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Article

Alcohol craving and severity are associated with dorsal anterior cingulate choline levels in individuals with an alcohol use disorder

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Abstract

Aims: Magnetic resonance spectroscopy (MRS) has been used to probe inflammation in the brain. While altered MRS metabolite levels have previously been found in individuals with alcohol use disorder (AUD), the relationship between potential metabolite markers of inflammation and the clinical correlates of AUD remains understudied. Therefore, this exploratory study sought to elucidate the clinical significance of inflammation in AUD by examining relationships between metabolites, AUD severity, alcohol consumption, and craving in individuals with AUD. Methods: Data for this secondary analysis are derived from a two-week clinical trial of ibudilast to treat AUD. Forty-three non-treatment-seeking individuals with an AUD (26M/17F) completed an MRS scan and alcohol-related questionnaires. MRS was performed using a multi-voxel array placed above the corpus callosum, extending from the pregnenual anterior cingulate to premotor cortex. The dorsal anterior cingulate was selected as the volume of interest. Metabolite levels of choline-compounds (Cho), *myo*-inositol (mI), and creatine+phosphocreatine (Cr) were quantified. Separate hierarchical regression models were used to evaluate the independent effects of metabolite levels on alcohol craving, alcohol problem severity, and alcohol consumption. Results: Dorsal anterior cingulate Cho predicted alcohol craving and alcohol problem severity over and above demographics, medication, and alcohol consumption measures. mI and Cr did not predict alcohol craving or alcohol problem severity. Metabolite markers were not predictive of alcohol consumption. Conclusions: This preliminary study indicates that dACC Cho is sensitive to clinical characteristics of AUD. This is a further step in advancing neurometabolites, particularly Cho, as potential biomarkers and treatment targets for AUD.

Introduction

Alcohol use disorder (AUD), characterized by an impaired ability to stop or control alcohol use despite clinically significant distress, impairment, or other adverse consequences, is a highly prevalent, chronic, relapsing condition with a significant public health impact. The World Health Organization estimates that alcohol consumption is responsible for nearly 6% of all deaths worldwide, and in 2016 100.4 million people globally were estimated to meet criteria for an AUD (World Health, 2014; Degenhardt et al., 2018).

A growing body of literature implicates the immune system in the development and maintenance of AUD. The neuroimmune hypothesis of AUD proposes a bidirectional relationship between inflammation and alcohol use (Cui et al., 2011; Cui et al., 2014; Mayfield and Harris, 2017). On the one hand, chronic alcohol exposure increases central and peripheral markers of inflammation (Mayfield et al., 2013; Crews et al., 2015); on the other hand, experimentally provoking inflammatory states results in increased motivation for alcohol consumption and enhanced alcohol-related reward in preclinical models (Blednov et al., 2011; Briones and Woods, 2013; Blednov et al., 2018). In humans, post-mortem brain tissue of individuals with AUD have increased proinflammatory gene expression (Liu et al., 2006; He and Crews, 2008), and individuals with AUD have heightened levels of peripheral proinflammatory cytokines relative to healthy controls (Achur et al., 2010; Adams et al., 2020). Proinflammatory protein levels positively correlate with clinical characteristics of AUD including alcohol consumption, alcohol craving, depression symptoms, anxiety symptoms, and AUD severity (Leclercq et al., 2012; Heberlein et al., 2014; Yen et al., 2017). However, evidence for heightened CNS levels of inflammatory signaling in clinical samples is limited. The degree to which AUD-associated inflammation can be detected in the human brain remains unclear, thus requiring further research to fully establish the neuroimmune hypothesis of AUD (Zahr et al., 2014; Kim et al., 2018).

One method by which human CNS inflammation may be probed *in vivo* is proton magnetic resonance spectroscopy (MRS). MRS detects neurometabolites thought to serve as

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neuroinflammatory biomarkers (Chang et al., 2013; Zahr et al., 2014). Neurometabolites of interest include *myo*-inositol (mI), a glial marker found primarily in gray matter (Hattingen et al., 2008); creatine+phosphocreatine (Cr), important compounds for energy metabolism and cryoprotection (Rackayova et al., 2017); and choline-containing compounds (Cho), metabolic biomarkers of cell membrane turnover which track with pain in human fibromyalgia patients and with reactive astroglyosis in non-human primates (Jung et al., 2020). Abnormal Cho levels have been linked to inflammation in central nervous system conditions including, multiple sclerosis, HIV, hepatitis-C, progressive multifocal leukoencephalopathy, cytomegalovirus, and other disorders (Chang et al., 2013). In frontal white-matter of Parkinson's patients, we recently found that Cho correlates positively with enlarged Virchow-Robin spaces, another putative sign of CNS inflammation (Donahue et al., 2022). However, there are competing mechanisms that may influence neurometabolites, including mI, Cr, and Cho; therefore, they may not solely represent neuroinflammation (Zahr et al., 2014).

