in metabolic rates of ethanol, which may vary as much as three- to four-fold from individual to individual [250]. Such inter-individual variability may in part due to genetic variations in the *ADH* and *ALDH* genes, which determines the metabolic rate of ethanol, and therefore impacts individual susceptibilities to the toxic effects of ethanol [251]. Factors that influences ethanol metabolism include age [252, 253], gender [254, 255], ethnicity and genetics [254, 256-259], body mass index (BMI) and liver size [255], and food intake [260].

3.5.2. Pathways of Ethanol Metabolism

3.5.2.1. ADH Pathway

ADH, an enzyme that facilitates the conversion of ethanol to acetaldehyde using nicotinamide adenine dinucleotide (NAD^+), is the first step of ethanol metabolism in the liver (Figure 4) [246, 249]. In humans, *AHD* genes cluster in a region of chromosome 4q21 covering approximately 370 KB [261]. Humans have seven *ADH* genes (i.e., *ADH1A*, *ADH1B*, *ADH1C*, *ADH4*, *ADH5*, *ADH6*, and *ADH7*) that have categorized into five classes based on their structural and kinetic properties (Table 6) [262].

Class I *ADH* genes (i.e., *ADH1A*, *ADH1B*, and *ADH1C*) are closely related. Class I *ADH* encodes α, β, Y subunits, which may form homodimers or heterodimers comprised of the three subunits (i.e., αα, αβ, ββ, β Y, YY, etc.) [263]. Class I genes are ubiquitous in the body, but 90% can be found in the liver. Class I genes metabolize most of the ethanol in the liver (almost 70% of total ethanol metabolizing capacity) [264, 265].

In humans, *ADH* genes are expressed differently in various tissues [266]. *ADH4* encodes π-*ADH*, is solely expressed in the liver, and contributes significantly to the oxidation of ethanol at higher concentrations. This gene plays a key role in ethanol metabolism by the liver, especially at high blood concentration levels, accounting for 30% of ethanol metabolism. *AHD5* encodes χ-*ADH*, is ubiquitously

expressed in human tissues (e.g., gastrointestinal tissues), and have very low affinity for ethanol. *ADH6* mRNA is expressed in fetal and adult liver, but its role in ethanol metabolism remains unknown. *ADH7* encodes σ-*ADH*, is not highly expressed in the liver, but contributes to ethanol oxidation [246].

3.5.2.2. ALDH Pathway

ALDH, an enzyme that catalyzes the conversion of acetaldehyde to acetate acetaldehyde using nicotinamide adenine dinucleotide (NAD^+) is the second step of ethanol metabolism in the liver (Figure 4) [267]. In humans, several isoforms of ALDH with different structural and kinetic properties have been identified in different organs and tissues (Table 7) [251]. However, the two main ALDH enzymes that are involved in the oxidation of acetaldehyde to acetate are: ALDH1 and ALDH2 [264, 268]. ALDH1 which is found in the cytosol is encoded by the Aldehyde Dehydrogenase 1 Family Member A1 *(ALDH1A1)* gene. The *ALDH1A1* gene covers approximately 52 kb on chromosome 9, displays relatively low catalytic activity (K_m ~30 μM) for acetaldehyde oxidation. The mitochondrial ALDH2 enzyme, although largely found in the liver and stomach, is extensively distributed in other bodily tissue including the brain. ALDH2, which covers approximately 43 kb on chromosome 9, plays a major in acetaldehyde oxidation largely due to its high catalytic activity (K_m ~3 μM) for acetaldehyde oxidation [246, 269].

3.5.2.3. CYP2E1 Pathway

Although most ethanol metabolism is accounted for by ADH, a small portion of ingested ethanol is metabolized by non-ADH enzymes [270, 271]. The microsomal ethanol oxidizing system (MEOS) which consists primarily of the cytochrome P450 isoform, P4502E1 (i.e., CYP2E1), accounts for the major non-ADH ethanol oxidation in the liver. CYP2E1 primary role in ethanol metabolism is the oxidization ethanol to acetaldehyde. Compared to ADH, CYP2E1 plays a small role in ethanol metabolism in the liver when a normal amount of ethanol in consumed due to its low catalytic activity. However, with chronic or prolonged ethanol intake, CYP2E1 can play a significant role in ethanol metabolism. Following chronic ethanol intake, CYP2E1 increases the rate of ethanol clearance contributes to the metabolic tolerance of ethanol intake, thereby facilitating to additional ethanol intake.

Because CYP2E1 plays a less important role in ethanol oxidation in the liver, it is not a focus in this work.

3.5.2.4. *Catalase*

Catalase is another enzyme that metabolizes ethanol to acetaldehyde. Catalase can be found in cell bodies called peroxisomes. In the presence of a hydrogen peroxide (H_2O_2) – generating system, catalase is capable of oxidizing ethanol intake *in vitro* producing acetaldehyde and water. The contribution of catalase to the elimination of ethanol *in vitro* is unclear. Most studies concluded that catalase is a minor pathway of ethanol oxidation because quantitatively it lacks the hydrogen peroxide that is needed to oxidize ethanol [272, 273]. As a result, catalase will not be of focus in this work.

3.5.3. Polymorphic Variants Affecting the Rate of Ethanol Metabolism

As mentioned, genetic variants exist in several classes of the ADH and ALDH enzymes that alter the rate of ethanol oxidation and have been associated with susceptibility to several morbidities which include cardiovascular disease, alcohol liver disease, alcoholism, and cognitive impairments.

3.5.3.1. *ADH* Variants

The *ADH* gene cluster includes many single-nucleotide polymorphisms (SNPs). Some of these genetic variants result in an altered amino acid sequence of the encoded enzyme and are therefore considered functional or coding SNPs (cSNP). Detailed functional studies are lacking for all these cSNPs except for those that produce the *ADH1B* and *ADH1C* alleles (Table 8) [274].

3.5.3.1.1. The *ADH1B* Alleles

The three most studied alleles of *ADH1B* usually are referred to as *ADH1B*1* (the reference allele, which encodes the β_1 form of the enzyme and carries the amino acid arginine [Arg] at positions 48 and 370 in the amino acid chain), *ADH1B*2* (encoding β_2 and carrying histidine [His] at position 48: His48Arg370 (rs1229984)), and *ADH1B*3* (encoding β_3 and carrying cysteine [Cys] at position 370: Arg48Cys370 (rs2066702)) [274]. In both the β_2 and β_3 subunits, amino acid substitutions occur at an amino acid that interacts with NAD^+, a requirement for ethanol oxidation. This results in a 70- to 80-fold

higher turnover rate (i.e., how many molecules of ethanol the enzyme will convert to acetaldehyde in 1 minute at saturating ethanol concentrations) for β_2 and β_3 subunits, respectively, than in β_1 because the coenzyme is released more rapidly at the end of the reaction (Table 8) [264]. Therefore, individuals who carry at least one *ADH1B*2* allele or at least one *ADH1B*3* allele experience rapid oxidation of ethanol and acetaldehyde accumulation levels in the body. Because acetaldehyde has harmful effects on the body, individuals carrying these alleles are less likely to drink and have a lower risk of ethanol dependence [274].

3.5.3.1.2. The *ADH1C* Alleles

ADH1C also has cSNPs, of which alleles *ADH1C*1* and *ADH1C*2* are the most studied. These two alleles differ at two sites, resulting in two amino acid changes: the enzyme encoded by *ADH1C*1* (γ1-*ADH*) has Arg at position 272 and isoleucine (Ile) at position 350, whereas that encoded by *ADH1C*2* (γ2-ADH) has glutamine (Gln) at position 272 and valine (Val) at position 350 [275]. Compared to the ADH1B isozymes, the kinetic differences between γ1-ADH and γ2-ADH are smaller (Table 8) [274]. In most instances, the *ADH1C*1* and *ADH1C*2* alleles are in very high linkage disequilibrium (occur together). *ADH* that consists of two γ1 (i.e., γ1 γ1) has a turnover rate that is ~70 percent higher than that of the γ2γ2 enzyme [276]. Therefore, individuals who carry the *ADH1C*1* allele are less likely to drink and have a lower risk of ethanol dependence [274].

ADH1B and *ADH1C* alleles differ in their rates of ethanol metabolism in the liver due to the differences in the amino acid that they encode. Presence of the *ADH1C*2* allele have been linked to lessen oxidizing capability (i.e., slow ethanol oxidation to acetaldehyde), while presence of the *ADH1B*2* and *ADH1B*3* alleles are related to significantly higher oxidative capability (i.e., rapid ethanol oxidation to acetaldehyde) [277] .

3.5.3.1.3. *ADH* Population Genetics

The frequency distribution of *ADH1B* coding variants differs by race/ethnic population, with the *ADH1B*1* allele being highly prevalent (~99%) in Caucasian and African populations; the *ADH1B*2*

allele highly prevalent in East Asians (60%-80%) (e.g., Chinese and Japanese), but uncommon in Caucasian (0%-10%) and African (<5%) populations; and the *ADH1B*3* allele having a prevalence of 25% of individuals of African ancestry, and not present in European populations [278]. With regards to the *ADH1C* gene, the *ADH1C*1* and *ADH1C*2* have an approximately equal frequency in Caucasians (40%-50%), but *ADH1C*1* predominates in African (~85%), and East Asian (~95%) populations, higher prevalence are found in the latter two populations [276, 279-281].

3.5.3.1.4. Noncoding *ADH* Variants

In vitro studies have shown that some non-coding SNPs affect the level of gene expression of the *ADH* gene (Table 9) [282-284]. It is possible that many different variations in the region that contains the *ADH* genes also affect the level of expression of the different ADH enzymes in the intact organism (i.e., *in vivo*), thereby influencing ethanol metabolism, its physiological effects, and ultimately; drinking behavior and risk for alcoholism. However, detailed analyses of which SNPs are functional are difficult because many of the *ADH* variations are inherited together with nearby SNPs as haplotypes [274].

3.5.3.2. *ALDH* Variants

The acetaldehyde produced by the action of one or more ADH enzymes must be oxidized by efficiently by one or more ALDH in order for the cell/tissue to maintain non-toxic level of acetaldehyde [274]. Even short-termed elevation of acetaldehyde can trigger an adverse reaction in individuals whose ALDH activity is reduced either genetically or pharmacologically [274]. Eighteen genes that encode members of the ALDH enzyme superfamily have been identified in humans. Three of these genes are most relevant to the acetaldehyde oxidation: *ALDH1A*, *ALDH1B1*, and *ALDH2* (Table 10). The *ALDH1A1* gene is located in the cytosol, whereas *ALDH1B* and *ALDH2* are produced in the nucleus. However, *ALDH1B* and *ALDH2* have leader sequences that direct them to mitochondria, where they exert their functions in the mitochondrial interior [285].

3.5.3.2.1. The *ALDH* Alleles

The best-known genetic polymorphism in the *ALDH* gene is *ALDH2*. *ALDH2* has the highest affinity for acetaldehyde and is the enzyme most responsible for acetaldehyde oxidation. The *ALDH2* genetic variant rs671(Glu504Lys) has two allelic variants, *ALDH2*1* (Glu504) and *ALDH2*2* (Lys504), encoding for the active and inactive subunits, respectively. The inactive *ALDH2*2* allele results from a single amino acid exchange, the substitution of lysine for glutamate at position 504 of the precursor protein (487 of the mature protein) [286, 287]. Studies of liver extracts suggest that the inactive *ALDH2*2* allele is dominant over the active *ALDH2*1*; individuals who are both homozygous and heterozygous for *ALDH2*2* lack detectable *ALDH2* activity in the liver [288, 289]. As a result, individuals who are homozygous or heterozygous for the *ALDH2*2* are likely to experience the accumulation of acetaldehyde levels in their blood causing toxic reactions that include severe facial flushing, nausea, increase skin temperature and heart rate [259, 261, 290, 291].

3.5.3.2.2. ALDH Population Genetics

The distribution of the *ADH2* and *ALDH2* genotypes and the frequencies for the respective alleles in various populations, grouped according to their racial origin, is shown in Table 11 [278]. The inactive *ALDH2*2* allele is not observed in Caucasian and African-American populations as these two ethnic groups predominately expresses the homozygote normal (*ALDH2*1/*1*). In contrast, the inactive allele is prevalent in East Asian populations (Chinese, Japanese, and Koreans), with ~30% are heterozygote (*ALDH2*1/*2*) and ~2% are homozygote atypical (*ALDH2*2/*2*) [278].

3.5.4. The Influence of *ADH* and *ALDH* Polymorphisms on Ethanol Metabolism

Functional polymorphisms of genes for the ethanol-metabolizing enzymes ADH and ALDH2, and differences in the prevalence of the polymorphic alleles in different ethnic populations, have resulted in several studies examining race-ethnic differences in ethanol metabolism and the influence of the *ADH1B*, *ADH1C*, and *ALDH2* genotypes. *In vitro*, the isozymes encoded by the polymorphic alleles have different catalytic properties and are expected to influence individual's ethanol metabolic rate.

A study of 68 Japanese subjects genotyped for both the *ADH1B* and *ALDH2* polymorphisms compared the ethanol disappearance rates (mg/ml/h) and elimination rates (mg/kg/h) among groups based on both ADH1B (*ADH1B*1/*1*, *ADH1B*1/*2*, and *ADH1B*2/*2*) and ALDH2 (*ALDH2*1/*1*, *ALDH2*1/*2, ALDH2*2/*2*) genotypes. This study found no differences in ethanol metabolism among *ADH* genotypes. However, significant differences in ethanol metabolism were observed among the ALDH2 genotypes. Study findings indicated that subjects homozygous for *ALDH2*1/*1* displayed no increase in acetaldehyde levels regardless of their *ADH1B* genotype. Also observed was a progressive increase in peak acetaldehyde levels in subjects with the *ALDH2*1/*2* and *ALDH2*2/*2* genotypes. Furthermore, disappearance rates and elimination were significantly different among the *ALDH2* genotypes, and in decreasing order the values were *ALDH2*1/*1*, *ALDH2*1/*2*, *ALDH2*2/*2* [256]. Similar findings were observed in other studies of Asians that reported no effect of the *ADH1B*1/*2* allele on ethanol metabolism once adjustment has been for the *ALDH2*2* allele.

A study of 109 young healthy Jewish men that assessed the effect of the *ADH1B* polymorphism on ethanol elimination rate (measured by an ethanol clamp) found significantly higher ethanol elimination rates among carriers of the *ADH1B*2* allele (heterozygotes - *ADH1B*1/*2* and homozygotes - *ADH1B*2/*2*) compared with the *ADH1B*1/*1* homozygotes. This effect of *ADH1B* genotypes on ethanol metabolism is considered to be direct since the *ALDH2* gene has not been observed in Jewish populations [292].

A study of 112 African- American men and women, selected by genotype, examined the influence of *ADH1B*3* polymorphism on ethanol metabolism. After receiving an oral dose of ethanol, participants' ethanol disappearance rates (mg% per h) were determined from the slope of the pseudo-linear portion of the blood ethanol concentration vs. time curves. Study findings indicate that carriers of the *ADH1B*3* allele (heterozygotes and homozygotes) had a higher ethanol disappearance rate than *ADH1B*1* homozygotes [254]. A more recent study of 91 African-Americans reported that the *ADH1B*3* polymorphism had no effect on breath ethanol concentrations following a moderate oral dose of ethanol [293]. A study of 39 Native American men found that subjects with *ADH1B*3* alleles had

faster ethanol elimination rates than those with the *ADH1B*1* alleles. However, this result was not significant which may be attributed to the study's number of subjects with the *ADH1B*3* alleles and the low frequency of the genotype in the Native American population [294].

The influence of *ALDH2* polymorphism on ethanol metabolism has been studied more extensively in Asian populations largely because of the high frequency of the polymorphisms in this population. Most studies compared peak concentrations of ethanol and acetaldehyde, peak responses on subjective and cardiovascular measures, and flushing across *AHD1B* and *ALDH2* genotypes. Study result are generally consistent with reporting that individuals who are heterozygous or homozygous for *ALDH2* showed increased acetaldehyde levels following ethanol intake [256, 258, 261, 291, 295, 296]. Some studies demonstrated the accumulation of acetaldehyde in carriers of the *ALDH2*2* allele without any difference in ethanol concentrations or elimination rates [258, 259]. A study of 100 Chinese men observed that the presence of the *ALDH2*2* allele was associated with slower ethanol metabolism. In individuals homozygous for *ALDH2*1*, the presence of two *ADH2*2 alleles correlated with slightly faster alcohol metabolism and more intense flushing [297]. Studies conducted by Peng et al demonstrated the effect of the *ALDH2* polymorphism on ethanol and acetaldehyde metabolism and the lack of effect of *ADH1B* polymorphism on acetaldehyde metabolism [259, 290, 295].

Recent efforts to understand the influence of genetic variations in ethanol-metabolizing enzymes on ethanol metabolism include the use of large-scale genetic association studies. Genome wide association studies (GWAS) or pathway-based candidate gene studies for many complex traits, like ethanol metabolism, have demonstrated an important role of intronic, intergenic, and non-coding variants in susceptibility to disease/phenotype. Where, in many cases, the underlying functional variant is not identified but a tag SNP marks a region of the genome as influential. Despite the discovery of the actual functional variant, the data in most of these studies are generally supportive or shown an important role of regulatory genetic variants influencing of phenotypic variation. One such candidate pathway study has been conducted for ethanol intake. In a large cohort of twin pairs of Caucasian ancestry103 single nucleotide polymorphisms (SNPs) across the chromosome 4q region were examined for allelic

associations with variation in blood and breath ethanol concentrations after an alcohol challenge. Study findings indicated significant associations between rate of elimination and SNPs in the *ADH1B*, *ADH1C*, and *ADH7* genes [298, 299].

3.5.5. Summary

In summary, genetic polymorphism in *ADH* and *ALDH* genes alters the metabolism of ethanol and/or acetaldehyde. Polymorphisms in *ADH1B* gene results in variants that code for isozymes that demonstrate faster rates of ethanol metabolism, whereas the *ALDH2*2* polymorphism results in a "deficient" form of *ALDH2* that demonstrates an accumulation of acetaldehyde and its associated physiological effects which include facial flushing.

3.6. Mechanisms Underlying the Ethanol Intake and Cognitive Decline Relationship

3.6.1. Mechanisms for Neurotoxic Effect of Ethanol

The brain is highly susceptible to the neurotoxic effects of ethanol. Chronic cerebral dysfunction may result from brain damage caused by long-term ethanol intake [300]. The neurotoxic effects of ethanol that cause cognitive deficits may be mediated directly through damage to brain structures or indirectly through malnutrition, ethanol metabolite toxicity, electrolyte imbalance, or accompanying physical illnesses including liver disease and infection [301].

Ethanol's direct neurotoxic effect on the brain is mediated through ethanol's effect on the N-methyl-D-aspartate receptors (NMDARs) of glutamatergic neurons. Glutamate (Figure 1) (green circles) exerts its effects by acting on various types of receptors, including the NMDARs and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptors (AMPARs), both of which are ion channels, and metabotropic glutamate receptors (mGluRs), which are coupled to G-proteins. G-proteins, in turn, indirectly activate protein kinase C (PKC) and activate or inhibit adenyl cyclase (AC), depending on the mGluR and G-protein involved. In the absence of ethanol, glutamate leads to the activation of the postsynaptic neuron and the generation of a new nerve signal [302]. In the presence of ethanol (Figure 2) (ethanol, purple circles), the activity of the NMDARs and AMPARs, is inhibited, reducing cation entry

into the cell. As a result, the activity of the neuron is reduced and no or fewer nerve signals are generated [302]. Because many glutamatergic cells are densely concentrated in the frontal lobes and subcortical cells such as the hippocampus, these brain structures are vulnerable to excitotoxicity of ethanol intake [300, 303]. An example of an indirect neurotoxicity is Korsakoff syndrome. Korsakoff syndrome is a neurological disorder caused by thiamine (Vitamin B_1) deficiency in the brain. Thiamine deficiency causes an excessive release of glutamate which may exert a neurotoxic effect that is similarly to ethanol. Chronic ethanol abuse and thiamine deficiency may have an additive or synergistic neurotoxic effect [304]. Studies of amnesia involving Korsakoff patients demonstrated that Korsakoff syndrome has an anterograde component (inability to learn information due to data not successfully transferred from short-term memory to long-term memory) and retrograde component (inability to recall pre-existing memories) [305] .

Genetic susceptibility to the neurotoxic effects of ethanol has been linked to the *APOE ε4* allele. Reports indicate that individuals with the *APOE ε4* allele have a neural repair mechanism that is less effective than individuals without the allele, and, therefore, are more vulnerable to the deleterious effects of ethanol [306, 307]. Homocysteine (Hcγ) is also implicated in ethanol neurotoxicity. Elevated serum levels of homocysteine overstimulate glutamate NMDARs, increasing NMDAR transmission and the potential for excitotoxity [308-311]. Ethanol neurotoxicity is also influenced by the immune system. Long-term ethanol use induces systemic cytokines such as tumor necrosis factor alpha (TNFα), which is involved in potentiating glutamate excitotoxicity and activating resident microglia, thereby inducing neuroinflammation [312]. Other mechanisms that may influence ethanol induced neurotoxicity include free radical toxicity, acetaldehyde toxicity, modulation of the nicotinic acetylcholine, however addition research is needed to confirm these findings [303, 313-315].

3.6.1.1. Acute Effect of Heavy Ethanol Intake on Cognition

Cognitive impairment, blackout, and hangover are common symptoms of acute ethanol neurotoxicity. Heavy ethanol intake causes acute intoxication, and blackouts that may not involve loss of consciousness (depends on the severity of cognitive impairment). Following intoxication, an individual

usually experiences hangover symptoms that may include headache, drowsiness, concentration problems, fatigue, nausea, vomiting, diarrhea, loss of appetite, depression, hyper-excitability and anxiety, which may persists for a considerable amount of time [316]. Blackouts and hangovers precede ethanol-related cognitive dysfunction and risk factors for brain damage that may cause transient or permanent cognitive dysfunction.[317, 318]

Ethanol intoxication during or following heavy ethanol intake causes clinical behavioral changes and physiological changes that may result in impairments in cognitive functions (memory, attention, executive function, and visuospatial). Most of these impairments are reversible after the withdrawal of ethanol [319-321].

A blackout is associated with impaired episodic memory [322]. Blackouts are consistently associated with accelerated increase in blood ethanol concentration. However, blackouts may not occur in all individuals who consume ethanol rapidly or excessively, thereby suggesting that genetics may modify the effects of ethanol on the brain. Ethanol-related blackouts may interfere with the various stages of memory (encoding, storage, and retrieval), and cause partial or complete deficits in retrieval. Blackouts may be caused by damage to the hippocampus which plays a role in memory encoding at the cellular level, and antagonization of the NMDARs, which are required for the induction of long-term potentiation in the hippocampus at the molecular level [323].

3.6.2. Mechanisms for Neuroprotective Effect of Ethanol

The neuroprotective effect of low-to-moderate ethanol intake (LMEI) is exemplified by its complex effect on coronary artery disease (CAD) and ischemic stroke. Several epidemiological studies have reported a J-shaped relationship between ethanol intake and CAD, with LMEI lowering risk of CAD compared to non-drinking, but increased risk for heavy ethanol intake [324-326]. A similar J-shaped curve reportedly describes the association of ethanol intake and ischemic stroke [327].

Several mechanisms appear to explain the protective effect of ethanol. Ethanol increases insulin sensitivity [328], prevents platelet aggregation [329], increases fibrinolysis [330], opposes thrombin activity [324], and reduces inflammatory markers [331].

Moreover, animal studies have linked the neuroprotective effects of ethanol to ethanol's interactions with protein kinase C (PKC), adenosine receptor, and cardio protection proteins that include superoxide dismutase, nitric oxide synthase, and heat shock proteins [324]. Furthermore, findings suggest that neuroprotection is correlated with down-regulation of inducible nitric oxide synthase and up-regulation of endothelial nitric oxide synthase [332]. The neuroprotective effect of ethanol also has been attributed to antioxidant polyphenols such as resveratrol, which is abundant in red wine [333-335].

3.7. Prospective Cohort Studies of Ethanol Intake and Cognitive Decline

3.7.1. Review of Prospective Cohort Studies of Ethanol Intake and Cognitive Decline

Fifteen prospective studies examined the association between ethanol intake and cognitive decline [32, 93, 336-348] (Tables 12 and 13). However, the relationship of ethanol intake with cognitive decline remains poorly understood due to inconsistent study findings. While heavy ethanol intake has been identified as a risk factor for cognitive decline [348], low-to-moderate ethanol intake has been associated with lesser cognitive decline [336, 339, 340, 342-344, 346, 347] and no cognitive decline [93, 341, 345, 348].

Of the fifteen studies, twelve studies studied the relationship between ethanol intake and cognitive decline [93, 336, 339-348]. Of the twelve studies that examined the low-to-moderate ethanol intake - cognitive decline relationship, eight reported that low-to-moderate ethanol intake is associated with lesser cognitive decline [336, 339, 340, 342-344, 346, 347]. The Epidemiology of Vascular Aging (EVA) study of 1,389 men and women ages 59-71 years in Western France reported that low (<2 glasses) or moderate (2-5 glasses) ethanol intake was associated with decreased risk of decline in MMSE (global cognitive function) in participants without the *APOE* ε4 allele, whereas moderate ethanol intake increased the risk of cognitive decline in those without the *APOE* ε4 allele [336]. The Women's Health Initiative Memory Study (WHIMS) of 4,461 postmenopausal women ages 65-79 years reported that moderate ethanol intake (<1 drink per day, and ≥1 drink per day) was associated with lesser decline in 3MSE (global cognitive function) over 4.2 years than no ethanol intake [339]. The Monongahela Valley

Independent Elders Survey (MoVIES project) study of 1,681 men and women ages 65 years and older reported that both minimal (ethanol intake once per month or less) and moderate ethanol intake (ethanol intake more than once per month) was associated with lesser decline on the MMSE (general mental status) and Trail making tests (executive function and psychomotor speed) over 7.3 when compared to those with no ethanol intake [340]. The Nurses' Health Study (NHS) of 12,480 female nurses aged 70 to 81 years old reported lower relative risk of substantial decline on the MMSE (general cognition) over 2 years among moderate (1.0-14.9g) drinkers than nondrinkers [342]. The Northern Manhattan Study (NOMAS) of 2,631 men and women ages 40 years and older reported that less than one drink per week, between one drink weekly up to two drinks daily, and more than two drinks daily were associated with a lesser decline in TICS-m scores over 2.2 years compared to never drinkers [343]. The Pravastatin in the Elderly at Risk (PROSPER) study of 5,804 men and women ages 70 to 82 years reported that in women, low (1 to 3 U per week) or moderate (> 3 U per week) intake was associated with lesser MMSE (general cognitive function) over 3.2 years than no ethanol intake [344]. The Baltimore Longitudinal Study of Aging (BLSA) of men and women aged 17-97 years of 628–1305 individuals (depending on the cognitive outcome) reported among participants less than age 70, ethanol intake was associated with faster decline or slower improvement on the MMSE (global cognition) and on the verbal fluency test - letter (VFT-L) [347]. Finally, the Seattle Longitudinal Study (SLS) of men and women ages 45 years and older reported that moderate ethanol intake (no more than 7 drinks per week) was associated with lesser decline in Thurstone Primary Mental Abilities (verbal memory) over 7 years [346].

In contrast to the previous studies, the remaining four studies reported that there is no association between levels of ethanol intake and cognitive decline (Tables 12 and 13). The Established Populations for Epidemiologic Studies of the Elderly (EPESE) of 3,809 men and women ages 65 years and older reported that moderate intake (>= 1 ounce) was not associated with lesser decline in memory over 3 years [93]. The Medical Research Council (MRC) National Survey of Health and Development (the British 1946 Cohort) of 1,764 men and women age 43 at study baseline and 53 at follow-up reported that compared to no ethanol intake, moderate ethanol intake (2.1 a 4.0 drinks per day) was associated with a

smaller decline in memory in men and greater decline in psychomotor speed in women over a follow-up period of 10 years [341]. The ZARADEMP Project study of 4,803 men and women ages 55 and older in Spain reported that low (< 12 grams per day) or moderate ethanol intake (12-24 grams per day) was not associated with reduced risk of decline in MMSE (global cognition) over 4.5 years[345]. Finally, the Whitehall ll Cohort Study of 10,308 British civilian workers ages 44-69 reported no association between moderate ethanol intake (< 20 g/d) and lesser decline in global cognition z-scores [348].

Few studies (N=2) examined the relationship between heavy drinking and cognitive decline due to small size [93, 342, 343, 345] or due to the underrepresentation of heavy drinkers in the study population [339, 340, 344]. While the Whitehall ll Cohort Study [348] reported that heavy ethanol intake was associated with greater decline in global z-scores, the NHS found no association [342].

3.7.2. Limitations of Prospective Cohort Studies of Ethanol Intake and Cognitive

Overall, the reported relationship between ethanol intake and cognitive decline is inconsistent. Inconsistent study findings may be attributable to methodological issues as they often include single measurements of ethanol intake [93, 336, 339-341, 344-347], non-standardized definitions of cognitive decline, short follow-up times (<5 years) [93, 336, 339, 342-345], and an analytic approach that does not appropriately consider confounders and effect modifiers [93, 336, 337, 339-348].

A short follow-up time (<5 years) may not capture changes in ethanol intake patterns that may occur, or in association with cognitive decline. Therefore, long-term studies are needed to study the relationship between ethanol intake and cognitive decline.

Most studies did not have data available on *APOE* ε4 allele status, which is an established strong risk factor for cognitive impairment.

Studies were also limited by an inconsistent use of outcome measures. Some studies use multidimensional measures of cognition (e.g., MMSE [336, 340, 344, 345, 347], 3MSE [339], TICS [342], TICS-m [343], and CASI [338]), whereas others examined only specific tasks (e.g., the DSST) [93, 341, 344, 346, 348]. As a result, the clinical meaning of changes in cognition associated with ethanol intake remains uncertain [11]. Assessment of cognitive function was often done by MMSE (global

cognitive function) [336, 340, 344, 345, 347], which have known "ceiling effects", thereby failing to capture differences in cognitive function among those with higher level of cognitive performance [349]. Few studies assessed decline in specific domains cognitive decline [342, 344, 347, 348], which is important since cognitive decline affects different domains differently [11]. Most studies evaluated change in cognitive performance at two points [93, 336, 339, 341, 342, 345-347], when multiple measurements of cognitive function performed at various points across the life span are needed to make more to firm conclusions on the effect of ethanol intake on cognition [11, 350].

Existing studies were conducted primarily in older populations (≥65 years at study baseline) [93, 336, 339, 340, 342-347], failing to capture the effect of ethanol intake on cognitive decline earlier in life. Only one study adjusted for attrition/missing data (differential), which may have produced less biased estimate of the effect of ethanol intake on cognitive function [346]. A limited number of studies investigated the effects of ethanol in African-Americans populations although the prevalence, incidence, and cumulative risk of AD, the most common form of dementia, appears to be much higher in African-Americans than in European-Americans [343, 347]. Furthermore, few studies investigated the effects of mid-life ethanol intake with late-life cognition [341, 348].

3.7.3. Summary of Prospective Studies of Ethanol Intake and Cognitive Decline

Although the relationship between ethanol intake and cognitive decline has been studied extensively, it remains poorly understood due to inconsistent study results. Methodological issues may account for inconsistent studies finding. Therefore, additional analyses of large, diverse populations with repeated measurements of ethanol intake and cognitive function are needed to better understand the relationship of ethanol intake with cognitive decline.

3.8. Genetic Association Studies of *ADH* and *ALDH* Polymorphisms with Ethanol Dependence and Ethanol Intake

Genetic polymorphisms that affects functional *ADH* and *ALDH* activities may be relevant for the biological actions of ethanol [259, 351]. Ethanol dependence syndrome is a complex behavioral disorder characterized by a preoccupation with ethanol and persistent drinking despite harmful effects [280, 352,

353]. Twin, adoption, and family studies demonstrated that genetic factors play a role in the determining drinking behavior and risk of ethanol use disorders [354, 355]. Ethanol dependence is moderately heritable, with heritability in most studies estimated to be 0.50-0.60 [355-360]. As previously mentioned, ethanol is metabolized in the liver to acetaldehyde and acetate primarily through the ADH and ALDH enzymes. Genetic polymorphisms in the genes encoding ADH and ALDH are associated with alteration enzyme kinetics [274, 361]. As a result, the roles of SNPs within ethanol-metabolizing genes, *ADH* (i.e., the Class I, low K_m *ADH*s *ADH1A*, *ADH1B* and *ALDH1C*) and *ALDH* (i.e., *ALDH2*), in ethanol dependence has been extensively examined, as well as quantitative ethanol intake measures. SNPs within ethanol-metabolizing genes have been associated with the risk of developing ethanol dependence and ethanol intake (Table 14). The significant association between *ALDH2* and *ADH1B* variants and ethanol dependence risks have been explained by the hypothesis that any increase in acetaldehyde production, or reduction in its subsequent elimination, will reduce and individual's vulnerability to ethanol abuse and ethanol dependency disorders due to the adverse effects associated with elevated blood and tissue acetaldehyde [352].

In this work, we focus on functional (e.g., intronic, exonic, intergenic, etc.) coding genetic variants within *ADH* and *ALDH* genes that have been identified by GWAS or pathway-based candidate gene studies to play an important role in ethanol metabolism and susceptibility to ethanol dependence (Table 14). For the *ADH* gene, the focus is placed on SNPs that produce the *ADH1B* and *ADH1C* alleles because studies on the functionality of other *ADH* coding SNPs are lacking. The *ALDH* missense SNP rs671 is not considered in this work because it is monomorphic in the ARIC study population. Although *in vitro* studies have shown that non-coding SNPS affect the levels of gene expression, they are not be considered in this work. Detailed analysis of which *ADH* non-coding SNPS are functional are difficult because many of the ADH variants are inherited together with nearby SNPs as haplotypes.

3.8.1. *ALDH* Polymorphisms and Ethanol Intake Phenotypes

The importance of *ALDH* genetic variation in risk for AD has been well established among Asian populations. The loss of function SNP rs671 (Glu504Lys) in the *ALDH2* gene results in an amino acid change from glutamic acid to lysine at position 504 in the ALDH enzyme. The *ALDH2*2* allele that contains the rs671 polymorphism is associated with reduced ALDH activity, which results in the accumulation of acetaldehyde following ethanol intake and a flushing response, thereby reducing the likelihood of ethanol abuse [362]. A meta-analysis of 53 studies, which included a total of 9,678 cases and 7,331 controls of East-Asian ancestry found that the risk allele *ALDH2 (*rs671*) *1* (Glu504) is significantly more prevalent individuals who are ethanol dependent. In contrast, the less active *ALDH2 (*rs671*) *2 (*Lys504*)* allele was found to be protective of ethanol abuse [363].

3.8.2. *ADH* Polymorphisms and Ethanol Intake Phenotypes

3.8.2.1. *ADH1A*

A study conducted by Zuccolo et al 2009 of 7,410 women of European ancestry, participants of the Avon Longitudinal Study of Parents and Children (ALSPAC), found evidence of an association between *ADH1A* intronic SNP rs2866151 and weekly drinking before pregnancy [364]. A genome-wide association study (GWAS) of ethanol dependence conducted in 2,379 European-American and 3,318 African-American subjects conducted by Gelernter et al in 2014 found that the intronic *ADH1A* SNP rs904092 is associated with ethanol dependence in European-American and African-American populations, with replication in an independent sample of Germans [365].

3.8.2.2. *ADH1B*

In the *ADH1B* gene, a SNP called rs1229984 (Arg48His), results in an amino change at position 48 in the β subunit of ethanol dehydrogenase from arginine to histidine [246]. *In vitro* studies have shown that rs1229984 increases the maximal velocity (V_{max}) at which ethanol is oxidize to acetaldehyde by over 100-fold [366, 367] .The *ADH1B* rs1229984 is common in East-Asian populations, with frequency ranging from 19% to 91% [360]. There is strong evidence that rs1229984 is linked to reduced ethanol

intake and a reduced risk of ethanol abuse in East-Asian populations [360, 362, 368]. Although the frequency (range: 0-10%) of rs1229984 variant is low in populations of European and African descent [278, 369] (0-10%), there is consistent evidence that this variant has a strong protective effect for ethanol abuse in Europeans [364, 365, 370-373] and African-Americans [365]. In European populations, rs1229984 variant is associated with lower ethanol intake (defined maximum number consumed in a 24-hour period) [371, 374], total number of drinks taken in the past year [374], and average ethanol intake and binge drinking during pregnancy [364]. Although, rs1229984 is rare among European and African populations, at an individual level the effect of this *ADH1B* variant on the level of ethanol consumed and the risk of developing ethanol dependence is the same irrespective of ethnicity [248].

A GWAS of 2,379 European-American and 3,318 African-American subjects conducted by Gelernter et al 2013 that was previously mentioned, found that the *ADH1B* SNP rs1789882 (Arg369Cys), is associated with ethanol dependence in African-Americans; the first Genome Wide Significant (GWS) finding for ethanol dependence in African-Americans, although the risk locus was previously known [375]. Associations with ethanol dependence were also found for the *ADH1B* SNPs rs2066702 (Arg370Cys) and rs1693457 in European and African-American populations with replication in an independent sample of Germans [365].

An association analysis conducted by Macgregor et al 2009 that included 4,597 Australian twins, participants of the Twin study conducted at the Queensland Institute of Medical Research (QIMR) study, observed an independent association between ADH1B SNP rs1042026 and alcohol intake, after controlling for rs1229984 [374].

3.8.2.3. *ADH1B/ADH1C*

A recent association analysis conducted by Way et al 2015 in a sample of 1,076 individuals of European ancestry (i.e., British and Irish) found significant associations between risk of ethanol dependency and the *ADH1B/1C* intergenic variant, rs1789891. This observed association was largely independent of *ADH1B* rs1229984 [372].

3.8.2.4 *ADH1C*

Polymorphisms in the gene encoding *ADH1C* have been implicated in the risk for developing ethanol dependency syndrome. Three non-synonymous *ADH1C* SNPs rs698 (IIe50Val) [248, 372, 376, 377], rs1693482 (Arg272Gln) [248, 372, 374, 376], and rs283413 (G78stop) [372, 378, 379] have been associated with ethanol dependence risk. However, these associations are not completely independent [368, 372] . GWASs observed an association between *ADH1C* SNPs rs2241894 [365] and rs1614972 [248, 365] and ethanol dependence.

3.8.2.5. *ADH4* and *ADH5*

Variants in *ADH4* and A*DH5* sporadically have been linked to ethanol dependence. Macgregor et al 2009 reported associations between *ADH4* SNPs rs3762894 and rs1126671 and ethanol dependence symptoms as well quantity, frequency, and maximum drinks [374]. A study of 715 European-Americans and 210 African Americans reported associations between *ADH4* SNPs and ethanol dependence [380]

3.8.3. Summary

In summary, genetic variations in *ADH1A , ADH1B, ADH1C, ADH4, ADH5,* and *ALDH2* contribute to differences in ethanol intake, and thus, the risk for development of ethanol dependence.

3.9. Gene-Environment Studies of *ADH* and *ALDH* Polymorphisms and Ethanol Intake on Cognitive Decline

Reports of the relationship between low-to-moderate ethanol intake and cognitive decline have been inconsistent. While some studies reported lower cognitive decline for low-to-moderate ethanol intake compared to no ethanol intake, others reported no association. Several mechanisms have been suggested to underlie the protective effect of low-to-moderate ethanol intake on cognition which include possible anti-inflammatory properties of ethanol [324]. The lack of consistent results has been attributed to methodological issues such as uncontrolled confounding. In vitro studies found that SNPs within ethanol-metabolizing genes (*ADH*, *ALDH*, *CYP2E1*) with different kinetic properties and ethnic distribution alters the rate of ethanol oxidation and therefore impacts individual susceptibilities to the toxic effects of ethanol [251].The direction of effect of ethanol intake on cognitive change may thus depend on genetic differences in the ability to metabolize ethanol.

To our knowledge, only one study has investigated an effect modification of the ethanol intake-cognitive decline relationship by SNPs within ethanol-metabolizing genes[381]. This study used a Mendelian randomization (MR) design, a method that allows testing for, or in certain cases to estimate, a potential causal effect from observational data in the presence of confounding factors. MR designs use common genetic polymorphisms with well-understood effects on exposure patterns (e.g., ethanol intake) or effects that mimic those produced by modifiable exposures. Importantly, the genotype must only affect the disease status indirectly via its effect on the exposure of interest. Because genotypes are assigned randomly when passed from parents to offspring during meiosis, the population genotype distribution should be unrelated to the confounders that typically plague observational epidemiology studies if one assumes that choice of mate is not associated with genotype. In this regard, MR has been described as a "natural" randomized controlled trial [382].

The study, conducted in 1,079 participants in the Lothian Birth Cohort 1936 observed a statistically significant interaction of a four-SNP score indexing alcohol dehydrogenase activity (*ADH7* rs284779, *ADH1B* rs4147536, *ADH1A* rs975833 and *ADH1A* rs2866151) with ethanol intake on lifetime

cognitive change (interaction beta parameter estimate= -1.13, p=0.007) [381]. This study used a population homogenous for age and culture, which may reduce potential confounding. Limitations of this study include the use of a single measurement of ethanol intake measured at ~age 70, the measurement of cognitive function at ages ~11 and ~70 years, and a marked attrition over the prolonged period of follow-up which was not accounted for in the analyses.

Therefore, there is need to study the effect modification of ethanol intake-associated 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 whether effect modification exists. Little is known about the effect of ethanol intake-associated genetic variants in other populations such as African-Americans; therefore, it is important that the role of these SNPs be assessed in ancestrally diverse populations. The rich data source of the ARIC study which includes repeated measurements of ethanol intake of cognitive function collected on African-American and European-American men and women from mid- to-late life that were genotyped for ethanol intake-associated SNPS is well-suited to address this current research need. Evidence of effect modification of the ethanol intake-cognitive decline relationship by SNPs within ethanol-metabolizing genes would inform the mechanisms by which ethanol affects cognition and be relevant to understanding the potential public health risk and benefit of ethanol.

3.10. Supporting Figures and Tables

Table 1. Cognitive domains [11]

Concepts	Definition
Language	Consists of an array of abilities, including understanding and producing speech, reading, writing, and naming
Visuospatial Skills	Abilities to make sense of the visual world—shapes, angles, larger gestalts vs details, the meaning of forms—and to reproduce what one sees.
Executive function	A variety of higher-order functions—planning, conceptualizing, organizing, and evaluating
Learning and memory	Process that allows to maintain and to immediately manipulate available information
Attention/Concentration	The ability to focus awareness on a given stimulus or task, to concentrate on that stimulus or task long enough to accomplish a goal, and to shift awareness if appropriate.
Social cognition	The ability to process, store, and apply information about other people and social situations.

