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PCSK9 Is Increased In Cerebrospinal Fluid Of Individuals With Alcohol Use Disorder

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Abstract

Background: Recent studies have shown that alcohol use affects the regulation and expression of proprotein convertase subtilisin/kexin 9 (PCSK9). While a major role of PCSK9 in hepatic function and lipid regulation has been clearly established, other pleiotropic effects remain poorly understood. Existing research suggests a positive association between PCSK9 expression in the brain and psychopathology, with increased levels of PCSK9 in the cerebrospinal fluid (CSF) of individuals with dementia and epigenetic modifications of *PCSK9* associated with Alcohol Use Disorder (AUD). In this study, we hypothesized that chronic alcohol use would increase PCSK9 expression in CSF.

Methods: PCSK9 levels in CSF were measured in individuals with AUD (n=42) admitted to an inpatient rehabilitation program and controls (n=25). CSF samples in AUD were assessed at two-time points, at day 5 and day 21 after admission. Furthermore, plasma samples were collected and measured from the individuals with AUD.

Results: PCSK9 in CSF was significantly increased in the AUD group at day 5 and day 21 compared to the controls ($p < 0.0001$). Plasma PCSK9 levels were correlated positively with CSF PCSK9 levels in AUD ($p = 0.0493$).

Conclusions: Our data suggest that PCSK9 is elevated in the CSF of individuals with AUD, which may indicate a potential role of PCSK9 in AUD. Additional studies are necessary to further elucidate the functions of PCSK9 in the brain.

Keywords

alcohol use disorder; proprotein convertase subtilisin/kexin 9; central nervous system; cerebrospinal fluid; lipid regulation

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Conflicts of Interest

The authors declare no conflict of interest.

Introduction

Alcohol Use Disorder (AUD) is characterized by chronic and uncontrolled alcohol consumption and is associated with high degrees of morbidity and mortality (Greenfield and Weisner, 1995, Harris, 1995, Stahre et al., 2014). Although the pathophysiology of AUD is multifaceted and remains elusive, strong evidence suggests a role for both genetic and environmental factors. From a genetic standpoint, research has shown that AUD is a complex disease, with many genes contributing small fractions to the overall risk (Enoch and Goldman, 2001, Agrawal and Lynskey, 2008, Tawa et al., 2016). Several environmental factors also likely contribute to the risk and pathophysiology of AUD, including socioeconomic factors, early life stress, trauma, and psychiatric comorbidities (Enoch, 2011, Sampson et al., 2015, Regier et al., 1990). Interestingly, one way the environment can interact with the genome is through epigenetic regulation—broadly defined as the modification of protein expression without DNA sequence changes. It is thought that various epigenetic mechanisms contribute to the pathophysiology of addictions. Some are specific to the drugs abused, while others are more generally involved in common pathways that lead to maladaptive and addictive behaviors (Ron and Barak, 2016, Robison and Nestler, 2011, Kessler et al., 1997).

We recently conducted an epigenome-wide association study in individuals with AUD and identified the gene encoding Proprotein Convertase Subtilisin/Kexin 9 (*PCSK9*) as a primary target (Lohoff et al., 2018). *PCSK9* is highly expressed in liver (Cariou et al., 2015, Zaid et al., 2008, Lambert et al., 2012) and forms a complex with the lipid-density lipoprotein receptor (LDL-R), which results in lysosomal degradation of LDL-R thus reducing hepatic clearance of LDL cholesterol (LDL-C) in blood (Ferri and Ruscica, 2016, Lambert et al., 2012, Poirier et al., 2008). The role of *PCSK9* in lipid regulation was initially identified by gain-of-function mutations in *PCSK9* which causes autosomal dominant hypercholesterolemia (Abifadel et al., 2003). Analogously, loss-of-function mutations are associated with a decrease in LDL-C levels and low rates of cardiovascular disease (CVD) (Cohen et al., 2005, Cohen et al., 2006). These findings led to the rapid development of *PCSK9* inhibitors as a new class of lipid-lowering medications, with recent U.S. Food and Drug Administration (FDA) approval of anti-*PCSK9* monoclonal antibodies for the treatment of adults with heterozygous familial hypercholesterolemia or clinical atherosclerotic cardiovascular disease, who require additional lowering of LDL-C (2015, 2017).