Alcohol use has been shown to affect MRS metabolites, albeit with mixed results. Higher mI correlated with lifetime alcohol consumption (Zahr et al., 2014) while hippocampal Cr correlated inversely with alcohol consumption in older adults (Demnitz et al., 2020). Another study found that recreational alcohol consumption correlated positively with both Cr and Cho (Tunc-Skarka et al., 2015). Evidence for elevated Cho in AUD has similarly been mixed. Frontal white matter and thalamic Cho correlated positively with alcohol consumption in social drinkers (Ende et al., 2006) and chronic heavy drinkers (Meyerhoff et al., 2004). Thalamic Cho correlated positively with duration of AUD (Zahr et al., 2016) and was higher in binge drinkers than in non-binge drinkers (Zahr et al., 2016). Rodent models of AUD and binge drinking have also shown elevated MRS Cho in the hippocampus and basal ganglia (Zahr et al., 2009; Zahr et al., 2010). In contrast, several studies found lower prefrontal, thalamic, or cerebellar Cho in individuals with AUD than in healthy controls (Parks et al., 2002; Zahr et al., 2016; de Souza et al., 2018).

One brain structure heavily implicated in alcohol use is the anterior cingulate cortex (ACC) (Mann et al., 2001). The ACC is a key component of reward processing circuitry thought to form associations between rewards and actions and to inform decision-making, emotion, and motivation (Koob and Le Moal, 2001; Zhao et al., 2021). In preclinical models, ACC signaling is amplified by alcohol consumption and is implicated in alcohol withdrawal symptoms (Smith et al., 2017; Cucinello-Ragland et al., 2021). In humans, heightened ACC cue-reactivity is associated with alcohol craving and ACC cue-induced connectivity predicts future relapse in AUD (Kühn and Gallinat, 2011; Fryer et al., 2013; Zakiniaeiz et al., 2017). The ACC has also been a frequent region of interest in MRS studies of AUD (Silveri et al., 2014; Cheng et al., 2018; Grecco et al., 2021). However, the relationship between levels of neurometabolites detected by MRS and clinical correlates of AUD remains understudied.

Our recent clinical trial (Grodin et al., 2021; Grodin et al., 2022) found that the neuroimmune modulator ibudilast reduces heavy drinking, alcohol cue-related neural activation, and frontal white-matter Cho. Based on these findings and the above-cited evidence for Cho, mI, and Cr as potential markers of central inflammation, we conducted a secondary analysis of the MRS data from our clinical trial. We used hierarchical regression to quantify possible associations of ACC Cho, mI, and Cr levels with AUD clinical variables, whilst accounting for effects of drug treatment. The aim was to further elucidate the clinical significance of inflammation in AUD by examining relationships between neurometabolites, AUD severity, alcohol consumption, and craving in our sample of heavy drinkers with current AUD. We hypothesized that ACC Cho would be higher in individuals with higher alcohol consumption, higher alcohol craving scores, and greater AUD severity.

Materials and methods

Participants

Fifty-two individuals with AUD who were not seeking alcohol treatment were enrolled in a clinical trial of ibudilast as a treatment for AUD for two weeks (see Grodin et al. (2021) for full study details). Forty-five participants completed the neuroimaging scan and 43 had usable MRS data after undergoing quality control monitoring. Eligible participants were between 21 and 50 years of age, met criteria for a current DSM-5 diagnosis of mild-to-severe AUD, and drank >14 drinks/week for males or >7 drinks/week for females. Exclusion criteria were: currently receiving or seeking treatment for AUD; past year DSM-5 diagnosis of a substance use disorder (other than AUD or tobacco use disorder); lifetime diagnosis of schizophrenia, bipolar disorder, or any psychotic disorder; nonremovable ferromagnetic objects in body; claustrophobia; and history of serious head injury or prolonged period of unconsciousness (>30 min). Participants were also excluded if they had a medical condition that had a potential to interfere with safe participation and if they reported recent use of medications contraindicated with ibudilast. Female participants of childbearing age had to be practicing effective contraception and could not be pregnant or nursing.