Table 2. MCI subtypes by etiology, pathology, presentation and outcomes [45]

Variable	Amnestic	Non-amnestic
Etiology	Neurodegenerative disease	Vascular damage
	APOE ε4 genotype	Cerebrovascular disease
Pathology	Neurodegenerative	Cerebrovascular
	Amyloid β plaques	Cortical infarctions
	Neurofibrillary tangles	Subcortical infarctions
	Hippocampal atrophy	White matter hyperintensities
	Reduced brain volume	
Presentation	Memory impairment present	Impairment in non-memory domains
Long term outcomes	Alzheimer's dementia	Non-Alzheimer dementias:
		Vascular dementia
		Lewy body, Frontotemporal

Abbreviations: MCI, mild cognitive impairment; *APOE*ε4, apolipoprotein E

Table 3. Summary of findings on potential risk factors for cognitive decline from observational studies and randomized controlled trials [62]

Factors	Direction of Association	Strength of Evidence
Demographic		
Age (Older)	Increased risk	Strong
Sex (Male)	Increased risk	Unclear
Race (African-American)	Increased risk	Unclear
Educational Attainment (Low)	Increased risk	Strong
Social Support	Decreased risk	Unclear
Lifestyle		
Smoking	Increased risk	Strong
Physical Activity	Decreased risk	Strong
ω-3 Fatty acids	Decreased risk	Moderate
Genetic		
APOE ε4 genotype	Increased risk	Low
Medical		
Diabetes	Increased risk	Strong
Hypertension	Increased risk	Strong
Dyslipidemia	Increased risk	Unclear
Obesity	Increased risk	Strong
Stroke	Increased risk	Strong
History of Depression	Increased risk	Lower

Abbreviations: ω-3, Omega-3; *APOE* ε4, apolipoprotein E ε4

Table 4. Types of memory [189]

Type of Memory	Type of Knowledge	Example
Episodic	Personal Experience	Imagery (sounds, smells, pictures) (space and time)
Semantic	General Knowledge	Meanings and Propositions (facts and general knowledge)
Declarative	How things are or were	"Knowledge knowing." How things are.
Procedural	How to do things	"Knowledge knowing." How to do things.
Explicit	Knowledge easily explained	Consciously recalled (How to add and subtract)
Implicit	Knowledge not easily explained	Unconscious recall (How to speak)

Table 5. Types of attention

Selective attention	Focus on specific aspects of experience that is relevant while ignoring others
Divided attention	Concentrating on more than one activity at a time
Sustained attention	Maintain focus on selected stimulus over prolonged period; called vigilance
Executive attention	Focus on attention planning, goals, errors, and compensation, monitoring, and unknown

Table 6. Alcohol dehydrogenase (ADH) genes and proteins [246, 274]

Official Gene Name*	Old Name†	Nonstandard Name‡	Sequence§	Protein	Class¶
ADH1A	*ADH1*	*ADH1A*	NM_000667	α	I
ADH1B	*ADH2*	*ADH1B*	NM_000668	β	I
ADH1C	*ADH3*	*ADH1C*	NM_000669	γ	I
ADH4	*ADH4*	*ADH2*	NM_000670	π	II
ADH5	*ADH5*	*ADH3*	NM_000671	χ	III
ADH6	*ADH6*	*ADH5*	NM_000672	ADH6	V
ADH7	*ADH7*	*ADH4*	NM_000673	σ	IV

Abbreviations: ADH1A, alcohol dehydrogenases class 1A; ADH1B, alcohol dehydrogenases class 1B; ADH1C, alcohol dehydrogenases class 1C; ADH4, alcohol dehydrogenases class 4; ADH5, alcohol dehydrogenases class 5; ADH6, alcohol dehydrogenases class 6; ADH7, alcohol dehydrogenases class 7; NM, nucleotide M; α, alpha; β, b

Table 7. Aldehyde dehydrogenase (ALDH) genes and proteins [246]

Official Gene Name	Older Names	Chromosomal Location	Protein	Sequence
ALDH1A1	*ALDH1, RALDH1*	9q21.13	Cytosolic aldehyde dehydrogenase 1, *ALDH1*	NM_000690
ALDH2		12q24.2	Mitochondrial aldehyde dehydrogenase 2, *ALDH2*	NM_000689

Abbreviations: ALDH1, aldehyde dehydrogenase 1, ALDH1A, aldehyde dehydrogenase 1 family member A1; ALDH2, aldehyde dehydrogenase 2; RALDH1, retinaldehyde dehydrogenase 1, and NM, nucleotide M. eta; γ, gamma; π, pie; χ, chi; and σ, sigma

Table 8. Alcohol dehydrogenase (ADH) genetic variants [246, 274]

ADH Class	Official Gene Name*	Amino Acid Difference Between Alleles	Protein	K_m (ethanol) mM	Turnover (min^{-1})
I	*ADH1A*		α	4	30
I	*ADH1B*1*	Arg48, Arg370	β1	0.05	4
I	*ADH1B*2*	His48, Arg370	β2	0.9	350
I	*ADH1B*3*	Arg48, Cys370	β3	40	300
I	*ADH1C*1*	Arg272, lle350	γ1	1	90
I	*ADH1C*2*	Gln272, Val350	γ2	0.6	40
II	*ADH4*		π	30	20
III	*ADH5*		χ	>1,000	100
IV	*ADH7*		σ	30	1800
V	*ADH6*		*ADH6*	?	?

Abbreviations: ADH, aldehyde dehydrogenase; ADH1A, aldehyde dehydrogenase 1 family member A1; ADH1B, aldehyde dehydrogenase class 1B; ADH1C, aldehyde dehydrogenase class 1C; ADH4, alcohol dehydrogenases class 4; ADH5, alcohol dehydrogenases class 5; ADH6, alcohol dehydrogenases class 6; ADH7, alcohol dehydrogenases class 7; Arg48, arginine 48; Arg272, arginine 272; Arg370, arginine 370; His48, histidine 48; Cys370, Cysteine 370; lle350, isoleucine 350; Gln272, glutamine 272; Val350, valine 350, K_m, Michaelis Constant; mM, millimeter; Min, minute; α, alpha; β, beta; γ, gamma; π, pie; χ, chi; and σ, sigma.

Table 9. Non-coding SNPs that affect the level of gene expression of the ADH gene

Gene	rs number	Functional Class	Allele A1	Allele A2	Reference	Region
ADH4	rs7678936	intron	G	T	[284]	Distal upstream enhancer (4E4)
	rs7678890	intron	T	G		
ADH1B	rs1159918	upstream	T	G	[283]	Proximal promoter
	rs1229982	upstream	A	C		
ADH1C	rs11499823	upstream	T	C	[282]	Regulatory
	rs1629838	upstream	C	T		
	rs11499824	upstream	G	A		
	rs11499825	upstream	T	C		
	rs11499826		C	T		
	rs4093924		A	G		
	rs11499830	intron	in	del		
	rs283408		A	C		
	rs10006545		A	G		
	rs2453980		G	A		
	rs11499828		A	G		
	rs1662036		T	G		

Abbreviation: rs, reference SNP**;** ADH4, aldehyde dehydrogenase 4; ADH1B, aldehyde dehydrogenase class 1B; ADH1C, aldehyde dehydrogenase class 1C; in, inversion; and del, deletion.

Table 10. Kinetic constants for acetaldehyde oxidation by human aldehyde dehydrogenases [274]

Enzyme	K_M (μM)	V_{max} (min^{-1})	V_{max} (min^{-1} μM^{-1})
ALDH1A1	180	380	2.1
ALDH1B1	55	40	0.7
*ALDH2*1*	0.2	280	1400
*ALDH2*2*	1.4	20	14

Abbreviations: V_{max}, maximal velocity; K_m, ***Michaelis*** Constant*;* ALDH1A, aldehyde dehydrogenase 1 family member A1; ALDHB1, aldehyde dehydrogenase 1 family member B1; ALDH2, aldehyde dehydrogenase 2; μM, micrometer; and min, minute.

Table 11. Distribution of the ADH2 and ALDH2 genotypes, by alleles and racial groups [278]

Population	ADH2						ALDH2					
	n	Genotype			Gene		*n*	Genotype			Gene frequency	
		1-1	1-2	2-2				1-1	1-2	2-2		
Caucasoids												
Germans	233	214	19	0	0.959	0.041	193	193	0	0	1	
Swedes	90	89	1	0	0.994	0.006	99	99	0	0	1	
Finns	85	83	2	0	0.988	0.012	100	100	0	0	1	
Hungarians	115	103	12	0	0.948	0.052	117	114	3	0	0.987	0.013
Turks	44	34	9	1	0.875	0.125	57	57	0	0	1	
Indians	167	142	17	8	0.901	0.099	179	173	5	1	0.980	0.020
Mongoloids												
Chinese	86	7	41	38	0.320	0.680	132	92	38	2	0.841	0.159
Japanese	32	5	16	11	0.406	0.594	53	29	23	1	0.764	0.236
Koreans	177	7	55	115	0.195	0.805	218	156	58	4	0.849	0.151
Thais	111	51	46	14	0.667	0.333	111	100	11	0	0.950	0.05
Filipinos	57	11	23	23	0.395	0.605	86	85	1	0	0.994	0.006
Malays	65	11	31	23	0.408	0.592	73	68	5	0	0.966	0.034
Negroids												
Africans	37	37	0	0	1		49	49	0	0	1	
Other populations												
Caboclos (Brazil)	20	18	0	2	0.900	0.100	23	15	8	0	0.826	0.174
Auracanians (South Chile)	27	27	0	0	1		7	7	0	0	1	
Mestizos (Mexico)	57	51	6	0	0.947	0.053	61	61	0	0	1	
Papua New Guineans	204	179	22	3	0.931	0.069	242	240	2	0	0.996	0.004
Australian Aborigines	22	10	9	3	0.659	0.341	37	37	0	0	1	
Swedish Lapps	100	99	1	0	0.995	0.005	100	100	0	0	1	
Eskimos (Alaska)	27	27	0	0	1		27	27	0	0	1	

Abbreviations: ADH, aldehyde dehydrogenase; ALDH2, aldehyde dehydrogenase 2.

Table 12. Review of prospective studies of the ethanol intake and cognitive decline relationship

Author (Year)	Study Design/ Population	Sample Size (N)	Follow-up Time (Year)	Ethanol Intake Assessment	Cognitive Status Assessment	Cognitive Domain Assessed
Herbert (1993) [93]	The Established Populations for Epidemiologic Studies of the Elderly ((EPESE) of men and women aged 65 years or over	3,809	3	Interview-administered questionnaire assessing ethanol intake in previous year (yes/no), and amount consumed in previous month (number of drinks)	Immediate Memory test, digit span test, and mental status questionnaire-assessed orientation	Immediate memory, digit span (memory), and orientation
Dufouil (2000) [336]	The Epidemiology of Vascular Aging (EVA) study prospective study of men and women ages 59-71 years in western France	1,389	4	Self-reported, beverage-specific number of glasses of ethanol consumed at 6 different times throughout the day	MMSE	Global cognition
Leroi (2002) [337]	The National Institute of Mental Health Epidemiologic Catchment Area of men and women ≥18 years	3,481	12.5	Self-reported alcohol use assessed at 3 waves	MMSE	MMSE score
Bond (2004) [338]	Pooled prospective cohort ofthe Kame Project -a population-based study of Japanese American adults aged persons aged 65 or older, and theAdult Changes in Thought (ACT) study of non-Hispanic Whites aged 65 and older	4,191	4	Kame Project-Structured interview asking about ethanol intake frequency and duration, and type consumed. ACT Study-Structured interview that assessed current and past drinking behaviors and type of alcohol drank	CASI	CASI score
Espeland (2005) [339]	The Women's Health Initiative Memory Study (WHIMS). Randomized Clinical Trial (39 US academic medical centers) of post-menopausal combination estrogen and progestin therapy of community-dwelling women aged 65-79 years	4,461	4.2	Food frequency questionnaire (FFQ) - assessed intake (beer, wine, and liquor separately) over the past 3 months.	3MS	Global cognitive function

Ganguli (2005) [340]	The Monongahela Valley Independent Elders Survey (MoVIES project), a prospective epidemiologic study of dementia in a largely rural, blue-collar community of men and women aged ≥65 years	1,681	7.3	Baseline Quantity/Frequency (QF) - lifetime history of alcohol use, alcohol use in the past year (yes/no), frequency of alcohol use during the past year, and number of drinks consumed per occasion	MMSE, Word List Learning test and Story Immediate Retell, Word List Delayed Recall and Story Delayed Recall, Clock Drawing and CERAD Con-structional Praxis, Verbal Fluency for Categories and for Initial letters, Trail Making A and B, and CERAD/Boston Naming Test alone	General mental status Learning Memory Visuospatial Fluency Trailmaking Naming
Richards (2005) [341]	The MRC NationalSurvey of Health and Development (the British 1946 birth cohort)	1,764	10	QF at aged 43 - frequency of type of alcohol drank per day	15 item word learning taskdevised by the NSHD-Verbal memory, Visual searchtask-speed and concentration	Verbal memory Speed and concentration
Stampfer (2005) [342]	Nurses' Health Study of men of female nurses aged 70 to 81 years old	12,480	2	FFQ completed in 1984, 1986. 1990, 1994, and 1998 - assessed beverage specific QF	Baseline-TICS, Follow-up - TICS, East Boston Memory Test, 10-word list, Verbal fluency, Digit span backward test	General cognition and verbal memory

Wright (2006) [343]	The Northern Manhattan Study (NOMAS). Community sample of men and women (Hispanic, black, white, and other groups) aged ≥40 years stroke free at study baseline	2,631	2.2	Structured interviews adapted from FFQ (Baseline - average amount consumed in the past year, Follow-up: intake over the prior six months)	TICS-m assesses orientation, attention, immediate recall of a ten-word list, calculations, judgment, language, finger tapping, and antonyms, and delayed recall of ten-word list resulting in a total score of 51 points	Changes in TICS-m scores over time from the baseline exam
Stott (2008) [344]	Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). Randomized placebo-controlled trial of pravastatin in men and women aged 70 to 82 with vascular risk factors or known vascular disease	5,804	3.2	Self-reported on usual ethanol intake in units perweek for the previous month.	MMSE, Stroop Color-Word Test, Letter-Digit Coding Test (LDCT), and Picture-Word Learning Test (PWRT)	MMSE - General cognitive impairment, Stroop Color-Word Test) and LDCT-attention and processing speed, and PWRT-memory
Yaffe (2009) [32]	Health, Aging and Body Composition (Health ABC) study, a prospective cohort study of 3,075 community-dwelling black and white men and women	3,075	Maximum 7	Interview-administered questionnaire assessing ethanol intake in previous year (yes/no), and amount consumed in previous month (number of drinks)	3MS	
Lobo (2010) [345]	The ZARADEMP Project, a prospective community-based study of men and women aged 55 years and older in Spain	4,803	4.5	Risk Factors Questionnaire - assessed Usual daily alcohol intake, present and past consumption, type, and quantity.	MMSE	Global cognition scores

Zanjani (2013) [346]	The Seattle Longitudinal Study (SLS) of men and women age ≥45 years	571	7	Questionnaire - assessed quantity of type of ethanol consumed	Thurstone Primary Mental Abilities-29 cognitive ability scores transformed into six standardized cognitive domain scores	Structural equation model used to represent latent variables for memory, reasoning, spatial, verbal, numeric, and perceptual speed abilities using well-validated instruments.
Beydoun (2014) [347]	Evaluate the independent association of alcohol intake and longitudinal cognitive performance in US older adults	628–1305 persons depending on the cognitive outcome; ~2 visits/person	46	7-d dietary records - assessed ethanol intake (g/d)	MMSE; BVRT; California Verbal Learning Test (CVLT); Digits span-backward (DS-B); Digits span-forward (DS-F); Nutrient adequacy score (NAS); Trails A, Trail Making Test, part A; Trails B, TrailMaking Test, part B; Verbal Fluency Test-Categorical (VFT-C); and Verbal Fluency Test-Letter (VFT-L)	Global cognition, verbal memory, visual memory/visuo-constructive ability, verbal fluency, attention, working memory, and executive function

Sabia (2014) [348]	Whitehall II cohort Study aged of British civilian workers aged 44-69 years at study baseline	10,308	10	QF for previous year and last 7 days	20-word free recall test (Verbal Memory). Executive function (3 tests), and Verbal Fluency. Global cognitive score created by averaging the z scores of each test	Verbal memory, executive function, and verbal fluency; global cognitive score created by averaging the z scores of each test

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Herbert (1993) [93]	The Established Populations for Epidemiologic Studies of the Elderly ((EPESE) of men and women aged 65 years or over	2; Baseline and 3 years follow-up visit	None in the previous year;< 0.5 ounce (15 ml) per day; 0.5 ounce to < 1 ounce (15 ml to < 30 ml) per day; and ≥ 1 ounces (30 ml or more) per day	Age, sex, education, and income.	No clear or consistent relation between moderate alcohol use and change in cognitive function. Individuals who consumed a very small amount of alcohol (< 0.5 ounce (15 ml) per day) had a normal change score that was 0.088 (95% CI: 0.015,0.160) better for digit span that did nondrinkers.
Dufouil (2000) [336]	The Epidemiology of Vascular Aging (EVA) study prospective study of men and women ages 59-71 years in western France	2; Baseline and 3 years follow-up visit	Never, fewer than two drinks, two to five drinks, or five drinks or more	Age, gender, education level, cognitive functions, hypertension, and depressive symptoms at study entry.	Alcohol consumption was associated with a decreased risk of cognitive deterioration in individuals without the ApoE ε4 allele (<2 glasses (RR=0.7, 95% CI=(0.5,1.1)), 2-5 glasses (RR=0.6,95% CI=(0.4,1.1)),≥5 glasses (RR=0.3,95% CI=(0.1,1.3))) whereas moderate drinking increased the risk of deterioration in ApoE ε4 allele (<2 glasses (RR=1.9, 95% CI=(0.7,5.0)), 2-5 glasses (RR=2.7,95% CI=(0.9,8.4)),≥5 glasses (RR=8.3,95% CI=(1.0,66.0))) carriers.

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Leroi (2002) [337]	The National Institute of Mental Health Epidemiologic Catchment Area of men and women ≥18 years	2; Baseline and 11 years	Nonusers, social users, habitual users, binge users, heavy/frequent	Age, race, and education.	Alcohol use was associated with significantly less cognitive decline in alcohol drinkers (Mean change in MMSE (β)=–1.25, 95% CI: –1.37, –1.13) when compared with nondrinkers (β=–1.99, 95% CI: –2.31, –1.67) for both sexes. When adjusted, a trend toward significantly less cognitive decline was seen in women drinkers (p<0.0001), but not in men (p=0.915).
Bond (2004) [338]	Pooled prospective cohort ofthe Kame Project -a population-based study of Japanese American adults aged persons aged 65 or older, and theAdult Changes in Thought (ACT) study of non-Hispanic Whites aged 65 and older	3; Baseline, 2- and 4-years follow-up	Current drinkers, past drinkers, and abstainers	Age, BMI, education and income, smoking, history of diagnosed stroke, hyper-tension, coronary heart disease, depression, and diabetes.	Drinkers had higher scores on cognition, measured by the CASI over the 4-yearfollow-up period than abstainers or past drinkers (p<0.05).

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Espeland (2005) [339]	The Women's Health Initiative Memory Study (WHIMS). Randomized Clinical Trial (39 US academic medical centers) of post-menopausal combination estrogen and progestin therapy of community-dwelling women aged 65-79 years	7; Baseline and annually up to 6 years	none, <1 drink per day, and ≥1 drink per day.	Age, years since menopause, education, ethnicity, family income, use of tobacco, body mass index (BMI), Hypertension (HTN), Cardiovascular Disease (CVD), diabetes (DM), statin therapy, aspirin; prior use of hormone therapy (HRT), and intervention assignment	Compared with no intake, intake of ≥1 drink per day was associated with higher baseline Modified Mini-Mental State Examination scores ($p < 0.001$) and a covariate-adjusted odds ratio of 0.40 (95% confidence interval: 0.28, 0.99) for significant declines in cognitive function.
Ganguli (2005) [340]	The Monongahela Valley Independent Elders Survey (MoVIES project), a prospective epidemiologic study of dementia in a largely rural, blue-collar community of men and women aged ≥65 years	Every 2 years	No drinking, minimal drinking, and moderate drinking	Age, sex, educational level, and recruitment status, MMSE baseline score, smoking, depressive symptoms, and subsequent new-onset dementia during follow-up.	Compared to no drinking, both minimal and moderate drinking were associated with lesser decline on the MMSE (minimal, Odds Ratio (OR)=0.30, 95% CI: 0.14,0.65); moderate, (OR=0.08, 95% CI: 0.02,0.28) and Trailmaking tests (minimal, OR=0.20,95% CI: 0.05,0.85; moderate, OR=0.05,95% CI: 0.01–0.45). Minimal drinking was also associated with lesser decline on tests of learning (OR=0.17,95%CI:0.05,0.57) and naming (OR=0.36,95%CI:0.15,0.84) .

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Richards (2005) [341]	The MRC NationalSurvey of Health and Development (the British 1946 birth cohort)	2; Aged 43 and 53	None (0/day), Very light (0.1–1.0/day), Light(1.1–2.0/day), Moderate (2.1–4.0/day), Heavy (4.1–8.0/day)	Educationalattainment, occupational social class, general cognitive abilityand a range of health indicators.	Alcohol consumption was associated with a slower memory decline from 43 to 53 years in men (F for equality of means = 2.35, P = 0.05), but a more rapid decline in visual search speed for the same interval in women (F = 2.94, P = 0.03), and a faster decline with increasing alcohol consumption (P for trend = 0.008).
Stampfer (2005) [342]	Nurses' Health Study of men of female nurses aged 70 to 81 years old	2; Baseline and two years follow-up visit	nondrinkers, 1.0 to 4.9	Age; education; hypertension, diabetes, high cholesterol levels, and heart disease; physical activity; age at menopause; HRT use, aspirin and ibuprofen, and vitamin E; BMI; smoking status; scores for the mental health and energy; and Social Network.	Lower relative risk of substantial decline in general cognition over two years among moderate (1.0-14.9g) drinkers compared to nondrinkers (β (95% CI): 0.85 (0.74,0.98). No significant association between higher levels of drinking (15.0 to 30.0g) risk of decline. No significance differences in risks according to beverage type.

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Wright (2006) [343]	The Northern Manhattan Study (NOMAS). Community sample of men and women (Hispanic, black, white, and other groups) aged ≥40 years stroke free at study baseline	Annually since 2001	never, past, less than one drink weekly, one drink weekly up to two daily, and more than two drinks daily.	Age, education, gender, race-ethnicity, insurance status, hypertension, diabetes, cardiac disease, physical inactivity, depression, current smoking, HDL-C level, and BMI.	Drinking less than one drink a week (β (95% CI): 0.9 (-1.2,1.9), P=0.09), between one drink weekly up to two drinks daily (1.5 (0.6,2.4), P=0.001), and more than two drinks daily (2.4 (0.8,4.0), P=0.003) were associated with less cognitive decline on the modified Telephone Interview for Cognitive Status (TICS-m) compared to never drinkers.
Stott (2008) [344]	Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). Randomized placebo-controlled trial of pravastatin in men and women aged 70 to 82 with vascular risk factors or known vascular disease	5; Baseline, 9,18,30 months, and final trial visit.	Women: Nondrinker, Low alcohol intake (1 to 3 U per week) and moderate intake (>3 U per week). Men: Nondrinker, Low alcohol intake (1 to 7 U per week), and moderate intake (>7 U per week).	Age, country, smoking status, body mass index, body weight, years of education, incident stroke, history of vascular disease, and version of test (if applicable).	Women: Mean difference were for female drinkers than nondrinkers across all cognitive domains, with the exception of PWLT. Decline similar across cognitive domains Less decline in MMSE in low or moderate female drinkers than nondrinkers (0.05 MMSE units per annum, P=0.001 Men: No significant association observed.

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Yaffe (2009) [32]	Health, Aging and Body Composition (Health ABC) study, a prospective cohort study of 3,075 community-dwelling black and white men and women	4; Baseline, 3, 5, and 8 years follow-up visit	Current drinking: >1 drink/day vs. ≤ 1 drink/day)	Age, sex, race, education, self-rated health, smoking, physical activity, depression, hypertension, diabetes, and history of myocardial infarction, stroke or TIA.	The odds of individuals who drinks >1 alcoholic drink/day being a cognitive maintainer is 1.33 (95% CI: 0.91,1.93) times than of individuals who drinks ≤ 1 alcoholic drink/day. The odds of individuals who drinks >1 alcoholic drink/day being a major cognitive decliner is 0.67 (95% CI: 0.36,1.27) times than of individuals who drinks ≤ 1 alcoholic drink/day.
Lobo (2010) [345]	The ZARADEMP Project, a prospective community-based study of men and women aged 55 years and older in Spain	2; Baseline and at follow-up visit	<12 alcohol/day, 12-24 g alcohol/day, >24-40 g alcohol/day, >40g alcohol/day, and former drinkers	Age, years of education, Mini-Mental State Examination (MMSE) score at baseline, marital status, smoking status, hypertension, depression, psychotropic medication use, and disability.	Men: Consumption of <40g/day was not associated with decreased risk of cognitive decline (<12 alcohol/day (OR=0.61, 95% CI: 0.31,1,20), < 24 alcohol g/day (OR=1.19, 95% CI: 0.61,2.32), and former drinkers (OR=1.03, 95% CI: 0.59,1.82)). Women: Consumption of <24 g/day was not associated with decreased risk of cognitive decline (<12 alcohol/day (OR=0.88, 95% CI: 0.45,1.72), <24 alcohol g/day (OR=2.38, 95% CI: 0.98,5.77), and former drinkers (OR=1.03 (95% CI: 0.48, 2.23)).

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Zanjani (2013) [346]	The Seattle Longitudinal Study (SLS) of men and women age ≥45 years	2; Baseline and at follow-up visit	Alcohol abstainer: no alcohol consumed, moderate alcoholconsumer: no more than 7 drinks/week, and at-risk alcohol consumers: more than 7drinks/week	Age, gender, income, education, baseline drinking level (beer, wine, liquor) and smoking status	Decline in verbal ability was seen among alcohol abstainers (Differences of Least Squares Means estimate (est.=1.54, P<0.0001) and moderate alcohol consumers (est.=1.18, P<0.0001), but at-risk drinkers (est.=0.34,0.44) displayed relative stability
Beydoun (2014) [347]	Evaluate the independent association of alcohol intake and longitudinal cognitive performance in US older adults	2; Baseline to follow-up visit	lower: <14 g/d, moderate alcohol consumption: 14 to 28 g/d, and higher: >28 g/d.	Age, gender, race, education, smoking, and BMI	Age <70 years: Alcohol intake was associated with faster decline or slower improvement on the MMSE (Global Cognition, P=0.008) and on the VFT-L test (Letter Fluency, P=0.001). Overall, among men, and for Age_{base} ≥70 y, lower alcohol intake compared with moderate consumption was associated with poorer performance on the DS-B (overall, ϒ031 = 20.76 6 0.28, P = 0.008).

Table 13. Review of prospective studies of the ethanol intake and cognitive decline relationship contd

Author (Year)	Study Design/ Population	Frequency of Cognitive Assessments	Exposure Definition	Covariates	Results
Sabia (2014) [348]	Whitehall II cohort Study aged of British civilian workers aged 44-69 years at study baseline	3; 1997-1999, 2002-2004, and 2007-2009	10-year abstainers, alcohol cessation in the last 10 years, occasional drinkers, drinkers: 0.1-9.9 g/d, drinkers: 20-35.9 g/d, and drinkers: ≥ 36 g/d	Age, sex, ethnicity, marital status, occupational position, and education.	Men consuming 36 g/d or more of alcohol in midlife were more likely to experience faster 10-year cognitive decline compared with consumption between 0.1 and 19.9 g/d (mean difference (95% CI) in 10-year decline in global cognition =-0.10 (-0.16, -0.04), executive function= -0.06 (-0.12,0.00), and memory=-0.16 (-0.26, -0.05). No differences in cognitive decline were observed for alcohol abstainers, quitters, and light or moderate alcohol drinkers (<20g/d). Weaker evidence of an association in women. In women, compared to those drinking 0.1 to 9.9 g/d of alcohol, 10-year abstainers shower fast decline in the global cognitive score (-0.21 (-0.37, -0.04) and executive function (-0.17 (-0.32, -0.01)).

Table 14. Ethanol metabolizing SNPs associated with ethanol dependence and intake [383]

Chr	Genes	rs number	Functional class	A1	A2	MAF* AA	MAF* EA	Effect direction of A1†	References	Available in ARIC	Final instruments
4	*ADH1A*	rs904092	upstream	A	G	0.246	0.147	NA	Gelernter et al, 2014	No	
4	*ADH1A*	rs2866151	intronic	T	A	0.254	0.520	+	Zuccolo et al, 2009	No	
4	*ADH1B*	rs1042026	downstream	G	A	0.066	0.278	-	Macgregor et al, 2009	Yes	No, high missing rate
4	*ADH1B*	rs1229984	exonic	A	G	0.000	0.015	-	Gelernter et al, 2014; Zuccolo et al, 2009; Agrawal et al, 2012; Ferrari et al, 2012; Li et al, 2011; Bierut et al, 2012; Way et al, 2015; Jorgensen et al, 2017	Yes	No, did not pass quality control‡
4	*ADH1B*	rs2066702	exonic	A	G	0.205	0.000	-	Gelernter et al, 2014	Yes	Yes
4	*ADH1B*	rs1789882	exonic	A	G	0.246	0.141	-	Gelernter et al, 2014	No	
4	*ADH1B*	rs1693457	intronic	C	T	0.246	0.147	-	Gelernter et al, 2014	Yes	Yes
4	*ADH1B/1C*	rs1789891	intergenic	A	C	0.025	0.167	+	Way et al, 2015; Agrawal et al, 2012	Yes	Yes
4	*ADH1C*	rs1693482	exonic	A	G	0.139	0.475	+	Macgregor et al, 2009; Agrawal et al, 2012; Way et al, 2015; Toth et al, 2011	Yes	No, in high LD with rs698 and has lower sample size
4	*ADH1C*	rs698	exonic	C	T	0.139	0.475	+	Agrawal et al, 2012; Way et al, 2015; Toth et al, 2011; Li et al, 2012	Yes	Yes
4	*ADH1C*	rs283413	exonic	T	G	0.000	0.005	-	Way et al, 2015; Biernacka et al, 2013; Norden-Krichmar et al, 2014	No	
4	*ADH1C*	rs2241894	exonic	C	T	0.475	0.207	-	Gelernter et al, 2014	No	

Table 14. Ethanol metabolizing SNPs associated with ethanol dependence and intake [383]

Chr	Genes	rs number	Functional class	A1	A2	MAF* AA	MAF* EA	Effect direction of A1†	References	Available in ARIC	Final instruments
4	*ADH1C*	rs1614972	intronic	T	C	0.459	0.768	-	Gelernter et al, 2014; Agrawal et al, 2012	Yes	No, violation of HWE assumptions
4	*ADH4*	rs3762894	upstream	G	A	0.205	0.131	NA	Macgregor et al, 2009	No	
4	*ADH4*	rs1042363	exonic	T	C	NA	NA	-	Luo et al, 2005	No	
4	*ADH4*	rs1126671	exonic	A	G	0.189	0.273	+	Luo et al, 2005	Yes	Yes
4	*ADH5*	rs1230165	downstream	C	T	0.082	0.152	NA	Macgregor et al, 2009	No	
12	*ALDH2*	rs671	exonic	A	G	0.000	0.000	-	Agrawal et al, 2012; Rietschel et al, 2013; Jorgensen et al, 2017	Yes	No, monomorphic

Abbreviations: Chr, chromosome; rs, reference SNP; A1, minor allele; A2, major allele; *, obtained from 1000 Genome; AA, African-Americans; EU, European-Americans†, effect direction; +, minor allele increases consumption, -, minor allele decreases consumption, NA, not available; ‡low call rate.

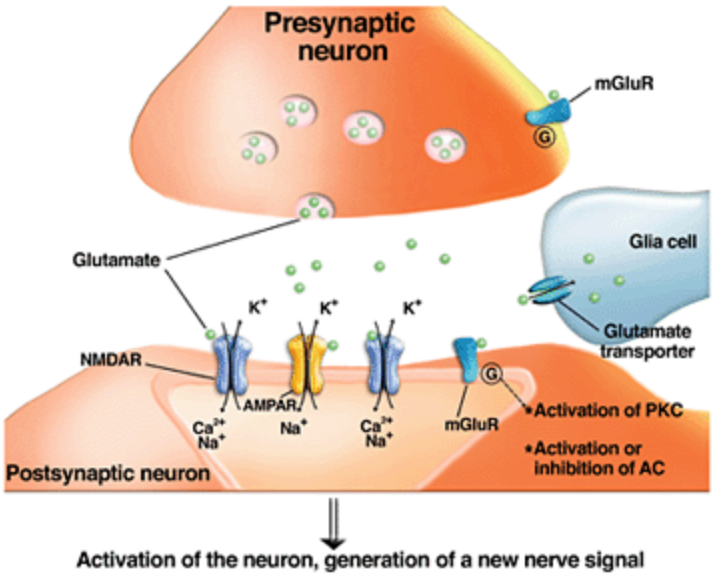


Figure 1. Actions of the brain's glutamate system in the absence of ethanol [302]

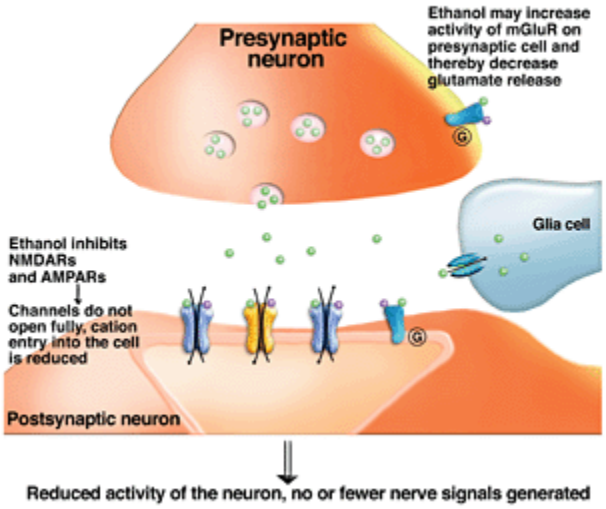


Figure 2. Actions of the brain's glutamate system in the presence of ethanol [302]

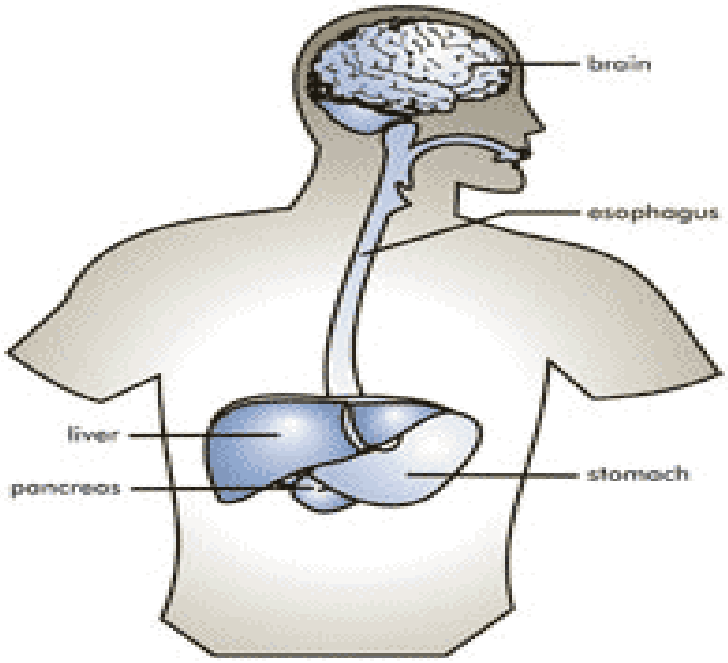


Figure 3. Ethanol metabolism (https://pubs.niaaa.nih.gov/publications/aa72/aa72.htm)

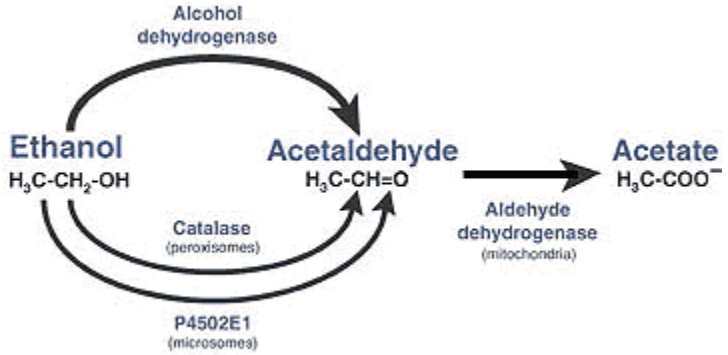


Figure 4. Ethanol metabolism pathways (https://pubs.niaaa.nih.gov/publications/aa72/aa72.htm)

CHAPTER IV: METHODS

4.1. Overview

This study benefited from the long follow-up of the ARIC cohort of African-Americans and European-Americans from mid-to-late life to examine the association of ethanol intake in mid-life and cognitive decline from mid-to-late life. The availability of repeated measures of ethanol intake and well-characterized cognitive function allowed for the characterization of long-term patterns of ethanol intake over nine-years in mid-life and the assessment of change in cognitive function over a 15-year period from mid-to-late life. The availability of genetic data for a majority of study population allowed for the exploration of possible mechanisms by which ethanol intake affects cognitive function by evaluating for possible effect modification of the ethanol intake-cognitive decline relation by ethanol intake-associated genetic variants. Fine-mapping and conditional analyses were used to identify genetic variants that are mostly strongly associated with ethanol intake among African-American participants. The potential for confounding was reduced by adjusting for of lifestyle, genetic, and clinical risk factors in all analyses. The potential of population stratification by controlling for principal components. Multiple imputations by chained equations (MICE) was used to account for attrition of the cohort during the course of follow-up.

4.2. Study Population

4.2.1. Description of the ARIC Study Cohort

The Atherosclerosis Risk in Communities (ARIC) study is a community-based, prospective cohort study established in 1987, designed to investigate the etiology of atherosclerosis and its clinical sequelae. From 1987 through 1989, 15,792 adults aged 45 to 64 years were recruited through probability sampling from 4 U.S. communities: Washington County, Maryland; Forsyth County, North Carolina; suburbs of Minneapolis, Minnesota; and Jackson, Mississippi. Participants were seen at 4 study visits

approximately 3 years apart from 1987-1989 through 1996-1998, and a fifth examination visit was conducted in 2011-2013 (Figure 5).

4.2.2. Inclusion Criteria

For Aim 1 analyses, we included participants with at least one measurement of ethanol intake from visits 1-4 and at least one measurement of cognitive function at visits 4 and 5.

For Aims 2 analyses, we included participants with ethanol intake measured at study baseline (visit 4) and those with genetic data that are associated with ethanol intake across ARIC visits 1-4.

4.2.3. Exclusion Criteria

Excluded from the proposed analyses were participants who prohibited use of their deoxyribonucleic acid (DNA) for research purposes, who did not self-identify as African-American or European-American, African-Americans residing in Washington County or Minneapolis (due to small numbers), participants missing one or more cognitive function tests at study baseline, and those with missing covariates at study baseline.

4.3. Exposures Assessment

4.3.1. Ethanol Intake

Ethanol intake was assessed at all visits by means of an interviewer-administered questionnaire [169]. During the exam, participants were asked the following questions: Do you presently drink alcoholic beverages?", and "Have you ever consumed alcoholic beverages?". Individuals replying no to both questions were classified as never drinkers. Those who replied "no" to the first question and "yes" to the second question were classified as 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. History of excessive ethanol intake (yes/no) was only assessed during ARIC visit 3 using the following question:

"Was there ever a time in your life when you consumed 5 or more drinks of any kind of alcoholic beverage almost every day?". We used this variable only for further description of the study sample. Categories of ethanol intake at each visit were created based on the U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2015-2020 guideline for low-to-moderate drinking (≤210 grams/week for men and ≤105 grams/week for women) and heavy drinking (> 210 grams/week for men and > 105 grams/week for women) [384].

4.4. Outcome Assessment

4.4.1. Assessment of Cognitive Status in ARIC

Cognitive function was assessed at visit 2 (1990-1992; ages 48-67), visit 4 (1996-1998; ages 54-73), and visit 5 (as part of the ARIC-NCS) (2011-2013; ages 70-89 years) using 3 standardized cognitive tests to assess different domains of cognition: verbal learning and short-term memory, executive function and processing speed, and executive function and expressive language.

4.4.2. Cognitive Function Tests

Verbal learning and recent memory were assessed by the delayed word recall test (DWRT). Participants were asked to learn 10 nouns, and after a five-minute delay were given 60 seconds to recall the words. The DWRT score is the number of words recalled (0-10) [201]. DWRT has a high test-retest reliability of 0.75 in older adults [201].

Executive function and processing speed were assessed by the digit symbol substitution test (DSST). Participants were given 90 seconds to fill in blank squares with symbols corresponding to digits from 1 to 9 using a key that matches digits to symbols [209]. DSST has high reliability (0.82-0.88) in older adults [209].

Executive function and expressive language were assessed by the word fluency test (WFT) , during which participants generate as many words starting with the letters F, A, and S as possible within 60 seconds, with one trial per letter [385]. The WFT score is the total number of acceptable words generated for the three letters [205]. WFT has a test-retest reliability of 0.88 in older adults [206]. All

three tests were administered by trained examiners using standardized protocols in a quiet room. Recordings were reviewed for quality control.

To facilitate comparison across cognitive tests, Z scores standardized to visit 2 were calculated for each test by subtracting the participant's overall mean test score at visit 2 from their test score at each visit and then dividing by the standard deviation of the visit 2 scores.

A factor score for general cognitive performance was previously derived using factor analysis [386, 387], and was scaled to have a mean of 50 and standard deviation of 10 at the 1990–92 visit [387].

4.5. Covariates Selection and Assessment

4.5.1. Selection of Covariates

Potential confounders were identified based on substantive knowledge of factors associated with ethanol [388] and risk of cognitive decline [10, 62, 63], and from existing literature on the association between ethanol and cognitive decline. Selection of confounders to include in primary analyses was based on directed acyclic graph analysis (DAG) (Figure 6) that included all potential confounders identified from the literature: demographic characteristics (age, sex, race, educational attainment, and social support), lifestyle factors (smoking status, physical activity, and omega-3 fatty acids), genetic risk factor (*APOE* ε4 allele) and medical history (diabetes, stroke, and depression) (Table 15).

Figure 6 presents the results of an assessment of potential confounding which may be present in the examination of the association of ethanol intake with change in cognitive function. The figure was created using the "DAGgity" software [389], which allows examining relationships between the exposure and outcome of interest, while accounting for all known associated factors and determining the minimal adjustment set needed to minimize confounding. Covariates in Figure 6 represented by pink circles were determined to be potential confounders, due to their direct or indirect association with the exposure and the outcome. Covariates represented by blue circles are those covariates that are associated with the outcome but are not on an "open path" (not causally associated) with the exposure. For the association of ethanol intake and cognitive decline, the minimal sufficient adjustment set includes the following

confounders: age, sex, SES (i.e., education attainment), social support, history of stroke, diabetes, and depression (Figure 7). By adjusting for those nine variables all the confounding paths are blocked. Additional adjustment could result in over adjusting and biasing the results.

4.5.2. Assessment of Covariates

4.5.2.1. Demographic Factors

Demographic factors associated with ethanol intake and cognitive decline that were treated as confounders in this work include age, sex, race, and educational attainment. Described below are the specific characteristics of each of these variables in the ARIC study.

4.5.2.1.1. Age, Sex, and Educational Attainment

Age, sex, and educational attainment (< high school, high school, >high school), and smoking status (current, former, never) were assessed at visit 4 via self-report from the home interviews.

4.5.2.2. Lifestyle Factors

Lifestyle factors associated with ethanol intake and cognitive decline that were treated as confounders in this work include smoking status, physical activity, and diet. Described below are the specific characteristics of each of these variables in the ARIC study.

4.5.2.2.1. Smoking Status

Cigarette smoking status was measured at visit 4 by self-report. Cigarette smoking status was categorized as current, former, and never.

4.5.2.2.2. Physical Activity

Physical activity in ARIC participants was measured at visits 1 and 3 using the modified Baecke questionnaire [390], which asks about three levels of physical activity (low, medium, and high intensity) in sports, during leisure time, and at work. The answers then were converted to minutes per week of moderate or vigorous activity based on Metabolic Equivalent of Task (MET) value [390].

4.5.2.2.3. Diet

Factor analysis were used to derive diet patterns and adjust for overall diet quality. The Healthy Food Score, adapted from Steffen et al. [391, 392] was created by summing the scores of food groups. Food groups included: dairy (low-fat and whole milk, cheese, yogurt, ice cream), vegetables, fruit (without juice), fruit juice, legumes, refined grain, whole grain, nuts, fish, meat (combined poultry, processed meat, beef, pork, and lamb), diet beverages, sugar-sweetened beverages, and coffee and tea. Daily intake of food groups was categorized into quintiles, except alcohol intake, legume, and beverages. Each quintile of food group intake was assigned a score: 0–4. For dairy, vegetables, fruit (without juice), fruit juice, refined grain, whole grain, nuts, and fish, scores were assigned in order (Quintile 1 = 0, Quintile 2 = 1, Quintile 3 = 2, Quintile 4 = 3, Quintile 5 = 4); for meat, the score was the reverse. Due to the limited range of intake, scoring for intake of legumes was 0, 1, and 2, if daily intake was 0, <1, and ≥1 serving, respectively. The score was reversed for diet beverages and sugar-sweetened beverages: 2, 1, and 0 for 0, >0 to <1, and one or more servings usually consumed per day, respectively. Daily coffee and tea intake were scored in five categories from 0 to 4, for 0, >0 to ≤2, >2 to ≤4, >4 to ≤6, and >6 cups per day, respectively. For alcohol intake, a score of 4 was assigned to the men who consumed between 10 and 50 g per day and to women who consumed between 5 and 30 g per day; otherwise a score of 0 was assigned [391].