Beside the action of *PCSK9* in the liver, extra-hepatic functions, especially in brain, are emerging areas of research. *PCSK9*, which was initially termed neural apoptosis-regulated convertase 1 (NARC-1), has been shown to be involved in the differentiation of cortical neurons (Seidah et al., 2003) and apoptosis of cerebellar (Kysenius et al., 2012, Do et al., 2016, Bingham et al., 2006) and hippocampal neurons (Zhao et al., 2017). Moreover, experiments using zebrafish as a model to investigate *PCSK9*'s involvement in central nervous system (CNS) development showed that *PCSK9* knockdown caused general cerebellar neuron disorganization and loss of hindbrain-midbrain boundaries, which led to early embryonic death (Poirier et al., 2006). Nevertheless, *PCSK9* null-mutant mice were

found to be viable with abnormalities in LDL regulations, glucose tolerance, and pancreas islet function (Rashid et al., 2005, Zaid et al., 2008). There has been no extensive behavioral or brain phenotyping of the PCSK9 null-mutant mice so far. Clinically, the use of PCSK9 inhibitors has raised questions about neurocognitive side effects (Koren et al., 2014, Walker, 2017), although this is controversial as recent data do not support major effects on cognitive function (Giugliano et al., 2017). Furthermore, a recent study suggested a possible link between PCSK9 inhibitors and depression (Alghamdi et al., 2018), which is in line with long-standing concerns about lipid-lowering drugs and their association with depression (Cham et al., 2016, Hyyppa et al., 2003, Morales et al., 2006, Lohoff, 2018).

Based on the potential role of PCSK9 in neuronal apoptosis and cholesterol regulation, with hypercholesterolemia being a major risk factor for late-onset Alzheimer's disease (ALZ), several studies have investigated underlying mechanisms of PCSK9 in ALZ (Burns and Duff, 2002, Jonas et al., 2008). Furthermore, two recent studies investigated PCSK9 concentrations in cerebrospinal fluid (CSF) from individuals with ALZ and controls: Zimetti et al. found that PCSK9 levels were higher in ALZ than in controls, while Courtemanche et al. did not find a difference between ALZ and non-ALZ patients with other neurodegeneration disorders but showed increased PCSK9 CSF levels in two subgroups, compared to age-matched controls (Zimetti et al., 2017, Courtemanche et al., 2018). In this study, we further explored the role of PCSK9 in CNS. First, we hypothesized that alcohol exposure would be associated with PCSK9 expression and assessed differences of the CSF PCSK9 levels between controls and AUD subjects. Second, given the hypothesis that multi-day-long periods of alcohol abstinence would affect the expression levels, we analyzed longitudinal CSF samples collected from AUD inpatients. Third, we examined the expression levels of PCSK9 in CSF and plasma in individuals with AUD and analyzed their relationship, expecting the PCSK9 expression in plasma could predict the levels in CSF.

Material and Methods

AUD Subjects

Individuals 21 years of age or older who were seeking inpatient treatment for alcohol drinking-related problems were recruited primarily from the Washington, D.C. metropolitan area through newspaper advertisements and healthcare organizations, particularly those that see patients with alcohol and drug problems throughout Northern Virginia, Maryland, West Virginia, and the Washington, D.C. and Baltimore metropolitan areas. Participants came to the National Institute on Alcohol Abuse and Alcoholism (NIAAA) inpatient treatment unit at the National Institutes of Health (NIH) Clinical Center in Bethesda, Maryland. All participants provided written informed consent in compliance with the Declaration of Helsinki and the Institutional Review Board of the NIH/NIAAA. Participants were excluded from participation if they met any of the following criteria: 1) presence of medical problems that could not be adequately managed at the NIH Clinical Center, as determined by the medical advisory investigator in consultation with subspecialty clinical service providers 2) presence of serious neuro-psychiatric conditions which impair judgment or cognitive function to an extent that precludes them from providing informed consent, such as acute psychosis or severe dementia; 3) history of seizures, head trauma (defined as a period of

unconsciousness exceeding 1h); 4) presence of a medical condition requiring chronic medications; 5) unlikely or unable to complete the treatment program because they were likely to be incarcerated while in the protocol; or 6) were required to receive treatment by a court of law or were involuntarily committed to treatment.