Study design

Data for this secondary analysis are derived from a two-week clinical trial of ibudilast (ClinicalTrials.gov identifier: NCT03489850). Baseline data for this study comes from the in-person screening visit, which assessed eligibility and evaluated recent drinking and substance use behavior. At midpoint in the study (Day 8), participants underwent magnetic resonance spectroscopy imaging. This trial was approved by the Institutional Review Board of the University of California, Los Angeles. All study participants provided written informed consent after discussing the study medication and procedures with the study physician.

Assessments

Participants completed a series of assessments for eligibility and individual differences. These measures included the Structured Clinical Interview for DSM-5 (SCID-5) (First et al., 2015), which assessed for AUD; the Clinical Institute Withdrawal Assessment for Alcohol Scale - Revised (CIWA-Ar) (Sullivan et al., 1989); which evaluated alcohol withdrawal symptoms, and the 30-day Timeline Followback Interview (TLFB) (Sobell and Sobell, 1992) for alcohol, cigarettes, and cannabis. Two measures of drinking were derived from the TLFB interview: drinks per drinking day (DPDD) and percent heavy drinking days (PHDD). Heavy drinking was assessed using the NIAAA definition of heavy drinking: ≥4 drinks/day

for females and ≥5 drinks/day for males. Participants also completed assessments regarding their alcohol use problem severity and craving, including the Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993) and Alcohol Dependence Scale (ADS) (Skinner and Horn, 1984), which measure severity of alcohol use problems; and the Penn Alcohol Craving Scale (PACS) (Flannery et al., 1999) and Obsessive Compulsive Drinking Scale (OCDS) (Anton et al., 1995), which measure alcohol craving.

Neuroimaging protocol

Magnetic resonance acquisition

Participants were scanned at study mid-point (Day 8) on a 3.0 Tesla Siemens Prisma Scanner (Siemens Medical Solutions USA, Inc.; Malvern, PA) at the UCLA Center for Cognitive Neuroscience. Anatomical T1 images were obtained through a magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2,530 ms, TE = 1.74 ms, time to inversion = 1,260 ms, flip angle = 7°, voxel size: 1 mm^3, FOV = 250x250 mm^2, ~6.2 minutes). This MPRAGE and coronal-oblique and axial-oblique resliced copies were used to prescribe MRS. MRS acquisition and post-processing have been described (Alger et al., 2021; O'Neill et al., 2021). Briefly, 2-dimensional water-suppressed proton magnetic resonance spectroscopic imaging was acquired with a stimulated-echo acquisition mode (STEAM) pulse-sequence with TR/TE/TM = 2000/20/10 ms, voxel dimensions 10×10×10 mm^3, and 4 excitations. The field of view was 160x160 mm^2 and the slab thickness was 10 mm. The prescription consisted of a coronal-oblique 16×16 matrix (voxel-array) oriented tangent to the dorsum of the corpus callosum as seen in the sagittal plane. The 8×8 subarray in the center of the slab constituted the "excitation box", resulting in an excitation volume of 80x80x10 mm^3, from which usable magnetic resonance spectra were recorded. Of note, a short-TE pulse-sequence was selected for the MRS data collection as mI is best measured using a short-TE sequence (≤ 20 ms). Moreover, some metabolites may undergo T2-shortening under conditions of inflammation, which further motivated the selection of a short-TE sequence (Mader et al., 2008).

Magnetic resonance spectroscopy post-processing

Processing details can be found in Grodin et al. (2022). Briefly, MPRAGE images were segmented into gray matter, white matter, and CSF and were parcellated into volumes of interest (VOI), including the dorsal anterior cingulate cortex (dACC) used for the present study (see **Figure 1**). Individual VOIs were converted into binary masks, visually inspected for each participant, and transformed into the native space of each MRS voxel using SVFit2016 (Alger et al., 2016). MR spectra were fit using model spectra for creatine, phosphocreatine, choline-compounds, and inositol compounds, among others (lactate, *N*-acetylaspartate, *N*-acetyl-aspartyl-glutamate, glutamate, glutamine, γ-aminobutyric acid, numerous low-level neurometabolites, residual water, lipids and macromolecules). Creatine and phosphocreatine signals were combined to total creatine (Cr). Metabolite levels were corrected for voxel CSF content. Fit quality for all spectra was reviewed by two experts (JRA, JON). Poor fits determined by visual inspection were resubmitted for fitting with different starting estimates of various parameters. Voxel spectra that showed poor fit quality after multiple retries were not included in further analyses. Spectra with signal-to-noise ratio <5 in the Cr region were excluded, as were spectra with voxel static magnetic field inhomogeneity >0.1 parts-per-million. For each participant, separately for left and right dACC, CSF-corrected metabolite levels for 1-4 voxels with spectra passing quality control were averaged. As they did not differ appreciably from each other, levels for left and right were again averaged to obtain the final MRS study endpoints.