4.5.2.3. Genetic Factor

4.5.2.3.1. Apolipoprotein E ε4 Polymorphism

Genotyping of the *APOE* polymorphisms at codons 112 and 158 in exon 4 was performed by using the TaqMan assay (Applied Biosystems, Foster City, California) [393, 394]. Allele detection and genotype calling were performed by using ABI 7900 and Sequence Detection System software (Applied Biosystems) [393]. The ARIC Study has extensive quality control measures for all genotyping assays, including but not limited to robotic liquid handling, separate pre– and post–polymerase chain reaction areas, standard protocols and quality control analyses, a blind duplicate program, positive and negative

controls, computerized sample tracking, and data validity checks [394]. The APOE ε4 polymorphism will be categorized as presence of 0,1, or 2 alleles.

4.5.2.4. Cardiovascular Disease Risk Factors

Medical risk factors associated with ethanol intake and cognitive decline that were treated as confounders in this work include diabetes, depression, and stroke. Described below are the specific characteristics of each of these variables in the ARIC study.

4.5.2.4.1. Diabetes

Diabetes (yes, no) at visit 4 was defined as self-reported history of a physician's diagnosis of diabetes, fasting blood glucose level of ≥126 mg/dL, or non-fasting blood glucose level of ≥200 mg/dL, or diabetes medication use in the past 2 weeks [395].

4.5.2.4.2. Stroke

Stroke was defined by a self-reported history at visit one or an adjudicated event between visits 1 and 4 [396].

4.6. Genotyping and SNP Selection

Consenting ARIC study participants were imputed separately by race using IMPUTE2 [397] with the 1000 Genomes Project phase 1 (March 2012) reference panel. Quality control excluded individuals based on single nucleotide polymorphism (SNP) mismatch, high discordance with previous TaqMan assay genotypes, genetic outlier status, and relatedness. SNPs with IMPUTE info score < 0.8 or minor allele frequency (MAF) < 0.05 were excluded. Only autosomal variants (on chromosomes 1–22) were considered [398]. Principal components analysis was used to estimate population substructure with EIGENSTRAT [399].

The GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) meta-analyses of 941,280 participants of European ancestry from 34 studies, including the ARIC study, identified 100 single nucleotide polymorphisms (SNPS) in 82 genetic loci to be independently associated with number of drinks per week (Appendix Table 1) [400]. In the ARIC 1000 Genome imputed dataset, 99 of the 100

SNPs were available for ARIC European-American participants (Appendix Table 1), and 74 SNPs were available for ARIC African-American participants (Appendix Table 1).

To determine if the GSCAN index SNPs replicate in the ARIC study population, we assessed association between the index SNPs and weekly ethanol intake across ARIC visits 1-4, separately, among European (99 SNPs) (Appendix Table 2) and African-American participants (74 SNPs) (Appendix Table 3). Among European-American participants, 11 SNPs direction of effect were consistent with the effect reported by GSCAN and were nominally significantly associated (P-value <0.05) with at least one measurement of weekly ethanol intake assessed across ARIC 1-4 (Appendix Table 4). These 11 SNPs were used to address Specific Aim 2 among ARIC European-American participants. Among ARIC African-American participants, one SNP ((rs12795042) was associated with weekly ethanol intake at study baseline (visit 4). However, this SNP's direction of effect was not consistent with the GSCAN SNP, and hence was not considered for this study (Appendix Table 3).

To characterize the best tag SNP in ARIC African-American participants, we conducted fine-mapping in the 1 MB region (± 500 kb windows surrounding each of the 99 GSCAN SNPs (index SNPs). Within each region, we identified the most strongly associated SNP with weekly ethanol intake at study baseline and in linkage disequilibrium (LD) (r^2>0.2) with the index SNP. We identified a total of 92 mostly strongly associated SNPs (Appendix Table 5). Of the 92 SNPs, 20 SNPs direction of effect were consistent with their index SNPs and were nominally significantly associated with at least one measurement of weekly ethanol intake assessed across ARIC 1-4 (Appendix Table 6). Conditional analyses were performed and determined that 20 SNPs are independent of their GSCAN index SNPs (Appendix Table 7). These 20 SNPs were used to address Specific Aim 2 among ARIC African-American participants (Appendix Table 6).

4.7. Statistical Approach

4.7.1. Specific Aim 1

Specific Aim 1: Characterize temporal trajectories of ethanol intake during mid-life in African-American and European-American adults and examine whether long-term trajectories of ethanol intake in mid-life

are associated with 15-year rate of decline in cognition from mid-to-late life among African-American and European-American adults.

We characterized temporal trajectories of ethanol intake during mid-life, separately in African-American and European-American adults, by first, categorizing study participants ethanol intake status at ARIC visits 1-4 as low-to-moderate drinkers ((≤210 grams/week for men and ≤105 grams/week for women), heavy drinkers (> 210 grams/week for men and > 105 grams/week for women), former drinkers and never drinkers. Second, we cross tabulated ethanol intake status variables from visits 1-4. Finally, from the cross tabulation of ethanol intake results, trajectories of ethanol intake were identified as 1) stable never drinkers, 2) stable low-to-moderate drinkers, 3) stable heavy drinkers, 4) stable former drinkers, 5) mostly low-to-moderate drinkers, 6) mostly heavy drinkers, and 7) mostly former drinkers. In creating the 'mostly' ethanol intake trajectories, ethanol intake status at study baseline (visit 4) was taken in account. Participants with non-current drinking status (i.e., never or former) across visits 1-3, but reported current drinking at visit 4 (i.e., low-to-moderate or heavy) were assigned to the "mostly" ethanol intake category at visit 4 (i.e., low-to-moderate or heavy). Participants with current drinking status (i.e., low-to-moderate or heavy) across visits 1-3 but reported former drinking at visit 4 were assigned to the "mostly" former long-term pattern of ethanol intake. Participants who reported 2 visits of current drinking (i.e., low-to-moderate or heavy) and 2 periods of former drinking were assigned to the "mostly" ethanol intake category at visit 4 (i.e., former, low-to-moderate or heavy).

To evaluate change in general cognitive performance, DSST, DWRT, and WFT tests between visits 4 and 5, multivariable linear regression models were used with the outcome being visit 5 z-score minus visit 4 z-score. All models were race-stratified and were adjusted for age, age squared, sex race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity, and the Apo lipoprotein E ε4 (APOE ε4) allele.

Temporal trajectories of ethanol intake were evaluated as a categorical variable in the analysis models, with stable never drinking as referent. Z-scores of DWRT, DSST, WFT, and global cognition were assessed as continuous measures of cognitive function.

4.7.1.1. Multivariate Imputation by Chained Equations (MICE) Models to Account for Attrition in ARIC

The long follow-up of the ARIC study resulted in some loss to follow-up due to refusal to participate or death. At Visit 2 13,351 participants underwent cognitive assessment, while at Visit 4 the number of participants dropped to 10,720 (80.3% of baseline number of participants) and to 5,987 (45.8% of baseline number of participants) at Visit 5. The number of participants who died was 1,350 (10.1% of baseline number of participants) between Visit 2 and Visit 4 and 2,037 (15.3% of baseline number of participants) between Visit 4 and Visit 5. The number of those who refused to participate was 1,281 (9.5% of baseline number of participants) at Visit 4 and 2,696 (20.2% of baseline number of participants) at Visit 5.

Missing data due to attrition were imputed by multiple imputation using chained equations (MICE)[401]. Missing ethanol intake and cognitive data across ARIC visits were imputed based on the observed values of key covariates for a given individual, as well as the relations observed in the data for other participants. To account for the uncertainty of the imputation and ensure correct standard error estimation [402], 25 datasets were imputed. Validation of the MICE approach for cognitive outcome in ARIC has been previously reported and it has been determined that MICE produced unbiased imputed values [403]. For this study, validation using observed data demonstrated MICE produces unbiased imputation of global cognition factor z-scores (Figure 8).

4.7.1.2. Statistical Power to Examine Association Hypothesized in Specific Aim 1

In Specific Aim 1, we estimated the relationship between ethanol intake and change in cognition function over a 15-year follow-up from Visit 4 to Visit 5. The R package 'longpower'[404] was utilized to estimate the power to detect the mean difference in the 15-year rate of change in global z-score between heavy drinking compared to never drinking and LM drinking compared to never drinking. Presented in Table 16 are parameter (i.e., sample size, parameter estimates, residual variance and working correlation matrix) values that were obtained from preliminary analyses and were used to estimate the

power of the proposed analyses. Also assumed in the power size calculation were a significant level of α=0.05 and a 'one-sided' test.

Power calculation results, assuming a significance level of α-0.05 and a 'one-sided" test, suggest this study had 1) excellent power (≥95%) to detect a mean difference of ≥0.02 in the 15-year rate of change in global cognition factor z-score between stable low-to-moderate drinking and never drinking among African-Americans and European-Americans participants, separately; 2) excellent power (≥94%) to detect a mean difference of ≥0.03 in the 15-year rate of change in global cognition factor z-score between stable heavy drinking and never drinking among African-Americans and European-Americans participants, separately; 3) excellent power (≥90%) to detect a mean difference of ≥0.02 in the 15-year rate of change in global cognition factor z-score between stable former drinking and never drinking among African-Americans and European-Americans participants, separately; 4) excellent power (≥97%) to detect a mean difference of ≥0.02 in the 15-year rate of change in global cognition factor z-score between mostly low-to-moderate drinking and never drinking among African-Americans and European-Americans participants, separately; 5) excellent power (≥95%) to detect a mean difference of ≥0.05 in the 15-year rate of change in global cognition factor z-score between mostly heavy drinking and never drinking among African-Americans and European-Americans participants, separately; and 6) excellent power (≥97%) to detect a mean difference of ≥0.03 in the 15-year rate of change in global cognition factor z-score between mostly former drinking and never drinking among African-Americans and European-Americans participants, separately (Table 16).

4.7.2. Specific Aim 2

Specific Aim 2: Evaluate for the effect modification of the ethanol intake-cognitive decline relationship by ethanol intake associated SNPs in African-American and European-American men and women from mid-to-late life.

4.7.2.1. Population Stratification Confounding

Although environmental exposures are not thought to influence genotype, confounding can still occur in genetic association studies. The reason for confounding is usually differences in ethnicity. At

many loci, distinct populations have different allele frequencies, and in an ethnically diverse population, ethnically-related differences in genotype may appear to be associated with a trait because genotype at an entirely different locus, also correlated with ethnicity, influences the trait. Different ethnic groups often share distinct lifestyle characteristics, and these differences may cause the trait to be associated with ethnic group. When this occurs, any genotype that is more prevalent in an ethnic group with higher or lower levels of the trait will appear to be associated with the trait in an ethnically diverse population. This population structure is referred to as "population stratification", meaning the population under study is comprised of strata formed by ethnic groups that have different trait distributions and different genotype distributions [405].

A consequence of population stratification is the potential for increased allelic associations and deviations from Hard-Weinberg equilibrium [406]. Another consequence of population stratification is bias in the estimate of genetic associations, which may lead to incorrect inferences and inconsistent study findings [407]. Studies have shown that the bias due to population stratification is small in magnitude [408, 409] and is bounded by the magnitude of the difference in background disease rates across the populations being compared [410]. Simulation studies have shown that the adverse effect of population stratification increases with increasing sample size [411, 412].

Several approaches exist to correct for the effects of population stratification, which includes adjustment for principal components methods [413-415]. In this method, the first principal component of the GWAS SNPs is computed and included as independent variable in regression models relating SNP genotype to trait. The first principal component is designed to summarize most of the variation in a large number of variables with many fewer variables. In general, for n variables X_1, …, Xn, the first principal component is the linear combination $a_1X_1+\ldots. + a_nX_n$ with the largest observed variance [414]. The second principal component is the linear combination $a_1'X_1+\ldots. + a_n'X_n$, with largest observed variance among linear combinations uncorrelated with the first principal component, and so on. Price et al 2006 recommend using the first ten principal components [414].

To minimize population stratification bias, we adjusted for principal components in the regression models that will be used to address Specific Aim 2.

4.7.2.2. Single Ethanol Intake-Associated SNPs

We used linear regression models to estimate the relationship of ethanol intake associated SNPs within with ethanol intake. In all models, genotypic effects will be modeled additively as the number of minor alleles increases. Ethanol intake was assessed as a continuous variable. All models were adjusted for age, sex, gender, education, center, and principal components. Regression coefficients and 95% confidence intervals (CIs) will be estimated. A Bonferroni correction (α/n) for number of independent tests (African-Americans, n=20; European-Americans, n=11) were used to adjust *P* values and control for type 1 error introduced by multiple testing.

4.7.2.3. Genetic Risk Scores of Ethanol Intake-Associated SNPs

Allele sores or genetic risk sores are a convenient way of summarizing multiple genetic variants that are associated with a risk factor [416]. The most common method sums the number of risk-conferring alleles that an individual has (0, 1, or 2) across all loci [417]. A genetic risk score (GRS) can be unweighted or weighted. An unweighted GRS is created as the total number of risk factor-increasing alleles present in the phenotype of an individual. The unweighted GRS and its construction is based on the assumption that each risk allele confers identical risk; thereby assigning equal weights to genetic variant [416]. However, for most complex traits, effect sizes across identified SNPs vary [418]. Therefore, GRSs are often weighted. A weighted GRS takes into consideration that each allele contributes a weight reflecting an estimate of the effect of the corresponding genetic variant on the risk factor. These can be derived internally from the data under-analysis or externally derived from prior knowledge or an independent data source, such as GWAS meta-analysis effect sizes, therefore giving more weight to variants with stronger effects [416, 417] . Weighted scores may increase statistical power compared to unweighted scores, provided that the weights are accurately determined [416, 417].

The weighting approach is utilized when the target population (the population in which GRS will be evaluated) is similar in demographic and ethnic composition as the meta-analysis population, from which the effect sizes were derived [417]. An unweighted GRS is the best option if stable effect size estimates are unavailable due to (1) no GWAS meta-analyses have been conducted on the trait of interest, and thus genetic variants are chosen from candidate gene studies or GWAS that are small and un-replicated, 2) the target population in which the GRS will be evaluated differs from the ethnicities in existing meta-analyses, and 3) genetic variants identified using multiple traits on different measurement scales are to be combined into a single GRS [417].

GRS are essential for the modelling of multifactorial polygenic traits, specifically when the GRS comprises of either may common genetic variants with small effects, or of rare variant [416]. The GRS may explain a substantial proportion of variation in the risk factor, even if none of the genetic variants individually does [416]. GRS have been constructed for various traits, which includes fasting [419], blood pressure [420], and high-density lipoprotein cholesterol [383, 421].

We will use linear regression models to estimate the association between unweighted genetic risk score of SNPs within ethanol-metabolizing genes and ethanol intake in race-stratified analyses. The five SNPs will be coded to ensure consistent effect direction of increasing ethanol intake, which will be combined to create the unweighted genetic risk score [422]. All models will be adjusted for age and sex. Regression coefficients and 95% confidence intervals (CIs) will be estimated. We will also adjust for principal components to account for population stratification within each race/ancestry group.

4.7.2.4. Gene-Environment Interaction

Our study outcome, fifteen-year cognitive performance change was calculated by subtracting visit 4 cognitive performance z-score from visit 5 neurocognitive exam z-score.

To evaluate if the relationship between ethanol intake and decline in general cognitive performance is modified by ethanol intake-associated SNPs, multivariable linear regression models were used and included the unweighted GRS, log-ethanol measured at study baseline, an interaction term between the unweighted GRS and log-ethanol intake, and covariates: age, age squared, sex, race-center,

education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity and *APOE* ε4 status. All models were race-stratified and further adjustments were made for principal components to account for population stratification.

Statistical tests were 2-sided, and the test for statistically significant interaction was set a priori at P<0.10. However, adjustment for multiple testing using the Bonferroni method was performed for the interaction analyses based on single SNPs (African-Americans: P<0.005, European-Americans, P<0.009).

Multiple Imputation were performed with Stata15 (StataCorp, College Station, TX, USA) [423], and statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) .The results from each imputed data set were summarized using Rubin's rule [424] into an overall estimate accounting for both within and between imputation variances.

4.7.2.5. Statistical Power to Examine Association Hypothesized in Specific Aim 2

4.7.2.4.1. Association between Single Genetic Variants and Ethanol Intake

In Specific Aim 2, we estimated the association between individual SNPs and weekly ethanol intake at ARIC visit 1-4. To estimate statistical power, preliminary estimates of effect allele frequency, beta estimate for the main effect of the SNPs, and mean (SD) for log-intake at each visit were inputted in the Quanto 1.2.4 [425] sample size software for gene association studies. Assuming an additive genetic model, a two-sided test, and a significance level of α=0.01/number of tested SNPs (African-Americans, 20; European-Americans, 11), this study has power ranging from 35%-94% to detect -0.12≤main genetic effect sizes≤0.17 log grams per week in African-American participants (Table 17). Among European-American participants, this study has power ranging from 50%-94% to detect -0.07≤main genetic effect sizes≤0.34 (Table 18).

4.7.2.4.2. Association between Unweighted Genetic Risk Score and Ethanol Intake

In Specific Aim 2, we also estimated the association between the unweighted GRS and weekly ethanol intake at ARIC visit 1-4 among ARIC African-American and European-American participants. To estimate statistical power, sample size (African-Americans, n=1733, European-Americans, n=7450),

percentage of variation in weekly ethanol intake explained by the unweighted GRS at each visit, and a significance level of α=0.05 were inputted in the 'AVENGEME' (Additive Variance Explained and Number of Genetic Effects) R software [426] (Tables 19 and 20). It was determined that this study has 99% power to detect an association between the unweighted genetic risk score and ethanol intake in African-Americans and European-Americans, separately.

4.7.2.4.3. Effect Modification of the Ethanol Intake-Cognitive Decline Association by GRS

In Specific Aim 2, we evaluated the effect modification of the ethanol intake-cognitive decline relationship by ethanol-associated SNPs. The MLPowSim Software Package [427] was used to generate R code that created simulations to estimate power to detect effect measure modification of the ethanol-intake cognitive decline by the GRS. We used the following assumptions from preliminary data analysis in estimating the power of the proposed analyses: sample size (African-Americans: N=1,733 and European-Americans: N=7,450); beta estimates for the model intercept, ethanol intake, GRS, and the GRS x ethanol intake interaction term (African-Americans: $\beta_{intercept}$=-0.829, $\beta_{Ethanol}$=0.008, β_{GRS}=-0.007, and $\beta_{Ethanol\ X\ GRS}$=-0.001, respectively; European-Americans:$\beta_{intercept}$=-0.728, $\beta_{Ethanol}$=-0.010, β_{GRS}=-0.001, and $\beta_{Ethanol\ X\ GRS}$=-0.0004, respectively), and mean (SD) estimates for ethanol intake, GRS, GRS x ethanol intake interaction term, and the mean square error (Table 21).

Assuming an additive genetic model, a two-sided test, and a significance level of α=0.05, it was determined that this study has 97% power to detect effect measure modification of the ethanol intake-cognitive decline relationship by a GRS of 11 ethanol intake-associated SNPs in European-Americans, and 83% power to detect effect measure modification by a GRS of 20 ethanol intake-associated SNPs among African-American participants.

4.8. Supporting Tables and Figures

Table 15. Summary of the covariates used in the analysis

Demographic factors	Age, sex, race, educational attainment
Lifestyle factors	Smoking status, physical activity, and diet
Genetic factors	*APOE*ε4 genotype
Medical History	Diabetes and stroke

Abbreviation: APOEε4, apolipoprotein epsilon 4.

Table 16. Parameters for power size calculation to estimate ethanol intake-cognitive decline association

Race Ethnicity/Drinking Contrast[+]	N	Difference in the 15-year rate of change in global z-score	Power
African-American			
Stable never drinking	529	Reference	
Stable low-to moderate drinking	197	0.03	99
Stable heavy drinking	19	0.08	94
Stable former drinking	225	-0.02	90
Mostly low-to moderate drinking	335	0.02	97
Mostly heavy drinking	60	0.15	95
Mostly former drinking	673	0.05	97
European-American			
Stable never drinking	1046	Reference	
Stable low-to moderate drinking	3136	0.02	95
Stable heavy drinking	185	-0.03	99
Stable former drinking	686	0.07	99
Mostly low-to moderate drinking	1068	0.02	99
Mostly heavy drinking	493	-0.05	99
Mostly former drinking	1602	0.03	99

[+]Referent group is stable never drinking; residual variance (African-Americans, 0.71; European-Americans, 0.50); and working correlation (African-Americans, 0.50; European-Americans, 0.12)

Abbreviation: N, sample size

Table 17. Power estimate for main genetic effect of ethanol intake-associated SNPs on weekly ethanol-intake at ARIC visits 1-4 for ARIC African-American participants

rsID	Effect Allele Frequency	Beta	Weekly Ethanol Intake	Power
rs6673687	0.359	-0.14	At visit 1	55
		-0.13	At visit 2	55
		-0.15	At visit 3	60
		-0.12	At visit 4	58
rs35608804	0.482	-0.14	At visit 2	65
rs7355953	0.058	-0.38	At visit 1	79
		-0.21	At visit 4	45
rs11940694	0.424	-0.12	At visit 1	45
		-0.14	At visit 3	56
rs10008281	0.293	0.16	At visit 1	62
		0.13	At visit 4	60
rs58440244	0.270	-0.14	At visit 1	49
		-0.11	At visit 4	44
rs78757076	0.242	-0.16	At visit 1	57
rs271085	0.304	0.17	At visit 1	68
		0.11	At visit 4	47
rs11768390	0.457	-0.14	At visit 2	65
		-0.12	At visit 4	61
rs10283354	0.096	-0.29	At visit 1	76
		-0.25	At visit 2	69
		-0.22	At visit 4	68
rs10840100	0.472	0.14	At visit 2	65
		0.15	At visit 3	63
rs1685404	0.245	-0.18	At visit 1	67
		-0.16	At visit 2	63
		-0.12	At visit 3	35
rs2514218	0.158	-0.26	At visit 1	84
		-0.18	At visit 4	69
rs1022084	0.496	0.12	At visit 2	52
		0.10	At visit 4	61
rs7940127	0.289	-0.14	At visit 1	51
		-0.11	At visit 4	46
rs12910841	0.096	0.32	At visit 2	88
		0.17	At visit 4	94
rs6496321	0.154	0.20	At visit 1	61
		0.22	At visit 4	85
rs4780836	0.355	0.14	At visit 2	61
rs62040427	0.306	-0.14	At visit 1	52
		-0.13	At visit 4	61
rs9929584	0.462	-0.15	At visit 2	71

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study

Log-ethanol intake, mean (SD): visit 1,1.06 (1.89); visit 2, 0.086 (1.76); visit 3, 0.99 (1.92); and visit 4, 0.66 (1.57)

Table 18. Power estimate for main genetic effect of ethanol intake-associated SNPs on weekly ethanol-intake at ARIC visits 1-4 for ARIC European-American participants

rsID	Effect Allele Frequency	Beta	Weekly Ethanol Intake	Power
rs1123285	0.338	-0.090	At visit 2	70
		-0.070	At visit 3	50
rs1229984	0.965	0.340	At visit 1	94
		0.240	At visit 2	72
		0.220	At visit 3	63
		0.260	At visit 4	79
rs12651313	0.441	-0.080	At visit 2	61
		-0.080	At visit 3	62
rs1713676	0.529	-0.070	At visit 1	50
		-0.080	At visit 2	63
		-0.080	At visit 4	64
rs2165670	0.104	0.120	At visit 3	55
rs55872084	0.232	0.110	At visit 1	74
		0.110	At visit 4	77
rs62250685	0.625	-0.080	At visit 2	60
		-0.110	At visit 3	86
rs7185555	0.141	-0.110	At visit 1	58
rs72859280	0.033	0.190	At visit 1	48
rs74664784	0.613	-0.070	At visit 2	50
		-0.100	At visit 3	79
rs7950166	0.635	-0.080	At visit 3	59

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study

Log-ethanol intake, mean (SD): visit 1,1.88 (2.17); visit 2, 1.63 (2.12); visit 3, 1.74 (2.14); and visit 4, 1.55(2.10)

Table 19. Power estimate for the association between unweighted genetic risk score (uGRS11) and weekly ethanol intake at ARIC visits 1-4 among ARIC African-American participants

Weekly Ethanol Intake	Beta	SE	P-value	Percent of variance explained (%)	Power
At visit 1	-0.045	0.016	0.006	0.41	99
At visit 2	-0.047	0.015	0.002	0.52	99
At visit 3	-0.037	0.017	0.029	0.28	99
At visit 4	-0.032	0.013	0.019	0.30	99

Abbreviations: ARIC, Atherosclerosis Risk in Communities (ARIC) Study; SE, Standard Error

Table 20. Power estimate for the association between unweighted genetic risk score (uGRS11) and weekly ethanol intake at ARIC visits 1-4 among ARIC European-American participants

Weekly Ethanol Intake	Beta	SE	P-value	Percent of variance explained (%)	Power
At visit 1	0.014	0.012	0.246	0.02	99
At visit 2	0.019	0.011	0.092	0.04	99
At visit 3	0.024	0.012	0.036	0.06	99
At visit 4	0.023	0.011	0.041	0.06	99

Abbreviations: ARIC, Atherosclerosis Risk in Communities (ARIC) Study; SE, Standard Error

Table 21. Power estimate for the GRS x ethanol intake effect on 15-year cognitive change

	African-American	European-American
Ethanol	4.16 (0.06)	4.17(0.02)
GRS	11.73 (0.15)	5.91 (0.04)
GRS x Ethanol	48.68 (0.89)	24.66 (0.21)
Mean Square Error	0.71	0.49

Abbreviation: GRS, genetic risk score

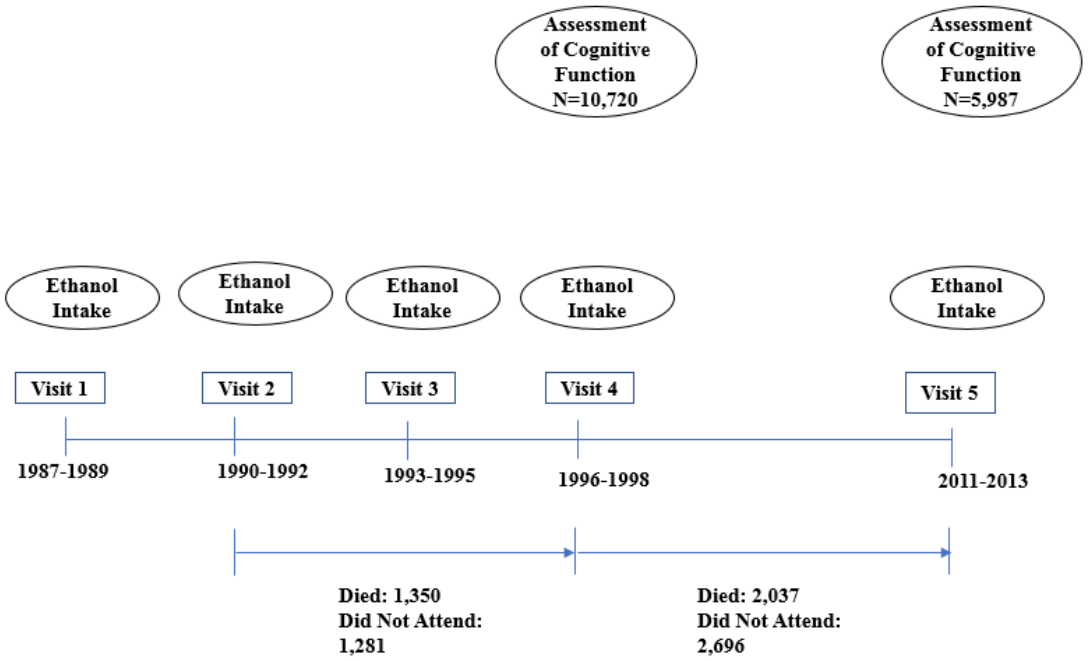


Figure 5. Timeline of the Atherosclerosis Risk in Communities (ARIC) Study, 1987-2013

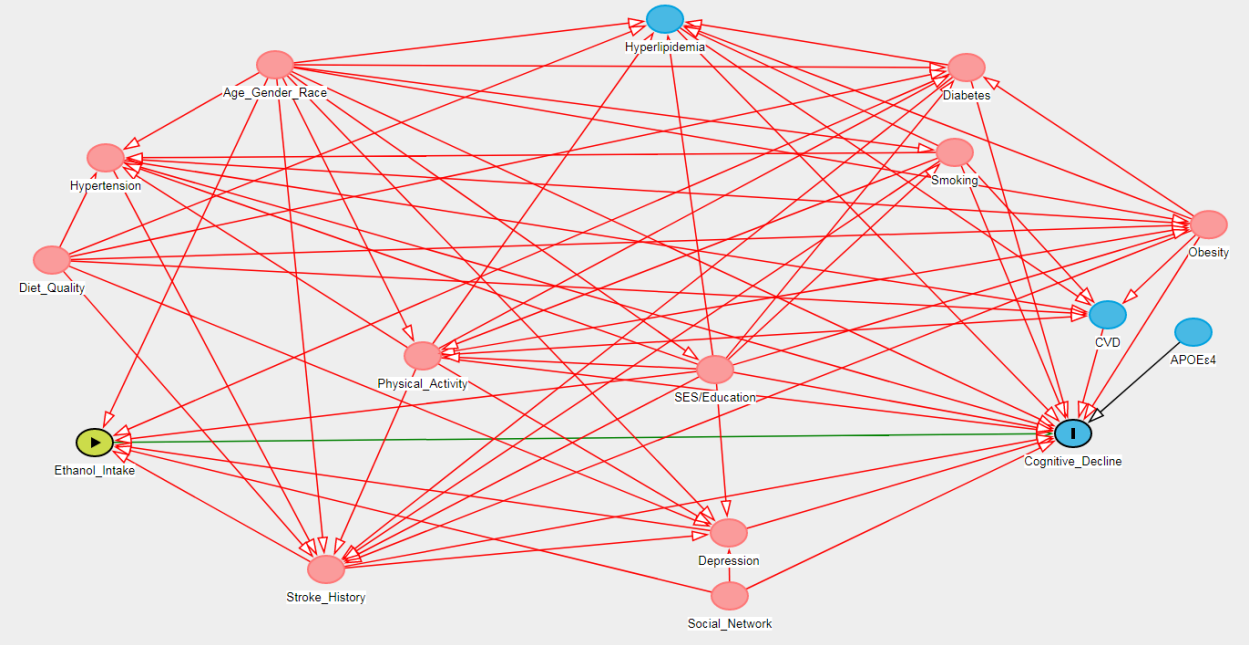


Figure 6. Direct acyclic graph (DAG) for confounders of the ethanol intake and cognitive decline relationship

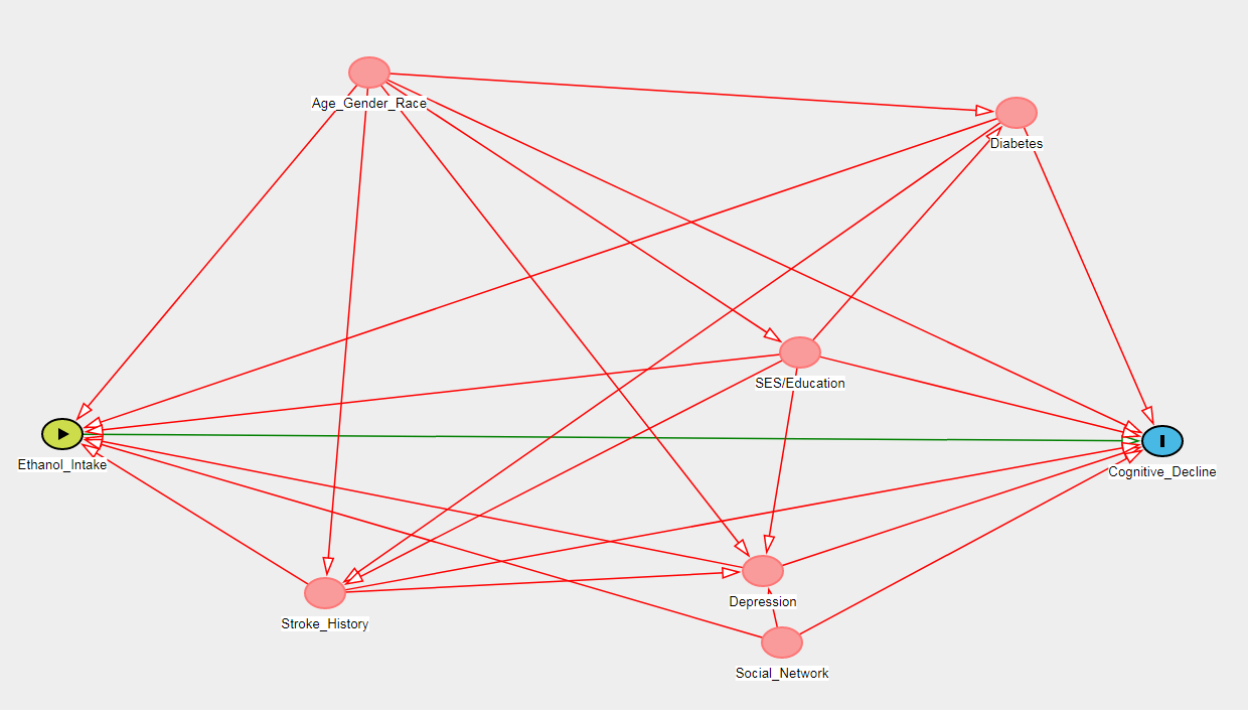


Figure 7. Direct acyclic graph (DAG) for the minimal sufficient adjustment set of confounders of the ethanol intake and cognitive decline relationship

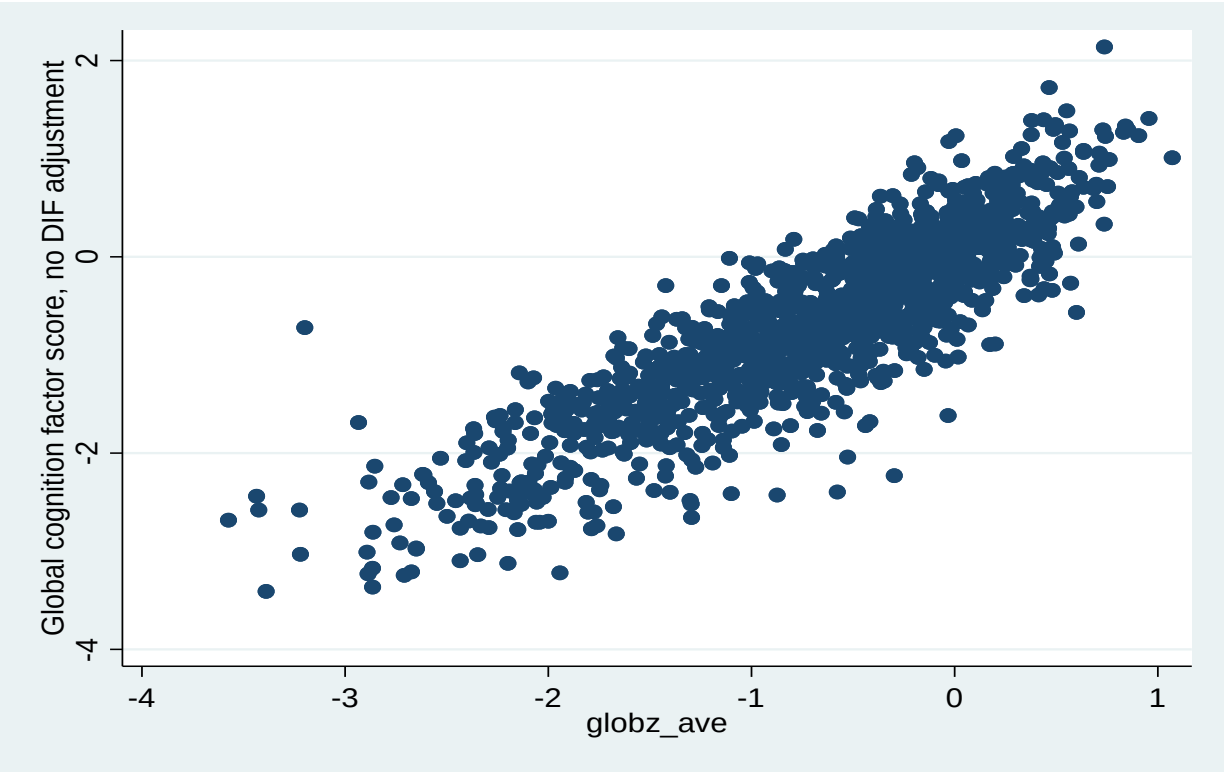


Figure 8.Validation of multiply imputed global Z score using existing data of multiply imputed global Z score using existing data

Note: Multiple imputation was done using chained equations, and 25 imputations were obtained and averaged for display in plot. 20% validation sample (N=1,247) to simulate missing completely at random (MCAR) data. All participants had a 0.2 probability of being selected. If selected, participants' Z scores at visit 5 were set to missing and imputed.

CHAPTER V: RESULTS

Manuscript A: Nine-year ethanol intake trajectories and their association with 15-year cognitive decline among African-American and European-American adults: The Atherosclerosis Risk in Communities Neurocognitive Study

1. Overview

Background: Faster rates of age-related cognitive decline may result in early onset of cognitive impairment and dementia. Ethanol use is highly prevalent (~70%) in the U.S., and although its relationship with cognitive decline has been extensively studied, it remains poorly understood. Previous studies used single measures of exposure to ethanol and few studies were conducted in diverse populations despite their disproportionate burden of Alzheimer's disease and other forms of cognitive impairment.

Objective: To assess the association of long-term trajectories of ethanol intake with 15-year rate of decline in cognitive function from mid-to-late life among African-American and European-American adults.

Methods: A total of 10,876 (n=2,169 African-Americans, n=8,707 European-Americans) participants of the Atherosclerosis Risk in Communities (ARIC) study completed repeated assessments of ethanol intake using an interviewer-administered questionnaire across a 9-year interval (1987-1998) and two neurocognitive examinations at 1996 and 2013. Multivariable linear regression was used to assess the association between long-term trajectories of ethanol intake and decline in z-score for general cognitive function. Multiple imputations by chained equations were used to account for attrition.

Results: Stable low-to-moderate drinking (African-Americans: (adjusted mean difference=0.03 (95% CI: -0.13, 0.19)), European-Americans: 0.02 (-0.05,0.08)), stable heavy drinking (African-Americans: 0.08 (-0.34, 0.50), European-Americans: -0.03 (-0.18, 0.11)), stable former drinking (African-

Americans: -0.02 (-0.18, 0.14), European-Americans: 0.07 (-0.02, 0.16)), mostly low-to-moderate drinking (African-Americans: 0.02 (-0.11, 0.16), European-Americans: 0.02 (-0.06, 0.10)), mostly heavy drinking (African-Americans: 0.15 (-0.10, 0.41), European-Americans: -0.05 (-0.15, 0.05)), and mostly former drinking (African-Americans: 0.05 (-0.07, 0.16), European-Americans: 0.03 (-0.04, 0.10)) in mid-life compared to stable never drinking were not associated with 15-year decline in general cognitive performance from mid-to-late life, after adjustment for attrition. Declines in cognitive performance were similar for long-term trajectories of ethanol intake and ethanol intake at study baseline.

Conclusions: Stable low-to-moderate and stable heavy drinking in mid-life are not associated with lesser and greater cognitive decline, respectively, from mid-to-late life among African-American and European-American adults.

2. Introduction

Cognitive decline refers to the decrease in mental processes, such as attention, short-term and long-term memory, reasoning, movement coordination, and planning of tasks, which are important for the conduct of daily living activities [10, 11]. Neurobiological and cognitive performance studies suggest that declines in cognitive function are gradual and begin in early adulthood [12-14]. Faster rates of cognitive decline may lead to earlier onset of cognitive impairment and dementia, which may result in significant burden for those experiencing decline and their caregivers [39]. By 2050, it is projected that the number of Americans aged 65 years and older will triple, and the U.S. will become more racially and ethnically diverse [1]. Racial ethnic disparities in dementia prevalence and incidence have been documented. Studies indicate that African-Americans and other racial minority groups are disproportionately burdened with Alzheimer's disease (AD), the most common form of dementia, and other forms of cognitive impairment compared to European-Americans [428-431]. To reduce the incidence of cognitive impairment and dementia, current research has focused on identifying modifiable lifestyle risk factors that can prevent or delay the progression of cognitive decline.

The relationship between ethanol intake and cognitive decline [32, 93, 336-348] has been previously studied but remains poorly understood due to inconsistent findings. While heavy ethanol

intake is associated with greater cognitive decline [348], low-to-moderate ethanol intake has been found associated with less cognitive decline [336, 339, 340, 342-344, 346, 347] or no cognitive decline [93, 345, 348]. Inconsistent findings may be attributable to the use of a single measurement of ethanol intake [93, 336, 339-341, 344-347] and short follow-up times (<5 years). A limited number of studies have investigated the effects of ethanol in African-Americans populations even though the prevalence, incidence, and cumulative risk of Alzheimer's Dementia (AD) is documented to be higher in African-Americans than in European-Americans [343, 347]. Furthermore, few studies investigated the effects of mid-life ethanol intake with late-life cognition [341, 348].

Studies that used a single measure of ethanol intake at study baseline to define the drinking behavior of participants assume that drinking behavior is static thereafter. However, individuals' drinking habits change over time [432, 433], which can affect their risk of developing disease [434, 435]. Therefore, not accounting for long-term drinking pattern or changes in ethanol intake can introduce bias in the study [436-438].

Using a repeat assessment of ethanol intake over 9 years and repeat measurements of global and multidimensional cognitive function over 15 years, our aims were 1) characterize temporal trajectories of ethanol intake during mid-life in African-American and European-American adults, 2) examine whether long-term trajectories of ethanol intake in mid-life are associated with 15-year rate of decline in cognition from mid-to-late life among African-American and European-American adults, and 3) examine if short-term ethanol intake measured in mid-life show comparable associations with 15-year cognitive decline.

3.Methods

Study Population

The Atherosclerosis Risk in Communities (ARIC) study is a community-based, prospective cohort study established in 1987, designed to investigate the etiology of atherosclerosis and its clinical sequelae. From 1987 through 1989, 15,792 adults aged 45 to 64 years were recruited through probability sampling from 4 U.S. communities: Washington County, Maryland; Forsyth County, North Carolina; suburbs of Minneapolis, Minnesota; and Jackson, Mississippi. Participants were seen at 4 study visits

approximately 3 years apart from 1987-1989 through 1996-1998, and a fifth examination visit was conducted in 2011-2013 (Figure 9).

The baseline for the present analysis was visit 4, which allows for the investigation of the association of trajectories of ethanol intake across 9 years in mid-life and subsequent 15-year cognitive decline from mid-to-late life (Figure 9). Of the 11,625 African-American and European-American participants who attended visit 4, we excluded African-Americans from Minnesota and Washington County due to small sample size (n=38), those who were missing one or more cognitive function tests at study baseline (n=625), and those with missing covariates (n=86), giving a final sample size of 10,876 participants at study baseline.

Assessment of Ethanol Intake

Ethanol intake was assessed at all visits by means of an interviewer-administered questionnaire [169]. During the exam, participants were asked the following questions: Do you presently drink alcoholic beverages?", and "Have you ever consumed alcoholic beverages?". Individuals replying no to both questions were classified as never drinkers. Those who replied "no" to the first question and "yes" to the second question were classified as 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. History of excessive ethanol intake (yes/no) was only assessed during ARIC visit 3 using the following question: "Was there ever a time in your life when you consumed 5 or more drinks of any kind of alcoholic beverage almost every day?". We used this variable only for further description of the study sample. Categories of ethanol intake at each visit were created based on the U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2015-2020 guideline for low-to-moderate drinking (≤210 grams/week for men and ≤105 grams/week for women) and heavy drinking (> 210 grams/week for men and > 105 grams/week for women) [384]. Utilizing ethanol intake

categories across visits 1-4, trajectories of ethanol intake were then classified as 1) stable never drinkers, 2) stable low-to-moderate drinkers, 3) stable heavy drinkers, 4) stable former drinkers, 5) mostly low-to-moderate drinkers, 6) mostly heavy drinkers, and 7) mostly former drinkers. In creating the 'mostly' ethanol intake trajectories, ethanol intake status at study baseline (visit 4) was taken in account. Participants with non-current drinking status (i.e., never or former) across visits 1-3, but reported current drinking at visit 4 (i.e., low-to-moderate or heavy) were assigned to the "mostly" ethanol intake category at visit 4 (i.e., low-to-moderate or heavy). Participants with current drinking status (i.e., low-to-moderate or heavy) across visits 1-3 but reported former drinking at visit 4 were assigned to the "mostly" former long-term pattern of ethanol intake. Participants who reported 2 visits of current drinking (i.e., low-to-moderate or heavy) and 2 periods of former drinking were assigned to the "mostly" ethanol intake category at visit 4 (i.e., former, low-to-moderate or heavy).