Participants were assessed for a diagnosis of alcohol dependence and any comorbid psychiatric disorders using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV Axis I Disorders Diagnosis (SCID IV) (First MB, 1996). Severity of alcohol dependence was assessed using the Alcohol Dependence Scale (ADS) (Skinner H, 1984) and the Addiction Severity Index (ASI), which quantifies problem severity in seven domains (McLellan AT, 1992). Alcohol consumption during the preceding three months was quantified using Time-Line Follow-Back (TLFB) (Sobell LC, 1992), and withdrawal symptoms were assessed using the revised Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-AR) (Sullivan JT, 1989). Alcohol craving was assessed using the Penn Alcohol Craving Scale (PACS) (Flannery et al., 1999). All participants had a structural MRI of the brain to rule-out central nervous system pathology.

AUD participants received oxazepam or diazepam to treat alcohol withdrawal symptoms (n=19 for oxazepam, 3 for diazepam). The average of equivalent benzodiazepine dose, total dosage regardless of what specific benzodiazepines they were given, was 83.64 mg, and the average treatment course was 2.4 days. The dosage was correlated with neither plasma nor CSF PCSK9 values at day 5. The diet or nutrition status of the inpatients was not reported.

CSF Collection of AUD Subjects and Controls

Lumbar punctures (LP) were performed on 42 participants with AUD to collect CSF. Participants had up to two LPs. The first LP was performed after the participant had a CIWA-AR score of <8, approximately five days (4.24 (average days) ± 2.98 (SD) days between the admission date and the first LP date) after each participant's last drink (n=42), and a second LP was performed 21 days after their last drink (n=33, completers). Nine individuals with AUD did not complete the second LP (dropouts) due to aborted procedures, subjects not giving consent for the second LP, or medical side effects after the first LP which prohibited a second LP. A separate consent was signed before each LP. Ten hours prior to the LP, all subjects were on overnight bedrest and received nothing by mouth, nil per os (NPO). The LPs were performed between 09:00am and 10:00am. Subjects were in the left lateral decubitus position and the procedure was performed between the L3/L4 or L4/L5 interspace following administration of local anesthetic. The first 5 mL of CSF collected were sent for clinical purposes to ensure patient evaluation and safety. Following that, 12 mL of CSF were collected in a single aliquot containing no additives. The 12 mL sample was immediately placed on wet ice. After being thoroughly mixed to avoid gradient effects, the CSF was quickly aliquoted into 1 mL aliquots and stored at -80°C . The LP procedure was part of a larger NIAAA protocol which was approved by the NIH Institutional Review Board (IRB). All participants provided written informed consent before participation. CSF of deidentified controls was purchased from Discovery Life Sciences, Inc. (Research Biospecimen Sample Bank, Los Osos, CA, USA). To represent the same age group as the AUD subjects, CSF of 25 individuals between the age of 21–65 was selected. Controls had no major psychiatric or

neurological disorders; however, detailed medical history was not available. A majority of CSF samples (22 out of 25 samples) from the controls were collected within 90 days, which did not affect variance of PCSK9 CSF values. Sample collection followed the standards for patient care.

Plasma Collection

Plasma samples were collected at approximate day 5 when the first LP was performed from each of the AUD inpatients for which CSF samples were obtained. All bloods were drawn around 08:00 am after overnight bed rest. Participants were NPO for ~ 10 h prior to blood collection. Blood was collected in prechilled ethylenediaminetetraacetic acid (EDTA) tubes which were promptly put into wet ice and taken to the laboratory to be processed. Samples were spun in a 4 °C centrifuge (Beckman-Coulter Allegra X-12R, Indianapolis, IN, USA) at 1880 g for 10 min; the plasma was then aliquoted into NUNC cryovials (Thermo Fisher Scientific, Waltham, MA, USA) and frozen at – 80 °C until assayed. Average drinks per day, drinking days and heavy drinking days were recorded for the 90 days prior to inpatient admission using the TLFB. Samples were obtained from two IRB approved protocols, collected on the date of admission to the NIAAA treatment program, and processed by the NIH Clinical Center Department of Laboratory Medicine.

Plasma PCSK9 Analysis

PCSK9 protein concentrations were determined in subjects with AUD using Luminex technology (Luminex Corporation, Austin, TX, USA). Luminex had a wide assay range (up to 97 ng/ml), which was suitable for the high protein concentration. A Human Magnetic Luminex Screening Assay was performed per the manufacturer's instructions (catalogue #LXSAHM, R&D Systems, Minneapolis, MN, USA), with plasma dilutions of 1:5. Data was analyzed using Milliplex Analyst software (EMD Millipore Corporation, Merck KGaA, Darmstadt, Germany). Samples were assayed in duplicates. One sample was excluded due to a coefficient of variance (CV) greater than 0.1 between duplicates. A separate sample was excluded as an outlier because it was more than three standard deviations below the mean. Average CV for the samples was 2.27 ± 2.00 (mean $\pm$ SD). Samples were randomized across four 96-well plates, and CV for two quality control samples run on all four plates were 0.069 and 0.065, respectively.