Data analysis

All analyses were conducted using SAS 9.4. Pearson correlations were calculated to examine the relationships between neurometabolite levels, alcohol craving, alcohol use severity, alcohol consumption, and demographics. Three separate hierarchical regressions were used to evaluate the independent effects of neurometabolites in the dACC on alcohol craving (i.e., PACS), alcohol use severity (i.e., AUDIT), and alcohol consumption (i.e., drinks per drinking day, DPDD). For each regression, demographic characteristics (age, sex, cigarette smoking, cannabis use, and body mass index (BMI)) were included in the first block. The second block included participant medication condition (ibudilast or placebo). For the craving and alcohol use severity regressions, the third block included baseline drinking characteristics (DPDD and PHDD). For the alcohol consumption regression, the third block included craving and severity measures (PACS and AUDIT scores). The final block included dACC neurometabolite levels (dACC Cho, dACC mI, and dACC Cr). Parameter estimates (β) were adjusted for all variables in the model as each block was added. Statistical significance was set at $p < 0.05$.

Given that our prior findings and the literature recommend Cho as the main significant neurometabolite predictor, additional hierarchical regression analyses were conducted with only Cho in the final block. These results are reported in the Supplementary Materials.

Results

Participant characteristics and correlation analyses

Participants were on average 32.36 years of age and were 62% male. Participants reported drinking 5.76 drinks per drinking day and drank heavily on 33% of the days assessed. Participants endorsed criteria, on average, for 5 symptoms of AUD, consistent with a moderate AUD; and had AUDIT scores of approximately 17, indicating a likelihood of moderate-to-severe AUD (see **Table 1**). Pearson's correlations among study variables included in the regression analyses can be found in **Table 2**. Plots of the pearson correlations can be found in the supplementary materials (see **Figures S1-S7**). dACC Cr, Cho, and mI levels were correlated with each other (r's > 0.332). dACC Cr was positively correlated significantly with age; additional positive correlations with PACS and AUDIT were found at trend level. dACC Cho correlated significantly with PACS and AUDIT scores, but not with drinking measures. dACC mI correlated with PACS scores, and had trend-level correlations with AUDIT scores and drinking measures.

Hierarchical regression analyses

Alcohol craving

The full hierarchical regression analysis for PACS scores is presented in **Table 3**. Demographic variables were not significantly predictive of PACS scores in the first block (Model 1:

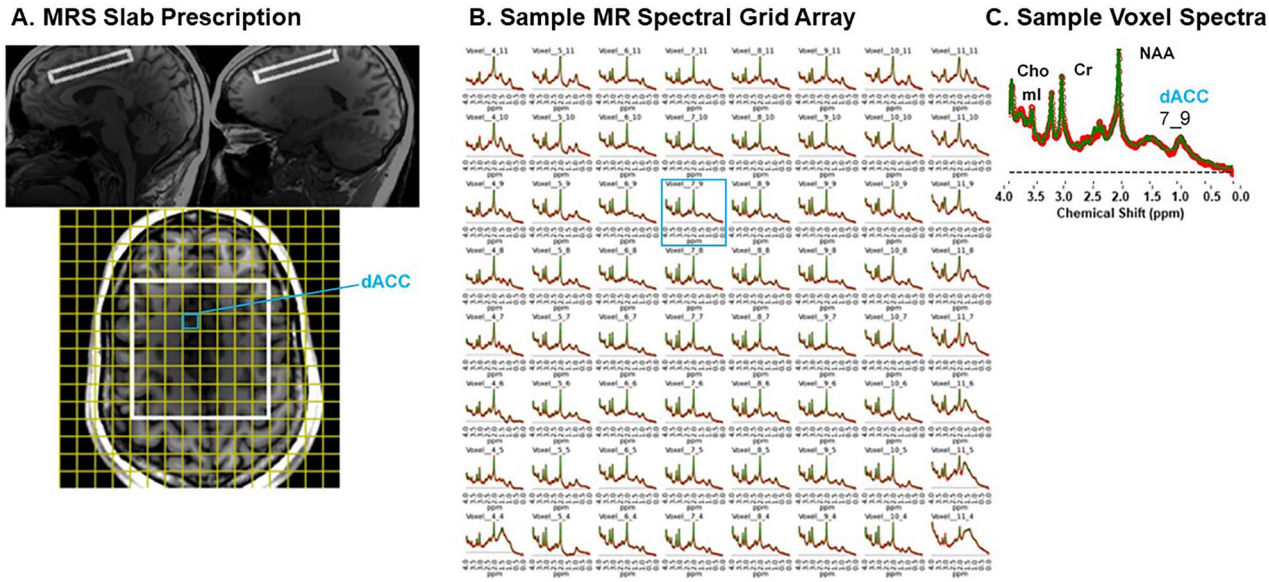

Figure 1. MRS Acquisition Volume Prescription and Data Quality From a Representative AUD Patient. Panel A features two sagittal (upper) and one transverse (lower) T1w MRI of the human brain showing position of the 8x8 subarray ("excitation box"; white-border) from which usable spectra are acquired within the 16x16 proton magnetic resonance spectroscopy (MRS) voxel grid (yellow). MRS was acquired with stimulated-echo acquisition mode (STEAM; TR/TE/TM=2000/20/10 ms, voxels 10x10x10 mm3, 4 excitation). A sample voxel from the dorsal anterior cingulate cortex (dACC) target volume-of-interest (VOI) is indicated. Panel B shows raw (red) and fit (green) spectra across the excitation grid with the spectrum from the sample voxel blown-up in Panel C. NAA=*N*-acetyl-compounds, Cr=creatine-compounds, Cho=choline-compounds, mI=myo-inositol.

Table 1. Participant Characteristics

	Mean or N	Std. Deviation or %
Age	32.36	8.34
Sex – Male	26	61.9
Cigarette Smoker	23	54.8
Cannabis User	11	26.2
Active Medication	19	45.2
BMI	26.64	4.58
AUD Symptoms	4.98	2.30
DPDD	5.76	3.36
PHDD	0.33	0.28
CIWA-Ar	0.57	1.43
PACS	12.24	6.56
AUDIT	16.98	6.15
ADS	12.76	6.46
OCDS	14.40	7.37
dACC Cr	26.50	4.24
dACC Cho	10.07	2.48
dACC mI	20.47	5.51

BMI = body mass index; AUD = alcohol use disorder; DPDD = drinks per drinking day; PHDD = percent heavy drinking days; CIWA-Ar = the Clinical Institute Withdrawal Assessment for Alcohol Scale – Revised; PACS = Penn Alcohol Craving Scale; AUDIT = Alcohol Use Disorders Identification Test; ADS = Alcohol Dependence Scale; OCDS = Obsessive Compulsive Drinking Scale; dACC = dorsal anterior cingulate cortex; Cr=creatine+phosphocreatine; Cho=choline-compounds; mI=*myo*-inositol. Metabolite levels are expressed in Institutional Units (IU) and are for midline (i.e, average of left and right) dorsal anterior cingulate cortex (dACC).

R^2 = 0.057, p = 0.831). Medication condition was also not significantly predictive of PACS scores (Model 2: R^2 = 0.064, p=0.882). The addition of drinking variables to the model significantly predicted an additional 35.1% of the variance (Model 3: R^2 = 0.415, p = 0.017). Drinks per drinking day was a significant predictor of PACS total scores (p = 0.027). The addition of the neurometabolite markers predicted an additional 13.5% of the variance (Model 4: R^2 = 0.550, p = 0.052). When this block was added, drink per drinking day was no longer a significant predictor (p = 0.114). Of the neurometabolite markers, Cho was a trend-level predictor of PACS scores (p = 0.052), while Cr and mI were non-significant (p's > 0.329).

AUD severity

The full hierarchical regression analysis for AUDIT scores is presented in Table 4. Demographic variables were not significantly predictive of AUDIT scores in the first block (Model 1: R^2 = 0.091, p = 0.628). Medication condition was also not significantly predictive of AUDIT scores (Model 2: R^2 = 0.091, p=0.998). The addition of drinking variables to the model significantly predicted an additional 40.2% of the variance (Model 3: R^2 = 0.493, p < 0.001). Drinks per drinking day was a significant predictor of AUDIT scores (p = 0.038). The addition of the neurometabolite markers predicted an additional 14.7% of the variance (Model 4: R^2 = 0.640, p = 0.018). When this block was added, drink per drinking day was no longer a significant predictor (p = 0.145); however, percent heavy drinking days became a significant predictor of AUDIT scores (p = 0.023). Of the neurometabolite markers, Cho was a significant predictor of AUDIT scores (p = 0.003), while Cr and mI were non-significant (p's > 0.914).