Definition and counts for this long-term categorization are presented in Table 1. Average ethanol intake across 9-years in mid-life was calculated for each participant by averaging weekly ethanol intake reported in ARIC visits 1-4.

Assessment of Cognitive Function

Cognitive function was assessed at visit 2 (1990-1992; ages 48-67), visit 4 (1996-1998; ages 54-73), and visit 5 (as part of the ARIC-NCS) (2011-2013; ages 70-89 years) using 3 standardized cognitive tests to assess different domains of cognition: verbal learning and short-term memory, executive function and processing speed, and executive function and expressive language.

Verbal learning and recent memory were assessed by the delayed word recall test (DWRT). Participants were asked to learn 10 nouns, and after a five-minute delay were given 60 seconds to recall the words. The DWRT score is the number of words recalled (0-10) [201]. DWRT has a high test-retest reliability of 0.75 in older adults [201].

Executive function and processing speed were assessed by the digit symbol substitution test (DSST). Participants were given 90 seconds to fill in blank squares with symbols corresponding to digits

from 1 to 9 using a key that matches digits to symbols [209]. DSST has high reliability (0.82-0.88) in older adults [209].

Executive function and expressive language were assessed by the word fluency test (WFT) , during which participants generate as many words starting with the letters F, A, and S as possible within 60 seconds, with one trial per letter [385]. The WFT score is the total number of acceptable words generated for the three letters [205]. WFT has a test-retest reliability of 0.88 in older adults [206]. All three tests were administered by trained examiners using standardized protocols in a quiet room. Recordings were reviewed for quality control.

To facilitate comparison across cognitive tests, Z scores standardized to visit 2 were calculated for each test by subtracting the participant's overall mean test score at visit 2 from their test score at each visit and then dividing by the standard deviation of the visit 2 scores.

A factor score for general cognitive performance was previously derived using factor analysis [386, 387], and was scaled to have a mean of 50 and standard deviation of 10 at the 1990–92 visit [387].

Covariates

Potential confounders were identified from the existing literature and the use of directed acyclic graphs (DAGs). Potential confounders included: demographic characteristics (age, sex, race-center, and educational attainment), lifestyle factors (smoking status, physical activity, and diet score), genetic risk factor (*APOE* ε4 genotype) and medical history (obesity, diabetes and history of stroke).

Age, sex, and educational attainment (< high school, high school, >high school), and smoking status (current, former, never) were assessed at visit 4 via self-report from the home interviews. Time spent in moderate to vigorous physical activity in MET-minutes/week was measured at visits 1 and 3 using the modified Baecke questionnaire [390]. APOE ε4 (0,1,2) was genotyped by TaqMan assay (Applied Biosystems, Foster City, California) [393, 394]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Diabetes (yes, no) was defined as self-reported history of a physician's diagnosis of diabetes, fasting blood glucose level of ≥126 mg/dL, or non-fasting blood glucose level of

≥200 mg/dL, or diabetes medication use in the past 2 weeks. Stroke was defined by a self-reported history at visit one or an adjudicated event between visits 1 and 4 [396]. Dietary factors were assessed using an interviewer-administered 66-item FFQ measuring usual intake of foods over the past year at visits 1 (1987–1989) and visit 3 (1993–1995). We calculated the Healthy Food Score, adapted from Steffen et al. described elsewhere [391, 392].

Statistical Analysis

Multiple Imputation

Missing data due to attrition were imputed by multiple imputation using chained equations (MICE)[401]. Missing ethanol intake and cognitive data across ARIC visits were imputed based on the observed values of key covariates for a given individual, as well as the relations observed in the data for other participants. To account for the uncertainty of the imputation and ensure correct standard error estimation [402], 25 datasets were imputed. Validation of the MICE approach for cognitive outcome in ARIC has been previously reported and it has been determined that MICE produced unbiased imputed values [403]. For this study, validation using observed data demonstrated MICE produces unbiased imputation of global cognition factor z-scores (Appendix Figure 1).

Statistical Modeling

To evaluate change in general cognitive performance, DSST, DWRT, and WFT tests between visits 4 and 5, multivariable linear regression models were used with the outcome being visit 5 z-score minus visit 4 z-score. All models were race-stratified and were adjusted for age, age squared, sex race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity, and the Apo lipoprotein E ε4 (APOE ε4) allele.

Statistical analyses (including multiple imputation) were performed with Stata15 (StataCorp, College Station, TX, USA) [423] and SAS version 9.4 (SAS Institute, Cary, NC, USA) . The results from each imputed dataset were summarized using Rubin's rule [424] into an overall estimate accounting for both within and between imputation variances.

Sensitivity analyses were undertaken to examine whether complete case analyses produced comparable results.

4. Results

Temporal trajectories of ethanol intake in study population

The trajectories of ethanol during mid-life observed in our study sample were stable never drinking (13.8%), stable low-to-moderate drinking (30.1%), stable heavy drinking (1.8%), stable former drinking (7.9%), mostly low-to-moderate drinking (12.9%), mostly heavy drinking (5.2%), and mostly former drinking (20.7%) (Table 22).

African-American participants had a higher proportion of never drinkers (stable never drinking, 21.8%), stable former drinking (8.8%), mostly low-to-moderate drinking (14.5%), and mostly former drinking (25.9%) than European-American participants (stable never drinking, 11.9%; stable former drinking, 7.6%; mostly low-to-moderate drinking, 12.5%; and mostly former drinking, 19.4%). European-American participants had a higher prevalence of stable low-to-moderate (35.5%), stable heavy drinking (2.0%), and mostly heavy drinking (5.9%) than African-American participants (stable low-to-moderate drinking, 8.2%; stable heavy drinking, 0.7%; and mostly heavy drinking, 2.4%). Overall, 7.7% of our study population long term ethanol intake could not be classified as stable never drinking, stable low-to-moderate drinking, stable heavy drinking, stable former drinking, mostly low-to-moderate drinking, mostly heavy drinking, and mostly former drinking, or any other ethanol intake category used in ethanol research literature.

Description of Baseline Characteristics

The socio-demographic and clinical characteristics of the 10,876 African-American and European-American participants at study baseline are presented in Tables 23 and 24, respectively, by ethanol intake trajectories. The mean age of participants at study baseline was 63 years (range:52-75 years), 56% were female, and 20% were African-Americans. Compared to European-Americans, African-Americans were less educated, had a higher proportion of currents smokers (16.6% vs 13.9%), higher prevalence of diabetes (25.4% vs. 13.6%) and stroke (3.4% vs. 2.5%), and lower baseline cognitive tests

scores. History of excessive drinking was more prevalent among stable former drinkers (African-Americans, 17.8%; European-Americans, 24.7%) ,stable heavy drinkers (African-Americans, 46.7%; European-Americans, 32.4%), and mostly heavy drinkers (African-Americans, 24.5%; European-Americans, 15.1%) than stable low-to-moderate drinkers (African-Americans, 7.3%; European-Americans, 3.1%), mostly low-to-moderate drinkers (African-Americans, 9.6%; European-Americans,10.3%), and mostly former drinkers (African-Americans, 8.0%; European-Americans, 6.0%).

Stable never drinkers, regardless of race, were most likely to be never smokers (African-Americans, 80.3%; European-Americans, 80.1%), had slightly higher prevalence of hypertension in European-Americans only (36.5%), and had lower mean levels of weekly physical activity **(**African-Americans, 7.1 (10.9); European-Americans, 8.9 (10.6)). Stable heavy drinkers (African-Americans, 66.7%; European-Americans, 30.1%) and mostly heavy drinkers (African-Americans, 58.5%; European-Americans, 26.8%) had the highest proportion of current smokers. Stable heavy drinkers had a higher prevalence of hypertension (66.7%) and stroke (6.7%) in African-Americans only. Whereas, stable former drinkers overall had the highest prevalence of diabetes (African-Americans, 22.6%; European-Americans 21.4%), and mostly former drinkers had a higher prevalence of stroke in European-Americans only (3.6%).

Higher baseline general cognitive performance, DSST, and WFT scores were observed for stable low-to-moderate drinkers, mostly low-to-moderate drinkers, stable heavy drinkers, and mostly never drinkers compared to stable never drinkers. Baseline scores for DWRT did not differ across categories of long-term ethanol intake.

Nine-year ethanol drinking trajectories and 15-year cognitive decline

Results from the multivariable linear regression models suggest no overall association between 9-year trajectories of ethanol intake in mid-life and 15-year change in general cognitive performance, DWRT, WFT and DSST z-scores (Tables 25 and 26, and Figure 10).

Among African-American participants, stable low-to-moderate drinkers (Adjusted 15-year decline: -0.61 (95% CI: -1.03, -0.20)), stable heavy drinkers (-0.57 (-1.14, 0.00)), mostly low-to-

moderate drinkers (-0.62 (-1.03, -0.21)), mostly heavy drinkers (-0.49 (-0.95, -0.03)), and mostly former drinkers (-0.60 (-1.00, -0.19)) had nominally lower 15-year decline in general cognitive performance z-scores than stable never drinkers (-0.64 (-1.05, -0.24)), equivalent to 5%, 12%, 4%, 24%, and 7% lesser decline, respectively. However, slightly greater 15-year decline in general cognitive performance were observed for stable former drinkers (-0.67 (-1.08, -0.25)) than stable never drinkers (-0.64 (-1.05, -0.24)), equivalent to 3% greater decline (Table 25).

Among European-American participants, 15-year decline in general cognitive performance for stable low-to-moderate drinkers (-0.84 (-1.08, -0.60)), stable former drinkers (-0.78 (-1.03, -0.54)), mostly low-to-moderate drinkers (-0.84 (-1.08, -0.59)), and mostly former drinkers (-0.83 (-1.07, -0.58)) had nominally lower 15-year decline in general cognitive performance than stable never drinkers (-0.86 (-1.10, -0.62)) , equivalent to 2%, 8%, 2%, and 4% lesser decline, respectively. However, among European-American participants, slightly greater 15-year decline in general cognitive performance were observed for stable heavy drinkers (-0.89 (-1.18, -0.60)) and mostly heavy drinkers (-0.90 (-1.15, -0.66)) than stable never drinkers (-0.86 (-1.10, -0.62)), equivalent to 4% and 5% greater decline, respectively. In addition, European-American participants who were mostly heavy drinkers (-0.89 (-1.18, -0.61)) had a greater 15-year decline in DSST z-scores than European-American who were stable never drinkers (-0.78 (-1.06, -0.51)), equivalent to 14% greater decline (Table 26).

Average ethanol intake across nine-years and 15-year cognitive decline

We observed no overall association between average ethanol intake across 9 years in mid-life and 15-year change in general cognitive performance, DWRT, DSST, and WFT z-scores from mid-to-late life among African-American (Table 27) and European-American (Table 28) participants. There was no evidence of trends of increased 15-year change in general cognitive performance, DWRT, WFT and DSST across quartiles of average ethanol intake across nine-years, among African-American and European-American participants (Tables 27 and 28) (Appendix Figure 2).

Ethanol intake measured at study baseline and 15-year cognitive change

We also determined whether ethanol intake levels measured at study baseline (visit 4) showed similar trajectories of associations with 15-year change in general cognitive performance, DWRT, WFT, and DSST z-scores. Findings based on ethanol intake levels at study baseline were similar to those observed using 9-year ethanol drinking trajectories for African-American and European-American participants (Tables 29 and 30) (Appendix Figure 3).

Among African-American participants, we observed no overall association between ethanol intake measured at study baseline and 15-year change in general cognitive performance (P= 0.697), DWRT (P=0.814), WFT (P= 0.609), and DSST z-scores (P= 0.614) (Table 29).

Among European-American participants, we observed no overall association between ethanol intake and 15-year change in general cognitive performance (P=0.072), DWRT (P=0.177), and WFT z-scores (P=0.323) (Table 30). However, an association was observed between ethanol intake measured at study baseline and 15-year change in DSST z-scores (P=0.026). The difference in 15-year change in DSST z-scores among heavy drinkers than never drinkers at study baseline was -0.10 (-0.19, -0.02), equivalent to 13% greater decline. Declines in cognitive performance were slighter higher for long-term trajectories of ethanol intake than ethanol intake at study baseline.

Sensitivity analysis

In sensitivity analysis, we conducted a complete case analyses of the long-term trajectories of ethanol intake and ethanol intake status at study baseline (Appendix Tables 10-15).

Nine-year ethanol drinking trajectories and 15-year cognitive decline

Among African-America participants, we observed no association between 9-year ethanol drinking trajectories and 15-year change in general cognitive performance z-score (P=0.314), DWRT z-score (P=0.132), WFT z-score (P=0.063), and DSST z-score (P=0.847) (Appendix Table 10). However, differences in 15-year change in general cognitive performance (0.46 (0.04, 0.88)) and DWRT z-score (1.14 (0.19, 2.08)) were observed for stable heavy drinkers and stable never drinkers, equivalent to 34%.and 57% lesser decline, respectively. Differences in 15-year change in WFT z-score were also

observed between stable former drinkers compared stable never drinkers (0.23 (0.05, 0.42)) and between mostly low-to-moderate drinkers and stable never drinkers (0.20 (0.05, 0.35), equivalent to 42% and 36% lesser decline, respectively (Appendix Table 10).

Among European-American participants, we observed an association between 9-year ethanol drinking and 15-year change in DWRT z-score (P=0.032) (Appendix Table 11). Differences in 15-year change in DSST z-score were observed for mostly low-to-moderate drinking and stable never drinking (-0.07 (-0.14, -0.01)) and mostly heavy drinking and stable never drinking (-0.10 (-0.18, -0.02)), equivalent to 7% and 10% greater decline, respectively (Appendix Table 11).

Average ethanol intake across nine-years and 15-year cognitive decline

Among African-Americans and European-Americans participants, we observed no association between average ethanol intake across 9-years in mid-life and general cognitive performance z-score (African-Americans: P=0.812, European-Americans: P=0.089), DWRT z-score (African-Americans: P=0.644, European-Americans: P=0.292), WFT z-score (African-Americans: P=0.904, European-Americans: P=0.406), and DSST z-score (African-Americans: P=0.567, European-Americans: P=0.384) (Appendix Tables 12 and 13). However, among European-American participants, we observed that participants in the highest quartile of cumulative average ethanol intake (-1.25 (-1.54, -0.96)) had greater 15-year change in general cognitive performance than participants in the lowest quartile of cumulative average ethanol intake (-1.18 (-1,47, -0.89)) (Appendix Table 13).

Ethanol intake measured at study baseline and 15-year cognitive decline

Among African-American participants, we observed no association between ethanol intake reported at baseline and 15-year cognitive performance change z-score (P=0.052), DWRT z-score (P=0.478) z-score, WFT z-score (P=0.084), and DSST z-score (P=0.529) (Appendix Table 14). However, differences in 15-year change in general cognitive performance were observed between low-to-moderate drinkers and never drinkers (0.12 (0.02, 0.22)) and between former drinkers and never drinkers (0.10 (0.01, 0.19)), equivalent to 10% and 8% lesser decline, respectively. Similarly, differences in 15-year change in WFT z-score was observed between low-to-moderate drinkers and never drinkers ((0.13 (0.01,

0.26)) and between former drinkers and never drinkers (0.13 (0.02, 0.24)), equivalent to 22% and 21% lesser decline, respectively (Appendix Table 14).

Among European-American participants, we observed associations between ethanol intake reported at study baseline and 15-year change in DWRT z-score (P=0.007) and DSST z-score (P=0.016) (Appendix Table 15). The difference in 15-year change in DWRT z-score for heavy drinkers and never drinkers was -0.19 (-0.36, -0.02)), equivalent to 11% greater decline. The difference in the 15-year change in DSST z-score for low-to-moderate drinks and never drinkers was -0.06 (-0.11, -0.01), equivalent to 6% greater decline (Appendix Table 15). The difference in the 15-year change in DSST z-score for heavy drinkers and former drinkers was -0.11 (-0.18, -0.04), equivalent to 11% greater decline (Appendix Table 15). Imputation produced smaller estimates of 15-year change in cognitive performance compared to complete case analyses, although confidence intervals overlapped.

5. Discussion

This study, conducted in a community cohort, found no evidence that stable low-to-moderate drinking and mostly low-to-moderate drinking in mid-life are associated with lesser 15-year cognitive decline from mid-to-late life compared to stable never drinking, after adjustment for attrition. There was no evidence that stable heavy drinking, mostly heavy drinking, stable former drinking, and mostly former drinking are associated with greater 15-year cognitive decline from mid-to-late life, after adjustment for attrition. However, the 15-year change in digit symbol substitution test (a test of executive function and processing speed) for mostly heavy drinkers was slightly higher that stable never drinkers, equivalent to a 14% greater decline. No association was found for ethanol intake averaged across 9 years during mid-life and 15-year cognitive decline from mid-to-late life. Further, we did not observe an association with ethanol intake at baseline and 15-year cognitive decline, except for digit symbol substitution test in European-American participants, after adjustment for attrition. The 15-year rate of change for heavy drinkers was slightly higher than never drinkers at study baseline, equivalent to a 13% greater decline. In African-Americans and European-Americans, we observed similar declines in cognitive performance for

stable drinking categories and drinking categories measured at study baseline. Overall, a slightly lesser rate of decline was observed among African-Americans.

Low-to-moderate ethanol intake is hypothetically associated with cognitive decline through cerebrovascular and cardiovascular pathways, involving effects that play out over an extended period of time [324, 439]. Heavy ethanol intake on the other hand has detrimental short- and long-term effects on the brain [440, 441], including direct neurotoxic effect [441], proinflammatory effects [441, 442], and indirect impact via cerebrovascular disease [327] and vitamin deficiency [443].

Cross-sectional studies finding on the relationship between ethanol intake and cognitive function have been mixed, with several suggesting a protective effect of moderate ethanol intake on cognitive function [444-446] . However, cross-sectional studies findings are inconclusive due to major concerns of reverse causation and their susceptibility to selection bias and residual confounding. A cross-sectional analysis of the association of ethanol intake with MRI-defined cerebral abnormalities conducted in the Atherosclerosis Risk in Community (ARIC) study reported no significant neuroprotective effect of low-to-moderate ethanol intake on white matter grade in middle-aged adults [447].

Prospective studies finding on the relationship between low-to-moderate ethanol intake and cognitive decline have been inconsistent [32, 93, 336-348], potentially due to their single measurement of ethanol intake [93, 336, 339-341, 344-347] and short follow-up times (<5 years). Our study, by focusing on the association of long-term trajectories of ethanol intake with cognitive decline adds to literature because it is unclear whether long-term ethanol intake influences cognitive decline.

Our observation that stable low-to-moderate drinking and mostly low-to-,moderate drinking during mid-life is not associated with lesser 15-year decline in general cognitive performance from mid-to-late life compared to stable never drinking is consistent with the findings of a recent Whitehall ll Cohort Study of 10,308 white European participants ages 44-69 years, which examined the relationship between ethanol intake (3 repeated measurements) averaged across 10 years in mid-life and subsequent 10-year cognitive decline [348]. The authors found that moderate drinking was not associated with lesser decline in 10-year decline in global cognitive score among men and women. Our study finding of no

association between stable heavy drinking with greater 15-year decline in global cognitive performance differed from the Whitehall II study, which found heavy drinking is associated with greater 10-year cognitive decline in men only. However, in our study, we found an association between mostly heavy drinking and greater 15-year cognitive change in digit symbol substitution test in European-Americans only.

Several limitations of this study should be mentioned. Our study findings of a lack of association between ethanol intake and cognitive decline in general cognitive performance may be the result of error in the measurement of ethanol intake that may have attenuated the effect estimates; the use of a standardized instrument administered by trained personnel and the availability of repeat measurements mitigates this concern. Cohort attrition over the prolonged follow up could have biased an association toward the null, if differentially related to ethanol intake. No clear pattern of association of ethanol intake with attrition was observed, and sensitivity analysis indicated that missing data patterns were effectively corrected by MICE imputation. The low prevalence of heavy drinking in our study population limited our ability to estimate the impact of heaving drinking on cognitive performance over time. Lastly, although community-based our results emerge from 4 geographically defined, closed cohorts and may not widely generalize to other populations.

Strengths of this study include the large population-based probability sample of middle-aged African Americans and European-Americans, a prospective design with 15 years of follow-up with repeated measurements of ethanol intake and well-characterized cognitive function. Ethanol intake was assessed using an instrument with beverage-specific questions (thus reducing under-reporting) that differentiated never from former drinkers. Additionally, we had rich covariate data that allowed adjustment of lifestyle, genetic, and clinical risk factors.

6. Conclusion

The results from this study suggest that stable low-to-moderate drinking and stable heavy drinking in mid-life are not associated with cognitive decline from mid-to late-life among African-American and European-American adults. Our findings are consistent with previous studies finding

demonstrating that moderate ethanol intake may not be protective of cognitive decline. Therefore, low-to-moderate drinking should not be recommended for slowing cognitive aging

.

7. Main Tables and Figures

Table 22. Long-term ethanol intake at study baseline with observed counts and percentage by race and overall†

		African-Americans	European-Americans	Overall
		N (%)	N (%)	N (%)
Long-term Ethanol Intake	Weekly Ethanol Intake	2169 (19.9)	8707 (80.1)	10876 (100.0)
Stable never	0 g/wk. at each visit	472 (21.8)	1033 (11.9)	1505 (13.8)
Stable low-to-moderate	≤210 g/wk. for men and ≤105 g/wk. for women at each visit	178 (8.2)	3095 (35.5)	3273 (30.1)
Stable heavy	>210 g/wk. for men and >105 g/wk. for women at each visit	15 (0.7)	176 (2.0)	191 (1.8)
Stable former	Classified as former drinker at each visit; visits 1-4	191 (8.8)	664 (7.6)	855 (7.9)
Mostly low-to-moderate	Majority of visits were low-to-moderate ethanol drinking	314 (14.5)	1091 (12.5)	1405 (12.9)
Mostly heavy	Majority of visits were heavy ethanol drinking	53 (2.4)	511 (5.9)	564 (5.2)
Mostly former	Majority of visits were former ethanol drinking	561 (25.9)	1687 (19.4)	2248 (20.7)
Unclassified‡		385 (17.7)	450 (5.2)	835 (7.7)

† Counts and percentage were calculated based on data prior to using multiple imputation by chains equation to impute missing weekly ethanol intake data for Atherosclerosis Risk in Communities (ARIC) Study visits 1-4.

Abbreviation: g/wk., grams per week.

‡Unclassified, study participants' long-term ethanol intake could not be classified as stable never drinking, stable low-to-moderate drinking, stable heavy drinking, stable former drinking, mostly low-to-moderate drinking, mostly heavy drinking, and mostly former drinking, or any well-established drinking category found in published literature.

Table 23. Baseline characteristics of Atherosclerosis Risk in Communities (ARIC) study African-American participants by 9-year ethanol drinking trajectories, 1987-1996 (N=2169) †

	Stable	Stable	Stable	Stable	Mostly	Mostly	Mostly		
	Never	Low-to-moderate	Heavy	Former	Low-to moderate	Heavy	Former	Unclassified ‡	Overall
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Characteristics	472 (21.8)	178 (8.2)	N=15 (0.2)	N=191 (8.8)	314 (14.5)	53 (2.4)	561 (25.9)	385 (17.7)	2169 (19.9)
Age (years), mean (SD)	62.8 (5.6)	60.1 (5.5)	60.3 (5.0)	62.8 (5.7)	60.2 (5.2)	60 (4.6)	61.6 (5.8)	62.4 (5.7)	61.7 (5.7)
Female, n (%)	411 (87.1)	72 (40.4)	6 (40.0)	97 (50.8)	159 (50.6)	21 (39.6)	355 (63.3)	304 (79.0)	1425 (65.7)
Study center, n (%)									
Forsyth County, NC	42 (8.9)	35 (19.7)	4 (26.7)	34 (17.8)	20 (6.4)	2 (3.8)	71 (12.7)	32 (8.3)	240 (11.1)
Jackson, MS	430 (91.1)	143 (80.3)	11 (73.3)	157 (82.2)	294 (93.6)	51 (96.2)	490 (87.3)	353 (91.7)	1929 (88.9)
Education, n (%)									
< high school	156 (33.1)	27 (15.2)	4 (26.7)	86 (45.0)	75 (23.9)	18 (34.0)	191 (34.0)	152 (39.5)	709 (32.7)
High school or vocational school	159 (33.7)	45 (25.3)	6 (40.0)	51 (26.7)	87 (27.7)	17 (32.1)	167 (29.8)	115 (29.9)	647 (29.8)
College or higher	157 (33.3)	106 (59.6)	5 (33.3)	54 (28.3)	152 (48.4)	18 (34.0)	203 (36.2)	118 (30.6)	813 (37.5)
Smoking, n (%)									
Never Smokers	379 (80.3)	65 (36.5)	1 (6.7)	46 (24.1)	103 (32.8)	10 (18.9)	203 (36.2)	220 (57.1)	1027 (47.3)
Former Smokers	67 (14.2)	66 (37.1)	4 (26.7)	114 (59.7)	126 (40.1)	12 (22.6)	268 (47.8)	125 (32.5)	782 (36.1)
Current Smokers	26 (5.5)	47 (26.4)	10 (66.7)	31 (16.2)	85 (27.1)	31 (58.5)	90 (16.0)	40 (10.4)	360 (16.6)
Body Mass Index (kg/m2), mean (SD)	31.6 (6.4)	28.8 (5.4)	26.4 (5.7)	31 (6.5)	29.7 (5.3)	27.4 (5.9)	30.7 (6.3)	31.5 (6.9)	30.7 (6.3)
Diabetes, n (%)	302 (20.1)	330 (10.1)	20 (10.5)	193 (22.6)	209 (14.9)	52 (9.2)	436 (19.4)	195 (23.4)	550 (25.4)
Hypertension, n (%)	288 (61.0)	91 (51.1)	10 (66.7)	113 (59.2)	150 (47.8)	31 (58.5)	314 (56.0)	245 (63.6)	1242 (57.3)
History of Stroke, n (%)	16 (3.4)	4 (2.2)	1 (6.7)	9 (4.7)	10 (3.2)	2 (3.8)	24 (4.3)	8 (2.1)	74 (3.4)
Diet score, mean (SD)	21.2 (5.3)	21.4 (4.7)	19 (5.8)	20.5 (5.4)	20.6 (6.3)	19.2 (6.7)	19.4 (6.2)	19.4 (6.9)	20.2 (6.0)
Physical activity (met-min/week), mean (SD)	7.1 (10.9)	12.2 (14.4)	9.2 (20.5)	9.2 (14.6)	10 (13.3)	9.4 (12.4)	8.3 (12.0)	7.2 (10.4)	8.5 (12.3)
APOEε4 allele present, n (%)	195 (41.3)	66 (37.1)	5 (33.3)	79 (41.4)	122 (38.9)	23 (43.4)	220 (39.2)	150 (39)	860 (39.6)
Ethanol Intake, median (25th-75th percentile)									
Frequency		12 (5-19)	97 (78-157)	8 (4-23)	8.5 (2-31)	52 (32-69)	2 (0-10)	0 (0-1)	1 (0-11)
Grams/week		162.3 (58.3-258.9)	1367.5 (1029.6-1979.6)		64.0 (0-345.2)	691.1 (451.8-953.1)	28.3 (0-115.1)	0 (0-26.2)	54.2 (0-263.2)
Averaged ethanol intake (g/wk.) (visits 1-4)		41 (14.6-64.7)	341.9 (257.4-494.9)		25.1 (0-101.3)	186.2 (118.8-252.4)	19.6 (0-66)	0 (0-19.1)	25.9 (0-85.1)
History of excessive drinking *, n (%)		13 (7.3)	7 (46.7)	34 (17.8)	30 (9.6)	13 (24.5)	45 (8.0)	3 (0.8)	145 (6.7)
Cognitive test scores, mean (SD)									
Global cognition factor z-score	-0.74 (0.71)	-0.41 (0.79)	-0.68 (0.90)	-0.86 (0.67)	-0.59 (0.77)	-0.57 (0.92)	-0.70 (0.77)	-0.89 (0.68)	0.72 (0.75)
DWRT	6.2 (1.6)	6.3 (1.6)	5.7 (1.5)	6.0 (1.5)	6.1 (1.8)	6.1 (1.8)	6.0 (1.7)	5.9 (1.7)	6.1 (1.7)
DSST	30.2 (12.8)	36.2 (13.8)	32.2 (13.3)	28.8 (11.9)	33.5 (12.9)	34.8 (15.9)	31.6 (13.6)	27.8 (13.0)	31.1 (13.3)
WFT	28.6 (12.8)	33.2 (14.6)	31.4 (12)	27.1 (13.1)	30.3 (13.5)	29.3 (14.3)	28.6 (13)	25.9 (12.3)	28.6 (13.2)

† Counts and percentage were calculated based on data prior to imputation for missing ethanol intake data for Atherosclerosis Risk in Communities (ARIC) Study visits 1-4.

Abbreviations: ‡ Unclassified, participants' ethanol intake pattern across visits 1-4 could not be classified due to missing ethanol intake. Missing ethanol data were later imputed, and participants' ethanol intake were determined; SD, standard deviation; n, number of participants; %, percent; NC, North Carolina, MS, Mississippi; GED, general educational development; kg/m2, grams per meter squared; met-min/week, metabolic equivalent of task per week; APOEε4, apolipoprotein epsilon 4 allele; g/wk., grams per week; self-reported at visit 3. "Was there ever a time in your life when you consumed 5 or more drinks of any kind of alcoholic beverage almost every day?"; DWRT, delayed word recall test; DSST, digit symbol substitution test; and WFT, word fluency test. Frequency, median (25th-75th percentile): stable

Table 24. Baseline characteristics of Atherosclerosis Risk in Communities (ARIC) study European-American participants by 9-year ethanol drinking trajectories, 1987-1996 (N=8707) †

	Stable	Stable	Stable	Stable	Mostly	Mostly	Mostly	Unclassified‡	Overall
	Never	Low-to-moderate	Heavy	Former	Low-to-moderate	Heavy	Former		
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Characteristics	1033 (11.9)	3095 (35.5)	176 (2.0)	664 (7.6)	1091 (12.5)	511 (5.9)	1687 (19.4)	450 (5.2)	8707 (80.1)
Age (years), mean (SD)	64.2 (5.5)	62.4 (5.6)	62.9 (5.7)	64 (5.5)	62.8 (5.6)	62.6 (5.4)	63.5 (5.8)	64.1 (5.5)	63.1 (5.6)
Female, n (%)	791 (76.6)	1517 (49.0)	77 (43.8)	256 (38.6)	547 (50.1)	250 (48.9)	927 (54.9)	305 (67.8)	4792 (53.5)
Study center, n (%)									
Forsyth County, NC	486 (47.0)	657 (21.2)	50 (28.4)	180 (27.1)	267 (24.5)	127 (24.9)	595 (35.3)	156 (34.7)	2518 (28.9)
Minneapolis, MN	94 (9.1)	1619 (52.3)	72 (40.9)	192 (28.9)	383 (35.1)	252 (49.3)	482 (28.6)	68 (15.1)	3162 (36.3)
Washington County, MD	453 (43.9)	819 (26.5)	54 (30.7)	292 (44.0)	441 (40.4)	132 (25.8)	610 (36.2)	226 (50.2)	3027 (34.8)
Education, n (%)									
Less than high school	233 (22.6)	178 (5.8)	18 (10.2)	175 (26.4)	156 (14.3)	45 (8.8)	301 (17.8)	120 (26.7)	1226 (14.1)
High school, GED, or vocational school	546 (52.9)	1325 (42.8)	77 (43.8)	290 (43.7)	498 (45.6)	209 (40.9)	851 (50.4)	223 (49.6)	4019 (46.2)
College, graduate, or professional school	254 (24.6)	1592 (51.4)	81 (46.0)	199 (30.0)	437 (40.1)	257 (50.3)	535 (31.7)	107 (23.8)	3462 (39.8)
Smoking, n (%)									
Never Smokers	827 (80.1)	1140 (36.8)	25 (14.2)	154 (23.2)	336 (30.8)	91 (17.8)	662 (39.2)	275 (61.1)	3510 (40.3)
Former Smokers	146 (14.1)	1603 (51.8)	98 (55.7)	410 (61.7)	592 (54.3)	283 (55.4)	752 (44.6)	102 (22.7)	3986 (45.8)
Current Smokers	60 (5.8)	352 (11.4)	53 (30.1)	100 (15.1)	163 (14.9)	137 (26.8)	273 (16.2)	73 (16.2)	1211 (13.9)
Body Mass Index (kg/m2), mean (SD)	28.4 (5.6)	28.1 (4.9)	27.2 (4.6)	29.1 (5.5)	28.3 (5.4)	26.9 (4.6)	28.5 (5.3)	29.1 (5.8)	28.3 (5.2)
Diabetes, n (%)	169 (16.4)	300 (9.7)	18 (10.2)	142 (21.4)	143 (13.1)	43 (8.4)	289 (17.1)	83 (18.4)	1187 (13.6)
Hypertension, n (%)	377 (36.5)	910 (29.4)	56 (31.8)	233 (35.1)	374 (34.3)	133 (26.0)	571 (33.8)	178 (39.6)	2832 (32.5)
History of Stroke n (%)	22 (2.1)	58 (1.9)	6 (3.4)	19 (2.9)	30 (2.7)	8 (1.6)	61 (3.6)	10 (2.2)	214 (2.5)
Diet score, mean (SD)	20.5 (5.0)	21.4 (5.0)	20.4 (5.2)	20.5 (5.1)	20.3 (5.9)	20.8 (5.4)	20.4 (5.5)	20.5 (5.8)	20.8 (5.3)
Physical activity (met-min/week), mean (SD)	8.9 (10.6)	14.3 (14.2)	12.2 (12.9)	10.9 (12.9)	13.2 (14.1)	14 (14.4)	10.6 (12.0)	9.6 (11.8)	12.2 (13.3)
APOEε4 allele present, n (%)	261 (25.3)	860 (27.8)	45 (25.6)	174 (26.2)	285 (26.1)	138 (27.0)	439 (26.0)	117 (26.0)	2319 (26.6)
Ethanol Intake, median (25th-75th percentile)									
Frequency		8 (2-17)	89.5 (58-113)	6 (1-22)	11 (1-36)	47 (31-66)	1 (0-6)	0 (0-0)	4 (0-19)
Grams/week		97.1 (25.9-218.9)	1214.5 (765.2-1582.2)		92.4 (0-430.8)	627.8 (421-884.4)	0 (0-66.9)	0 (0-0)	82.3 (0-296.3)
Averaged ethanol intake (g/wk.) (visits 1-4)		24.3 (6.5-54.8)	303.6 (191.3-395.5)		38.9 (0-119.3)	162.3 (108.9-231)	0 (0-28.8)	0 (0-0)	24.3 (0-80.2)
History of excessive drinking *, n (%)		95 (3.1)	57 (32.4)	164 (24.7)	112 (10.3)	77 (15.1)	101 (6.0)	0 (0.0)	606 (7.0)
Cognitive test scores, mean (SD)									
Global cognition factor z-score	0.02 (0.68)	0.30 (0.67)	0.14 (0.69)	-0.20 (0.74)	0.13 (0.74)	0.25 (0.69)	0.02 (0.69)	-0.11 (0.73)	0.12 (0.71)
DWRT	6.7 (1.5)	6.8 (1.5)	6.8 (1.6)	6.4 (1.5)	6.7 (1.5)	6.8 (1.5)	6.6 (1.6)	6.4 (1.5)	6.7 (1.5)
DSST	45.5 (11.0)	49.7 (10.7)	46.4 (10.6)	41.8 (11.3)	46.9 (11.5)	48.2 (10.6)	45.3 (11.1)	43.9 (11.4)	46.9 (11.2)
WFT	32.1 (11.1)	36.8 (11.6)	37.7 (11.8)	32.1 (11.8)	35.8 (12.2)	37.4 (12.4)	33.4 (11.7)	31 (11.5)	34.9 (11.9)

† Counts and percentage were calculated based on data prior to imputation for missing ethanol intake data for Atherosclerosis Risk in Communities (ARIC) Study visits 1-4.

Abbreviations: ‡ Unclassified, participants' ethanol intake pattern across visits 1-4 could not be classified due to missing ethanol intake. Missing ethanol data were later imputed and participants' ethanol intake were determined; SD, standard deviation; n, number of participants; %, percent; NC, North Carolina; MN, Minnesota; MD, Maryland; GED, general educational development; kg/m2, grams per meter squared; met-min/week, metabolic equivalent of task per week; APOEε4, apolipoprotein epsilon 4 allele; g/wk., grams per week; *, self-reported at visit 3. "Was there ever a time in your life when you consumed 5 or more drinks of any kind of alcoholic beverage almost every day?"; DWRT, delayed word recall test; DSST, digit symbol substitution test; and WFT, word fluency test.

Table 25. Adjusted mean difference in 15-year change in cognitive performance by long-term ethanol intake category for Atherosclerosis Risk in Communities (ARIC) study African-American participants

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Long-Term Drinking Category	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	P for Difference
Global Factor Score z-score	2342	-0.73 (0.75)				
Stable never drinking	529	-0.76 (0.71)	-0.64 (-1.05, -0.24)	Reference	Reference	0.862
Stable low-to-moderate drinking	197	-0.42 (0.78)	-0.61 (-1.03, -0.20)	0.03 (-0.13, 0.19)	-5%	
Stable heavy drinking	19	-0.69 (0.90)	-0.57 (-1.14, 0.00)	0.08 (-0.34, 0.50)	-12%	
Stable former drinking	225	-0.90 (0.67)	-0.67 (-1.08, -0.25)	-0.02 (-0.18, 0.14)	3%	
Mostly low-to-moderate drinking	335	-0.67 (0.76)	-0.62 (-1.03, -0.21)	0.02 (-0.11, 0.16)	-4%	
Mostly heavy drinking	60	-0.59 (0.91)	-0.49 (-0.95, -0.03)	0.15 (-0.10, 0.41)	-24%	
Mostly former drinking	673	-0.73 (0.76)	-0.60 (-1.00, -0.19)	0.05 (-0.07,0.16)	-7%	
Delayed Word Recall z-score	2340	-0.37 (1.11)				
Stable never drinking	528	-0.27 (1.09)	-1.00 (-1.82, -0.18)	Reference	Reference	0.425
Stable low-to-moderate drinking	196	-0.20 (1.05)	-0.93 (-1.76, -0.10)	0.08 (-0.23, 0.39)	-8%	
Stable heavy drinking	19	-0.48 (1.08)	-0.82 (-1.89, 0.24)	0.18 (-0.69, 1.05)	-18%	
Stable former drinking	225	-0.46 (1.05)	-1.11 (-1.96, -0.26)	-0.11 (-0.41, 0.19)	11%	
Mostly low-to-moderate drinking	335	-0.43 (1.23)	-0.80 (-1.64, 0.03)	0.20 (-0.07, 0.46)	-20%	
Mostly heavy drinking	59	-0.33 (1.05)	-0.79 (-1.71, 0.12)	0.21 (-0.31, 0.73)	-21%	
Mostly former drinking	673	-0.38 (1.07)	-0.85 (-1.65, -0.04)	0.16 (-0.07, 0.38)	-16%	
Word Fluency z-score	2334	-0.39 (1.06)				
Stable never drinking	528	-0.40 (1.02)	-0.44 (-0.89, 0.02)	Reference	Reference	0.634
Stable low-to-moderate drinking	196	-0.02 (1.14)	-0.41 (-0.86, 0.05)	0.03 (-0.14, 0.20)	-6%	
Stable heavy drinking	19	-0.12 (0.99)	-0.37 (-0.98, 0.24)	0.06 (-0.40, 0.53)	-15%	
Stable former drinking	223	-0.56 (1.08)	-0.39 (-0.86, 0.08)	0.05 (-0.12, 0.21)	-10%	
Mostly low-to-moderate drinking	331	-0.30 (1.09)	-0.31 (-0.78, 0.17)	0.13 (-0.01, 0.28)	-30%	
Mostly heavy drinking	59	-0.31 (1.15)	-0.27 (-0.80, 0.25)	0.16 (-0.12, 0.45)	-37%	
Mostly former drinking	672	-0.40 (1.04)	-0.37 (-0.83, 0.09)	0.07 (-0.05, 0.19)	-15%	
Digit Symbol Substitution z-score	2325	-0.97 (0.94)	-0.45 (-0.90, 0.01)			
Stable never drinking	526	-1.04 (0.91)	-0.39 (-0.84, 0.06)	Reference	Reference	0.954
Stable low-to-moderate drinking	196	-0.58 (0.94)	-0.44 (-0.88, 0.01)	-0.05 (-0.22, 0.12)	12%	
Stable heavy drinking	18	-0.83 (0.90)	-0.43 (-1.03, 0.18)	-0.04 (-0.48, 0.40)	10%	
Stable former drinking	221	-1.16 (0.84)	-0.48 (-0.93, -0.02)	-0.09 (-0.24, 0.06)	23%	
Mostly low-to-moderate drinking	331	-0.88 (0.94)	-0.45 (-0.90, 0.00)	-0.06 (-0.20, 0.08)	16%	
Mostly heavy drinking	59	-0.72 (1.11)	-0.41 (-0.90, 0.09)	-0.02 (-0.29, 0.25)	5%	
Mostly former drinking	668	-0.95 (0.95)	-0.42 (-0.86, 0.02)	-0.03 (-0.15, 0.08)	8%	

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4; 1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker). All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances. CI, confidence interval. Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. † Percent, positive values represent % greater decline relative to the referent group. P for difference, p-value for t-test of equality of mean difference in 15-year change in cognitive performance across categories of long-term ethanol intake.

Table 26. Adjusted mean difference in 15-year change in cognitive performance by long-term ethanol intake category for Atherosclerosis Risk in Communities (ARIC) study European-American participants

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Long-Term Drinking Category	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	P for difference
Global Factor Score z-score	8709	0.12 (0.72)				
Stable never drinking	1046	0.02 (0.68)	-0.86 (-1.10, -0.62)	Reference	Reference	0.275
Stable low-to-moderate drinking	3136	0.30 (0.67)	-0.84 (-1.08, -0.60)	0.02 (-0.05, 0.08)	-2%	
Stable heavy drinking	185	0.13 (0.69)	-0.89 (-1.18, -0.60)	-0.03 (-0.18, 0.11)	4%	
Stable former drinking	686	-0.21 (0.74)	-0.78 (-1.03, -0.54)	0.07 (-0.02, 0.16)	-8%	
Mostly low-to-moderate drinking	1068	0.12 (0.73)	-0.84 (-1.08, -0.59)	0.02 (-0.06, 0.10)	-2%	
Mostly heavy drinking	493	0.23 (0.70)	-0.90 (-1.15, -0.66)	-0.05 (-0.15, 0.05)	5%	
Mostly former drinking	1602	0.02 (0.70)	-0.83 (-1.07, -0.58)	0.03 (-0.04, 0.10)	-4%	
Delayed Word Recall z-score	8706	0.06 (1.00)				
Stable never drinking	1047	0.08 (1.01)	-1.38 (-1.94, -0.82)	Reference	Reference	0.435
Stable low-to-moderate drinking	3136	0.14 (0.96)	-1.37 (-1.93, -0.81)	0.01 (-0.12, 0.13)	-1%	
Stable heavy drinking	184	0.14 (1.02)	-1.58 (-2.16, -1.00)	-0.20 (-0.49, 0.09)	15%	
Stable former drinking	687	-0.13 (1.01)	-1.46 (-2.05, -0.87)	-0.08 (-0.26, 0.11)	5%	
Mostly low-to-moderate drinking	1067	0.04 (1.01)	-1.41 (-1.98, -0.84)	-0.02 (-0.18, 0.13)	2%	
Mostly heavy drinking	492	0.13 (1.01)	-1.52 (-2.12, -0.93)	-0.14 (-0.34, 0.05)	10%	
Mostly former drinking	1602	0.02 (1.04)	-1.39 (-1.95, -0.82)	0.00 (-0.16, 0.15)	0.30%	
Word Fluency z-score	8702	0.14 (0.95)				
Stable never drinking	1047	-0.08 (0.89)	-0.47 (-0.79, -0.15)	Reference		0.361
Stable low-to-moderate drinking	3135	0.29 (0.93)	-0.45 (-0.76, -0.14)	0.02 (-0.05, 0.09)	-5%	
Stable heavy drinking	185	0.34 (0.95)	-0.55 (-0.88, -0.22)	-0.08 (-0.23, 0.07)	17%	
Stable former drinking	685	-0.10 (0.96)	-0.50 (-0.82, -0.17)	-0.03 (-0.12, 0.07)	6%	
Mostly low-to-moderate drinking	1068	0.19 (0.98)	-0.50 (-0.82, -0.18)	-0.04 (-0.12, 0.05)	7%	
Mostly heavy drinking	492	0.34 (0.98)	-0.51 (-0.83, -0.19)	-0.04 (-0.15, 0.06)	9%	
Mostly former drinking	1599	0.03 (0.95)	-0.49 (-0.8, -0.17)	-0.02 (-0.10, 0.06)	4%	
Digit Symbol Substitution z-score	8691	0.17 (0.79)				
Stable never drinking	1045	0.07 (0.78)	-0.78 (-1.06, -0.51)	Reference	Reference	0.114
Stable low-to-moderate drinking	3134	0.36 (0.75)	-0.84 (-1.11, -0.56)	-0.05 (-0.12, 0.01)	7%	
Stable heavy drinking	184	0.14 (0.74)	-0.86 (-1.15, -0.58)	-0.08 (-0.22, 0.05)	10%	
Stable former drinking	683	-0.20 (0.80)	-0.77 (-1.05, -0.49)	0.02 (-0.08, 0.11)	-2%	
Mostly low-to-moderate drinking	1066	0.15 (0.80)	-0.83 (-1.1, -0.56)	-0.05 (-0.13, 0.03)	6%	
Mostly heavy drinking	492	0.25 (0.76)	-0.89 (-1.18, -0.61)	-0.11 (-0.21, -0.01)	14%	
Mostly former drinking	1597	0.05 (0.79)	-0.80 (-1.07, -0.53)	-0.02 (-0.09, 0.05)	2%	

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4; 1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker). All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances. CI, confidence interval. Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. † Percent, positive values represent % greater decline relative to the referent group. P for difference, p-value for t-test of equality of mean difference in 15-year change in cognitive performance across categories of long-term ethanol intake.