CSF PCSK9 Analysis

PCSK9 levels in the CSF samples were measured using a high-sensitivity, quantitative sandwich enzyme immunoassay (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. ELISA had a high sensitivity (0.219 ng/mL), which was suitable for the low protein concentration. All CSF samples were randomized and run in duplicate. Standards were also included in duplicates on each plate. Briefly, assay diluent was added to each well followed by addition of standards and samples. After incubation for two hours at room temperature, wells were washed four times with a diluted ELISA wash buffer. Conjugate was added to each well, and plates were incubated for two hours. After the washing, substrate solution was added and incubated without light for 30 minutes. Finally, stop solution was added and then absorbance was read at 540 nm with a spectrophotometer.

Data Analysis

Statistical analysis was performed using GraphPad Prism Software program (GraphPad Software Inc. La Jolla, CA, USA) for the analysis of the CSF and plasma samples. Unpaired t-test and Pearson's correlation were used for normally distributed data. In order to analyze data for CSF, separate unpaired t-tests were used to compare control CSF samples to AUD CSF samples collected at day 5 and at day 21, and Bonferroni correction was applied to control multiple comparisons ($P < 0.05/2$). Statistical analysis of the longitudinal CSF data was done by a paired t-test. The correlation of CSF and Plasma was attained using linear regression and Pearson's correlation. Grubbs' test was used to determine outliers, and we found no outliers. Data were expressed as the means $\pm$ SEM. A p-value of < 0.05 or < 0.025 (Bonferroni correction) was considered to indicate statistical significance.

Results

Baseline Characteristics of Participants

Demographic characteristics of the subjects are shown in Table 1. CSF samples were collected from 42 alcohol-dependent individuals and 25 controls. There were more males than females in both groups (78.57% for AUD and 60% for controls), and mean ages of the groups were similar (44.18 for AUD and 43.4 for controls). Two CSF samples were collected from AUD inpatients, one at day 5 and one at day 21 after their last drink. Only 33 out of the 42 inpatients, however, received the second LP at day 21. There were no statistically significant differences between completers and dropouts with regard to demographics or clinical characteristics.

PCSK9 Concentrations in CSF and Correlation Between CSF and Plasma PCSK9

First, we investigated the difference of PCSK9 in CSF between the control and the AUD subjects. The AUD inpatients showed markedly higher CSF PCSK9 levels than the controls ($n=25$ for controls, 42 for AUD at day 5, and $n=33$ for AUD at day 21; Control vs AUD at day 5: $t(66) = 4.191$, $p < 0.0001$; Control vs AUD at day 21: $t(56) = 4.213$, $p < 0.0001$). However, CSF PCSK9 levels in each group did not show any gender differences ($n=15$ for male controls, 10 for female controls, 33 for male AUD at day 5, 9 for female AUD at day 5, 27 for male AUD at day 21, and 6 for female AUD at day 21; male vs female controls: $t(23) = 0.4214$, $p = 0.6773$; male vs female AUD at day 5: $t(40) = 0.1652$, $p = 0.8696$; male vs female AUD at day 21: $t(31) = 0.6677$, $p = 0.5093$) (Figure 1A). We further analyzed longitudinal changes of PCSK9 in CSF obtained from the AUD inpatients over 14 days after abstinence from alcohol consumption. Remarkably, most of the PCSK9 levels in CSF were constant at the two-time points, and the changes of PCSK9 levels over 14 days did not show a statistical difference ($n=33$; $t(32) = 1.675$, $p = 0.1037$) (Figure 1B).

Next, we compared PCSK9 concentrations in CSF with those in plasma of the AUD inpatients, both of which were collected from the same subjects at day 5. The levels of PCSK9 between plasma and CSF were positively associated ($n=41$ for AUD at day 5; $r=0.3091$, $p=0.0493$) (Figure 2). However, the levels of plasma PCSK9 were not correlated with alcohol dependence scale (ADS) scores, a quantitative measure of the severity of alcohol dependence ($n=40$; Pearson $r=-0.1549$, $p=0.3692$).