Drinks per drinking day

The full hierarchical regression analysis for DPDD is presented in Table 5. Demographic variables were not significantly predictive of DPDD scores in the first block (Model 1: R^2 = 0.063, p = 0.796). Medication condition was also not significantly predictive of AUDIT scores (Model 2: R^2 = 0.063, p = 0.892). The addition of craving and severity variables to the model significantly predicted an additional 38.3% of the variance (Model 3: R^2 = 0.446, p < 0.001). AUDIT scores were a trend-level predictor of DPDD (p =

Table 2. Pearson Correlations between study variables

	Age	BMI	PACS	AUDIT	DPDD	PHDD	dACC Cr	dACC Cho	dACC mI
Age	–								
BMI	.266^	–							
PACS	.145	.096	–						
AUDIT	−.014	−.107	**.765*****	–					
DPDD	−.008	.188	**.588*****	**.592*****	–				
PHDD	.119	−.059	**.519*****	**.553*****	**.627*****	–			
dACC Cr	**.346***	−.115	.297^	.265^	.087	.212	–		
dACC Cho	.025	−.018	**.409****	**.490****	.218	.062	**.332***	–	
dACC mI	.017	−.070	**.361***	.267^	.278^	.290^	**.460****	**.351***	–

***p<0.001. **p<0.005. *p<0.05. ^p<.09. dACC = dorsal anterior cingulate cortex; Cr=creatine+phosphocreatine; Cho=choline-compounds; mI=*myo*-inositol.

0.084). The addition of the neurometabolite markers only predicted an additional 2.6% of the variance (Model 4: R^2 = 0.472, p = 0.704). When this block was added, AUDIT scores remained a trend-level predictor of DPDD (p = 0.059). All of the neurometabolite markers were non-significant predictors of DPDD (p's > 0.381).

Cho only results

Given that all of the significant results were driven solely by Cho, additional regression models were conducted with Cho alone in the 4th block. These results largely mirrored those above (see Supplement for details), such that Cho was a significant predictor of PACS scores (Model 4: R^2 = 0.525, ΔR^2 = 0.111, p = 0.011) and AUDIT scores (Model 4: R^2 = 0.640, ΔR^2 = 0.147, p = 0.001), but not DPDD (Model 4: R^2 = 0.457, ΔR^2 = 0.011, p = 0.436).

Discussion

The goal of this study was to evaluate the clinical significance of neurometabolite markers of inflammation in individuals with an AUD. Results revealed that dorsal anterior cingulate MRS choline-compounds (Cho) levels predicted alcohol craving and alcohol use severity over and above demographics, medication, and alcohol consumption measures. Conversely, mI and Cr did not predict alcohol craving or alcohol use severity. Neurometabolite markers (Cho, mI, and Cr) were not predictive of alcohol consumption. This study indicates that dACC Cho is sensitive to clinical characteristics implicated in the phenomenology of AUD. This represents further evidence for MRS neurometabolites, particularly Cho, as potential biomarkers and treatment targets in AUD.

This study found that dACC Cho predicted craving and AUD severity, but not alcohol consumption, in individuals with an AUD. Alcohol cue-reactivity paradigms potentiate neural activity in ACC, implicating this region in processing alcohol craving (Kühn and Gallinat, 2011; Fryer et al., 2013; Huang et al., 2018). Activity in the ACC has also been associated with alcohol use severity and failures in control, such that individuals with AUD who have higher ACC activation in response to alcohol cues report greater alcohol use severity and more failures in control over drinking (Claus et al., 2011). Regarding MRS metabolites, glutamate levels in ACC and dorsolateral prefrontal cortex have been shown to correlate with craving in AUD (Bauer et al., 2013; Frye et al., 2016). Longer duration of AUD, typically associated with greater severity, correlates with higher thalamic Cho and Cr (Zahr et al., 2016). Of note, the present study detected no significant relationship between dACC Cho and alcohol consumption while two previous studies found associations between alcohol consumption and frontal white matter and thalamic Cho (Meyerhoff et al., 2004; Ende et al., 2006). The latter associations, however, were reported in social drinkers (Ende et al., 2006) and heavy drinkers (Meyerhoff et al., 2004), while the present sample consisted of individuals with diagnosed AUD. It is possible that as individuals transition to more severe alcohol use, the relationship between Cho levels and alcohol consumption is altered or that this relationship varies by brain region.