Table 27. Adjusted mean difference in 15-year change in cognitive performance by quartiles of cumulative average ethanol intake for Atherosclerosis Risk in Communities (ARIC) study African-American participants

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Quartile‡	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	P for trend
Global Factor Score z-score	2342	-0.73 (0.75)				
Quartile 1	300	-0.55 (0.75)	-0.66 (-1.23, -0.09)	Reference	Reference	0.791
Quartile 2	271	-0.56 (0.79)	-0.69 (-1.26, -0.11)	-0.02 (-0.17, 0.13)	3%	
Quartile 3	263	-0.66 (0.82)	-0.67 (-1.24, -0.10)	0.00 (-0.15, 0.14)	1%	
Quartile 4	279	-0.77 (0.79)	-0.67 (-1.24, -0.10)	-0.01 (-0.17, 0.15)	1%	
P for difference						0.992
Delayed Word Recall z-score	2340	-0.37 (1.11)				
Quartile 1	301	-0.30 (1.08)	-0.78 (-1.85, 0.28)	Reference	Reference	0.828
Quartile 2	270	-0.27 (1.11)	-0.75 (-1.81, 0.32)	0.04 (-0.25, 0.32)	-4%	
Quartile 3	261	-0.38 (1.08)	-0.86 (-1.90, 0.19)	-0.07 (-0.38, 0.23)	9%	
Quartile 4	279	-0.49 (1.21)	-0.84 (-1.88, 0.20)	-0.06 (-0.39, 0.27)	7%	
P for difference						0.885
Word Fluency z-score	2334	-0.39 (1.06)				
Quartile 1	300	-0.15 (1.05)	-0.64 (-1.23, -0.05)	Reference	Reference	0.712
Quartile 2	269	-0.24 (1.06)	-0.62 (-1.2, -0.05)	0.02 (-0.13, 0.17)	-2%	
Quartile 3	259	-0.32 (1.14)	-0.62(-1.21, -0.03)	0.02 (-0.14, 0.18)	-3%	
Quartile 4	279	-0.41 (1.08)	-0.66 (-1.23, -0.09)	-0.02 (-0.19, 0.15)	3%	
P for difference						0.96
Digit Symbol Substitution z-score	2325	-0.97 (0.94)				
Quartile 1	299	-0.74 (0.91)	-0.60 (-1.2, 0.00)	Reference	Reference	0.866
Quartile 2	267	-0.72 (0.94)	-0.62 (-1.22, -0.03)	-0.02 (-0.17, 0.13)	4%	
Quartile 3	266	-0.90 (1.03)	-0.58 (-1.17, 0.00)	0.02 (-0.14, 0.18)	-3%	
Quartile 4	273	-1.00 (0.97)	-0.61 (-1.19, -0.03)	-0.01 (-0.17, 0.16)	1%	
P for difference						0.963

‡ Global Factor Score z-score: Quartile 1: 0g/wk., Quartile 2: 2.7-26.4 g/wk., Quartile 3: 26.4-86.2 g/wk., and Quartile 4: 86.3-1559.4 g/wk.; Delayed Word Recall z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-26.4 g/wk., Quartile 3: 26.4-85.8 g/wk., and Quartile 4: 86.1-1526.1 g/wk.; Word Fluency z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-26.4 g/wk., Quartile 3: 26.4-85.8 g/wk., and Quartile 4: 86.0-1565.1 g/wk.; and Digit Symbol Substitution z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-26.4 g/wk., Quartile 3: 26.4-87.3 g/wk., and Quartile 4: 87.9-1566.9 g/wk.

All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances. CI, confidence interval.

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† Percent, positive values represent % greater decline relative to the referent group.

P for trend, p-value for trend obtained from a linear regression model with average ethanol intake across 9-years modeled as an ordinal variable. P for difference-, p-value for t-test of equality of mean difference in 15-year change cognitive performance across quartiles of cumulative average ethanol intake

Table 28. Adjusted mean difference in 15-year change in cognitive performance by quartiles of cumulative average ethanol intake for Atherosclerosis Risk in Communities (ARIC) study European-American participants

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Quartile‡	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	P for trend
Global Factor Score z-score	8709	0.12 (0.72)				
Quartile 1	1706	0.21 (0.70)	-0.84 (-1.10, -0.57)	Reference	Reference	0.831
Quartile 2	1520	0.25 (0.69)	-0.84 (-1.10, -0.58)	0.00 (-0.06, 0.05)	1%	
Quartile 3	1609	0.21 (0.70)	-0.83 (-1.09, -0.57)	0.00 (-0.06, 0.06)	-0.30%	
Quartile 4	1612	0.09 (0.71)	-0.87 (-1.13, -0.60)	-0.03 (-0.09, 0.03)	4%	
P for difference						0.637
Delayed Word Recall z-score	8706	0.06 (1.00)				
Quartile 1	1704	0.15 (0.96)	-1.46 (-2.05, -0.86)	Reference	Reference	0.394
Quartile 2	1518	0.12 (0.99)	-1.44 (-2.02, -0.86)	0.02 (-0.10, 0.14)	-1%	
Quartile 3	1611	0.08 (0.98)	-1.40 (-1.99, -0.81)	0.06 (-0.06, 0.18)	-4%	
Quartile 4	1612	0.00 (1.03)	-1.50 (-2.09, -0.92)	-0.05 (-0.17, 0.08)	3%	
P for difference						0.342
Word Fluency z-score	8702	0.14 (0.95)				
Quartile 1	1704	0.14 (0.91)	-0.53 (-0.85, -0.21)	Reference	Reference	0.531
Quartile 2	1517	0.25 (0.93)	-0.52 (-0.84, -0.20)	0.01 (-0.06, 0.08)	-2%	
Quartile 3	1611	0.24 (0.97)	-0.52 (-0.83, -0.20)	0.02 (-0.05, 0.08)	-3%	
Quartile 4	1610	0.23 (1.00)	-0.56 (-0.88, -0.23)	-0.02 (-0.09, 0.04)	5%	
P for difference						0.641
Digit Symbol Substitution z-score	8691	0.17 (0.79)				
Quartile 1	1702	0.27 (0.78)	-0.80 (-1.07, -0.52)	Reference	Reference	0.892
Quartile 2	1524	0.30 (0.77)	-0.80 (-1.08, -0.53)	-0.01 (-0.06, 0.05)	1%	
Quartile 3	1603	0.27 (0.77)	-0.79 (-1.06, -0.51)	0.01 (-0.04, 0.06)	-1%	
Quartile 4	1607	0.10 (0.77)	-0.82 (-1.10, -0.54)	-0.02 (-0.09, 0.04)	3%	
P for difference						0.701

‡ Global Factor Score z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.0 g/wk., Quartile 3: 24.3-80.2 g/wk., and Quartile 4: 80.3-1071.5 g/wk.; Delayed Word Recall z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.0 g/wk., Quartile 3: 24.0-80.2 g/wk., and Quartile 4: 80.2-1072.8 g/wk.; Word Fluency z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.0 g/wk., Quartile 3: 24.0-80.2 g/wk., and Quartile 4: 80.3-1071.5 g/wk.; and Digit Symbol Substitution z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.3 g/wk., Quartile 3: 24.3-80.1 g/wk., and Quartile 4: 80.2-1072.8 g/wk..

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4;1996-1998) z-score. Negative values correspond to greater decline compared to the reference (lowest quartile).

All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances.

CI, confidence interval.

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† Percent, positive values represent % greater decline relative to the referent group.

P for trend, p-value for trend obtained from a linear regression model with average ethanol intake across 9-years modeled as an ordinal variable. P for difference-, p-value for t-test of equality of mean difference in 15-year change cognitive performance across quartiles of cumulative average ethanol intake

Table 29. Adjusted mean difference in 15-year change in cognitive performance by visit 4 ethanol intake status for Atherosclerosis Risk in Communities (ARIC) study African-American participants

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Drinking status at visit 4	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	P for difference
Global Factor Score z-score	2342	-0.73 (0.75)				
Never drinking	817	-0.85(0.71)	-0.53 (-0.91, -0.15)	Reference	Reference	0.697
Low-to-moderate drinking	531	-0.55(0.77)	-0.52 (-0.90, -0.14)	0.01 (-0.10, 0.13)	-2%	
Heavy drinking	78	-0.62(0.91)	-0.40 (-0.82, 0.03)	0.13 (-0.09, 0.35)	-25%	
Former drinking	915	-0.75(0.74)	-0.52 (-0.90, -0.14)	0.01 (-0.09, 0.11)	-1%	
Delayed Word Recall z-score	2340	-0.37 (1.11)				
Never drinking	816	-0.39(1.12)	-0.95 (-1.72, -0.18)	Reference	Reference	0.814
Low-to-moderate drinking	530	-0.31(1.16)	-0.86 (-1.63, -0.09)	0.09 (-0.12, 0.31)	-10%	
Heavy drinking	78	-0.38(1.13)	-0.79 (-1.62, 0.04)	0.16 (-0.30, 0.62)	-17%	
Former drinking	916	-0.38(1.06)	-0.89 (-1.65, -0.12)	0.06 (-0.13, 0.26)	-6%	
Word Fluency z-score	2334	-0.39 (1.06)				
Never drinking	814	-0.53(1.02)	-0.40 (-0.83, 0.03)	Reference	Reference	0.609
Low-to-moderate drinking	529	-0.16(1.1)	-0.34 (-0.77, 0.10)	0.06 (-0.06 ,0.18)	-16%	
Heavy drinking	77	-0.28(1.13)	-0.27 (-0.75, 0.22)	0.13 (-0.12, 0.38)	-33%	
Former drinking	914	-0.41(1.05)	-0.38 (-0.80, 0.05)	0.02 (-0.08, 0.13)	-6%	
Digit Symbol Substitution z-score	2325	-0.97 (0.94)				
Never drinking	812	-1.13(0.91)	-0.26 (-0.69, 0.17)	Reference	Reference	0.614
Low-to-moderate drinking	528	-0.74(0.94)	-0.32 (-0.74, 0.11)	-0.06 (-0.17, 0.06)	21%	
Heavy drinking	77	-0.75(1.06)	-0.27 (-0.74, 0.20)	-0.01 (-0.23, 0.22)	3%	
Former drinking	908	-0.98(0.92)	-0.32 (-0.74, 0.10)	-0.06 (-0.15, 0.03)	22%	

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4;1996-1998) z-score. Negative values correspond to greater decline compared to the reference (never drinker).

All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances.

CI, confidence interval

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† Percent, positive values represent % greater decline relative to the referent group.

P for difference, p-value for t-test of equality of mean difference in 15-year change cognitive performance across categories of ethanol intake status at study baseline

Table 30. Adjusted mean difference in 15-year change in cognitive performance by visit 4 ethanol intake status for Atherosclerosis Risk in Communities (ARIC) study European-American participants

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Drinking status at visit 4	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	P for difference
Global Factor Score z-score	8709	0.12 (0.72)				
Never drinking	1450	-0.02 (0.70)	-0.84 (-1.07, -0.61)	Reference	Reference	0.072
Low-to-moderate drinking	4247	0.25 (0.69)	-0.83 (-1.06, -0.59)	0.01 (-0.04, 0.07)	-1%	
Heavy drinking	629	0.23 (0.69)	-0.90 (-1.14, -0.65)	-0.06 (-0.15, 0.03)	7%	
Former drinking	2383	-0.05 (0.72)	-0.80 (-1.04, -0.57)	0.04 (-0.02, 0.10)	-4%	
Delayed Word Recall z-score	8706	0.06 (1.00)				
Never drinking	1450	0.01 (1.01)	-1.37 (-1.91, -0.83)	Reference		0.177
Low-to-moderate drinking	4246	0.12 (0.98)	-1.36 (-1.89, -0.83)	0.02 (-0.09, 0.13)	-1%	
Heavy drinking	627	0.15 (1.01)	-1.53 (-2.08, -0.98)	-0.16 (-0.34, 0.03)	11%	
Former drinking	2383	-0.02 (1.03)	-1.38 (-1.91, -0.84)	0.00 (-0.13, 0.12)	0.40%	
Word Fluency z-score	8702	0.14 (0.95)				
Never drinking	1450	-0.11 (0.90)	-0.43 (-0.74, -0.12)	Reference	Reference	0.323
Low-to-moderate drinking	4245	0.27 (0.95)	-0.43 (-0.73, -0.13)	0.00 (-0.07, 0.06)	1%	
Heavy drinking	628	0.36 (0.96)	-0.50 (-0.80, -0.20)	-0.07 (-0.17, 0.02)	17%	
Former drinking	2379	-0.01 (0.95)	-0.45 (-0.75, -0.15)	-0.03 (-0.09, 0.04)	6%	
Digit Symbol Substitution z-score	8691	0.17 (0.79)				
Never drinking	1447	0.03 (0.78)	-0.77 (-1.04, -0.50)	Reference	Reference	0.026
Low-to-moderate drinking	4242	0.30 (0.77)	-0.81 (-1.07, -0.55)	-0.04 (-0.10, 0.02)	5%	
Heavy drinking	627	0.24 (0.75)	-0.87 (-1.15, -0.6)	**-0.10 (-0.19, -0.02)**	13%	
Former drinking	2375	-0.02 (0.80)	-0.77 (-1.04, -0.51)	0.00 (-0.06, 0.06)	0%	

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4;1996-1998) z-score. Negative values correspond to greater decline compared to the reference (never drinker).

All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances.

CI, confidence interval.

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† Percent, positive values represent % greater decline relative to the referent group.

P for difference, p-value for t-test of equality of mean difference in 15-year change cognitive performance across categories of ethanol intake status at study baseline

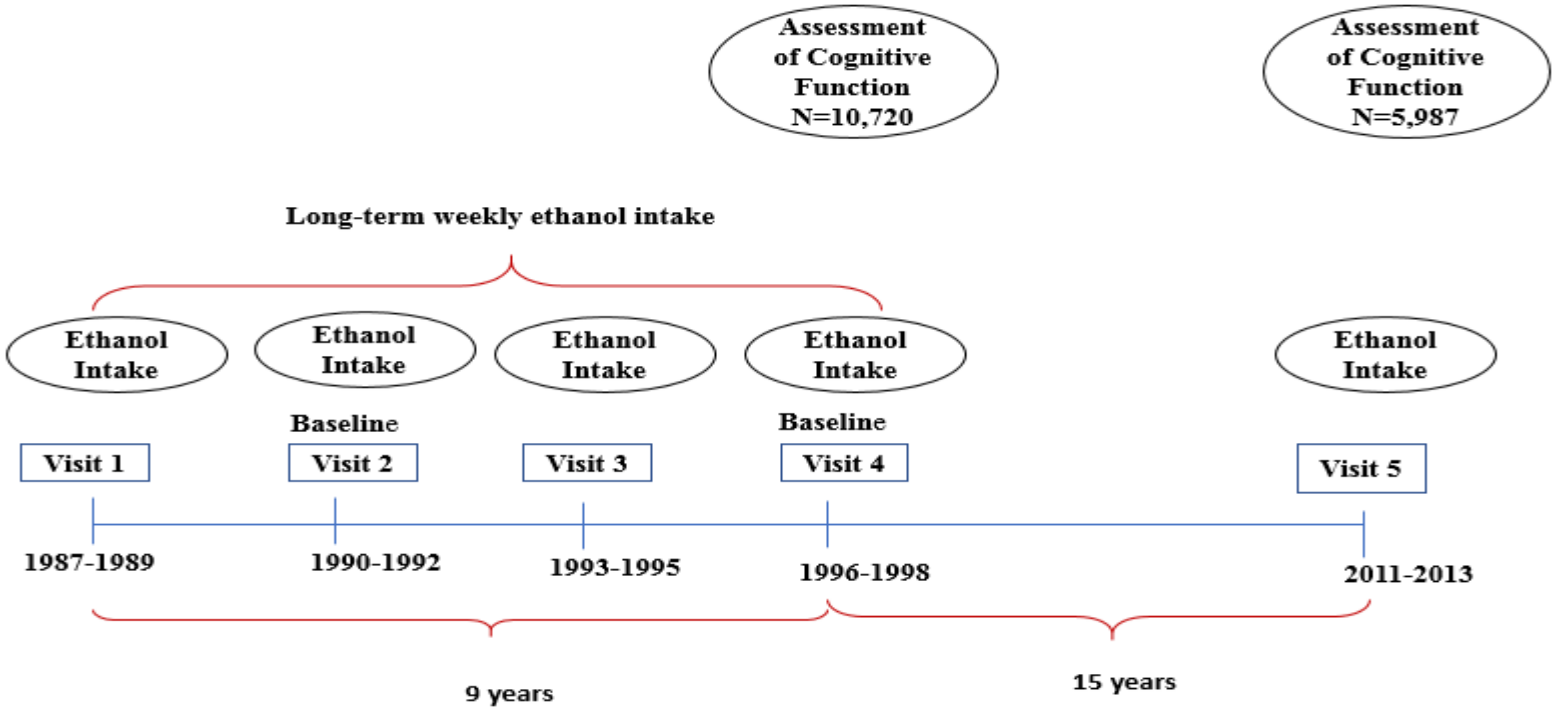


Figure 9. Timeline for the Atherosclerosis Risk in Communities (ARIC) Study for Specific Aim 1

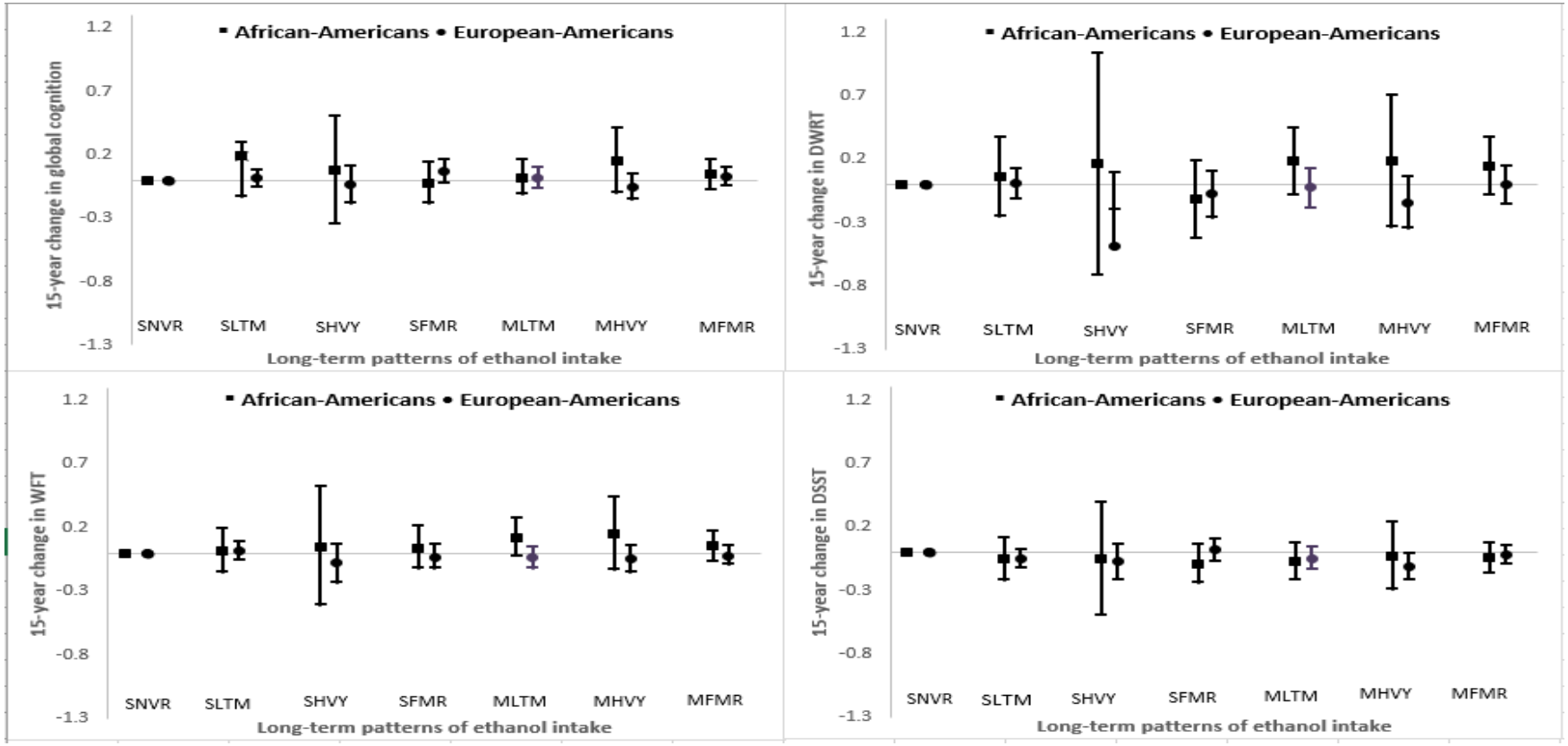


Figure 10. Estimated mean difference in the 15-year change in cognitive performance by long-term trajectories of ethanol intake in mid-life relative to those who reported stable never drinking

All models were adjusted for sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances. Abbreviations: SNVR, stable never drinker; SLTM, stable low-to-moderate drinker; SHVY, stable heavy drinker; SFMR, stable former drinker; MLTM, mostly low-to-moderate drinker; MHVY, mostly heavy drinker; MFMR, mostly former drinker; DWRT, delayed word recall test; DSST, digit symbol substitution test; and WFT, word fluency test. Sample sizes: African-Americans (global cognition, n=2342; DWRT, n=2340; WFT, n=2334; and DSST, n=2325) and European-Americans (global cognition, n=8709; DWRT, n=8706; WFT, n=8702; and DSST, n=8691).

Manuscript B: Mid-life Ethanol Intake and Cognitive Decline: A Gene x Environment Interaction Study

1. Overview

Background: Previous reports of the relationship between ethanol intake and cognitive decline have been inconsistent, yet possible interaction between ethanol intake and genetic susceptibility on the risk of cognitive decline has often not been considered.

Objective: To investigate whether unweighted genetic risk scores (GRSs) based on ethanol intake-associated single nucleotide polymorphisms (SNPs) modify the relationship between weekly ethanol intake in mid-life and 15-year rate of decline in general cognitive performance from mid-to-late life among African-American and European-American adults.

Methods: A total of 9,183 participants (n=1,733 African-Americans and n=7,450 European-Americans) of the Atherosclerosis Risk in Communities (ARIC) study completed an interviewer-administered questionnaire on habitual ethanol intake and neurocognitive assessments at 1996 and 2013. Twenty ethanol intake-associated SNPs for African-Americans and 11 for European-Americans served to create unweighted GRSs, $uGRS_{20}$ and $uGRS_{11}$, respectively. Multivariable linear regression was used to assess modification of the ethanol intake-cognitive decline association by $uGRS_{20}$ and $uGRS_{11}$. Multiple imputation by chained equations (MICE) were used to account for attrition.

Results: Ethanol intake (log grams per week) in mid-life was not associated with 15-year decline in general cognitive performance from mid-to-late life (African-American: β for log-ethanol intake (log grams per week)= -0.011 ((95% CI: -0.052,0.031), European-Americans: -0.010 (-0.021,0.002)). The $uGRS_{20}$ and $uGRS_{11}$ did not modify the association of ethanol intake in mid-life with 15-year change in general cognitive performance from mid-to-late life among African-Americans (P= 0.811) and European-Americans (P= 0.847), respectively.

Conclusions: Ethanol intake in mid-life is not associated with cognitive decline from mid-to-late. There is no indication that an association between ethanol intake and cognitive depends on genetic susceptibility to ethanol intake among African-American and European-American adults.

2. Introduction

Reports from prospective studies on the relationship between ethanol intake and cognitive decline have been inconsistent [32, 93, 336-348]. While heavy ethanol intake is associated with greater cognitive decline [348], low-to-moderate ethanol intake has been associated with less cognitive decline [336, 339, 340, 342-344, 346, 347] or no cognitive decline [93, 345, 348]. Inconsistent findings may be attributable to potential effect modifiers that were not taken into account. A limited number of studies have investigated the effects of ethanol in African-Americans populations even though the prevalence, incidence, and cumulative risk of Alzheimer's Dementia (AD) is documented to be higher in African-Americans than in European-Americans [343, 347]. Furthermore, few studies investigated the effects of mid-life ethanol intake with late-life cognition [341, 348].

Genome-wide association studies (GWAS) have shown that ethanol intake is influenced by hundreds of common genetic variants [383]. Recently, the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) study of 941,280 participants of European ancestry, identified 100 single nucleotide polymorphisms (SNPS) in 82 genetic loci to be independently associated with number of drink per week [400]. Little is known about the effect of these genetic variants in other populations such as African-Americans. It is well known that SNPs identified in European descent individuals do not transfer well to African ancestry populations, especially given the differences in linkage disequilibrium (LD) across ancestral populations. To date, only one study has investigated the modification of the ethanol intake-cognitive decline relationship by genetic variants identified by GWAS to be associated with ethanol intake [381]. This study was conducted in a primarily white European ancestry sample. The purpose of this investigation is to determine if ethanol intake-associated loci modify the association between ethanol intake in mid-life and 15-year cognitive decline from mid-to-late life in American-American and European-American adults.

3. Methods

Study Population

The Atherosclerosis Risk in Communities (ARIC) study is a community-based, prospective cohort study established in 1987, designed primarily to investigate the etiology of atherosclerosis and its clinical sequelae. From 1986 through 1990, 15,792 adults aged 45 to 64 years were recruited through probability sampling from 4 U.S. communities: Washington County, Maryland; Forsyth County, North Carolina; the suburbs of Minneapolis, Minnesota; and Jackson, Mississippi. Participants were seen at 4 study visits approximately 3 years apart from 1987-1989 through 1996-1998, and a fifth examination visit in 2011-2013 (Figure 11). All study participants provided written informed consent, and study protocols were approved by the relevant institutional review boards.

The baseline for the present analysis was visit 4, which allows for the investigation of the association of ethanol intake in mid-life and subsequent 15-year cognitive decline from mid-to-late life, and assessment of modification of this association by genetic variants that are associated with weekly ethanol intake (Figure 11). Of the 9,576 African-American and European-American participants for whom we had genetic data at visit 4**,** we excluded participants who were missing general cognitive function measures at study baseline (n=354), and those with missing covariates (n=39), giving a final sample size of 9,183 participants at study baseline.

Exposures

Assessment of Ethanol Intake

Ethanol intake was assessed at all visits by means of an interviewer-administered questionnaire (Figure 11) [448]. Participants were asked if they currently or formerly drank alcoholic beverages. 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. Ethanol intake was recorded as 0 g/wk. for current drinkers having less than one drink per week. Total ethanol intake was analyzed as the natural log of (ethanol use in g/wk. +1) given the skewed distribution.

Assessment of Cognitive Function

Cognitive function was assessed at visit 4 (1996-1998; ages 54-73) and visit 5 (as part of the ARIC-NCS) (2011-2013; ages 70-89 years) using 3 standardized cognitive tests to assess different domains of cognition: verbal learning and short-term memory, executive function and processing speed, and executive function and expressive language (Figure 11).

Verbal learning and recent memory were assessed by the delayed word recall test (DWRT). Participants were asked to learn 10 nouns, and after a five-minute delay were given 60 seconds to recall the words. The DWRT score is the number of words recalled (0-10) [201]. Executive function and processing speed were assessed by the digit symbol substitution test (DSST). Participants were given 90 seconds to fill in blank squares with symbols corresponding to digits from 1 to 9 using a key that matches digits to symbols [199]. Executive function and expressive language were assessed by the word fluency test (WFT) , during which participants generate as many words starting with the letters F, A, and S as possible within 60 seconds, with one trial per letter [385]. The WFT score is the total number of acceptable words generated for the three letters [205]. All three tests were administered by trained examiners using standardized protocols in a quiet room. Recordings were reviewed for quality control.

Using data from these tests in a factor analysis, factor scores for general cognitive performance were derived [387]. Briefly, the factor analysis is a structured approach for identifying common covariation between specific indicators, in this case the cognitive tests, to reduce measurement error when combining data across multiple cognitive tests. The interpretations of factor scores are similar to that for z scores because they were scaled to have a mean of 0 and standard deviation of 1 at ARIC visit 2 when the participant's cognitive function was first tested [387].

Covariates

Age, sex, and educational attainment (< high school, high school, >high school), and smoking status (current, former, never) were assessed at visit 4 via self-report from the home interviews. Time spent in moderate to vigorous physical activity in MET-minutes/week was measured at visits 1 and 3 using the modified Baecke questionnaire [390]. APOE ε4 (0,1,2) was genotyped by TaqMan assay

(Applied Biosystems, Foster City, California) [393, 394]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Diabetes (yes, no) was defined as self-reported history of a physician's diagnosis of diabetes, fasting blood glucose level of ≥126 mg/dL, or non-fasting blood glucose level of ≥200 mg/dL, or diabetes medication use in the past 2 weeks. Stroke was defined by a self-reported history at visit one or an adjudicated event between visits 1 and 4 [396]. Dietary factors were assessed using an interviewer-administered 66-item FFQ measuring usual intake of foods over the past year. We calculated the Healthy Food Score, adapted from Steffen et al. described elsewhere [391, 392].

SNP selection

Genotyping

ARIC study participants were imputed separately by race using IMPUTE2 [397] with the 1000 Genomes Project phase 1 (March 2012) reference panel. Quality control excluded individuals based on single nucleotide polymorphism (SNP) mismatch, high discordance with previous TaqMan assay genotypes, genetic outlier status, and relatedness. SNPs with IMPUTE info score < 0.8 or minor allele frequency (MAF) < 0.05 were excluded. Only autosomal variants (on chromosomes 1–22) were considered [398]. Principal components analysis was used to estimate population substructure with EIGENSTRAT [399].

Investigation of previously reported GSCAN regions

The GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) meta-analyses of 941,280 participants of European ancestry from 34 studies, including the ARIC study, identified 100 single nucleotide polymorphisms (SNPS) in 82 genetic loci to be independently associated with number of drinks per week (Appendix Table 1) [400]. In the ARIC 1000 Genome imputed dataset, 99 of the 100 SNPs were available for ARIC European-American participants (Appendix Table 1), and 74 SNPs were available for ARIC African-American participants (Appendix Table 1).

Replication of index variants

Among ARIC European-American participants, we assessed the association between the 99 GSCAN SNPs and weekly ethanol intake assessed across ARIC visits 1-4 (Appendix Table 2). Of the 99

SNPs, 11 SNPs direction of effect were consistent with the effect reported by GSCAN and were nominally significantly associated (P-value <0.05) with at least one measurement of weekly ethanol intake assessed across ARIC 1-4 (Appendix Table 4).

Among ARIC African-American participants, we assessed the association between the 74 GSCAN SNPs and weekly ethanol intake assessed at study baseline (Appendix Table 3). We identified one SNP (rs12795042 (*LOC646522*)) that was nominally significantly associated with weekly ethanol intake at study baseline; however, the direction of effect for SNP s12795042 effect was not consistent the effect reported by GSCAN (Appendix Table 3). Consequently, this SNP was not included in our study.

Identification of population appropriate tag SNPs

To characterize the best tag SNP in ARIC African-American participants, we conducted fine-mapping in the 1 MB region (± 500 kb windows surrounding each of the 99 GSCAN SNPs (index SNPs). Within each region, we identified the most strongly associated SNP with weekly ethanol intake at study baseline and in linkage disequilibrium (LD) (r^2>0.2) with the index SNP. We identified a total of 92 mostly strongly associated SNPs (Appendix Table 5). Of the 92 SNPs, 20 SNPs direction of effect were consistent with their index SNPs and were nominally significantly associated with at least one measurement of weekly ethanol intake assessed across ARIC 1-4 (Appendix Table 6). Conditional analyses were performed and determined that 20 SNPs are independent of their GSCAN index SNPs (Appendix Table 7). These 20 SNPs were used to address Specific Aim 2 among ARIC African-American participants (Appendix Table 6).

Genetic Risk Score

To study the cumulative effect of multiple gene loci, an unweighted genetic risk score (GRS) for ethanol intake was computed for each study participant. Race-specific unweighted GRS were calculated by summing the number of ethanol intake risk alleles for the 20 SNPs for African-American participants ($uGRS_{20}$), and 11 SNPs for European-American participants ($uGRS_{11}$). We defined a risk allele as the allele that is associated with a unit increase in log-ethanol intake level. By construction, a higher GRS score indicated an estimated greater predisposition to ethanol intake.

Statistical Analysis

Multiple Imputation

Missing data due to attrition were imputed by multiple imputation using chained equations (MICE)[401]. Missing ethanol intake and cognitive data across ARIC visits were imputed based on the observed values of key covariates for a given individual, as well as the relations observed in the data for other participants. To account for the uncertainty of the imputation and ensure correct standard error estimation [402], 25 datasets were imputed. Validation of the MICE approach for cognitive outcome in ARIC has been previously reported and it has been determined that MICE produced unbiased imputed values [403]. For this study, validation using observed data demonstrated MICE produces unbiased imputation of global cognition factor z-scores (Appendix Figure 1).

Statistical Modeling

Our study outcome, fifteen-year cognitive performance change was calculated by subtracting visit 4 cognitive performance z-score from visit 5 neurocognitive exam z-score.

To evaluate if the association between ethanol intake during mid-life and cognitive decline from mid-to-late life is modified by predisposition to ethanol intake, multivariable linear regression models were used and included the unweighted GRS, log-ethanol measured at study baseline, an interaction term between the unweighted GRS and log-ethanol intake, and covariates: age, age squared, sex, race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity and *APOE* ε4 status. All models were race-stratified and further adjustments were made for principal components to account for population stratification.

Statistical tests were 2-sided, and the test for statistically significant interaction was set a priori at $P<0.10$. However, adjustment for multiple testing using the Bonferroni method was performed for the interaction analyses based on single SNPs (African-Americans: $P<0.005$, European-Americans, $P<0.009$).

Multiple Imputation were performed with Stata15 (StataCorp, College Station, TX, USA) [423], and statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) .The

results from each imputed data set were summarized using Rubin's rule [424] into an overall estimate accounting for both within and between imputation variances.

4. Results

Description of Baseline Characteristics

The mean age of participants at study baseline was 63 years (52-75 years), 56% were female, and 19% were African-American (Table 31). Compared to European-Americans, African-Americans were less educated, had a higher proportion of current smokers (17.0% vs 14.2%), higher prevalence of diabetes (25.8% vs. 13.4%) and stroke (3.9% vs. 2.7%), and lower levels of physical activity (8.2 (11.9) vs.12.3 (13.5)). In addition, African-Americans had lower levels of weekly ethanol intake (15.9 (56.6) vs. 38.3 (86.0)), lower prevalence of excessive drinking (6.5% vs. 7.2%), and lower general cognitive performance factor scores compared to European-American participants .

The frequency distributions of $uGRS_{20}$ and $uGRS_{11}$ were approximately normally distributed (Figure 12). The mean for $uGRS_{20}$ was 12.1 (SD=2.7) for African-Americans, and the mean for $uGRS_{11}$ was 5.8 (2.1) for European-American participants. Among African-American participants, $uGRS_{20}$ was associated with decreased ethanol intake at study baseline (β= -0.032, P=0.019) (Appendix Table 8) and explained approximately 0.30% of the variation in ethanol intake at study baseline. Among European-American participants, $uGRS_{11}$ was associated with increased ethanol intake at study baseline (β=0.023, P=0.041) (Appendix Table 9**)** and explained approximately 0.06% of the variation in ethanol intake at study baseline.

Association of log-ethanol intake and 15-year cognitive decline

The association between log-ethanol intake in mid-life and 15-year change in general cognitive performance from mid-to-late life is presented in Table 32. The multivariable linear regression models do not support an association between log-ethanol intake and 15-year change in general cognitive performance (African-American: β for log ethanol intake (log grams per week)= -0.011 (95% CI: -0.052,0.031), European-Americans: β for log ethanol intake (log grams per week)=-0.01 (-0.021,0.002)).

Interaction between log-ethanol intake and 15-year cognitive decline

Results from the multivariable linear regression models suggest no interaction of $uGRS_{20}$ with log-ethanol intake in mid-life on 15-year change in general cognitive performance from mid-to-late life among African-American participants (P=0.811) (Table 33). No interaction was observed of $uGRS_{11}$ and log-ethanol intake in mid-life on 15-year change in general cognitive performance from mid-to-late life among European-American participants (P=0.847) (Table 33).

Furthermore, after adjustment for multiple testing, no significant interaction was observed for any of the individual SNPs in relations to 15-year change in general cognitive performance among African-American (Appendix Table 16) and European-American participants (Appendix Table 17).

5. Discussion

This study investigated the association between ethanol intake in mid-life and 15-year change in general cognitive performance from mid-to-late life, and possible effect modification by genetic predisposition to ethanol intake. We found no evidence that ethanol intake in mid-life is associated with lesser 15-year cognitive decline from mid-to-late life. In addition, we found no evidence that the association between ethanol intake at mid-life and 15-year change in general cognitive performance from mid-to-late life is modified by an unweighted genetic risk score of ethanol intake-associated SNPs, or by any individual SNPs that is associated with ethanol intake among African-American and European-American participants.

The mechanisms underlying the association of ethanol intake and cognitive decline are complex. Low-to-moderate ethanol intake is hypothetically associated with cognitive decline through cerebrovascular and cardiovascular pathways, involving effects that play out over an extended period of time [324, 439]. Heavy ethanol intake on the other hand has detrimental short- and long-term effects on the brain [440, 441], including direct neurotoxic effect [441], proinflammatory effects [441, 442], and indirect impact via cerebrovascular disease [327] and vitamin deficiency [443].

Results from cross-sectional studies on the relationship between ethanol intake and cognitive function have been mixed, with several suggesting a protective effect of moderate ethanol intake on cognitive function [444-446]. To be considered, cross-sectional studies are open to bias due to reverse causation and are susceptible to selection bias and residual confounding[449]. A cross-sectional analysis of the association of ethanol intake with MRI-defined cerebral abnormalities conducted in the Atherosclerosis Risk in Community (ARIC) study reported no significant neuroprotective effect of low-to-moderate ethanol intake on white matter grade in men and women in middle-aged adults [447].

Findings on the relationship between ethanol intake and cognitive decline reported by prospective studies also have been inconsistent [32, 93, 336-348]. Our results add to the knowledge base by overcoming some of the limitations of previous studies, which includes short follow-up times (<5 years), homogeneous study populations (primarily white Europeans), and an analytic approach that does not appropriately consider effect modifiers and attrition.

Our observation of a lack of association of ethanol intake in mid-life with 15-year cognitive decline from mid-to late life is consistent with the results reported by the Lothian Birth Cohort 1936 Study of 1,079 white European participants, which examined the association between ethanol intake in late-life and lifetime cognitive change in cognitive ability, and possible effect modification by a four-SNP score indexing alcohol dehydrogenase activity (*ADH7* rs284779, *ADH1B* rs4147536, *ADH1A* rs975833 and *ADH1A* rs2866151) [381].In contrast to our study results, a significant interaction between ethanol intake in late-life and a four-SNP score influenced lifetime change in cognitive ability in the Lothian Birth Cohort 1936 study (interaction beta parameter estimate= -1.13, p=0.007). Unlike our study that investigated ethanol intake during mid-life, the Lothian Birth Cohort 1936 study examined ethanol intake measured in late-life, a period that may not reflect the most critical exposure window for disease risk and that may be influenced by other medical conditions developing in later life [450]. Adding uncertainty, cognitive function was measured ages at ~11 years and ~70 years in the Lothian Birth Cohort 1936 study. Despite the study's extended follow-up, attrition was not accounted for in the analyses.

Previous studies provide evidence that genetic risk scores (GRS) derived from European-based GWAS are biased towards Europeans and are less accurate and predictive when applied to racially/ethnically diverse populations [451]. As a result, race-specific GRSs were created to measure an individual's predisposition to ethanol intake among ARIC African-American and European-American participants. However, our GRS have not been validated in other populations and likely overestimate the effect of these variants in the population. Validation of the GRS developed in ARIC African-American participant is needed in a separate dataset to prove that the GRS did not overfit this study's data and produced inflated results [452]. Moreover, we acknowledge that genotyping arrays considered herein are European biased. Nonetheless, it is well-established that GRSs are statistically powerful for detection of gene-environment (GxE) interactions in comparison to the common univariate single-variant approaches[453, 454].

Limitations and Strengths

Several limitations of this study should be mentioned. The lack of association between ethanol intake in mid-life and cognitive decline in general cognitive performance from mid-to-late life observed in our study may be the result of measurement variability, such as in the assessment of ethanol intake, which could have attenuated the effect estimates. The use of a standardized instrument administered by trained personnel and the availability of repeat measurements mitigates this concern. Cohort attrition over the prolonged follow up could have biased an association toward the null, if differentially related to ethanol intake [455]. No clear pattern of association of ethanol intake with attrition was observed, and sensitivity analysis indicated that missing data patterns were effectively corrected by MICE imputation. The low prevalence of heavy drinking in our study population limited our ability to estimate the impact of heaving drinking on cognitive performance over time. The unweighted GRS for African-American (0.30%) and European-American (0.06%) participants only explain a modest proportion of the total variation in weekly ethanol intake. Given the modest sample size, this study may be insufficiently powered to detect effect measure modification by the genetic risk scores for African-Americans and

European-American participants. Lastly, although community-based our results emerge from 4 geographically defined, closed cohorts and may not widely generalize to other populations.

Strengths of this study include the large population-based probability sample of middle-aged African Americans and European-Americans, a prospective design with 15 years of follow-up with repeated measurements of ethanol intake and well-characterized cognitive function. Ethanol intake was assessed using an instrument with beverage-specific questions (thus reducing under-reporting) that differentiated never from former drinkers. The rich covariate data that allowed adjustment of lifestyle, genetic, and clinical risk factors is a further strength. The potential of population stratification was addressed by controlling for principal components all analyses.

6. Conclusion

This results from this study suggest that ethanol intake in mid-life is not associated with cognitive decline from mid-to late-life among African-American and European-American adults. Furthermore, this study suggests the association of ethanol intake with cognitive decline is not influenced by genetic variants known to influence ethanol metabolism.