Discussion

In this study, we investigated the PCSK9 levels in CSF collected from individuals with AUD and controls. We found that levels of CSF PCSK9 were increased in the AUD subjects compared to the control group (Figure 1A). This is in line with existing research suggesting a potential increase in CSF PCSK9 levels in patients with ALZ (Zimetti et al., 2017) or neurodegenerative disorders more broadly. The associations between PCSK9 in both AUD and neurodegenerative disorders may have similar causes—in particular, they may be linked to the role of PCSK9 in neuronal apoptosis (Kysenius et al., 2012, Wu et al., 2014, Wu et al., 2012, Bingham et al., 2006). Neurodegeneration and brain atrophy are found in both ALZ and AUD (Sullivan et al., 2010). The atrophy in AUD is highly associated with hyperlipidemia (Namura, 2006) induced by excessive alcohol intake (Baraona and Lieber, 1979). Given the role of PCSK9 in regulation of lipid metabolism and its proapoptotic effect on neurons, it is possible that the increased CSF PCSK9 levels are in part responsible for the deleterious impact of alcohol on CNS in AUD. Furthermore, individuals with AUD often show chronic inflammation partially due to increased oxidative stress and lipopolysaccharides derived from gut microflora (Wang et al., 2010). It has been reported that inflammation promotes *PCSK9* expression (Feingold et al., 2008), and vice versa (Ricci et al., 2018). Based on our finding of enhanced CSF PCSK9 levels in AUD, it could be postulated that alcohol-mediated inflammation may induce *PCSK9* expression, which could exacerbate inflammation.

While under normal circumstances PCSK9 cannot cross the blood-brain barrier (BBB), inflammation can cause changes in BBB function (Varatharaj and Galea, 2017, Lopez-Ramirez et al., 2014). In addition, alcohol-mediated oxidative stress weakens BBB integrity, resulting in the transport of large molecules from blood to brain (Haorah et al., 2005, Haorah et al., 2007, Banks, 2009). Thus, it is possible that BBB permeability due to inflammation and oxidative stress allows PCSK9 to pass into the brain, which would explain why plasma PCSK9 is correlated with CSF PCSK9 in AUD individuals (Figure 2), but not in healthy individuals (Chen, Troutt et al. 2014). We also found the CSF PCSK9 levels remained high for 14 days after alcohol withdrawal (Figure 1B). While plasma PCSK9 levels change dynamically over the course of 24 hours, CSF PCSK9 levels have been shown to be stable over the course of the day in healthy individuals (Chen et al., 2014). Thus, our findings add to existing research of PCSK9 level stability in CSF by suggesting it is also stable on two-week timescales. This may indicate that PCSK9 in the brain is more tightly regulated and less sensitive to external changes, including alcohol abstinence, than it is in peripheral tissues.

We previously found that *PCSK9* expression was affected by epigenetic regulation of *PCSK9* and dependent on levels of alcohol intake. In the study, we showed that methylomic variation in the promoter region of *PCSK9* was associated with expression changes in a dynamic fashion, with initial alcohol exposure leading to lower *PCSK9* expression while chronic exposure led to higher *PCSK9* expression. End-stage liver disease states and acute liver toxicity models showed increased methylation but decreased expression likely due to impaired liver synthesis of PCSK9. Possible mechanisms include alcohol-induced methylation changes of the *PCSK9* promoter region either leading directly to expression

changers or via disruption of transcription factor binding sites involved in PCSK9 and lipid homeostasis, such as the regulatory element-binding protein-2 (SREBP-2) and hepatocyte nuclear factor-1 α (HNF-1 α) (Lohoff et al., 2018). This discovery provided a potential explanation for the strong epidemiological trends observed between alcohol use and cardiovascular outcomes, with total mortality of CVD being inversely associated with light to moderate alcohol consumption but positively associated with excessive consumption of alcohol, depicted as a J-shaped relationship (Costanzo et al., 2010). Remarkably, epidemiological studies also have indicated a similarly J-shaped relationship between the amount of alcohol consumption and the incidence of dementia and ALZ (Anstey et al., 2009, Panza et al., 2012, Piazza-Gardner et al., 2013, Pinder and Sandler, 2004, Anttila et al., 2004, Garcia et al., 2010, Zhou et al., 2014, Mukamal et al., 2003). Given the associations between alcohol consumption, PCSK9 levels, and ALZ, further research into the role of PCSK9 in ALZ as it relates to alcohol use is warranted. Additionally, more research is needed into the effects of PCSK9 inhibitors, as a new drug class for the treatment of hypercholesterolemia, on depression and cognition, which so far have been studied with mixed results (Koren et al., 2014, Walker, 2017, Alghamdi et al., 2018, Giugliano et al., 2017). Additionally, PCSK9's involvement with alcohol intake, memory, and mental disorders through cholesterol homeostasis should be investigated, as cholesterol homeostasis is a critical component of CNS function.