Choline-containing compounds are metabolic biomarkers of cell membrane turnover. Ethanol disrupts the physical structure of the cell membrane, which can increase membrane fluidity and membrane damage, ultimately leading to membrane turnover (Tóth et al., 2014). Therefore, an alternative explanation to these findings is that ethanol consumption leads to membrane turnover, which was detected as an increase in Cho. However, the lack of direct relationship between alcohol consumption and Cho, and rather a relationship between Cho and craving and severity, indicates that other processes, including inflammatory mechanisms, may be at play.

While the neuroimmune hypothesis of AUD has garnered support over the past decade (Crews et al., 2017; Coleman and Crews, 2018), the consistent use of brain-based biomarkers has tremendous potential to accelerate the translation of these findings into clinically useful discoveries (Heilig et al., 2016). In the field of AUD, we have seen that alcohol neural cue-reactivity has become a reproducible and treatment responsive biomarker of AUD (Schacht et al., 2013; Courtney et al., 2016; Grodin and Ray, 2019). MRS neurometabolites represent candidate non-invasive biomarkers for neuroimmune contributions to AUD. MRS also has the potential to play a role in drug discovery and development (Mason and Krystal, 2006). In order to get to the point of applying MRS assessments in AUD more consistently and to build reproducibility, it is critical to ground the interpretation of MRS-based assessments of neuroinflammation on robust clinical variables indexing AUD phenomenology. In this preliminary study, we leveraged clinical and biomarker data from a clinical trial to establish associations between MRS neurometabolites and AUD clinical endpoints. The main finding was that dACC Cho was sensitive to alcohol craving and AUD problem severity. Additional studies are needed to replicate these findings in larger samples. The clinical trial found that MRS Cho measured at treatment mid-point was sensitive to treatment (ibudilast vs. placebo). Further treatment studies with MRS scans are

Table 3. Hierarchical Regression Predictors of Craving

	Model 1			Model 2			Model 3			Model 4		
	R^2 / Adj. R^2 / Δ R^2 .057	β	95% CI	R^2 / Adj. R^2 / Δ R^2 .064/-.101/.007	β	95% CI	**R^2 / Adj. R^2 / Δ R^2 .415/.269/.351****	β	95% CI	***R^2 / Adj. R^2 / Δ R^2 .550/.379/.135^***	β	95% CI
Block 1 - Demographics												
Age		.098	[-.207, .362]		.092	[-.215, .361]		.096	[-.169, .361]		.025	[-.227, .267]
Sex		.083	[-3.721, 5.955]		.084	[-3.760, 6.029]		.035	[-3.540, 5.653]		.079	[-2.743, 4.867]
Cigarette Smoker		−.031	[-5.087, 4.260]		−.041	[-5.300, 4.212]		−.035	[-4.520, 3.594]		−.057	[-4.659, 3.171]
Cannabis User		.164	[-2.922, 7.787]		.153	[-3.202, 7.719]		.070	[-3.519, 5.583]		.054	[-3.420, 5.015]
BMI		.108	[-.373, .682]		.104	[-.385, .684]		.020	[-.436, .495]		.105	[-.299, .600]
Block 2 - Medication												
Ibudilast					.087	[-3.362, 5.653]		.067	[-2.810, 4.583]		.134	[-1.790, 5.303]
Block 3 – Baseline Drinking												
DPDD								**.443***	[.104, 1.634]		.303	[-.152, 1.343]
PHDD								.217	[-4.108, 14.344]		.240	[-3.156, 14.476]
Block 4 - Metabolites												
dACC Cr											.033	[-.522, .623]
dACC Cho											***.294^***	[-.008, 1.579]
dACC mI											.162	[-.253, .678]