7. Main Tables and Figures

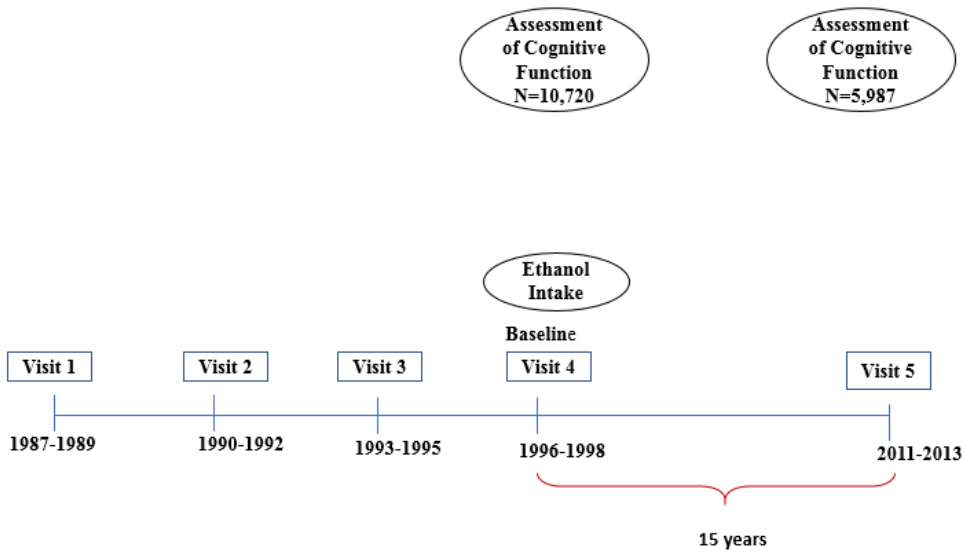


Figure 11. Timeline for the Atherosclerosis Risk in Communities (ARIC) Study for Specific Aim 2

Table 31. Population characteristics, by race ethnicity, Atherosclerosis Risk in Communities (ARIC) study visit 4, 1996-1999 (N=9183)†

	African-Americans	European-Americans	Total
Characteristics	(N=1733)	(N=7450)	(N=9183)
Age (years), mean (SD)	61.8 (5.7)	63.1 (5.6)	62.8 (5.7)
Female, n (%)	5105 (55.6)	1125 (64.9)	3980 (53.4)
Study center, n (%)			
Forsyth County, NC	182 (10.5)	2109 (28.3)	2291 (24.9)
Jackson, MS	1551 (89.5		1551 (16.9)
Minneapolis, MN		2842 (38.1)	2842 (30.9)
Washington County, MD			
Education, n (%)		2499 (33.5)	2499 (27.2)
Less than high school	506 (29.2)	3390 (45.5)	3896 (42.4)
High school, GED, or vocational school	590 (34.0)	1012 (13.6)	1602 (17.4)
College, graduate, or professional school	637 (36.8)	3048 (40.9)	3685 (40.1)
Smoking, n (%)			
Never Smokers	797 (46.0)	2953 (39.6)	3750 (40.8)
Former Smokers	642 (37.0)	3436 (46.1)	4078 (44.4)
Current Smokers	294 (17.0)	1061 (14.2)	1355 (14.8)
Body Mass Index (kg/m2), mean (SD)	30.8 (6.4)	28.3 (5.3)	28.8 (5.6)
Diabetes, n (%)	447 (25.8)	1001 (13.4)	1448 (15.8)
Hypertension, n (%)	1005 (58.0)	2435 (32.7)	3440 (37.5)
History of Stroke, n (%)	67 (3.9)	203 (2.7)	310 (3.2)
Diet score, mean (SD)	20.2 (6.0)	20.8 (5.3)	20.7 (5.5)
Physical activity (met-min/week), mean (SD)	8.2 (11.9)	12.3 (13.5)	11.6 (13.3)
APOEε4 allele present, n (%)	675 (38.9)	1987 (26.7)	2662 (29.0)
Ethanol Intake (Grams/Week), mean (SD)	15.9 (56.6)	38.3 (86.0)	34.0 (81.7)
History of excessive drinking *, n (%)	113 (6.5)	533 (7.2)	646 (7.0)
General cognitive function factor score, mean (SD)	-0.73 (0.74)	0.13 (0.71)	-0.04 (0.79)

† Counts and percentage were calculated based on data prior to imputation for missing ethanol intake data for Atherosclerosis Risk in Communities (ARIC) Study visits 1-4.

Abbreviations: SD, standard deviation; n, number of participants; %, percent; NC, North Carolina, MS, Mississippi; GED, general educational development; kg/m2, grams per meter squared; met-min/week, metabolic equivalent of task per week; APOEε4, apolipoprotein epsilon 4 allele; g/wk., grams per week; self-reported at visit 3. “Was there ever a time in your life when you consumed 5 or more drinks of any kind of alcoholic beverage almost every day?”

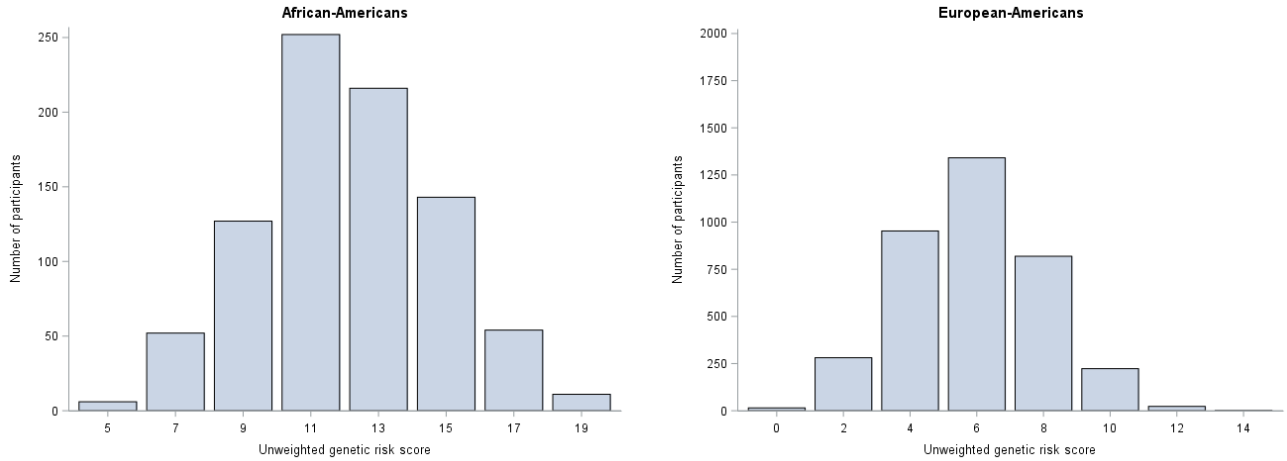


Figure 12. Distribution of the unweighted genetic risk score for Atherosclerosis Risk in Communities (ARIC) study African-American (uGRS20) and European-American (uGRS11) populations

Table 32. Linear regression model results for the association of log ethanol intake at study baseline with 15-cognitive change from ARIC visits 4 and 5, by race

Race/Ethnicity	N	Beta (95% CI)	SE	P-value
African-Americans	1733	-0.011 (-0.052,0.031)	0.021	0.619
European-Americans	7450	-0.010 (-0.021,0.002)	0.006	0.089

All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances.
Abbreviations: ARIC, Atherosclerosis Risk in Communities Study; CI, Confidence Intervals; and SE, Standard Error.

Table 33. Linear regression results for the interaction of the unweighted genetic risk score (GRS) x log-ethanol intake interaction in relation to 15-year cognitive change in general cognitive performance, by race*

Race/Ethnicity	β_E	P_E	β_{GRS}	P_{GRS}	β_{GRSXE}	P_{GRSXE}
African-Americans	0.008	0.901	-0.007	0.432	-0.001	0.811
European-Americans	-0.010	0.457	-0.001	0.822	-0.0004	0.847

* Unweighted genetic risk score (GRS) was created by summing together the number of ethanol intake -increasing alleles for 20 single nucleotide polymorphism (SNPs) among African-Americans and 11 SNPs among European-Americans that are associated with weekly ethanol intake

All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances.

Abbreviations: β_E, estimate for log-ethanol intake with corresponding p-value (P_E) ; β_{GRS}, estimate for the unweighted GRS with corresponding p-value (P_{GRS}); β_{GRSXE}, estimate for the interaction term between the unweighted GRS and log-ethanol intake with corresponding p-value (P_{GRSXE})

Adjusted for age, sex, race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity, APOE ε4 status, and principal components

CHAPTER VI: CONCLUSIONS

6.1. Recapitulations of Specific Aims

Cognitive impairment is a growing public health problem in the U.S. due to a rapidly aging and increasingly diverse population [1]. To reduce associated disability and morbidity [2], caretakers' burden [3-7], and high health care costs [8], it is important to identify and intervene upon modifiable factors that may prevent or reduce the risk of cognitive impairment. One such factor is ethanol use, which has a high prevalence of use (70%) and misuse (14%) in the U.S. [9]. The relationship between ethanol intake and cognitive decline has been studied extensively, but findings have been inconsistent. While heavy ethanol intake is associated with greater cognitive decline [348], low-to-moderate ethanol intake has been associated with less cognitive decline [336, 339, 340, 342-344, 346, 347] or no cognitive decline [93, 345, 348]. Limitations of previous studies include 1) single measurement of ethanol intake [93, 336, 339-341, 344-347] that does not capture changes in individuals' drinking habits over time [432, 433], which may affect their risk of developing disease [434, 435]; 2) the assessment of ethanol intake late in life, a period that may not reflect the most critical exposure window for disease risk and that may be influenced by other medical conditions developing in later life; and 3) not taking potential confounders and effect modifiers into account. In addition, few studies have been conducted in diverse populations despite their disproportionate burden of Alzheimer's disease and other forms of cognitive impairment.

Therefore, the goal of this doctoral research was to provide clear antecedent-consequent estimates of the ethanol intake-cognitive decline association by studying the relationship of long-term patterns of ethanol intake in mid-life and cognitive decline from mid-to-late life, and to inform mechanisms by which ethanol affects cognition by exploring possible effect modification of this association by ethanol intake-associated genetic variants. To achieve this goal, we: 1) Characterized the temporal trajectories of ethanol intake during mid-life in African-American and European-American adults and examined whether long-

term trajectories of ethanol intake in mid-life were associated with 15-year rate of decline in cognition from mid-to-late life among African-American and European-American adults; and 2) evaluated the evidence for the effect modification of the ethanol intake-cognitive decline relationship by ethanol intake associated SNPs in African-American and European-American men and women from mid-to-late life.

We addressed our study aims using data from the Atherosclerosis Risk in Communities (ARIC) study, a large population-based probability sample of middle-aged African-Americans and European-Americans recruited from 4 U.S. communities in 1987-1989 and followed through 2013. Participants had repeated assessment of ethanol intake by an interviewer-administered questionnaire [169] and assessment of cognitive function at two time points using 3 cognitive tests that assessed different domains of cognition. In addition, participants were well-characterized on lifestyle and clinical risk factors. Genetic data was available for the majority of participants.

6.2. Main Findings

The final analytic sample for Specific Aim 1 included 10,876 (n=2,169 African-Americans, n=8,707 European-Americans) participants of the ARIC study who completed repeated assessments of ethanol intake using an interviewer-administered questionnaire across a 9-year interval (1987-1998) and two neurocognitive examinations at 1996 and 2013. We found no evidence that stable low-to-moderate drinking and mostly low-to-moderate alcohol consumption in mid-life was associated with l5-year cognitive decline from mid-to-late life compared to stable never drinking, after adjustment for attrition. In addition, we found no evidence that stable heavy drinking, mostly heavy drinking, stable former drinking, and mostly former drinking were associated with greater 15-year cognitive decline from mid-to-late life, after adjustment for attrition. In contrast, the 15-year change in digit symbol substitution test (a test of executive function and processing speed) for mostly heavy drinkers was slightly higher than that of stable never drinkers, equivalent to a 14% greater decline. No association was found for ethanol intake averaged across 9 years during mid-life and 15-year cognitive decline from mid-to-late life. Further, we did not observe an association of ethanol intake at baseline with 15-year cognitive decline, except for digit symbol substitution test in European-American participants, after adjustment for attrition. The 15-

year rate of change for heavy drinkers was slightly higher than never drinkers at study baseline, equivalent to a 13% greater decline. We observed similar declines in cognitive performance for stable drinking categories and drinking categories measured at study baseline in African-Americans and European-Americans. Overall, a slightly lower rate of decline was observed among African-Americans.

The final analytic sample for Specific Aim 2 included 9,183 participants (n=1,733 African-Americans and n=7,450 European-Americans) who had genetic data available. At study baseline, we observed lower levels of weekly ethanol intake, lower prevalence of excessive drinking, and lower general cognitive performance factor score among African-Americans compared to European-American participants. We found no evidence that ethanol intake in mid-life is associated with lesser 15-year cognitive decline from mid-to-late life. In addition, we found no evidence that the association between ethanol intake at mid-life and 15-year change in general cognitive performance from mid-to-late life is modified by an unweighted genetic risk scores of ethanol intake-associated SNPs or by any individual SNPs that is associated with ethanol intake among African-American and European-American participants.

6.2.1. Strengths

Our study is the second prospective study to examine the association between ethanol intake and cognitive decline using repeated measurements of ethanol intake. It is the only study to examine the association of long-term patterns of ethanol in mid-life and cognitive decline from mid-to-late life in African-American and European-American populations. Strengths of this study include the large population-based bi-racial probability sample of middle-aged African-Americans and European-Americans, a prospective design with 15 years of follow-up with repeated measurements of ethanol intake and well-characterized cognitive function in 3 cognitive domains. Ethanol intake was assessed using an instrument with beverage-specific questions that differentiated never from former drinkers. Additionally, we had rich covariate data that allowed adjustment of lifestyle, genetic, and clinical risk factors.

We were also able to address an additional limitation of previous studies, namely accounting for differential susceptibility and vulnerabilities to the effects of alcohol consumption on cognitive decline.

Our study was only the second study to evaluate for effect modification of the association of ethanol intake and cognitive decline by ethanol intake-associated genetic variants, although it is the first study to evaluate for effect modification using mid-life ethanol intake as exposure and the first to be conducted in an African-American population. As our understanding of the molecular mechanisms that regulate and metabolize ethanol continues to grow, studies like ours will grow in importance. Further strengths of this study include the measurement of ethanol intake by an interviewer-administered questionnaire, repeated measurements of cognitive decline which allowed the study of changes in cognitive function from mid-to-late life, and detailed assessments of lifestyle, genetic, and clinical risk factors. Additional strengths of this study include the availability of quality-controlled genetic variants in most study participants, and the ability to address the potential of population stratification by controlling for principal components in all analyses.

6.2.2. Limitations

Several limitations should also be highlighted. The observation of a lack of association between ethanol intake in mid-life and cognitive decline in general cognitive performance from mid-to-late life could be the result of error in the measurement of ethanol intake, that may have attenuated the effect estimates; however, the use of a standardized instrument administered by trained personnel and the availability of repeat measurements mitigates this concern. Cohort attrition over the prolonged follow up could have biased an association toward the null, if non-differentially related to ethanol intake [455]. No clear pattern of association of ethanol intake with attrition was observed, and sensitivity analysis indicated that missing data patterns were effectively corrected by MICE imputation. The low prevalence of heavy drinking in our study population limited our ability to estimate the impact of heaving drinking on cognitive performance over time. In addition, although the genetic risk scores were based on information from well-established ethanol intake associated SNPs, these variants only explain a limited proportion of the total variation in weekly ethanol intake (African-Americans=0.30%, European-Americans=0.06%). Lastly, although community-based our results emerge from 4 geographically defined, closed cohorts and may not widely generalize to other populations.

6.3. Overall Conclusions

The results from this study suggest that stable low-to-moderate drinking and stable heavy drinking in mid-life are not associated with cognitive decline from mid-to late-life among African-American and European-American adults. Slightly higher 15-year rate of cognitive decline was observed for stable heavy drinkers than stable never drinkers. Our findings are consistent with previous reports indicating that moderate ethanol intake likely is not be protective of cognitive decline. Our results support the American Heart Association (AHA) recommendation that adults who consume alcohol should do so at moderate level (≤2 drinks per day for men; ≤1 for women) while cautioning non-drinkers not to start drinking alcohol in order to reduce their risk of cardiovascular and certain types of cancer [456].

Our study also suggests that the association of ethanol intake with cognitive decline does not depend on genetic variants that influence alcohol metabolism, among African-American as well as European-American adults. This finding is inconsistent with a previous report of evidence of effect modification. Therefore, larger, longitudinal studies in populations of diverse ancestry are needed to improve our understanding of the mechanisms by which ethanol intake affects cognitive function.

APPENDIX A: SNPS IDENTIFIED BY THE GWAS SEQUENCING CONSORTIUM OF ALCOHOL AND NICOTINE USE (GSCAN) TO BE GENOME-WIDE SIGNIFICANTLY ASSOCIATED WITH DRINKS PER WEEK IN A META-ANALYIS OF 941, 280 INDIVIDUALS OF EUROPEAN-AMERICAN ANCESTRY FROM 34 STUDIES

Appendix Table 1. SNPs identified by the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) to be genome-wide significantly† associated with drinks per week in a meta-analysis of 941, 280 individuals of EA ancestry from 34 studies [407]

Locus	Gene	rsID	Chr	Position	EA	OT	EAF	Beta	SE	P-value	Available in ARIC
1:4048453-5048453	*Intergenic*	rs705687	1	4548453	G	A	0.785	-0.011	0.002	8.20E-10	AA, EUR
1:33337334-34337334	*PHC2*	rs58107686	1	33837334	A	C	0.328	-0.010	0.002	7.80E-10	AA, EUR
1:65907700-66907700	*PDE4B*	rs12088813	1	66407700	C	A	0.267	-0.009	0.002	1.60E-08	AA, EUR
1:70991890-71991890	*PTGER3*	rs5024204	1	71491890	T	A	0.278	0.010	0.002	2.60E-09	AA, EUR
1:96481736-97481736	*Intergenic*	rs184083806	1	96981736	C	T	0.007	-0.048	0.009	3.40E-08	EUR
1:164619792-165619792	*Intergenic*	rs10753661	1	165119792	A	G	0.684	-0.009	0.002	3.80E-08	AA, EUR
1:173348808-175396299	*ZBTB37*	rs28680958	1	173848808	A	G	0.217	-0.011	0.002	5.10E-10	AA, EUR
1:205219532-206219532	*Intergenic*	rs823114	1	205719532	A	G	0.553	0.009	0.001	2.30E-09	AA, EUR
2:-69025-930975	*Intergenic*	rs77165542	2	430975	T	C	0.035	-0.026	0.004	5.60E-11	EUR
2:27230940-28746841	*GCKR*	rs1260326	2	27730940	C	T	0.601	0.021	0.001	8.10E-45	AA, EUR
2:27230940-28746841	*GPN1*	rs2178197	2	27860551	G	A	0.569	-0.009	0.001	2.50E-09	AA, EUR
2:43771496-45655276	*LINC01833*	rs13383034	2	45155276	T	C	0.329	0.015	0.002	6.30E-22	AA, EUR
2:43771496-45655276	*LINC01833*	rs1004787	2	45159091	A	G	0.551	0.008	0.001	8.40E-09	AA, EUR
2:62478981-64081507	*WDPCP*	rs13032049	2	63581507	G	A	0.283	0.010	0.002	3.00E-10	AA, EUR
2:73834462-74834462	*Utr3:TET3*	rs828867	2	74334462	A	G	0.545	0.009	0.001	2.20E-09	AA, EUR
2:97168945-98775354	*ACTR1B*	rs11692435	2	98275354	A	G	0.085	0.017	0.003	2.50E-11	EUR
2:143725215-144725215	*ARHGAP15*	rs13024996	2	144225215	A	C	0.364	-0.011	0.002	5.70E-13	AA, EUR
2:147456293-148456293	*Intergenic*	rs72859280	2	147956293	T	G	0.036	0.023	0.004	4.40E-09	EUR
2:224975560-225975560	*Intergenic*	rs56337305	2	225475560	C	T	0.383	-0.010	0.001	1.60E-10	AA, EUR
3:70468431-71468431	*Intergenic*	rs13094887	3	70968431	T	A	0.301	-0.010	0.002	8.60E-11	AA, EUR
3:84408785-85957240	*CADM2*	rs62250685	3	85457240	G	A	0.614	-0.014	0.002	1.10E-21	AA, EUR
3:84408785-85957240	*CADM2*	rs74664784	3	85475292	C	T	0.359	-0.013	0.002	1.60E-14	AA, EUR
3:93494255-94494255	*Intergenic*	rs13066454	3	93994255	T	C	0.398	-0.009	0.001	4.10E-09	AA, EUR
3:131076287-132076287	*CPNE4*	rs9838144	3	131576287	C	G	0.209	-0.010	0.002	2.70E-08	EUR

Appendix Table 1. SNPs identified by the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) to be genome-wide significantly† associated with drinks per week in a meta-analysis of 941, 280 individuals of EA ancestry from 34 studies [407]

Locus	Gene	rsID	Chr	Position	EA	OT	EAF	Beta	SE	P-value	Available in ARIC
3:140767295-141767295	*ZBTB38*	rs2011092	3	141124607	C	T	0.339	-0.009	0.002	7.40E-09	AA, EUR
3:140767295-141767295	*RASA2*	rs60654199	3	141267295	A	C	0.063	-0.017	0.003	2.90E-08	AA, EUR
3:157687811-158687811	*RSRC1*	rs6787172	3	158187811	G	T	0.554	-0.008	0.001	4.30E-08	AA, EUR
4:2946091-3946091	*HGFAC*	rs3748034	4	3446091	T	G	0.143	-0.012	0.002	1.70E-08	EUR
4:38914993-39914993	*Intergenic*	rs7682824	4	39406254	T	C	0.548	0.008	0.002	2.80E-08	AA, EUR
4:38914993-39914993	*KLB*	rs11940694	4	39414993	G	A	0.597	0.026	0.001	3.00E-68	AA, EUR
4:38914993-39914993	*KLB*	rs35538052	4	39418965	A	G	0.379	-0.009	0.002	1.40E-08	AA, EUR
4:41651306-42651306	*BEND4*	rs4501255	4	42151306	G	C	0.235	0.011	0.002	4.80E-10	AA, EUR
4:96764066-101983024	*Intergenic*	rs12499107	4	99678691	G	A	0.131	0.013	0.002	4.50E-09	EUR
4:96764066-101983024	*Intergenic*	rs144198753	4	99713350	T	C	0.016	-0.042	0.006	1.40E-12	EUR
4:96764066-101983024	*ADH5*	rs1154414	4	100000136	C	T	0.141	0.018	0.002	3.70E-17	EUR
4:96764066-101983024	*ADH1B*	rs1229984	4	100239319	C	T	0.963	0.151	0.004	0.00E+00	EUR
4:96764066-101983024	*Intergenic*	rs10028756	4	100254520	A	G	0.129	-0.019	0.002	1.20E-17	EUR
4:96764066-101983024	*ADH1C*	rs561222871	4	100260679	T	C	0.047	-0.039	0.004	6.60E-27	AA, EUR
4:96764066-101983024	*ADH1C*	rs36052336	4	100273594	G	A	0.061	-0.018	0.003	1.20E-09	EUR
4:96764066-101983024	*Intergenic*	rs2165670	4	100286085	A	G	0.106	0.023	0.002	1.70E-22	EUR
4:96764066-101983024	*Synonymous:C4 orf17*	rs17029090	4	100443853	G	A	0.020	-0.049	0.005	4.80E-21	AA, EUR
4:96764066-101983024	*C4orf17*	rs79139602	4	100444363	T	A	0.021	0.060	0.005	1.80E-32	AA, EUR
4:96764066-101983024	*Intergenic*	rs4699791	4	101243023	A	G	0.096	0.019	0.002	6.60E-14	AA, EUR
4:102688709-103688709	*SLC39A8*	rs13107325	4	103188709	T	C	0.072	-0.028	0.003	1.50E-22	EUR
4:143148579-144148579	*INPP4B*	rs4690727	4	143648579	G	C	0.718	0.011	0.002	2.40E-11	AA, EUR
4:152468372-153468372	*Intergenic*	rs10004020	4	152968372	A	G	0.720	0.009	0.002	2.40E-08	AA, EUR
4:170586393-171586393	*Intergenic*	rs12651313	4	171086393	G	C	0.443	-0.009	0.001	3.80E-09	AA, EUR
5:86827886-88354395	*LINC00461*	rs4916723	5	87854395	C	A	0.416	-0.010	0.001	1.70E-11	AA, EUR
5:143912335-144912335	*Intergenic*	rs12655091	5	144412335	A	G	0.530	-0.008	0.001	1.30E-08	AA, EUR
5:155402003-156402003	*SGCD*	rs55872084	5	155902003	T	G	0.235	0.010	0.002	6.30E-09	EUR
5:166303321-167303321	*TENM2*	rs11739827	5	166803321	T	G	0.451	-0.008	0.001	1.20E-08	NA

Appendix Table 1. SNPs identified by the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) to be genome-wide significantly† associated with drinks per week in a meta-analysis of 941, 280 individuals of EA ancestry from 34 studies [407]

Locus	Gene	rsID	Chr	Position	EA	OT	EAF	Beta	SE	P-value	Available in ARIC
7:68574768-70283020	*AUTS2*	rs10085696	7	69783020	G	A	0.186	-0.011	0.002	1.10E-09	AA, EUR
7:72542443-73542443	*Intergenic*	rs6460047	7	73042443	C	T	0.208	0.012	0.002	9.70E-11	AA, EUR
7:98477515-99477515	*ARPC1B*	rs10236149	7	98977515	G	A	0.123	-0.013	0.002	1.20E-09	AA, EUR
7:103340115-104340115	*ORC5*	rs35034355	7	103840115	A	G	0.521	-0.008	0.001	2.90E-08	AA, EUR
7:152989744-153989744	*Intergenic*	rs6951574	7	153489744	C	T	0.458	0.013	0.001	1.60E-19	AA, EUR
8:20449917-21449917	*Intergenic*	rs13250583	8	20949917	T	C	0.213	-0.010	0.002	4.70E-08	AA, EUR
8:64027399-65027399	*Intergenic*	rs1217091	8	64527399	C	T	0.812	0.012	0.002	7.10E-11	AA, EUR
8:126000031-127000031	*Intergenic*	rs28601761	8	126500031	G	C	0.420	0.009	0.001	7.20E-10	AA, EUR
9:108255622-109845993	*Intergenic*	rs55932213	9	108755622	G	A	0.736	0.009	0.002	9.60E-09	AA, EUR
9:108255622-109845993	*Intergenic*	rs10978550	9	109345993	C	T	0.206	-0.012	0.002	7.20E-11	EUR
10:110007806-111007806	*Intergenic*	rs7074871	10	110507806	A	G	0.255	-0.009	0.002	1.90E-08	AA, EUR
10:124593880-125593880	*Intergenic*	rs17665139	10	125093880	T	C	0.149	-0.012	0.002	1.60E-08	EUR
11:8142218-9142218	*TRIM66*	rs7950166	11	8642218	T	C	0.637	-0.010	0.002	9.90E-11	AA, EUR
11:27143725-28143725	*BDNF-AS|LINC00678*	rs11030084	11	27643725	T	C	0.184	-0.011	0.002	1.70E-08	EUR
11:46897353-48410823	*SPI1*	rs56030824	11	47397353	A	G	0.322	-0.012	0.002	1.20E-13	AA, EUR
11:112924042-113924042	*Intergenic*	rs10750025	11	113424042	T	C	0.686	0.010	0.002	4.90E-11	AA, EUR
11:112924042-113924042	*Intergenic*	rs1713676	11	113660576	G	A	0.522	-0.008	0.001	4.30E-08	AA, EUR
11:115575001-116575001	*Intergenic*	rs4938230	11	116075001	A	C	0.842	0.013	0.002	1.50E-10	AA, EUR
11:121044285-122044285	*Intergenic*	rs682011	11	121544285	C	T	0.559	0.008	0.001	2.20E-08	AA, EUR
11:133158168-134158168	*LOC646522*	rs12795042	11	133658168	C	A	0.623	-0.008	0.002	3.30E-08	AA, EUR
12:51395882-52395882	*SLC4A8*	rs10876188	12	51895882	T	C	0.457	-0.008	0.001	4.80E-08	AA, EUR
12:54174235-55174235	*Intergenic*	rs3809162	12	54674235	G	A	0.397	0.009	0.001	1.20E-09	AA, EUR
12:81101464-82875393	*ACSS3*	rs10506274	12	81601464	T	G	0.484	-0.009	0.001	5.80E-10	AA, EUR
12:91670791-92670791	*Intergenic*	rs4842786	12	92170791	A	G	0.584	-0.009	0.001	2.70E-09	AA, EUR
13:26624360-27624360	*Intergenic*	rs500321	13	27124360	T	A	0.736	-0.010	0.002	4.90E-09	AA, EUR
14:56774519-57774519	*OTX2*	rs1123285	14	57274519	G	C	0.335	-0.009	0.002	8.10E-09	AA, EUR
14:58282779-59282779	*ARID4A*	rs2180870	14	58782779	C	T	0.135	-0.012	0.002	1.10E-08	AA, EUR

Appendix Table 1. SNPs identified by the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) to be genome-wide significantly† associated with drinks per week in a meta-analysis of 941, 280 individuals of EA ancestry from 34 studies [407]

Locus	Gene	rsID	Chr	Position	EA	OT	EAF	Beta	SE	P-value	Available in ARIC
14:94344947-95344947	*SERPINA1*	rs28929474	14	94844947	T	C	0.018	-0.037	0.005	1.30E-11	EUR
14:104110138-105110138	*KIF26A*	rs11625650	14	104610138	A	G	0.233	-0.010	0.002	2.90E-08	EUR
15:74527880-75527880	*Intergenic*	rs2472297	15	75027880	T	C	0.249	0.011	0.002	3.10E-10	EUR
15:86296012-87296012	*AGBL1*	rs12907323	15	86796012	G	A	0.411	0.008	0.001	9.90E-09	AA, EUR
16:19513793-20513793	*Intergenic*	rs2764771	16	20013793	A	G	0.307	0.010	0.002	4.00E-10	AA, EUR
16:24310681-25310681	*TNRC6A*	rs17177078	16	24810681	T	C	0.063	-0.022	0.003	1.30E-13	EUR
16:28254684-29254684	*Intergenic*	rs378421	16	28754684	A	G	0.404	-0.011	0.001	4.80E-14	AA, EUR
16:29392184-30974856	*SEZ6L2*	rs113443718	16	29892184	A	G	0.305	-0.010	0.002	1.20E-10	AA, EUR
16:64372590-65372590	*Intergenic*	rs62044525	16	64872590	G	C	0.184	-0.012	0.002	1.00E-10	EUR
16:68631281-69631281	*Intergenic*	rs7185555	16	69131281	C	G	0.153	-0.011	0.002	4.20E-08	AA, EUR
16:71279310-72838507	*LINC01572*	rs79616692	16	72338507	C	G	0.108	0.016	0.002	4.10E-12	AA, EUR
16:73412588-74412588	*Intergenic*	rs1104608	16	73912588	C	G	0.425	-0.011	0.001	1.10E-13	EUR
17:1709888-2709888	*SRR*	rs4548913	17	2209888	A	G	0.632	-0.008	0.002	3.10E-08	AA, EUR
17:6962969-7962969	*TNFSF13|TNFSF13*	rs3803800	17	7462969	G	A	0.786	0.011	0.002	1.50E-10	AA, EUR
17:29215500-30215500	*Intergenic*	rs2854334	17	29715500	G	A	0.615	0.009	0.001	7.50E-10	AA, EUR
17:43159975-45273783	*KANSL1*	.	17	44246624	A	C	0.215	-0.022	0.003	1.60E-17	AA, EUR
17:78024597-79024597	*RPTOR*	rs10438820	17	78524597	T	C	0.702	0.009	0.002	1.80E-08	AA, EUR
18:52552169-53552169	*TCF4*	rs9950000	18	53052169	T	C	0.395	-0.009	0.001	9.40E-10	AA, EUR
18:54580437-55580437	*Intergenic*	rs4092465	18	55080437	G	A	0.635	-0.008	0.002	4.40E-08	AA, EUR
19:48714274-49714274	*Intergenic*	rs281379	19	49214274	A	G	0.508	0.014	0.001	4.90E-21	AA, EUR
20:24535711-25535711	*ACSS1*	rs4815364	20	25035711	A	G	0.616	0.009	0.001	1.00E-08	AA, EUR
22:41446519-42446519	*Intergenic*	rs9607814	22	41946519	A	C	0.200	-0.010	0.002	4.30E-08	AA, EUR

†, p-value threshold $<5.0 \times 10^{-8}$. Abbreviations: SNP, single nucleotide polymorphisms; GWAS, genome-wide association studies; Chr, chromosome; SE, standard error; EA, effect allele, OA, other allele; EAF, effect allele frequency; AA, African-American; EUR, European-American; NA, unavailable

APPENDIX B: ASSOCIATION OF GCAN SNPS WITH WEEKLY ETHANOL INTAKE AT STUDY BASELINE AMONG ARIC EUROPEAN-AMERICAN PARTICIPANTS

Appendix Table 2. Association of GSCAN SNPS (N=99) with weekly ethanol intake at study baseline (visit 4) among ARIC European-American Participants.

				ARIC						GSCAN					
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs705687	Intergenic	1	4548453	G	A	0.789	-0.016	0.040	0.695	G	A	0.785	-0.011	0.002	8.15E-10
rs58107686	PHC2	1	33837334	A	C	0.340	-0.036	0.035	0.303	A	C	0.328	-0.010	0.002	7.79E-10
rs12088813	PDE4B	1	66407700	C	A	0.273	-0.060	0.037	0.111	C	A	0.267	-0.009	0.002	1.58E-08
rs5024204	PTGER3	1	71491890	T	A	0.279	0.011	0.037	0.759	T	A	0.278	0.010	0.002	2.55E-09
rs184083806	Intergenic	1	96981736	C	T	0.008	-0.063	0.187	0.738	C	T	0.007	-0.048	0.009	3.42E-08
rs10753661	Intergenic	1	165119792	A	G	0.696	0.002	0.036	0.962	A	G	0.684	-0.009	0.002	3.76E-08
rs28680958	ZBTB37	1	171086393	A	G	0.217	-0.023	0.040	0.571	A	G	0.217	-0.011	0.002	5.13E-10
rs823114	Intergenic	1	173848808	A	G	0.547	-0.007	0.033	0.837	A	G	0.553	0.009	0.001	2.31E-09
rs77165542	Intergenic	2	430975	T	C	0.023	0.080	0.111	0.468	T	C	0.035	-0.026	0.004	5.63E-11
rs1260326	GCKR	2	27730940	C	T	0.594	-0.047	0.034	0.172	C	T	0.601	0.021	0.001	8.05E-45
rs2178197	GPN1	2	27860551	G	A	0.575	-0.033	0.034	0.322	G	A	0.569	-0.009	0.001	2.45E-09
rs13383034	LINC01833	2	45155276	T	C	0.318	-0.050	0.036	0.158	T	C	0.329	0.015	0.002	6.31E-22
rs1004787	LINC01833	2	45159091	A	G	0.553	-0.002	0.033	0.958	A	G	0.551	0.008	0.001	8.40E-09
rs13032049	WDPCP	2	63581507	G	A	0.284	0.044	0.037	0.235	G	A	0.283	0.010	0.002	3.00E-10
rs828867	Utr3:TET3	2	74334462	A	G	0.552	0.018	0.033	0.583	A	G	0.545	0.009	0.001	2.15E-09
rs11692435	ACTR1B	2	98275354	A	G	0.081	0.017	0.061	0.785	A	G	0.085	0.017	0.003	2.53E-11
rs13024996	ARHGAP15	2	144225215	A	C	0.368	-0.056	0.035	0.103	A	C	0.364	-0.011	0.002	5.72E-13
rs72859280	Intergenic	2	147956293	T	G	0.033	0.059	0.092	0.522	T	G	0.036	0.023	0.004	4.44E-09
rs56337305	Intergenic	2	205719532	C	T	0.385	-0.020	0.034	0.563	C	T	0.383	-0.010	0.001	1.63E-10
rs13094887	Intergenic	3	70968431	T	A	0.299	-0.036	0.036	0.324	T	A	0.301	-0.010	0.002	8.57E-11
rs62250685	CADM2	3	85457240	G	A	0.625	-0.064	0.034	0.063	G	A	0.614	-0.014	0.002	1.05E-21
rs74664784	CADM2	3	85475292	C	T	0.613	-0.061	0.034	0.074	C	T	0.359	-0.013	0.002	1.58E-14
rs13066454	Intergenic	3	93994255	T	C	0.397	-0.041	0.034	0.230	T	C	0.398	-0.009	0.001	4.13E-09
rs9838144	CPNE4	3	131576287	C	G	0.209	-0.036	0.041	0.375	C	G	0.209	-0.010	0.002	2.65E-08
rs2011092	ZBTB38	3	141124607	C	T	0.342	-0.024	0.035	0.487	C	T	0.339	-0.009	0.002	7.35E-09
rs60654199	RASA2	3	141267295	A	C	0.065	0.026	0.067	0.700	A	C	0.063	-0.017	0.003	2.85E-08
rs6787172	RSRC1	3	158187811	G	T	0.558	-0.039	0.033	0.243	G	T	0.554	-0.008	0.001	4.27E-08
rs3748034	HGFAC	4	3446091					0.047	0.031	T	G	0.143	-0.012	0.002	1.67E-08
rs7682824	Intergenic	4	39406254	T	C	0.842	0.010	0.046	0.832	T	C	0.548	0.008	0.002	2.77E-08

Appendix Table 2. Association of GSCAN SNPS (N=99) with weekly ethanol intake at study baseline (visit 4) among ARIC European-American Participants.

				ARIC						GSCAN					
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs11940694	KLB	4	39414993	G	A	0.585	0.031	0.034	0.351	G	A	0.597	0.026	0.001	3.03E-68
rs35538052	KLB	4	39418965	A	G	0.399	-0.021	0.034	0.544	A	G	0.379	-0.009	0.002	1.39E-08
rs4501255	BEND4	4	42151306	G	C	0.229	0.049	0.039	0.213	G	C	0.235	0.011	0.002	4.83E-10
rs12499107	Intergenic	4	99678691	G	A	0.128	-0.023	0.050	0.654	G	A	0.131	0.013	0.002	4.45E-09
rs144198753	Intergenic	4	99713350	T	C	0.011	-0.227	0.158	0.150	T	C	0.016	-0.042	0.006	1.35E-12
rs1154414	ADH5	4	100000136	C	T	0.141	-0.050	0.048	0.297	C	T	0.141	0.018	0.002	3.74E-17
rs1229984	ADH1B	4	100239319	C	T	0.965	0.234	0.091	0.010	C	T	0.963	0.151	0.004	0.00E+00
rs10028756	Intergenic	4	100254520	A	G	0.128	-0.080	0.051	0.116	A	G	0.129	-0.019	0.002	1.16E-17
rs561222871	ADH1C	4	100260679	T	C	0.067	-0.095	0.068	0.161	T	C	0.047	-0.039	0.004	6.56E-27
rs36052336	ADH1C	4	100273594	G	A	0.056	-0.067	0.072	0.356	G	A	0.061	-0.018	0.003	1.23E-09
rs2165670	Intergenic	4	100286085	A	G	0.104	0.035	0.055	0.518	A	G	0.106	0.023	0.002	1.67E-22
rs17029090	Synonymous: C4orf17	4	100443853	G	A	0.020	0.023	0.120	0.846	G	A	0.020	-0.049	0.005	4.75E-21
rs79139602	C4orf17	4	100444363	T	A	0.022	0.038	0.115	0.745	T	A	0.021	0.060	0.005	1.80E-32
rs4699791	Intergenic	4	101243023	A	G	0.096	-0.031	0.057	0.582	A	G	0.096	0.019	0.002	6.58E-14
rs13107325	SLC39A8	4	103188709	T	C	0.081	-0.007	0.061	0.914	T	C	0.072	-0.028	0.003	1.53E-22
rs4690727	INPP4B	4	143648579	G	C	0.729	0.008	0.038	0.822	G	C	0.718	0.011	0.002	2.43E-11
rs10004020	Intergenic	4	152968372	A	G	0.729	0.022	0.037	0.556	A	G	0.720	0.009	0.002	2.43E-08
rs12651313	Intergenic	4	166803321	G	C	0.441	-0.063	0.033	0.060	G	C	0.443	-0.009	0.001	3.79E-09
rs4916723	LINC00461	5	87854395	C	A	0.421	-0.009	0.034	0.780	C	A	0.416	-0.010	0.001	1.72E-11
rs12655091	Intergenic	5	144412335	A	G	0.528	-0.030	0.033	0.368	A	G	0.530	-0.008	0.001	1.25E-08
rs55872084	SGCD	5	155902003	T	G	0.232	0.104	0.039	0.009	T	G	0.235	0.010	0.002	6.32E-09
rs10085696	AUTS2	7	69783020	G	A	0.181	-0.031	0.043	0.479	G	A	0.186	-0.011	0.002	1.12E-09
rs6460047	Intergenic	7	73042443	C	T	0.207	0.063	0.041	0.121	C	T	0.208	0.012	0.002	9.69E-11
rs10236149	ARPC1B	7	98977515	G	A	0.125	-0.008	0.050	0.878	G	A	0.123	-0.013	0.002	1.18E-09
rs35034355	ORC5	7	103840115	A	G	0.524	-0.042	0.033	0.208	A	G	0.521	-0.008	0.001	2.87E-08
rs6951574	Intergenic	7	153489744	C	T	0.468	0.035	0.033	0.295	C	T	0.458	0.013	0.001	1.58E-19
rs13250583	Intergenic	8	20949917	T	C	0.207	0.026	0.041	0.522	T	C	0.213	-0.010	0.002	4.70E-08
rs1217091	Intergenic	8	64527399	C	T	0.803	-0.032	0.042	0.449	C	T	0.812	0.012	0.002	7.05E-11
rs28601761	Intergenic	8	126500031	G	C	0.427	0.062	0.034	0.064	G	C	0.420	0.009	0.001	7.17E-10
rs55932213	Intergenic	9	108755622	G	A	0.731	0.015	0.038	0.692	G	A	0.736	0.009	0.002	9.55E-09
rs10978550	Intergenic	9	109345993	C	T	0.198	0.084	0.042	0.044	C	T	0.206	-0.012	0.002	7.15E-11

Appendix Table 2. Association of GSCAN SNPS (N=99) with weekly ethanol intake at study baseline (visit 4) among ARIC European-American Participants.

				ARIC						GSCAN					
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs7074871	Intergenic	10	110507806	A	G	0.255	-0.036	0.038	0.342	A	G	0.255	-0.009	0.002	1.86E-08
rs17665139	Intergenic	10	125093880	T	C	0.154	-0.011	0.046	0.807	T	C	0.149	-0.012	0.002	1.59E-08
rs7950166	TRIM66	11	8642218	T	C	0.635	-0.068	0.034	0.049	T	C	0.637	-0.010	0.002	9.89E-11
rs11030084	BDNF-AS|LINC00678	11	27643725	T	C	0.190	0.002	0.042	0.963	T	C	0.184	-0.011	0.002	1.72E-08
rs56030824	SPI1	11	47397353	A	G	0.334	-0.006	0.036	0.876	A	G	0.322	-0.012	0.002	1.15E-13
rs10750025	Intergenic	11	113424042	T	C	0.691	0.053	0.036	0.147	T	C	0.686	0.010	0.002	4.89E-11
rs1713676	Intergenic	11	113660576	G	A	0.529	-0.080	0.033	0.017	G	A	0.522	-0.008	0.001	4.29E-08
rs4938230	Intergenic	11	116075001	A	C	0.844	-0.023	0.046	0.615	A	C	0.842	0.013	0.002	1.48E-10
rs682011	Intergenic	11	121544285	C	T	0.555	-0.028	0.034	0.412	C	T	0.559	0.008	0.001	2.22E-08
rs12795042	LOC646522	11	133658168	C	A	0.623	0.020	0.034	0.571	C	A	0.623	-0.008	0.002	3.25E-08
rs10876188	SLC4A8	12	51895882	T	C	0.444	-0.021	0.033	0.523	T	C	0.457	-0.008	0.001	4.84E-08
rs3809162	Intergenic	12	54674235	G	A	0.394	0.014	0.034	0.677	G	A	0.397	0.009	0.001	1.19E-09
rs10506274	ACSS3	12	81601464	T	G	0.486	-0.007	0.033	0.835	T	G	0.484	-0.009	0.001	5.78E-10
rs4842786	Intergenic	12	92170791	A	G	0.582	-0.043	0.034	0.202	A	G	0.584	-0.009	0.001	2.73E-09
rs500321	Intergenic	13	27124360	T	A	0.742	-0.013	0.038	0.739	T	A	0.736	-0.010	0.002	4.92E-09
rs1123285	OTX2	14	57274519	G	C	0.338	-0.046	0.035	0.186	G	C	0.335	-0.009	0.002	8.14E-09
rs2180870	ARID4A	14	58782779	C	T	0.138	-0.047	0.048	0.330	C	T	0.135	-0.012	0.002	1.12E-08
rs28929474	SERPINA1	14	94844947	T	C	0.019	0.003	0.122	0.981	T	C	0.018	-0.037	0.005	1.34E-11
rs11625650	KIF26A	14	104610138	A	G	0.064	0.079	0.068	0.247	A	G	0.233	-0.010	0.002	2.89E-08
rs2472297	Intergenic	15	75027880	T	C	0.253	-0.011	0.038	0.773	T	C	0.249	0.011	0.002	3.10E-10
rs12907323	AGBL1	15	86796012	G	A	0.410	-0.029	0.034	0.386	G	A	0.411	0.008	0.001	9.93E-09
rs2764771	Intergenic	16	20013793	A	G	0.301	0.057	0.036	0.118	A	G	0.307	0.010	0.002	4.02E-10
rs17177078	TNRC6A	16	24810681	T	C	0.069	0.001	0.066	0.992	T	C	0.063	-0.022	0.003	1.27E-13
rs378421	Intergenic	16	28754684	A	G	0.409	-0.012	0.034	0.717	A	G	0.404	-0.011	0.001	4.83E-14
rs113443718	SEZ6L2	16	29892184	A	G	0.288	-0.021	0.037	0.566	A	G	0.305	-0.010	0.002	1.19E-10
rs62044525	Intergenic	16	64872590	G	C	0.184	0.001	0.043	0.990	G	C	0.184	-0.012	0.002	1.03E-10
rs7185555	Intergenic	16	69131281	C	G	0.141	-0.045	0.048	0.348	C	G	0.153	-0.011	0.002	4.24E-08
rs79616692	LINC01572	16	72338507	C	G	0.108	-0.079	0.053	0.137	C	G	0.108	0.016	0.002	4.11E-12
rs1104608	Intergenic	16	73912588	C	G	0.428	-0.003	0.034	0.928	C	G	0.425	-0.011	0.001	1.05E-13
rs4548913	SRR	17	2209888	A	G	0.622	0.037	0.034	0.280	A	G	0.632	-0.008	0.002	3.11E-08

Appendix Table 2. Association of GSCAN SNPS (N=99) with weekly ethanol intake at study baseline (visit 4) among ARIC European-American Participants.