There are some limitations of our study, including the lack of detailed drinking information and cholesterol data for the CSF control samples, which makes the comparison to the AUD group challenging. However, we observed an increase in PCSK9 CSF levels in the AUD group for which cholesterol levels were within the normal range (average 177mg/dl), making it unlikely that this effect is solely driven by peripheral cholesterol. While it is possible that the control group might have had extremely low cholesterol levels resulting in lower PCSK9 CSF levels, it is unlikely in particular as PCSK9 levels of our controls samples were in line with what has been previously reported for control populations in CSF (Courtemanche et al., 2018). Future studies are needed to address this limitation and should include detailed drinking information and lipid characterization data to avoid potential confounding factors.

In conclusion, we found that PCSK9 CSF levels were increased in individuals suffering from AUD compared to controls, and that these increased levels were stable over 14 days despite alcohol abstinence. CSF PCSK9 was also positively correlated with plasma PCSK9 in AUD, which, to the best of our knowledge, has not previously been demonstrated. Further studies to determine the effects of PCSK9 on CNS will be important to better understand the pathophysiology of AUD and its related symptoms.

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Figure 1. PCSK9 levels in CSF collected from subjects of the study

(A) CSF control samples were purchased from Discovery Life Sciences (Los Osos, California research biospecimen sample bank). AUD CSF samples were collected from inpatients seeking inpatient treatment for alcohol drinking-related problems at the NIH Clinical Center. Two AUD CSF samples were collected at inpatient day 5 and 21. Among 42 inpatients, 9 people were withdrawn during the study and did not receive the second LP at day 21. The graph represents pooled data of CSF samples in AUD at inpatient day 5 (controls, $n=25$, 1.92 ± 0.27 ; AUD at day 5, $n=42$, 3.14 ± 0.16 ; AUD at day 21 $n=33$, 3.34 ± 0.21 ; Control vs AUD at day 5: $t(66) = 4.191$ $p < 0.0001$; Control vs AUD at day 21: $t(56) = 4.213$ $p < 0.0001$, separate unpaired t-tests followed by Bonferroni correction). (B) longitudinal data of CSF samples in AUD patients. The spaghetti plot shows CSF PCSK9 levels at inpatient day 5 and 21 from the same subjects ($n=33$ each, $p=0.1037$, paired t-test).

Figure 2. Association between CSF PCSK9 and plasma PCSK9

Levels of PCSK9 in CSF collected at inpatient day 5 were compared with plasma PCSK9 level. Plasma PCSK9 are positively correlated with CSF PCSK9 (n=41, $r = 0.3091$, $p=0.0493$).

Table 1.

Demographics and clinical characteristics of AUD participants and controls.

	AUD n = 42	Controls n = 25
Gender, n(%)		
Male	33 (78.57)	15 (60)
Female	9 (21.43)	10 (40)
Age, mean years (SD)	44.18 (8.79)	43.4 (12.28)
Ethnicity, n (%)		
Black/African American	25 (59.52)	1 (4)
European American	14 (33.33)	2 (8)
Multiracial	1 (2.38)	-
Unknown	2 (4.76)	22 (88)
Smokers, n (%)	29 (76.32) (n= 38)	NR
Body mass index, mean kg/m ² (SD)	24.05 (3.15) (n= 41)	NR
Total Blood Cholesterol, mean mg/dl (SD)	177.74 (45.95)	NR
Alcohol Dependence Severity Score, mean (SD)	18.49 (7.44) (n= 41)	NR
Number of Drinking Days in Past 90 Days, mean (SD)	67.81 (24.43)	NR
Number of Heavy Drinking Days in Past 90 Days, mean (SD)	61.88 (29.14)	NR
Average Number of Drinks per Drinking Day, mean (SD)	14.1 (10.91)	NR

Note. NR indicates “not reported” because the information was not available for this study.