Table 4. Hierarchical Regression Predictors of Alcohol Problem Severity

	Model 1			Model 2			Model 3			Model 4		
	R^2 / Adj. R^2 / Δ R^2 .091	β	95% CI	R^2 / Adj. R^2 / Δ R^2 .091 / -.070 /.000	β	95% CI	**R^2 / Adj. R^2 / Δ R^2 .493 / .366 / .402*****	β	95% CI	**R^2 / Adj. R^2 / Δ R^2 .640 / .503 / .147***	β	95% CI
Block 1 - Demographics												
Age		−.030	[-.284, .239]		−.030	[-.289, .244]		−.049	[-.250, .177]		−.090	[-.275, .140]
Sex		.097	[-3.230, 5.677]		.097	[-3.300, 5.747]		.044	[-2.945, 4.058]		.070	[-2.307, 4.074]
Cigarette Smoker		.172	[-2.183, 6.421]		.172	[-2.275, 6.514]		.202	[-1.053, 6.030]		.181	[-1.050, 5.515]
Cannabis User		.293	[-.862, 8.996]		.293	[-.978, 9.144]		.218	[-.955, 6.991]		.197	[-.803, 6.269]
BMI		−.065	[-.574, .398]		−.065	[-.581, .406]		−.126	[-.576, .237]		−.080	[-.484, .269]
Block 2 – Medication												
Ibudilast					−.001	[-4.172, 4.159]		−.025	[-3.525, 2.911]		.074	[-2.067, 3.880]
Block 3 – Baseline Drinking												
DPDD								**.384***	[.038, 1.374]		.250	[-.168, 1.086]
PHDD								*.331^*	[-.751, 15.357]		**.392***	[1.273, 16.056]
Block 4 – Metabolites												
dACC Cr											.004	[-.475, .485]
dACC Cho											**.414****	[.374, 1.705]
dACC mI											−.016	[-.403, .362]

Table 5. Hierarchical Regression Predictors of Alcohol Consumption

	Model 1			Model 2			Model 3			Model 4		
	R^2 / Adj. R^2 / Δ R^2	β	95% CI	R^2 / Adj. R^2 / Δ R^2	β	95% CI	R^2 / Adj. R^2 / Δ R^2	β	95% CI	R^2 / Adj. R^2 / Δ R^2	β	95% CI
	.063			.063 / -.102 / .001			**.446 / .308 / .383*****			.472 / .272 / .026		
Block 1 – Demographics												
Age		−.081	[-.177, .112]		−.083	[-.180, .114]		−.095	[-.157, .081]		−.060	[-.160, .111]
Sex		.076	[-1.936, 2.981]		.077	[-1.972, 3.022]		.014	[-1.956, 2.109]		−.004	[-1.941, 2.090]
Cigarette Smoker		.073	[-1.883, 2.867]		.071	[-1.475, 2.900]		.011	[-1.951, 2.900]		.031	[-1.941, 2.356]
Cannabis User		.177	[-1.387, 4.056]		.174	[-1.475, 4.096]		.013	[-2.223, 2.412]		.006	[-2.342, 2.436]
BMI		.229	[-.100, .436]		.228	[-.105, .440]		.227	[-.057, .390]		.231	[-.076, .415]
Block 2 – Medication												
Ibudilast					.023	[-2.144, 2.454]		.000	[-1.847, 1.846]		−.029	[-2.181, 1.796]
Block 3 – Baseline Craving Severity												
PACS								.269	[-.098, .372]		.232	[-.138, .375]
AUDIT								***.410***^	[-.032, .478]		***.485***^	[-.011, .539]
Block 4 – Metabolites												
dACC Cr											−.106	[-.394, .226]
dACC Cho											−.137	[-.660, .285]
dACC mI											.160	[-.145, .367]

called for to determine whether Cho levels change pre-post treatment and whether baseline levels predict clinical course, with and without intervention. That could open possibilities for developing prognostic and therapeutic biomarkers for AUD research.

This study has several limitations. The sample size was modest, further research in larger samples is warranted. Additionally, data from healthy controls was not included in this study; future studies should include a control group to confirm the relationships identified herein. Data for this secondary analysis were drawn from a medication study. While we controlled for medication condition within our analyses, treatment with ibudilast (or placebo) may have influenced our results. Relatedly, MRS metabolites were only assessed at a single time point, one-week into the medication regimen. Future studies should assess MRS metabolites and clinical characteristics concurrently and longitudinally. While most studies we have cited acquired MRS with PRESS, we used STEAM for better fitting at short-TE. Results of the two techniques can be compared only imperfectly due to variations in metabolite yield. These variations, however, are likely to affect only the magnitude and not the direction of findings. Finally, this study did not adjust for multiple comparisons, i.e., for three separate hierarchical regression analyses, as the study was exploratory in nature. Hypothesis-driven studies using MRS metabolite markers should be conducted in the future to replicate and to extend the present findings.

In closing, this exploratory study indicates that MRS Cho in the dorsal anterior cingulate cortex is sensitive to clinical characteristics implicated in the phenomenology of AUD. Specifically, dACC Cho predicted alcohol craving and alcohol problem severity over and above effects of demographics, medication, and alcohol consumption. MRS dACC Cho, mI, and Cr were not predictive of alcohol consumption. The utility of MRS-based markers in AUD research hinges on establishing their clinically significance. To that end, these results provide further support for the development of Cho as a potential biomarker for AUD.

CRediT authors statement

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Supplementary material

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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