				ARIC						GSCAN					
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs3803800	TNFSF12-TNFSF13|TNFSF13	17	7462969	G	A	0.793	0.021	0.041	0.606	G	A	0.786	0.011	0.002	1.50E-10
rs2854334	Intergenic	17	29715500	G	A	0.611	-0.030	0.034	0.382	G	A	0.615	0.009	0.001	7.51E-10
.	KANSL1	17	44246624	A	C	0.211	0.031	0.040	0.439	A	C	0.215	-0.022	0.003	1.62E-17
rs10438820	RPTOR	17	78524597	T	C	0.699	0.011	0.036	0.756	T	C	0.702	0.009	0.002	1.76E-08
rs9950000	TCF4	18	53052169	T	C	0.401	-0.014	0.034	0.675	T	C	0.395	-0.009	0.001	9.38E-10
rs4092465	Intergenic	18	55080437	G	A	0.625	-0.023	0.034	0.503	G	A	0.635	-0.008	0.002	4.39E-08
rs281379	Intergenic	19	49214274	A	G	0.486	0.058	0.033	0.080	A	G	0.508	0.014	0.001	4.91E-21
rs4815364	ACSS1	20	25035711	A	G	0.619	0.050	0.034	0.144	A	G	0.616	0.009	0.001	1.02E-08
rs9607814	Intergenic	22	41946519	A	C	0.200	0.071	0.041	0.083	A	C	0.200	-0.010	0.002	4.31E-08

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study; Chr, Chromosome; EA, effect allele, OA, other allele; EAF, effect allele frequency; SE, Standard Error; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use.

APPENDIX C: ASSOCIATION OF GSCAN SNPS WITH WEEKLY ETHANOL INTAKE AT STUDY BASELINE (VISIT 4) AMONG ARIC AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 3. Association of GSCAN SNPS (N=74) with weekly ethanol intake at study baseline (visit 4) among ARIC African-American participants

						ARIC						GSCAN			
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs705687	Intergenic	1	4548453	G	A	0.849	0.055	0.066	0.404	G	A	0.785	-0.011	0.002	8.15E-10
rs58107686	PHC2	1	33837334	A	C	0.137	-0.003	0.069	0.969	A	C	0.328	-0.010	0.002	7.79E-10
rs12088813	PDE4B	1	66407700	C	A	0.084	0.131	0.085	0.122	C	A	0.267	-0.009	0.002	1.58E-08
rs5024204	PTGER3	1	71491890	T	A	0.242	-0.016	0.056	0.775	T	A	0.278	0.010	0.002	2.55E-09
rs10753661	Intergenic	1	165119792	A	G	0.882	0.024	0.075	0.753	A	G	0.684	-0.009	0.002	3.76E-08
rs28680958	ZBTB37	1	173848808	A	G	0.406	-0.024	0.048	0.620	A	G	0.217	-0.011	0.002	5.13E-10
rs823114	Intergenic	1	205719532	A	G	0.236	-0.084	0.057	0.145	A	G	0.553	0.009	0.001	2.31E-09
rs1260326	GCKR	2	27730940	C	T	0.858	-0.012	0.068	0.854	C	T	0.601	0.021	0.001	8.05E-45
rs2178197	GPN1	2	27860551	G	A	0.604	0.007	0.048	0.887	G	A	0.569	-0.009	0.001	2.45E-09
rs13383034	LINC01833	2	45155276	T	C	0.206	0.105	0.059	0.073	T	C	0.329	0.015	0.002	6.31E-22
rs1004787	LINC01833	2	45159091	A	G	0.849	0.107	0.068	0.117	A	G	0.551	0.008	0.001	8.4E-09
rs13032049	WDPCP	2	63581507	G	A	0.056	-0.009	0.106	0.933	G	A	0.283	0.010	0.002	3E-10
rs828867	Utr3TET3	2	74334462	A	G	0.230	-0.110	0.057	0.054	A	G	0.545	0.009	0.001	2.15E-09
rs13024996	ARHGAP15	2	144225215	A	C	0.122	0.080	0.075	0.289	A	C	0.364	-0.011	0.002	5.72E-13
rs56337305	Intergenic	2	225475560	C	T	0.300	0.073	0.051	0.157	C	T	0.383	-0.010	0.001	1.63E-10
rs13094887	Intergenic	3	70968431	T	A	0.267	0.062	0.054	0.247	T	A	0.301	-0.010	0.002	8.57E-11
rs62250685	CADM2	3	85457240	G	A	0.152	-0.063	0.071	0.378	G	A	0.614	-0.014	0.002	1.05E-21
rs74664784	CADM2	3	85475292	C	T	0.367	-0.070	0.049	0.157	C	T	0.359	-0.013	0.002	1.58E-14
rs13066454	Intergenic	3	93994255	T	C	0.083	-0.055	0.090	0.539	T	C	0.398	-0.009	0.001	4.13E-09
rs2011092	ZBTB38	3	141124607	C	T	0.129	0.045	0.071	0.523	C	T	0.339	-0.009	0.002	7.35E-09
rs60654199	RASA2	3	141267295	A	C	0.221	0.016	0.058	0.778	A	C	0.063	-0.017	0.003	2.85E-08
rs6787172	Intergenic	3	158187811	T	A	0.739	-0.021	0.053	0.688	T	A	0.301	-0.010	0.002	8.57E-11
rs7682824	Intergenic	4	39406254	T	C	0.791	-0.027	0.058	0.645	T	C	0.548	0.008	0.002	2.77E-08

Appendix Table 3. Association of GSCAN SNPS (N=74) with weekly ethanol intake at study baseline (visit 4) among ARIC African-American participants

						ARIC						GSCAN			
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs11940694	KLB	4	39414993	G	A	0.576	0.091	0.047	0.057	G	A	0.597	0.026	0.001	3.03E-68
rs35538052	KLB	4	39418965	A	G	0.098	-0.040	0.081	0.623	A	G	0.379	-0.009	0.002	1.39E-08
rs4501255	BEND4	4	42151306	G	C	0.683	0.057	0.052	0.274	G	C	0.235	0.011	0.002	4.83E-10
rs561222871	ADH1C	4	100260679	T	C	0.059	-0.012	0.100	0.905	T	C	0.047	-0.039	0.004	6.56E-27
rs17029090	C4orf17	4	100443853	G	A			0.055	0.959	G	A	0.020	-0.049	0.005	4.75E-21
rs79139602	C4orf17	4	100444363	T	A	0.183	-0.040	0.061	0.515	T	A	0.021	0.060	0.005	1.8E-32
rs4699791	Intergenic	4	101243023	A	G	0.101	-0.003	0.078	0.971	A	G	0.096	0.019	0.002	6.58E-14
rs4690727	INPP4B	4	143648579	G	C	0.834	0.045	0.063	0.480	G	C	0.718	0.011	0.002	2.43E-11
rs10004020	Intergenic	4	152968372	A	G	0.726	0.094	0.052	0.070	A	G	0.720	0.009	0.002	2.43E-08
rs12651313	Intergenic	4	171086393	A	G	0.683	-0.088	0.051	0.085	A	G	0.530	-0.008	0.001	1.25E-08
rs4916723	LINC00461	5	87854395	C	A	0.171	-0.005	0.063	0.932	C	A	0.416	-0.010	0.001	1.72E-11
rs12655091	Intergenic	5	144412335	G	C	0.765	0.041	0.057	0.467	G	C	0.443	-0.009	0.001	3.79E-09
rs10085696	AUTS2	7	69783020	G	A	0.219	-0.015	0.057	0.795	G	A	0.186	-0.011	0.002	1.12E-09
rs6460047	Intergenic	7	73042443	C	T	0.291	0.018	0.052	0.729	C	T	0.208	0.012	0.002	9.69E-11
rs10236149	ARPC1B	7	98977515	G	A	0.815	0.020	0.066	0.766	G	A	0.123	-0.013	0.002	1.18E-09
rs35034355	ORC5	7	103840115	A	G	0.160	-0.025	0.065	0.706	A	G	0.521	-0.008	0.001	2.87E-08
rs6951574	Intergenic	7	153489744	C	T	0.399	-0.007	0.048	0.877	C	T	0.458	0.013	0.001	1.58E-19
rs13250583	Intergenic	8	20949917	T	C	0.088	-0.118	0.083	0.152	T	C	0.213	-0.010	0.002	4.7E-08
rs1217091	Intergenic	8	64527399	C	T	0.774	0.027	0.056	0.625	C	T	0.812	0.012	0.002	7.05E-11
rs28601761	Intergenic	8	126500031	G	C	0.307	-0.033	0.052	0.525	G	C	0.420	0.009	0.001	7.17E-10
rs55932213	Intergenic	9	108755622	G	A	0.237	0.038	0.055	0.498	G	A	0.736	0.009	0.002	9.55E-09
rs7074871	Intergenic	10	110507806	A	G	0.268	-0.041	0.053	0.439	A	G	0.255	-0.009	0.002	1.86E-08
rs7950166	TRIM66	11	8642218	T	C	0.504	-0.074	0.048	0.121	T	C	0.637	-0.010	0.002	9.89E-11
rs56030824	SPI1	11	47397353	A	G	0.117	0.039	0.077	0.610	A	G	0.322	-0.012	0.002	1.15E-13
rs10750025	Intergenic	11	113424042	T	C	0.802	0.032	0.059	0.592	T	C	0.686	0.010	0.002	4.89E-11

Appendix Table 3. Association of GSCAN SNPS (N=74) with weekly ethanol intake at study baseline (visit 4) among ARIC African-American participants

						ARIC						GSCAN			
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs1713676	Intergenic	11	113660576	G	A	0.202	0.001	0.060	0.993	G	A	0.522	-0.008	0.001	4.29E-08
rs4938230	Intergenic	11	116075001	A	C	0.817	0.038	0.060	0.529	A	C	0.842	0.013	0.002	1.48E-10
rs682011	Intergenic	11	121544285	C	T	0.742	-0.082	0.053	0.119	C	T	0.559	0.008	0.001	2.22E-08
rs12795042	LOC646522	11	133658168	C	A	0.746	0.169	0.054	0.002	C	A	0.623	-0.008	0.002	3.25E-08
rs10876188	SLC4A8	12	51895882	T	C	0.325	0.017	0.050	0.741	T	C	0.457	-0.008	0.001	4.84E-08
rs3809162	Intergenic	12	54674235	G	A	0.770	0.055	0.057	0.338	G	A	0.397	0.009	0.001	1.19E-09
rs10506274	ACSS3	12	81601464	T	G	0.199	-0.052	0.059	0.382	T	G	0.484	-0.009	0.001	5.78E-10
rs4842786	Intergenic	12	92170791	A	G	0.843	0.054	0.066	0.412	A	G	0.584	-0.009	0.001	2.73E-09
rs500321	Intergenic	13	27124360	T	A	0.848	0.010	0.066	0.879	T	A	0.736	-0.010	0.002	4.92E-09
rs1123285	OTX2	14	57274519	G	C	0.453	0.004	0.047	0.934	G	C	0.335	-0.009	0.002	8.14E-09
rs2180870	ARID4A	14	58782779	C	T	0.269	0.054	0.053	0.308	C	T	0.135	-0.012	0.002	1.12E-08
rs12907323	AGBL1	15	86796012	G	A	0.555	-0.079	0.048	0.101	G	A	0.411	0.008	0.001	9.93E-09
rs2764771	Intergenic	16	20013793	A	G	0.494	0.035	0.048	0.459	A	G	0.307	0.010	0.002	4.02E-10
rs378421	Intergenic	16	28754684	A	G	0.293	-0.015	0.052	0.773	A	G	0.404	-0.011	0.001	4.83E-14
rs113443718	SEZ6L2	16	29892184	A	G	0.071	0.005	0.095	0.959	A	G	0.305	-0.010	0.002	1.19E-10
rs7185555	Intergenic	16	69131281	C	G	0.243	0.010	0.055	0.853	C	G	0.153	-0.011	0.002	4.24E-08
rs1104608	Intergenic	16	73912588	C	G	0.621	0.015	0.049	0.766	C	G	0.425	-0.011	0.001	1.05E-13
rs4548913	SRR	17	2209888	A	G	0.341	0.053	0.050	0.286	A	G	0.632	-0.008	0.002	3.11E-08
rs3803800	TNFSF12-TNFSF13|TNFSF13	17	7462969	G	A	0.369	0.027	0.049	0.584	G	A	0.786	0.011	0.002	1.5E-10
rs2854334	Intergenic	17	29715500	G	A	0.148	0.091	0.068	0.182	G	A	0.615	0.009	0.001	7.51E-10
rs10438820	RPTOR	17	78524597	T	C	0.487	0.065	0.048	0.172	T	C	0.702	0.009	0.002	1.76E-08
rs9950000	TCF4	18	53052169	T	C	0.768	0.019	0.058	0.744	T	C	0.395	-0.009	0.001	9.38E-10
rs4092465	Intergenic	18	55080437	G	A	0.138	-0.013	0.069	0.848	G	A	0.635	-0.008	0.002	4.39E-08
rs281379	Intergenic	19	49214274	A	G	0.195	0.030	0.059	0.615	A	G	0.508	0.014	0.001	4.91E-21

Appendix Table 3. Association of GSCAN SNPS (N=74) with weekly ethanol intake at study baseline (visit 4) among ARIC African-American participants

						ARIC						GSCAN			
rsID	Gene	Chr	Position	EA	OA	EAF	Beta	SE	P-value	EA	OA	EAF	Beta	SE	P-value
rs4815364	ACSS1	20	25035711	A	G	0.370	0.000	0.049	0.999	A	G	0.616	0.009	0.001	1.02E-08
rs9607814	Intergenic	22	41946519	A	C	0.115	-0.096	0.072	0.186	A	C	0.200	-0.010	0.002	4.31E-08

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study; Chr, Chromosome; EA, effect allele, OA, other allele; EAF, effect allele frequency; SE, Standard Error; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use.

APPENDIX D: FINAL GENETIC INSTRUMENTS FOR UNWEIGHTED GENETIC RISK SCORE FOR ARIC EUROPEAN-AMERICAN PARTICIPANTS

Appendix Table 4. ARIC European-American participants 1000 G SNPs that are nominally significant and directionally consistent with GSCAN SNPs across visits 1-4 - Final genetic instruments for unweighted genetic risk score ($uGRS_{11}$)

						ARIC								GSCAN			
rsID	Region	Chr	Position	EA	OA	EAF	Beta	SE	P	VE (%)	Weekly Ethanol Intake	EA	OA	EAF	Beta	SE	P
rs1123285	*OTX2*	14	57274519	G	C	0.338	-0.090	0.040	0.015	-0.090	V2	G	C	0.335	-0.009	0.002	8.14E-09
							-0.070	0.040	0.039	-0.070	V3						
rs1229984	*ADH1B*	4	100239319	C	T	0.965	0.340	0.090	0.000	0.340	V1	C	T	0.963	0.151	0.004	0.00E+00
							0.240	0.090	0.010	0.240	V2						
							0.220	0.090	0.017	0.220	V3						
							0.260	0.090	0.005	0.260	V4						
rs12651313	*Intergenic*	4	171086393	G	C	0.441	-0.080	0.030	0.017	-0.080	V2	G	C	0.443	-0.009	0.001	3.79E-09
							-0.080	0.030	0.018	-0.080	V3						
rs1713676	*Intergenic*	11	113660576	G	A	0.529	-0.070	0.030	0.043	-0.070	V1	G	A	0.522	-0.008	0.001	4.29E-08
							-0.080	0.030	0.017	-0.080	V2						
							-0.080	0.030	0.020	-0.080	V4						
rs2165670			100286085	A	G	0.104	0.120	0.060	0.033	0.120	V3	A	G	0.106	0.023	0.002	1.67E-22
rs55872084	*Intergenic*	5	155902003	T	G	0.232	0.110	0.040	0.008	0.110	V1	T	G	0.235	0.010	0.002	6.32E-09
							0.110	0.040	0.005	0.110	V4						
rs62250685	*CADM2*	3	85457240	G	A	0.625	-0.080	0.030	0.028	-0.080	V2	G	A	0.614	-0.014	0.002	1.05E-21
							-0.110	0.030	0.002	-0.110	V3						
rs7185555	*Intergenic*	16	69131281	C	G	0.141	-0.110	0.050	0.026	-0.110	V1	C	G	0.153	-0.011	0.002	4.24E-08
rs72859280	*Intergenic*	2	147956293	T	G	0.033	0.190	0.090	0.048	0.190	V1	T	G	0.036	0.023	0.004	4.44E-09
rs74664784	*CADM2*	3	85475292	C	T	0.613	-0.070	0.030	0.032	-0.070	V2	C	T	0.359	-0.013	0.002	1.58E-14
							-0.100	0.030	0.004	-0.100	V3						
rs7950166	*TRIM66*	11	8642218	T	C	0.635	-0.080	0.030	0.019	-0.080	V3	T	C	0.637	-0.010	0.002	9.89E-11

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study; Chr, Chromosome; SE, Standard Error; P, P-value; VE, variance explained; EA, effect allele, OA, other allele; EAF, effect allele frequency; V1, visit 1; V2, visit 2; V3, visit 3; V4, visit4; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use.

APPENDIX E: AFRICAN-AMERICAN 1000 GENOME SNPS MOST STRONGLY ASSOCIATED WITH ETHANOL INTAKE AT STUDY BASELINE AND IN LD WITH GSCAN INDEX SNP

Appendix Table 5. ARIC African-American 1000 Genome SNPs† most strongly associated with weekly ethanol intake at study baseline (visit 4) and are in LD with their GSCAN index SNP

			ARIC								GSCAN						
rsID	Chr	Position	EA	OA	EAF	Beta	SE	P	rsID	Position	EA	OA	EAF	Beta	SE	P	LD
rs10915562	1	4630379	A	G	0.235	-0.137	0.056	0.014	rs705687	4548453	G	A	0.785	-0.011	0.002	1.00E-09	0.315
rs6703350	1	33732772	T	G	0.241	-0.111	0.055	0.046	rs58107686	33837334	A	C	0.328	-0.010	0.002	1.00E-09	0.306
rs1354060	1	66511404	G	A	0.431	0.115	0.047	0.015	rs12088813	66407700	C	A	0.267	-0.009	0.002	1.60E-08	0.283
rs3932455	1	71511990	A	C	0.124	-0.220	0.072	0.002	rs5024204	71491890	T	A	0.278	0.010	0.002	3.00E-09	0.408
rs12084359	1	165139653	A	G	0.239	-0.129	0.055	0.020	rs10753661	165119792	A	G	0.684	-0.009	0.002	3.80E-08	0.202
rs10912751	1	174232130	G	T	0.352	0.097	0.050	0.050	rs28680958	173848808	A	G	0.217	-0.011	0.002	1.00E-09	0.207
rs6673687	1	205670369	T	A	0.359	-0.112	0.049	0.023	rs823114	205719532	A	G	0.553	0.009	0.001	2.00E-09	0.725
rs780096	2	27741072	C	G	0.432	0.095	0.047	0.041	rs1260326	27730940	C	T	0.601	0.021	0.001	8.05E-45	0.730
rs1317580	2	27961344	C	G	0.199	-0.162	0.058	0.006	rs2178197	27860551	G	A	0.569	-0.009	0.001	2.00E-09	0.315
rs11692742	2	45112453	A	G	0.315	0.120	0.050	0.017	rs13383034	45155276	T	C	0.329	0.015	0.002	0.00E+00	0.458
rs494904	2	45141180	C	T	0.470	0.111	0.047	0.018	rs1004787	45159091	A	G	0.551	0.008	0.001	8.00E-09	0.361
rs17348120	2	63434271	A	T	0.137	-0.226	0.070	0.001	rs13032049	63581507	G	A	0.283	0.010	0.002	0.00E+00	0.331
rs828867	2	74334462	A	G	0.230	-0.110	0.057	0.054	rs828867	74334462	A	G	0.545	0.009	0.001	2.00E-09	1.000
rs2544471	2	98241213	T	G	0.420	-0.072	0.049	0.143	rs11692435	98275354	A	G	0.085	0.017	0.003	0.00E+00	0.386
rs35608804	2	144271545	T	C	0.482	-0.066	0.047	0.158	rs13024996	144225215	A	C	0.364	-0.011	0.002	0.00E+00	0.399
rs17720710	2	148091377	C	T	0.060	0.054	0.101	0.590	rs72859280	147956293	T	G	0.036	0.023	0.004	4.00E-09	0.202
rs1908252	2	225484736	A	G	0.478	0.089	0.048	0.064	rs56337305	225475560	C	T	0.383	-0.010	0.001	0.00E+00	0.331
rs6790743	3	70957896	C	A	0.489	0.101	0.048	0.036	rs13094887	70968431	T	A	0.301	-0.010	0.002	0.00E+00	0.765
rs7355953	3	85792137	C	T	0.058	-0.229	0.103	0.027	rs62250685	85457240	G	A	0.614	-0.014	0.002	0.00E+00	0.286
rs7355953	3	85792137	C	T	0.058	-0.229	0.103	0.027	rs74664784	85475292	C	T	0.359	-0.013	0.002	0.00E+00	0.262
rs13062355	3	93688511	A	G	0.288	0.090	0.052	0.084	rs13066454	93994255	T	C	0.398	-0.009	0.001	4.00E-09	0.337
rs1913287	3	131469850	A	G	0.427	0.081	0.049	0.097	rs9838144	131576287	C	G	0.209	-0.010	0.002	2.70E-08	0.254
rs7613516	3	141079309	T	G	0.121	-0.210	0.077	0.006	rs2011092	141124607	C	T	0.339	-0.009	0.002	7.00E-09	0.387
rs16851438	3	141169112	G	A	0.103	-0.112	0.077	0.145	rs60654199	141267295	A	C	0.063	-0.017	0.003	2.90E-08	0.332

Appendix Table 5. ARIC African-American 1000 Genome SNPs† most strongly associated with weekly ethanol intake at study baseline (visit 4) and are in LD with their GSCAN index SNP

			ARIC								GSCAN						
rsID	Chr	Position	EA	OA	EAF	Beta	SE	P	rsID	Position	EA	OA	EAF	Beta	SE	P	LD
rs12634907	3	158226886	G	A	0.181	0.110	0.061	0.075	rs6787172	158187811	G	T	0.554	-0.008	0.001	4.30E-08	0.358
rs13108218	4	3443931	A	G	0.483	-0.060	0.048	0.212	rs3748034	3446091	T	G	0.143	-0.012	0.002	1.70E-08	0.255
rs4974995	4	39218123	C	T	0.176	0.138	0.063	0.029	rs11940694	39414993	G	A	0.597	0.026	0.001	3.03E-68	0.220
rs4974995	4	39218123	C	T	0.176	0.138	0.063	0.029	rs11940694	39414993	G	A	0.597	0.026	0.001	3.03E-68	0.220
rs7682824	4	39406254	C	T	0.209	0.027	0.058	0.645	rs7682824	39406254	T	C	0.548	0.008	0.002	2.80E-08	1.000
rs11940694	4	39414993	A	G	0.424	-0.091	0.047	0.057	rs35538052	39418965	A	G	0.379	-0.009	0.002	1.40E-08	0.864
rs79754951	4	42160026	A	T	0.273	0.126	0.053	0.017	rs4501255	42151306	G	C	0.235	0.011	0.002	0.00E+00	0.598
rs62325466	4	99625032	T	G	0.057	-0.177	0.101	0.080	rs12499107	99678691	G	A	0.131	0.013	0.002	4.00E-09	0.316
rs7692974	4	100051161	A	C	0.306	0.191	0.051	0.000	rs561222871	100260679	T	C	0.047	-0.039	0.004	6.56E-27	0.228
rs10008281	4	100142302	A	C	0.293	0.154	0.052	0.003	rs1154414	100000136	C	T	0.141	0.018	0.002	0.00E+00	0.436
rs10008281	4	100142302	A	C	0.293	0.154	0.052	0.003	rs10028756	100254520	A	G	0.129	-0.019	0.002	0.00E+00	0.204
rs1693457	4	100236762	C	T	0.240	0.150	0.056	0.007	rs2165670	100286085	A	G	0.106	0.023	0.002	0.00E+00	0.316
rs58440244	4	100378680	A	G	0.270	-0.123	0.053	0.021	rs17029090	100443853	G	A	0.020	-0.049	0.005	0.00E+00	0.694
rs58440244	4	100378680	A	G	0.270	-0.123	0.053	0.021	rs79139602	100444363	T	A	0.021	0.060	0.005	1.80E-32	0.694
rs113930074	4	100529342	A	G	0.052	-0.167	0.105	0.113	rs36052336	100273594	G	A	0.061	-0.018	0.003	1.00E-09	0.228
rs3077043	4	101361211	A	C	0.101	-0.160	0.077	0.038	rs4699791	101243023	A	G	0.096	0.019	0.002	0.00E+00	0.544
rs238449	4	103112813	A	C	0.348	-0.123	0.050	0.014	rs13107325	103188709	T	C	0.072	-0.028	0.003	0.00E+00	0.215
rs6831562	4	143716975	T	C	0.467	0.104	0.047	0.026	rs4690727	143648579	G	C	0.718	0.011	0.002	0.00E+00	0.868
rs78757076	4	153010001	T	C	0.242	-0.106	0.056	0.059	rs10004020	152968372	A	G	0.720	0.009	0.002	2.40E-08	0.205
rs578402	4	171047179	G	A	0.257	0.120	0.055	0.028	rs12651313	171086393	G	C	0.443	-0.009	0.001	4.00E-09	0.269
rs7706932	5	87775691	T	C	0.249	-0.078	0.055	0.159	rs4916723	87854395	C	A	0.416	-0.010	0.001	0.00E+00	0.472
rs271085	5	144543593	A	G	0.304	0.118	0.051	0.020	rs12655091	144412335	A	G	0.530	-0.008	0.001	1.20E-08	0.358
rs56235470	5	155945315	A	G	0.110	-0.153	0.075	0.043	rs55872084	155902003	T	G	0.235	0.010	0.002	6.00E-09	0.296
rs11959347	5	166815244	T	C	0.491	0.165	0.048	0.001	rs11739827	166803321	T	G	0.451	-0.008	0.001	1.20E-08	0.370
rs11768390	7	69742936	G	A	0.457	-0.108	0.048	0.025	rs10085696	69783020	G	A	0.186	-0.011	0.002	1.00E-09	0.613
rs42238	7	72830546	T	C	0.172	0.153	0.063	0.015	rs6460047	73042443	C	T	0.208	0.012	0.002	0.00E+00	0.230

Appendix Table 5. ARIC African-American 1000 Genome SNPs† most strongly associated with weekly ethanol intake at study baseline (visit 4) and are in LD with their GSCAN index SNP

			ARIC								GSCAN						
rsID	Chr	Position	EA	OA	EAF	Beta	SE	P	rsID	Position	EA	OA	EAF	Beta	SE	P	LD
rs13438288	7	98916414	G	T	0.129	0.147	0.071	0.039	rs10236149	98977515	G	A	0.123	-0.013	0.002	1.00E-09	0.283
rs2385142	7	103869191	G	A	0.111	-0.185	0.075	0.014	rs35034355	103840115	A	G	0.521	-0.008	0.001	2.90E-08	0.222
rs199626887	7	153404124	T	G	0.281	-0.073	0.052	0.163	rs6951574	153489744	C	T	0.458	0.013	0.001	0.00E+00	0.277
rs10283354	8	21016340	C	T	0.096	-0.212	0.079	0.008	rs13250583	20949917	T	C	0.213	-0.010	0.002	4.70E-08	0.501
rs1234627	8	64574537	A	T	0.383	-0.100	0.051	0.049	rs1217091	64527399	C	T	0.812	0.012	0.002	0.00E+00	0.547
rs2980860	8	126485337	G	A	0.338	0.089	0.050	0.078	rs28601761	126500031	G	C	0.420	0.009	0.001	1.00E-09	0.725
rs10978391	9	108803650	G	C	0.165	0.157	0.063	0.013	rs55932213	108755622	G	A	0.736	0.009	0.002	1.00E-08	0.293
rs4743016	9	109377233	G	T	0.382	0.108	0.048	0.025	rs10978550	109345993	C	T	0.206	-0.012	0.002	0.00E+00	0.310
rs67127636	10	110474056	G	A	0.122	-0.199	0.073	0.006	rs7074871	110507806	A	G	0.255	-0.009	0.002	1.90E-08	0.574
rs35217446	10	124954612	A	C	0.064	-0.199	0.097	0.041	rs17665139	125093880	T	C	0.149	-0.012	0.002	1.60E-08	0.231
rs10840100	11	8669437	A	G	0.472	0.103	0.048	0.031	rs7950166	8642218	T	C	0.637	-0.010	0.002	0.00E+00	0.943
rs11030024	11	27508681	C	T	0.450	0.110	0.047	0.020	rs11030084	27643725	T	C	0.184	-0.011	0.002	1.70E-08	0.424
rs1685404	11	47243665	C	G	0.245	-0.115	0.054	0.033	rs56030824	47397353	A	G	0.322	-0.012	0.002	0.00E+00	0.344
rs2514218	11	113392994	T	C	0.158	-0.193	0.064	0.003	rs10750025	113424042	T	C	0.686	0.010	0.002	0.00E+00	0.665
rs1022084	11	113508425	G	A	0.496	0.118	0.047	0.013	rs1713676	113660576	G	A	0.522	-0.008	0.001	4.30E-08	0.278
rs7940127	11	116102388	T	C	0.289	-0.124	0.052	0.018	rs4938230	116075001	A	C	0.842	0.013	0.002	0.00E+00	0.845
rs532585	11	121542675	C	T	0.253	0.095	0.053	0.075	rs682011	121544285	C	T	0.559	0.008	0.001	2.20E-08	0.992
rs12795042	11	133658168	A	C	0.254	-0.169	0.054	0.002	rs12795042	133658168	C	A	0.623	-0.008	0.002	3.30E-08	1.000
rs4578438	12	51939749	T	C	0.268	-0.099	0.053	0.063	rs10876188	51895882	T	C	0.457	-0.008	0.001	4.80E-08	0.245
rs6580980	12	54692061	A	G	0.068	-0.241	0.096	0.012	rs3809162	54674235	G	A	0.397	0.009	0.001	1.00E-09	0.308
rs10862233	12	81483052	C	T	0.132	-0.139	0.073	0.056	rs10506274	81601464	T	G	0.484	-0.009	0.001	1.00E-09	0.329
rs12828474	12	92226816	A	G	0.171	0.161	0.063	0.011	rs4842786	92170791	A	G	0.584	-0.009	0.001	3.00E-09	0.326
rs525956	13	27101573	A	T	0.229	0.080	0.056	0.157	rs500321	27124360	T	A	0.736	-0.010	0.002	5.00E-09	0.899
rs1483107	14	57318956	A	G	0.281	-0.084	0.054	0.119	rs1123285	57274519	G	C	0.335	-0.009	0.002	8.00E-09	0.289
rs1190979	14	58805862	C	A	0.276	0.108	0.052	0.038	rs2180870	58782779	C	T	0.135	-0.012	0.002	1.10E-08	0.923
rs941948	14	104553521	A	G	0.269	0.104	0.053	0.050	rs11625650	104610138	A	G	0.233	-0.010	0.002	2.90E-08	0.210

Appendix Table 5. ARIC African-American 1000 Genome SNPs† most strongly associated with weekly ethanol intake at study baseline (visit 4) and are in LD with their GSCAN index SNP

			ARIC								GSCAN						
rsID	Chr	Position	EA	OA	EAF	Beta	SE	P	rsID	Position	EA	OA	EAF	Beta	SE	P	LD
rs12910841	15	74725822	T	C	0.096	0.136	0.083	0.101	rs2472297	75027880	T	C	0.249	0.011	0.002	0.00E+00	0.308
rs6496321	15	86858420	T	G	0.154	0.217	0.065	0.001	rs12907323	86796012	G	A	0.411	0.008	0.001	1.00E-08	0.211
rs4780836	16	19985393	C	A	0.355	0.119	0.049	0.015	rs2764771	20013793	A	G	0.307	0.010	0.002	0.00E+00	0.536
rs8054332	16	24861927	T	C	0.411	-0.107	0.049	0.029	rs17177078	24810681	T	C	0.063	-0.022	0.003	0.00E+00	0.392
rs12919058	16	28927818	A	C	0.476	-0.105	0.047	0.027	rs378421	28754684	A	G	0.404	-0.011	0.001	0.00E+00	0.628
rs3815822	16	29872361	G	A	0.463	-0.076	0.047	0.110	rs113443718	29892184	A	G	0.305	-0.010	0.002	0.00E+00	0.326
rs62040427	16	64839701	T	C	0.306	-0.112	0.051	0.029	rs62044525	64872590	G	C	0.184	-0.012	0.002	0.00E+00	0.888
rs9929584	16	69489576	T	C	0.462	-0.077	0.047	0.102	rs7185555	69131281	C	G	0.153	-0.011	0.002	4.20E-08	0.219
rs62058280	16	72285039	C	A	0.051	-0.167	0.107	0.119	rs79616692	72338507	C	G	0.108	0.016	0.002	0.00E+00	0.400
rs1492559	16	73868230	G	T	0.343	-0.049	0.049	0.320	rs1104608	73912588	C	G	0.425	-0.011	0.001	0.00E+00	0.254
rs17761864	17	2171637	A	C	0.127	0.228	0.071	0.001	rs4548913	2209888	A	G	0.632	-0.008	0.002	3.10E-08	0.265
rs4578723	17	7437845	C	T	0.281	0.101	0.053	0.059	rs3803800	7462969	G	A	0.786	0.011	0.002	0.00E+00	0.257
rs178840	17	29737612	G	A	0.267	0.118	0.053	0.027	rs2854334	29715500	G	A	0.615	0.009	0.001	1.00E-09	0.205
rs78011262	17	43837917	C	T	0.152	-0.211	0.065	0.001	.	44246624	A	C	0.215	-0.022	0.003	0.00E+00	0.623
rs6565473	17	78643206	T	C	0.408	0.187	0.048	0.000	rs10438820	78524597	T	C	0.702	0.009	0.002	1.80E-08	0.288
rs7231748	18	53109035	G	A	0.403	0.107	0.048	0.025	rs9950000	53052169	T	C	0.395	-0.009	0.001	1.00E-09	0.421
rs221876	18	55066438	C	G	0.467	0.051	0.047	0.281	rs4092465	55080437	G	A	0.635	-0.008	0.002	4.40E-08	0.245
rs281392	19	49164952	G	A	0.411	0.098	0.048	0.043	rs281379	49214274	A	G	0.508	0.014	0.001	0.00E+00	0.286
rs6083730	20	25027526	G	A	0.152	-0.115	0.066	0.082	rs4815364	25035711	A	G	0.616	0.009	0.001	1.00E-08	0.771
rs139568	22	42210985	T	C	0.296	0.134	0.051	0.009	rs9607814	41946519	A	C	0.200	-0.010	0.002	4.30E-08	0.397

†Single Nucleotide Polymorphisms (SNP)s were identified by fine-mapping in ± 500 kb windows surrounding each of the 99 index SNPs that were independently associated with drinks per week in those of European ancestry

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study; Chr, Chromosome; EA, effect allele, OA, other allele; EAF, effect allele frequency; SE, Standard Error; P, P-value; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use; LD, Linkage Disequilibrium.

Linear regression models adjusted for age, sex, race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity, APOE ε4 status, and principal components

APPENDIX F: FINAL GENETIC INSTRUMENTS FOR UNWEIGHTED GENETIC RISK SCORE FOR ARIC AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 6. ARIC African-American participants 1000 G SNPs that are nominally significant and directionally consistent with GSCAN SNPs across visits 1-4 - Final genetic instruments for unweighted genetic risk score (uGRS20)

			ARIC									GSCAN						
rsID	Chr	Position	EA	OA	EAF	Beta	SE	P	VE (%)	Weekly Ethanol Intake	rsID	EA	OA	EAF	Beta	SE	P	LD
rs6673687	1	205670369	T	A	0.359	-0.140	0.060	0.019	0.28	V1	rs823114	A	G	0.553	0.009	0.001	2.00E-09	0.725
						-0.130	0.060	0.021	0.30	V2								
						-0.150	0.060	0.020	0.29	V3								
						-0.120	0.050	0.020	0.27	V4								
rs35608804	2	144271545	T	C	0.482	-0.140	0.050	0.010	0.24	V2	rs13024996	A	C	0.364	-0.011	0.002	0.00E+00	0.399
rs7355953	3	85792137	C	T	0.058	-0.380	0.130	0.003	0.28	V1	rs62250685	G	A	0.614	-0.014	0.002	0.00E+00	0.286
						-0.210	0.110	0.047	0.13	V4								
rs11940694	4	39414993	A	G	0.424	-0.120	0.060	0.041	0.15	V1	rs35538052	A	G	0.379	-0.009	0.002	1.40E-08	0.864
						-0.140	0.060	0.019	0.22	V3								
rs10008281	4	100142302	A	C	0.293	0.160	0.060	0.011	0.30	V1	rs1154414	C	T	0.141	0.018	0.002	0.00E+00	0.436
						0.130	0.050	0.017	0.26	V4								
rs58440244	4	100378680	A	G	0.270	-0.140	0.070	0.035	0.18	V1	rs17029090	G	A	0.020	-0.049	0.005	0.00E+00	0.694
						-0.110	0.060	0.046	0.15	V4								
rs78757076	4	153010001	T	C	0.242	-0.160	0.070	0.018	0.17	V1	rs10004020	A	G	0.720	0.009	0.002	2.40E-08	0.205
rs271085	5	144543593	A	G	0.304	0.170	0.060	0.005	0.26	V1	rs12655091	A	G	0.530	-0.008	0.001	1.20E-08	0.358
						0.110	0.050	0.029	0.15	V4								
rs11768390	7	69742936	G	A	0.457	-0.140	0.060	0.010	0.28	V2	rs10085696	G	A	0.186	-0.011	0.002	1.00E-09	0.613
						-0.120	0.050	0.014	0.26	V4								
rs10283354	8	21016340	C	T	0.096	-0.290	0.100	0.003	0.29	V1	rs13250583	T	C	0.213	-0.010	0.002	4.70E-08	0.501
						-0.250	0.090	0.007	0.25	V2								
						-0.220	0.080	0.007	0.24	V4								
rs10840100	11	8669437	A	G	0.472	0.140	0.060	0.013	0.31	V2	rs7950166	T	C	0.637	-0.010	0.002	0.00E+00	0.943
						0.150	0.060	0.019	0.30	V3								
rs1685404	11	47243665	C	G	0.245	-0.180	0.070	0.007	0.36	V1	rs56030824	A	G	0.322	-0.012	0.002	0.00E+00	0.344
						-0.160	0.060	0.012	0.31	V2								
						-0.120	0.060	0.039	0.25	V3								

Appendix Table 6. ARIC African-American participants 1000 G SNPs that are nominally significant and directionally consistent with GSCAN SNPs across visits 1-4 - Final genetic instruments for unweighted genetic risk score (uGRS20)

			ARIC									GSCAN						
rsID	Chr	Position	EA	OA	EAF	Beta	SE	P	VE (%)	Weekly Ethanol Intake	rsID	EA	OA	EAF	Beta	SE	P	LD
rs2514218	11	113392994	T	C	0.158	-0.260	0.080	0.001	0.61	V1	rs10750025	T	C	0.686	0.010	0.002	0.00E+00	0.665
						-0.180	0.070	0.006	0.43	V4								
rs1022084	11	113508425	G	A	0.496	0.120	0.060	0.026	0.32	V2	rs1713676	G	A	0.522	-0.008	0.001	4.30E-08	0.278
						0.100	0.050	0.047	0.26	V4								
rs7940127	11	116102388	T	C	0.289	-0.140	0.060	0.025	0.28	V1	rs4938230	A	C	0.842	0.013	0.002	0.00E+00	0.845
						-0.110	0.050	0.034	0.28	V4								
rs12910841	15	74725822	T	C	0.096	0.320	0.110	0.003	0.10	V2	rs2472297	T	C	0.249	0.011	0.002	0.00E+00	0.308
						0.170	0.090	0.044	0.11	V4								
rs6496321	15	86858420	T	G	0.154	0.200	0.080	0.012	0.24	V1	rs12907323	G	A	0.411	0.008	0.001	1.00E-08	0.211
						0.220	0.070	0.001	0.40	V4								
rs4780836	16	19985393	C	A	0.355	0.140	0.060	0.010	0.29	V2	rs2764771	A	G	0.307	0.010	0.002	0.00E+00	0.536
rs62040427	16	64839701	T	C	0.306	-0.140	0.060	0.028	0.40	V1	rs62044525	G	C	0.184	-0.012	0.002	0.00E+00	0.888
						-0.130	0.050	0.014	0.44	V4								
rs9929584	16	69489576	T	C	0.462	-0.150	0.050	0.006	0.30	V2	rs7185555	C	G	0.153	-0.011	0.002	4.20E-08	0.219

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study; Chr, Chromosome; SE, Standard Error; P, P-value; EA, effect allele, OA, other allele; EAF, effect allele frequency; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use; LD, Linkage Disequilibrium.

APPENDIX G: CONDITIONAL ANALYSES RESULTS FOR GENETIC VARIANTS ASSOCIATED WITH ETHANOL INTAKE AT ARIC VISITS 1-4 AMONG AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 7. Conditional analyses results variants associated with ethanol intake at ARIC visits 1-4 among African-Americans

									Unconditioned			Conditioned on GSCAN		
Trait	rsID	Chr	Position	Gene	EA	OA	EAF	GSCAN Reference SNP	Beta	SE	P	Beta	SE	P
Ethanol Intake at visit 1	rs6673687	1	205670369	*Intergenic*	T	A	0.359	rs823114	-0.140	0.060	0.019	-0.120	0.060	0.051
	rs7355953	3	85792137	*CADM2*	C	T	0.058	rs74664784	-0.381	0.128	0.003	-0.410	0.130	0.001
	rs11940694	4	39414993	*KLB*	A	G	0.424	rs35538052	-0.119	0.058	0.041	-0.130	0.060	0.034
	rs58440244	4	100378680	*C4orf17*	A	G	0.270	rs17029090	-0.139	0.066	0.035	-0.150	0.070	0.032
	rs78757076	4	153010001	*Intergenic*	T	C	0.242	rs10004020	-0.162	0.069	0.018	-0.160	0.070	0.020
	rs271085	5	144543593	*Intergenic*	A	G	0.304	rs12655091	0.175	0.062	0.005	0.170	0.060	0.006
	rs10283354	8	21016340	*Intergenic*	C	T	0.096	rs13250583	-0.295	0.098	0.003	-0.350	0.110	0.002
	rs1685404	11	47243665	*SPI1*	C	G	0.245	rs56030824	-0.180	0.067	0.007	-0.200	0.070	0.003
	rs2514218	11	113392994	*Intergenic*	T	C	0.158	rs10750025	-0.255	0.079	0.001	-0.260	0.090	0.006
	rs7940127	11	116102388	*Intergenic*	T	C	0.289	rs4938230	-0.144	0.064	0.025	-0.250	0.090	0.004
	rs6496321	15	86858420	*AGBL1*	T	G	0.154	rs12907323	0.201	0.080	0.012	0.200	0.080	0.012
Ethanol Intake at visit 2	rs6673687	1	205670369	*Intergenic*	T	A	0.359	rs823114	-0.130	0.057	0.021	-0.120	0.060	0.046
	rs35608804	2	144271545	*ARHGAP15*	T	C	0.482	rs13024996	-0.139	0.054	0.010	-0.160	0.060	0.007
	rs11768390	7	69742936	*AUTS2*	G	A	0.457	rs10085696	-0.143	0.055	0.010	-0.190	0.060	0.003
	rs10283354	8	21016340	*Intergenic*	C	T	0.096	rs13250583	-0.248	0.092	0.007	-0.170	0.100	0.110
	rs10840100	11	8669437	*TRIM66*	A	G	0.472	rs7950166	0.137	0.055	0.013	0.350	0.180	0.045
	rs1685404	11	47243665	*SPI1*	C	G	0.245	rs56030824	-0.158	0.063	0.012	-0.160	0.060	0.013
	rs1022084	11	113508425	*Intergenic*	G	A	0.496	rs1713676	0.123	0.055	0.026	0.130	0.060	0.025
	rs4780836	16	19985393	*Intergenic*	C	A	0.355	rs2764771	0.144	0.056	0.010	0.150	0.060	0.014
	rs9929584	16	69489576	*Intergenic*	T	C	0.462	rs7185555	-0.150	0.055	0.006	-0.150	0.060	0.005
Ethanol Intake at visit 3	rs6673687	1	205670369	*Intergenic*	T	A	0.359	rs823114	-0.148	0.063	0.020	-0.140	0.070	0.028
	rs11940694	4	39414993	*KLB*	A	G	0.424	rs35538052	-0.143	0.061	0.019	-0.150	0.060	0.015
	rs10840100	11	8669437	*TRIM66*	A	G	0.472	rs7950166	0.147	0.063	0.019	0.650	0.200	0.001
	rs1685404	11	47243665	*SPI1*	C	G	0.245	rs56030824	-0.147	0.071	0.037	-0.170	0.070	0.018
Ethanol Intake at visit 4	rs6673687	1	205670369	*Intergenic*	T	A	0.359	rs823114	-0.117	0.050	0.020	-0.110	0.050	0.037
	rs7355953	3	85792137	*CADM2*	C	T	0.058	rs74664784	-0.214	0.108	0.047	-0.230	0.110	0.031
	rs58440244	4	100378680	*C4orf17*	A	G	0.270	rs17029090	-0.110	0.055	0.046	-0.130	0.060	0.033
	rs271085	5	144543593	*Intergenic*	A	G	0.304	rs12655091	0.115	0.052	0.029	0.110	0.050	0.033

									Unconditioned			Conditioned on GSCAN		
Trait	rsID	Chr	Position	Gene	EA	OA	EAF	GSCAN Reference SNP	Beta	SE	P	Beta	SE	P
	rs11768390	7	69742936	*AUTS2*	G	A	0.457	rs10085696	-0.122	0.049	0.014	-0.160	0.060	0.006
	rs10283354	8	21016340	*Intergenic*	C	T	0.096	rs13250583	-0.221	0.082	0.007	-0.210	0.090	0.022
	rs2514218	11	113392994	*Intergenic*	T	C	0.158	rs10750025	-0.184	0.066	0.006	-0.250	0.080	0.002
	rs1022084	11	113508425	*Intergenic*	G	A	0.496	rs1713676	0.098	0.049	0.047	0.100	0.050	0.042
	rs7940127	11	116102388	*Intergenic*	T	C	0.289	rs4938230	-0.115	0.054	0.034	-0.160	0.070	0.024
	rs6496321	15	86858420	*AGBL1*	T	G	0.154	rs12907323	0.216	0.067	0.001	0.210	0.070	0.003

Abbreviations: SNP, Single Nucleotide Polymorphisms; ARIC, Atherosclerosis Risk in Communities (ARIC) Study; Chr, Chromosome; SE, Standard Error; P, P-value; EA, effect allele, OA, other allele; EAF, effect allele frequency; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use.

APPENDIX H: ASSOCIATION BETWEEN UNWEIGHTED GENETIC RISK SCORE AND ETHANOL INTAKE AT ARIC VISIT 1-4 AMONG AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 8. Association between unweighted genetic risk score (uGRS20) and weekly ethanol intake at ARIC visits 1-4 among African-American participants

Weekly Ethanol Intake	Beta	SE	P-value	Percent of variance explained (%)
At visit 1	-0.045	0.016	0.006	0.41
At visit 2	-0.047	0.015	0.002	0.52
At visit 3	-0.037	0.017	0.029	0.28
At visit 4	-0.032	0.013	0.019	0.30

Abbreviations: ARIC, Atherosclerosis Risk in Communities (ARIC) Study; SE, Standard Error

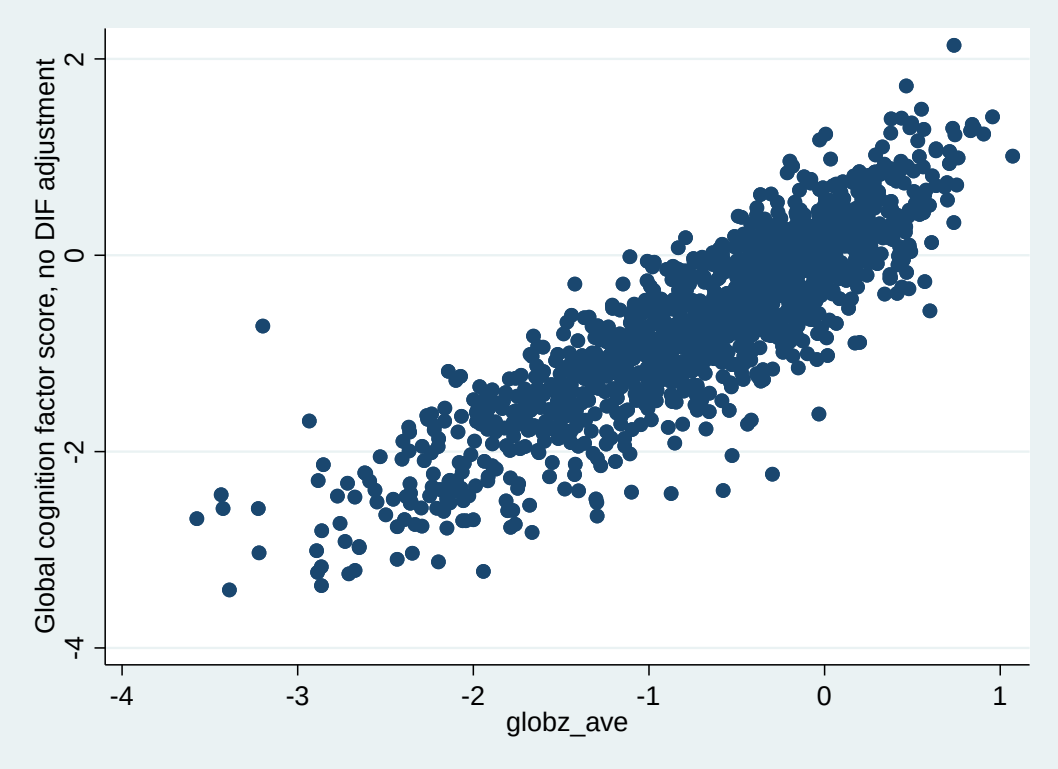
APPENDIX I: ASSOCIATION BETWEEN UNWEIGHTED GENETIC RISK SCORE AND ETHANOL INTAKE AT ARIC VISITS 1-4 AMONG EUROPEAN-AMERICAN PARTICIPANTS

Appendix Table 9. Association between unweighted genetic risk score (uGRS11) and weekly ethanol intake at ARIC visits 1-4 among European-American participants

Weekly Ethanol Intake	Beta	SE	P-value	Percent of variance explained (%)
At visit 1	0.014	0.012	0.246	0.02
At visit 2	0.019	0.011	0.092	0.04
At visit 3	0.024	0.012	0.036	0.06
At visit 4	0.023	0.011	0.041	0.06

Abbreviations: ARIC, Atherosclerosis Risk in Communities (ARIC) Study; SE, Standard Error

APPENDIX J: VALIDATION OF MULTIPLE IMPUTED GLOBAL Z FACTOR SCORES USING EXISTING DATA



Appendix Figure 1. Validation of multiple imputed global Z score using existing data
Note: Multiple imputation was done using chained equations, and 25 imputations were obtained and averaged for display in plot.
20% validation sample (N=1,247) to simulate missing completely at random (MCAR) data. All participants had a 0.2 probability of being selected. If selected, participants' Z scores at visit 5 were set to missing and imputed.

APPENDIX K: ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY LONG-TERM ETHANOL INTAKE CATEGORY FOR AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 10. Adjusted mean difference in 15-year change in cognitive performance by long-term ethanol intake category for ARIC African-American participants*

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Long-Term Drinking Category	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	$P_{overall}$
Global Factor Score z-score	2169	-0.72 (0.75)				
Stable never drinking	472	-0.74 (0.71)	-1.36 (-1.77, -0.95)	Reference	Reference	0.314
Stable low-to-moderate drinking	178	-0.41 (0.79)	-1.27 (-1.69, -0.86)	0.08 (-0.06, 0.23)	-6%	
Stable heavy drinking	15	-0.68 (0.90)	-0.89 (-1.46, -0.33)	0.46 (0.05, 0.88)	-34%	
Stable former drinking	191	-0.86 (0.67)	-1.26 (-1.68, -0.85)	0.10 (-0.05, 0.25)	-7%	
Mostly low-to-moderate drinking	314	-0.59 (0.77)	-1.26 (-1.67, -0.84)	0.10 (-0.02, 0.22)	-8%	
Mostly heavy drinking	53	-0.57 (0.92)	-1.26 (-1.71, -0.81)	0.10 (-0.13, 0.33)	-7%	
Mostly former drinking	561	-0.70 (0.77)	-1.26 (-1.67, -0.86)	0.10 (-0.01, 0.20)	-7%	
Delayed Word Recall z-score	2169	-0.35 (1.11)				
Stable never drinking	472	-0.25 (1.08)	-1.99 (-2.92, -1.06)	Reference	Reference	0.132
Stable low-to-moderate drinking	178	-0.20 (1.06)	-1.89 (-2.83, -0.95)	0.10 (-0.22, 0.42)	-5%	
Stable heavy drinking	15	-0.62 (1.02)	-0.85 (-2.13,0.43)	1.14 (0.19, 2.08)	-57%	
Stable former drinking	191	-0.39 (1.01)	-1.96 (-2.90, -1.02)	0.03 (-0.31, 0.36)	-1.30%	
Mostly low-to-moderate drinking	314	-0.33 (1.21)	-1.78 (-2.72, -0.84)	0.21 (-0.06, 0.49)	-11%	
Mostly heavy drinking	53	-0.33 (1.17)	-2.01 (-3.04, -0.99)	-0.02 (-0.55, 0.50)	1%	
Mostly former drinking	561	-0.40 (1.12)	-1.75 (-2.67, -0.84)	0.24 (-0.01, 0.48)	-12%	
Word Fluency z-score	2169	-0.36 (1.06)				
Stable never drinking	472	-0.37 (1.03)	-0.55 (-1.06, -0.04)	Reference	Reference	0.063
Stable low-to-moderate drinking	178	0.00 (1.17)	-0.53 (-1.04, -0.01)	0.03 (-0.15, 0.20)	-5%	
Stable heavy drinking	15	-0.14 (0.96)	-0.21 (-0.90, 0.49)	0.35 (-0.16, 0.86)	-62%	
Stable former drinking	191	-0.49 (1.05)	-0.32 (-0.84, 0.2)	0.23 (0.05, 0.42)	-42%	
Mostly low-to-moderate drinking	314	-0.23 (1.08)	-0.35 (-0.87, 0.16)	0.20 (0.05, 0.35)	-36%	
Mostly heavy drinking	53	-0.31 (1.14)	-0.46 (-1.02, 0.10)	0.10 (-0.19, 0.38)	-17%	
Mostly former drinking	561	-0.37 (1.04)	-0.44 (-0.95, 0.06)	0.11 (-0.02, 0.24)	-19%	
Digit Symbol Substitution z-score	2169	-0.95 (0.94)				
Stable never drinking	2169	-0.95 (0.94)	-0.96 (-1.41, -0.51)	Reference	Reference	0.847
Stable low-to-moderate drinking	472	-1.02 (0.90)	-0.99 (-1.44, -0.54)	-0.03 (-0.18, 0.12)	3%	
Stable heavy drinking	178	-0.60 (0.97)	-0.87 (-1.5, -0.25)	0.08 (-0.37, 0.54)	-9%	
Stable former drinking	15	-0.87 (0.94)	-0.95 (-1.41, -0.5)	0.00 (-0.16, 0.16)	-0.3%	
Mostly low-to-moderate drinking	191	-1.12 (0.84)	-0.95 (-1.4, -0.5)	0.01 (-0.12, 0.14)	-0.9%	
Mostly heavy drinking	314	-0.79 (0.91)	-1.13 (-1.63, -0.64)	-0.18 (-0.43, 0.08)	18%	
Mostly former drinking	53	-0.69 (1.12)	-0.99 (-1.43, -0.55)	-0.03 (-0.14, 0.09)	3%	

* Difference. modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4;1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker). * Complete case analysis. Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. † CI, confidence interval; percent, positive values represent % greater decline relative to the referent group.

APPENDIX L: ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY LONG-TERM ETHANOL INTAKE CATEGORY FOR EUROPEAN-AMERICAN PARTICIPANTS

Appendix Table 11. Adjusted mean difference in 15-year change in cognitive performance by long-term ethanol intake category for ARIC for European-American participants*

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Long-Term Drinking Category	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	$P_{overall}$
Global Factor Score z-score	8707	0.12 (0.71)				
Stable never drinking	1033	0.02 (0.68)	-1.16 (-1.42, -0.91)	Reference	Reference	0.301
Stable low-to-moderate drinking	3095	0.30 (0.67)	-1.14 (-1.39, -0.88)	0.03 (-0.03, 0.09)	-2%	
Stable heavy drinking	176	0.14 (0.69)	-1.23 (-1.51, -0.95)	-0.07 (-0.20, 0.07)	6%	
Stable former drinking	664	-0.20 (0.74)	-1.14 (-1.4, -0.88)	0.02(-0.07, 0.10)	-2%	
Mostly low-to-moderate drinking	1091	0.13 (0.74)	-1.19 (-1.44, -0.93)	-0.02 (-0.09, 0.05)	2%	
Mostly heavy drinking	511	0.25 (0.69)	-1.20 (-1.46, -0.94)	-0.04 (-0.12, 0.05)	3%	
Mostly former drinking	1687	0.02 (0.69)	-1.17 (-1.42, -0.91)	0.00 (-0.07, 0.06)	0.3%	
Delayed Word Recall z-score	8707	0.06 (1.01)				
Stable never drinking	1033	0.08(1.02)	-1.74 (-2.29, -1.18)	Reference	Reference	0.032
Stable low-to-moderate drinking	3095	0.14 (0.97)	-1.71 (-2.26, -1.16)	0.02 (-0.10 ,0.15)	-1%	
Stable heavy drinking	176	0.13 (1.05)	-2.02 (-2.63, -1.42)	-0.29 (-0.58, 0.01)	17%	
Stable former drinking	664	-0.13 (1.01)	-1.90 (-2.46, -1.34)	-0.17 (-0.35, 0.01)	10%	
Mostly low-to-moderate drinking	1091	0.04 (1.01)	-1.78 (-2.33, -1.23)	-0.04 (-0.19, 0.11)	2%	
Mostly heavy drinking	511	0.14 (1.01)	-1.85 (-2.42, -1.29)	-0.12 (-0.30, 0.07)	7%	
Mostly former drinking	1687	0.01 (1.04)	-1.82 (-2.37, -1.28)	-0.09 (-0.22, 0.05)	5%	
Word Fluency z-score	8707	0.13 (0.95)				
Stable never drinking	1033	-0.09 (0.89)	-0.51 (-0.82, -0.20)	Reference	Reference	0.249
Stable low-to-moderate drinking	3095	0.29 (0.93)	-0.50 (-0.81, -0.19)	0.01 (-0.06, 0.08)	-2%	
Stable heavy drinking	176	0.36 (0.95)	-0.61 (-0.96, -0.27)	-0.10 (-0.27, 0.07)	20%	
Stable former drinking	664	-0.08 (0.95)	-0.55 (-0.87, -0.23)	-0.04 (-0.14, 0.06)	8%	
Mostly low-to-moderate drinking	1091	0.21 (0.98)	-0.57 (-0.88, -0.25)	-0.06 (-0.14, 0.03)	11%	
Mostly heavy drinking	511	0.33 (0.99)	-0.56 (-0.88, -0.24)	-0.05 (-0.16, 0.06)	10%	
Mostly former drinking	1687	0.02 (0.94)	-0.54 (-0.85, -0.23)	-0.03 (-0.10, 0.05)	5%	
Digit Symbol Substitution z-score	8707	0.16 (0.79)				
Stable never drinking	1033	0.06 (0.77)	-0.98 (-1.21, -0.74)	Reference	Reference	0.228
Stable low-to-moderate drinking	3095	0.35 (0.75)	-1.03 (-1.26, -0.79)	-0.05 (-0.11, 0.00)	5%	
Stable heavy drinking	176	0.13 (0.74)	-1.07 (-1.33, -0.80)	-0.09 (-0.22, 0.04)	9%	
Stable former drinking	664	-0.20 (0.80)	-1.00 (-1.25, -0.76)	-0.03 (-0.11, 0.05)	3%	
Mostly low-to-moderate drinking	1091	0.16 (0.81)	-1.05 (-1.29, -0.81)	-0.07 (-0.14, -0.01)	7%	
Mostly heavy drinking	511	0.25 (0.75)	-1.08 (-1.32, -0.83)	-0.10 (-0.18, -0.02)	10%	
Mostly former drinking	1687	0.05 (0.78)	-1.02 (-1.26, -0.79)	-0.05 (-0.11, 0.01)	5%	

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4; 1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker). * Complete case analysis. Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. † CI, confidence interval; percent, positive values represent % greater decline relative to the referent group.

APPENDIX M: ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY QUARTILES OF CUMULATIVE AVERAGE ETHANOL INTAKE FOR ATHERSOCLEROSIS RISK IN COMMUNITIES (ARIC) FOR AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 12. Adjusted Mean difference in 15-year change in cognitive performance by quartiles of cumulative average ethanol intake for Atherosclerosis Risk in Communities (ARIC) for African-American participants*

		Baseline Cognitive Score	15-Year Decline	Difference[*]		
Test/Quartile[‡]	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent[†]	$P_{overall}$
Global Factor Score z-score	2169	-0.72 (0.75)				
Quartile 1	286	-0.54 (0.75)	-1.18 (-1.77, -0.58)	Reference	Reference	0.812
Quartile 2	223	-0.50 (0.79)	-1.17 (-1.77, -0.58)	0.00 (-0.13, 0.14)	-0.4%	
Quartile 3	254	-0.68 (0.83)	-1.12 (-1.71, -0.53)	0.05 (-0.09, 0.20)	-4%	
Quartile 4	254	-0.72 (0.79)	-1.19 (-1.77, -0.61)	-0.01 (-0.17, 0.14)	1%	
Delayed Word Recall z-score	2169	-0.35 (1.11)				
Quartile 1	286	-0.28 (1.10)	-0.98 (-2.3,0.33)	Reference	Reference	0.644
Quartile 2	223	-0.24 (1.11)	-0.86 (-2.17,0.45)	0.13 (-0.17, 0.43)	-13%	
Quartile 3	254	-0.38 (1.09)	-0.95 (-2.25,0.34)	0.03 (-0.29, 0.35)	-3%	
Quartile 4	254	-0.46 (1.20)	-1.07 (-2.35,0.21)	-0.08 (-0.43, 0.27)	8%	
Word Fluency z-score	2169	-0.36 (1.06)				
Quartile 1	286	-0.15 (1.06)	-0.73 (-1.46,0.00)	Reference	Reference	0.904
Quartile 2	223	-0.16 (1.06)	-0.76 (-1.48, -0.03)	-0.03 (-0.20, 0.14)	4%	
Quartile 3	254	-0.34 (1.14)	-0.69 (-1.41, 0.02)	0.04 (-0.14, 0.21)	-5%	
Quartile 4	254	-0.36 (1.08)	-0.74 (-1.45, -0.03)	-0.01 (-0.20, 0.18)	1%	
Digit Symbol Substitution z-score	2169	-0.95 (0.94)				
Quartile 1	286	-0.73 (0.91)	-0.90 (-1.57, -0.24)	Reference	Reference	0.567
Quartile 2	223	-0.66 (0.91)	-0.87 (-1.53, -0.21)	0.03 (-0.11, 0.18)	-4%	
Quartile 3	254	-0.91 (1.04)	-0.84 (-1.49, -0.19)	0.06 (-0.09, 0.22)	-7%	
Quartile 4	254	-0.94 (0.97)	-0.95 (-1.60, -0.30)	-0.05 (-0.21, 0.12)	5%	

[‡] Global Factor Score z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-25.9g/wk., Quartile 3: 26.4-85.1g/wk., and Quartile 4: 85.5-2106.9g/wk.; Delayed Word Recall z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-25.9 g/wk., Quartile 3: 26.4-85.1 g/wk., and Quartile 4: 85.5-2106.9 g/wk.; Word Fluency z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-25.9 g/wk., Quartile 3: 26.4-85.1 g/wk., and Quartile 4: 85.5-2106.9 g/wk.; and Digit Symbol Substitution z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-25.9 g/wk., Quartile 3: 26.4-85.1 g/wk., and Quartile 4: 85.5-2106.9 g/wk.

[*] Difference. modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4;1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker).

[*] Complete case analysis

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

[†] CI, confidence interval; percent, positive values represent % greater decline relative to the referent group.

APPENDIX N: ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY QUARTILES OF CUMULATIVE AVERAGE ETHANOL FOR EUROPEAN-AMERICAN PARTICIPANTS

Appendix Table 13. Adjusted Mean difference in 15-year change in cognitive performance by quartiles of cumulative average ethanol intake for Atherosclerosis Risk in Communities (ARIC) for European-American participants*

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Quartile‡	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	$P_{overall}$
Global Factor Score z-score	8707	0.12 (0.71)				
Quartile 1	1712	0.20 (0.70)	-1.18 (-1.47, -0.89)	Reference	Reference	0.089
Quartile 2	1512	0.26 (0.69)	-1.20 (-1.49, -0.91)	-0.02 (-0.07, 0.03)	2%	
Quartile 3	1610	0.22 (0.70)	-1.22 (-1.51, -0.93)	-0.04 (-0.09, 0.01)	3%	
Quartile 4	1611	0.09 (0.70)	-1.25 (-1.54, -0.96)	-0.07 (-0.13, -0.01)	6%	
Delayed Word Recall z-score	8707	0.06 (1.01)				
Quartile 1	1712	0.14 (0.97)	-1.84 (-2.46, -1.21)	Reference	Reference	0.292
Quartile 2	1512	0.12 (1.00)	-1.84 (-2.47, -1.21)	0.00 (-0.11, 0.11)	0.2%	
Quartile 3	1610	0.08 (0.99)	-1.81 (-2.43, -1.19)	0.02 (-0.09, 0.14)	-1%	
Quartile 4	1611	0.00 (1.03)	-1.92 (-2.55, -1.30)	-0.09 (-0.21, 0.04)	5%	
Word Fluency z-score	8707	0.13 (0.95)				
Quartile 1	1712	0.14 (0.91)	-0.64 (-0.99, -0.28)	Reference	Reference	0.406
Quartile 2	1512	0.24 (0.94)	-0.66 (-1.02, -0.30)	-0.02 (-0.08, 0.04)	3%	
Quartile 3	1610	0.24 (0.97)	-0.69 (-1.05, -0.34)	-0.05 (-0.12, 0.01)	8%	
Quartile 4	1611	0.23 (0.99)	-0.68 (-1.03, -0.32)	-0.04 (-0.11, 0.03)	6%	
Digit Symbol Substitution z-score	8707	0.16 (0.79)				
Quartile 1	1712	0.26 (0.78)	-1.06 (-1.34, -0.79)	Reference	Reference	0.384
Quartile 2	1512	0.31 (0.77)	-1.09 (-1.36, -0.82)	-0.03 (-0.07, 0.02)	2%	
Quartile 3	1610	0.26 (0.78)	-1.08 (-1.35, -0.81)	-0.02 (-0.07, 0.03)	1%	
Quartile 4	1611	0.10 (0.77)	-1.11 (-1.38, -0.84)	-0.05 (-0.10, 0.01)	4%	

‡ Global Factor Score z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.3 g/wk., Quartile 3: 24.3-80.1 g/wk., and Quartile 4: 80.2-1071.5 g/wk.; Delayed Word Recall z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.3 g/wk., Quartile 3: 24.3-80.1 g/wk., and Quartile 4: 80.2-1071.5 g/wk.; Word Fluency z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.3 g/wk., Quartile 3: 24.3-80.1 g/wk., and Quartile 4: 80.2-1071.5 g/wk.; and Digit Symbol Substitution z-score: Quartile 1: 0 g/wk., Quartile 2: 2.7-24.3 g/wk., Quartile 3: 24.3-80.1 g/wk., and Quartile 4: 80.2-1071.5 g/wk.

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4;1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker).

* Complete case analysis

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† CI, confidence interval; percent, positive values represent % greater decline relative to the referent group.

APPENDIX O: ADJUSTED MEAN DIFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY VISIT 4 ETHANOL INTAKE STATUS FOR AFRICAN-AMERICAN PARTICIPANTS

Appendix Table 14. Adjusted Mean difference in 15-year change in cognitive performance by visit 4 ethanol intake status for Atherosclerosis Risk in Communities (ARIC) for African-American participants*

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Drinking status at visit 4	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	$P_{overall}$
Global Factor Score z-score	2169	-0.72 (0.75)				
Never drinking	753	-0.83 (0.70)	-1.21 (-1.59, -0.83)	Reference	Reference	0.052
Low-to-moderate drinking	498	-0.54 (0.78)	-1.09 (-1.47, -0.71)	0.12 (0.02, 0.22)	-10%	
Heavy drinking	68	-0.59 (0.91)	-1.03 (-1.45, -0.61)	0.18 (-0.03, 0.39)	-15%	
Former drinking	848	-0.73 (0.75)	-1.11 (-1.48, -0.74)	0.10 (0.01, 0.19)	-8%	
Delayed Word Recall z-score	2169	-0.35 (1.11)				
Never drinking	753	-0.37 (1.11)	-1.88 (-2.73, -1.03)	Reference	Reference	0.478
Low-to-moderate drinking	498	-0.30 (1.17)	-1.76 (-2.61, -0.90)	0.12 (-0.10,0.34)	-6%	
Heavy drinking	68	-0.39 (1.13)	-1.71 (-2.65, -0.77)	0.17 (-0.30, 0.64)	-9%	
Former drinking	848	-0.37 (1.08)	-1.72 (-2.56, -0.88)	0.16 (-0.04, 0.36)	-8%	
Word Fluency z-score	2169	-0.36 (1.06)				
Never drinking	753	-0.49 (1.02)	-0.61 (-1.08, -0.14)	Reference	Reference	0.084
Low-to-moderate drinking	498	-0.14 (1.11)	-0.47 (-0.95, 0.00)	0.13 (0.01, 0.26)	-22%	
Heavy drinking	68	-0.28 (1.1)	-0.45 (-0.97, 0.07)	0.16 (-0.10, 0.41)	-26%	
Former drinking	848	-0.39 (1.03)	-0.48 (-0.95, -0.01)	0.13 (0.02, 0.24)	-21%	
Digit Symbol Substitution z-score	2169	-0.95 (0.94)				
Never drinking	753	-1.11 (0.91)	-0.80 (-1.22, -0.38)	Reference	Reference	0.529
Low-to-moderate drinking	498	-0.74 (0.94)	-0.78 (-1.2, -0.36)	0.02 (-0.09, 0.12)	-2%	
Heavy drinking	68	-0.73 (1.08)	-0.92 (-1.39, -0.46)	-0.12 (-0.34, 0.10)	15%	
Former drinking	848	-0.96 (0.92	-0.83 (-1.25, -0.42)	-0.03 (-0.12, 0.07)	4%	

* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4; 1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker).

* Complete case analysis

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† CI, confidence interval; percent, positive values represent % greater decline relative to the referent group.

APPENDIX P: ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY VISIT 4 ETHANOL INTAKE STATUS FOR EUROPEAN-AMERICAN PARTICIPANTS

Appendix Table 15. Adjusted Mean difference in 15-year change in cognitive performance by visit 4 ethanol intake status for Atherosclerosis Risk in Communities (ARIC) for European-American participants*

		Baseline Cognitive Score	15-Year Decline	Difference*		
Test/Drinking status at visit 4	N	Mean (SD)	Estimate (95% CI)	Estimate (95% CI)	Percent†	$P_{overall}$
Global Factor Score z-score	8707	0.12 (0.71)				
Never drinking	1450	-0.02 (0.69)	-1.16 (-1.41, -0.92)	Reference	Reference	0.249
Low-to-moderate drinking	4254	0.25 (0.69)	-1.17 (-1.41, -0.92)	0.00 (-0.05, 0.05)	0.3%	
Heavy drinking	629	0.23 (0.69)	-1.23 (-1.49, -0.98)	-0.07 (-0.15, 0.01)	6%	
Former drinking	2373	-0.04 (0.71)	-1.18 (-1.42, -0.93)	-0.01 (-0.07, 0.04)	1%	
Delayed Word Recall z-score	8707	0.06 (1.01)				
Never drinking	1450	0.01 (1.01)	-1.77 (-2.30, -1.24)	Reference	Reference	0.007
Low-to-moderate drinking	4254	0.11 (0.98)	-1.77 (-2.30, -1.24)	0.00 (-0.11, 0.11)	0%	
Heavy drinking	629	0.14 (1.03)	-1.96 (-2.50, -1.41)	-0.19 (-0.36, -0.02)	11%	
Former drinking	2373	-0.03 (1.04)	-1.88 (-2.41, -1.36)	-0.11 (-0.23, 0.00)	6%	
Word Fluency z-score	8707	0.13 (0.95)				
Never drinking	1450	-0.11 (0.9)	-0.44 (-0.74, -0.14)	Reference	Reference	0.348
Low-to-moderate drinking	4254	0.27 (0.95)	-0.46 (-0.76, -0.16)	-0.02 (-0.08, 0.04)	4%	
Heavy drinking	629	0.35 (0.96)	-0.51 (-0.82, -0.21)	-0.08 (-0.17, 0.02)	18%	
Former drinking	2373	-0.01 (0.94)	-0.48 (-0.78, -0.18)	-0.04 (-0.11, 0.03)	9%	
Digit Symbol Substitution z-score	8707	0.16 (0.79)				
Never drinking	1450	0.03 (0.78)	-0.97 (-1.20, -0.74)	Reference	Reference	0.016
Low-to-moderate drinking	4254	0.30 (0.77)	-1.03 (-1.26, -0.80)	-0.06 (-0.11, -0.01)	6%	
Heavy drinking	629	0.23 (0.75)	-1.08 (-1.32, -0.85)	-0.11 (-0.18, -0.04)	11%	
Former drinking	2373	-0.02 (0.79)	-1.02 (-1.25, -0.79)	-0.05 (-0.10, 0.00)	5%	

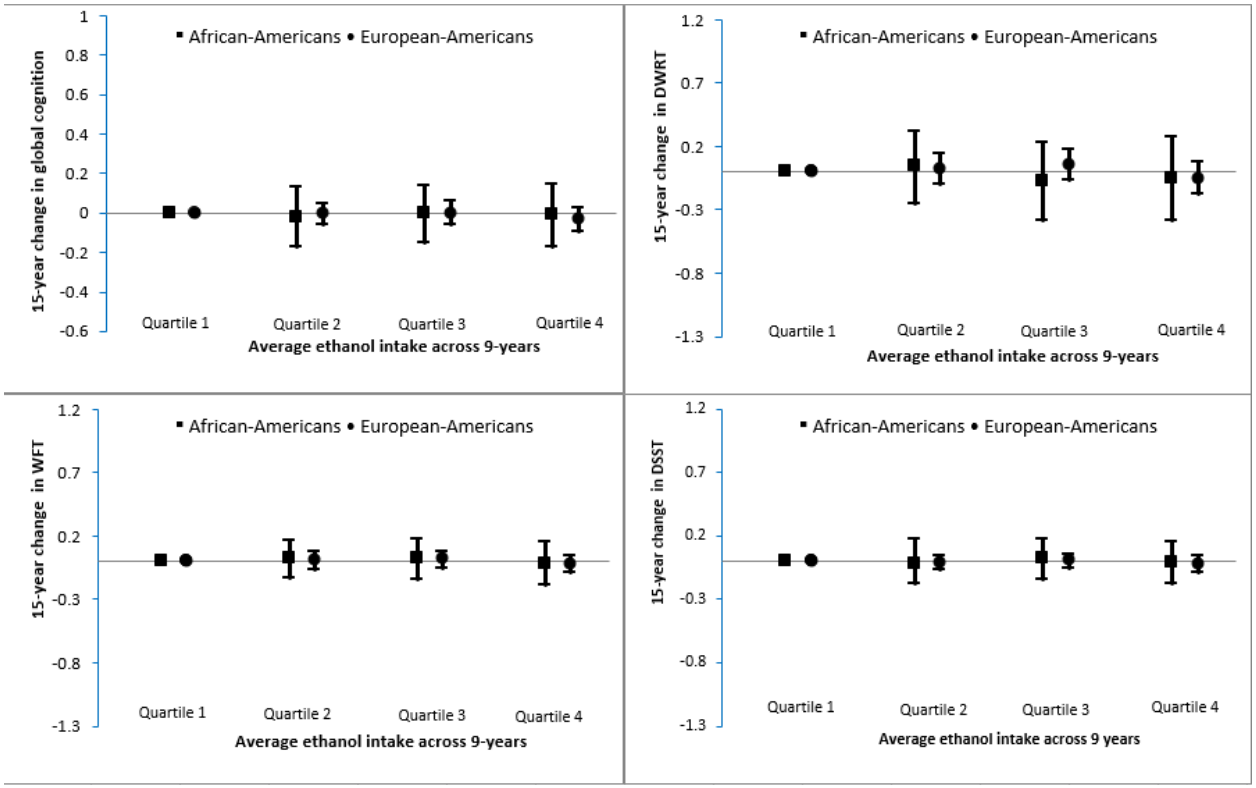
* Difference modeled as the follow-up neurocognitive exam (visit 5; 2011-2013) z-score minus study baseline (visit 4; 1996-1998) z-score. Negative values correspond to greater decline compared to the reference (stable never drinker).

* Complete case analysis

Adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele.

† CI, confidence interval; percent, positive values represent % greater decline relative to the referent group.

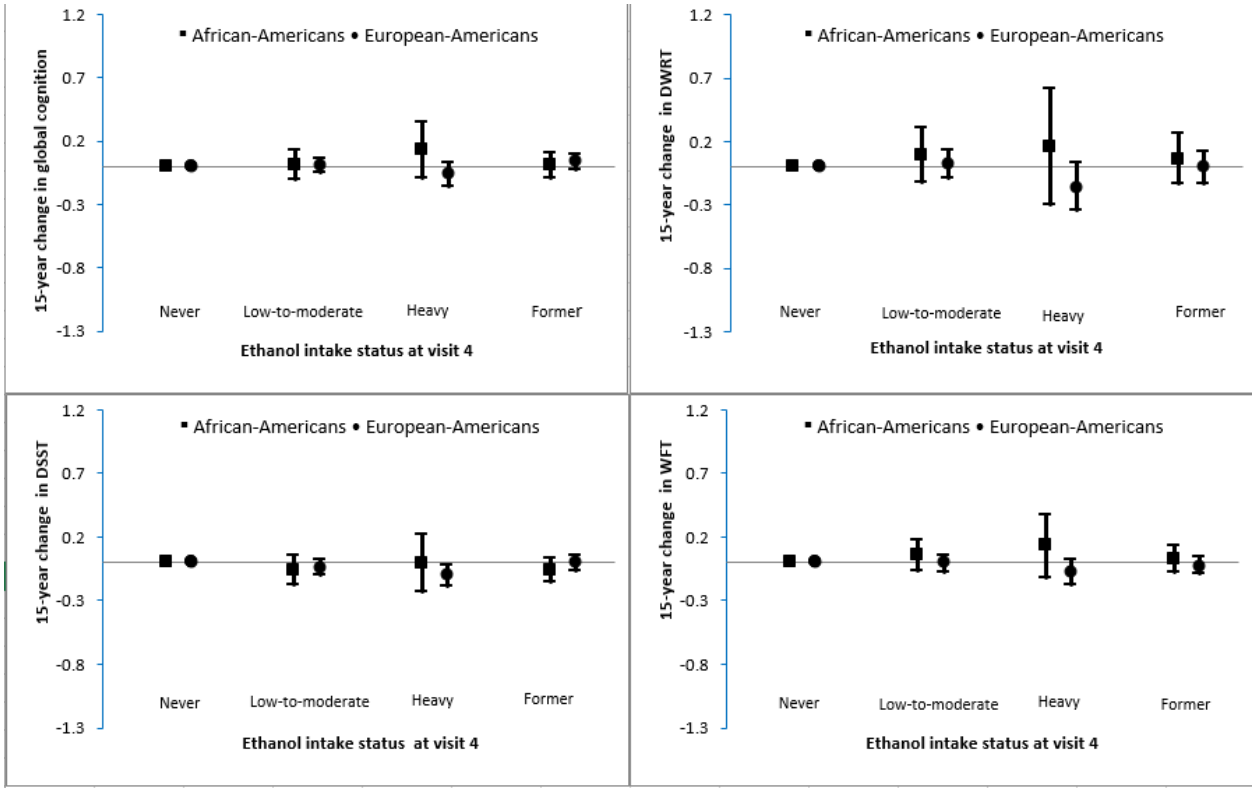
APPENDIX Q: PLOT OF ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY VISIT 4 ETHANOL INTAKE STATUS BY RACE/ETHNICITY



Appendix Figure 2. Estimated mean difference in the 15-year change in cognitive performance by average ethanol intake across 9-years in mid-life relative to quartile 1

All models were adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances. Abbreviations: DWRT, delayed word recall test; DSST, digit symbol substitution test; and WFT, word fluency test. Sample sizes: African-Americans (global cognition, n=2362; DWRT, n=2,380; WFT, n=2,375; and DSST, n=2367) and European-Americans (global cognition, n=8709; DWRT, n=8,723; WFT, n=8719; and DSST, n=8716).

APPENDIX R: PLOT OF ADJUSTED MEAN DIFFERENCE IN 15-YEAR CHANGE IN COGNITIVE PERFORMANCE BY VISIT 4 ETHANOL INTAKE STATUS BY RACE/ETHNICITY



Appendix Figure 3. Estimated mean difference in the 15-year change in cognitive performance by ethanol intake at study baseline relative to those who reported never drinking

All models were adjusted for age, age squared, sex, race-center, education attainment, diet quality score, physical activity, smoking status, body mass-index (BMI), diabetes, history of stroke and the Apo lipoprotein E ε4 (APOE ε4) allele. All estimates were averages from 25 rounds of multiple imputation combined using Rubin's rule and the variance of a function of the within and between completed data set variances. Abbreviations: DWRT, delayed word recall test; DSST, digit symbol substitution test; and WFT, word fluency test. Sample sizes: African-Americans (global cognition, n=2360; DWRT, n=2378; WFT, n=2373; and DSST, n=2365) and European-Americans (global cognition, n=8,708; DWRT, n=8722; WFT, n=8718; and DSST, n=8715).

APPENDIX S: SINGLE SNPS INTERACTION RESULTS FOR AFRICAN-AMERICANS

Appendix Table 16. Linear regression results for the interaction of ethanol intake-associated genetic variants and log-ethanol intake in relation to 15-year cognitive change in general cognitive performance for African-Americans

SNP	β_E	P_E	β_G	P_G	β_{GXE}	P_{GXE}
rs10008281	0.003	0.891	0.006	0.869	-0.013	0.558
rs1022084	-0.038	0.143	-0.013	0.701	0.029	0.129
rs10283354	-0.006	0.715	-0.065	0.237	-0.006	0.860
rs10840100	-0.014	0.555	-0.007	0.831	0.009	0.647
rs11768390	-0.016	0.476	-0.045	0.183	0.011	0.557
rs11940694	0.007	0.738	0.026	0.430	-0.015	0.426
rs12910841	-0.013	0.444	-0.017	0.776	0.033	0.285
rs1685404	-0.010	0.592	-0.040	0.283	0.010	0.685
rs2514218	0.000	0.991	0.010	0.815	-0.024	0.396
rs271085	0.005	0.801	0.032	0.381	-0.017	0.417
rs35608804	-0.005	0.822	0.001	0.970	0.000	0.998
rs4780836	-0.007	0.745	0.023	0.496	0.002	0.931
rs58440244	-0.004	0.836	0.022	0.542	-0.003	0.907
rs62040427	0.001	0.954	-0.001	0.983	-0.014	0.523
rs6496321	-0.006	0.727	-0.022	0.631	0.003	0.893
rs6673687	-0.007	0.724	-0.011	0.743	0.002	0.910
rs7355953	-0.004	0.795	-0.060	0.393	-0.024	0.624
rs78757076	0.004	0.813	-0.012	0.763	-0.023	0.310
rs7940127	0.001	0.939	-0.004	0.904	-0.014	0.528
rs9929584	-0.012	0.587	-0.033	0.310	0.007	0.710

Abbreviations: ARIC, Atherosclerosis Risk in Communities (ARIC) Study; SNP, Single nucleotide polymorphism; β_E, estimate for log-ethanol intake with corresponding p-value (P_E) ; β_G, estimate for the SNP with corresponding p-value (P_G); β_{GXE}, Estimate for the interaction term between the SNP and log-ethanol intake with corresponding p-value (P_{GXE}).

Adjusted for age, sex, race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity, APOE ε4 status, and principal components

APPENDIX T: SINGLE SNPS INTERACTION RESULTS FOR EUROPEAN-AMERICANS

Appendix Table 17. Linear regression results for the interaction of ethanol intake-associated genetic variants and log-ethanol intake in relation to 15-year cognitive change in general cognitive performance for European-Americans

SNP	β_E	P_E	β_G	P_G	β_{GXE}	P_{GXE}
rs1123285	-0.011	0.088	-0.001	0.952	-0.001	0.850
rs1229984	-0.013	0.011	-0.036	0.443	0.002	0.927
rs12651313	-0.015	0.034	-0.024	0.134	0.003	0.584
rs1713676	-0.011	0.150	0.007	0.674	-0.002	0.766
rs2165670	-0.015	0.006	-0.033	0.233	0.011	0.270
rs55872084	-0.012	0.053	-0.010	0.607	-0.001	0.839
rs62250685	-0.014	0.034	0.009	0.572	0.002	0.754
rs7185555	-0.011	0.046	0.013	0.592	-0.006	0.490
rs72859280	-0.012	0.020	0.008	0.867	-0.010	0.585
rs74664784	-0.012	0.059	0.014	0.392	0.000	0.994
rs7950166	-0.008	0.279	0.001	0.937	-0.006	0.340

Abbreviations: SNP, Single nucleotide polymorphism; β_E, estimate for log-ethanol intake with corresponding p-value (P_E) ; β_G, estimate for the SNP with corresponding p-value (P_G); β_{GXE}, Estimate for the interaction term between the SNP and log-ethanol intake with corresponding p-value (P_{GXE}).

Adjusted for age, sex, race-center, education attainment, smoking status, body mass index (BMI), diabetes, history of stroke, diet score, physical activity, APOE ε4 status, and principal components

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