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BDNF rs6265 Met carriers with alcohol use disorder show greater age-related decline of N-acetylaspartate in left dorsolateral prefrontal cortex

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is implicated in neuronal and glial cell growth and differentiation, synaptic plasticity, and apoptotic mechanisms. A single-nucleotide polymorphism of the *BDNF* rs6265 gene may contribute to the pattern and magnitude of brain metabolite abnormalities apparent in those with an Alcohol Use Disorder (AUD). We predicted that methionine (Met) carriers would demonstrate lower magnetic resonance spectroscopy (MRS) measures of N-acetylaspartate level (NAA) and greater age-related decline in NAA than valine (Val) homozygotes.

Methods: Veterans with AUD (n=95; 46±12 years of age, min = 25, max = 71) were recruited from VA Palo Alto residential treatment centers. Single voxel MRS, at 3 Tesla, was used to obtain NAA, choline (Cho) and creatine (Cr) containing compounds from the left dorsolateral prefrontal cortex (DLPFC). Metabolite spectra were fit with LC Model and NAA and Cho were standardized to total Cr level and NAA was also standardized to Cho.

Results: Val/Met (n=35) showed markedly greater age-related decline in left DLPFC NAA/Cr level than Val/Val (n=60); no differences in mean metabolite levels were observed between Val/Met and Val/Val. Val/Met demonstrated greater frequency of history of MDD and higher frequency of cannabis use disorder over 12 months prior to study.

Conclusions: The greater age-related decline in left DLPFC NAA/Cr and the higher frequency of MDD history and Cannabis Use disorder in *BDNF* rs6265 Met carriers with AUD are novel and may have implications for non-invasive brain stimulation targeting the left DLFPC and other psychosocial interventions typically utilized in the treatment of AUD.

1. Introduction

Numerous premorbid and/or comorbid conditions, including variations in single-nucleotide polymorphisms (SNPs), may contribute to the pattern and magnitude of neurobiological and/or neurocognitive abnormalities apparent in those with an Alcohol Use Disorder (AUD) (Durazzo et al., 2012; Hoefer et al., 2014; Mon et al., 2013). Brain-derived neurotrophic factor (BDNF) is implicated in neuronal and glial cell growth and differentiation, neurotransmission and synaptic plasticity, as well as neuronal apoptotic mechanisms [see (Szarowicz et al., 2022) for review]. Within the *BDNF* gene, on chromosome 11p13, a common SNP (rs6265) G>A substitution at nucleotide 196 results in a substitution of valine (Val) for methionine (Met) in the 5' prodomain at codon 66 (Val66Met) (Bath and Lee, 2006). Studies with non-AUD samples have related *BDNF* genotypes to magnetic resonance spectroscopy (MRS) measures of N-acetylaspartate level (NAA); NAA is a surrogate marker of neuronal integrity/viability and NAA detected by MRS largely represents concentrations within mature neurons (Meyerhoff et al., 2013; Oz et al., 2014). Egan and colleagues (Egan et al., 2003) reported lower ratio of NAA to total creatine (NAA/Cr) in rs6265 Met carriers (Val/Met), relative to Val homozygotes (Val/Val), in the bilateral hippocampi of healthy controls and those with schizophrenia. In

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healthy adult controls, *BDNF* rs6265 Met carriers showed decreased NAA/Cr concentration in the left hippocampus compared to Val/Val (Stern et al., 2008). Met homozygotes (Met/Met) showed lower NAA/Cr levels in the left hippocampus across a combined sample of healthy controls and individuals diagnosed with schizophrenia and bipolar disorder than Val homozygotes (Gruber et al., 2012). Alternately, Gallinat and colleagues reported higher NAA level in the anterior cingulate of healthy adult rs6265 Met carriers compared to Val homozygotes (Gallinat et al., 2010). Similarly, Martins and colleagues reported male, but not female, rs6265 Met carriers demonstrated higher NAA than Val homozygotes in the perigenual anterior cingulate cortex (Martens et al., 2021). Our group and others have observed NAA levels show a linear decrease with increasing age in multiple brain regions (Meyerhoff et al., 2013). Additionally, individuals with AUD demonstrate lower frontal/anterior frontal NAA levels relative to controls [see (Kirkland et al., 2022) for review], and we previously reported that those with AUD demonstrate significant cortical thinning and decreased NAA concentration in the bilateral DLPFC (Durazzo et al., 2013). However, to our knowledge, there are no published studies that reported NAA level, in any brain region, was moderated by rs6265 genotype and age in AUD.

Depressive disorders are common in those with AUD (Conway et al., 2006; Durazzo et al., 2008) and Major Depressive Disorder (MDD) diagnosis (Durazzo et al., 2008; Durazzo and Meyerhoff, 2017) and anhedonic depressive symptomatology (Nguyen et al., 2020) are related to resumed alcohol consumption in those seeking treatment for AUD. MDD is also associated with decreased NAA level in the frontal/anterior frontal regions in non-AUD samples [see (Kahl et al., 2020) and references therein]. The association between *BDNF* rs6265 and depressive disorders is not consistent across clinical populations (Hartig and Nemeș, 2022). However, Su and Colleagues observed a significantly greater frequency of rs6265 A allele carriers (producing greater rate of Met carriers) in those with concurrent MDD and AUD.

The effect of the rs6265 Met allele on NAA level in hippocampal and anterior cingulate cortex in healthy controls and individuals with Schizophrenia and Bipolar disorder is not uniform. Additionally, in these studies, the age ranges were largely restricted to younger-to-middle-aged adults, so the potential interactive effect of rs6265 genotype and age across the adult lifespan is not clear. Furthermore, the previous rs6265 studies did not include those with AUD, therefore, the association of rs6265 genotype with NAA and other neurometabolites levels and MDD frequency in AUD is unknown.

Accordingly, this study examined the associations between *BDNF* rs6265 genotypes and neurometabolite levels in the left dorsolateral prefrontal cortex (DLPFC) of Veterans seeking treatment for AUD. Neurometabolites from left DLPFC were chosen as this cortical region has frequently been targeted by repetitive transcranial magnetic stimulation (rTMS) for the treatment of MDD (Peng et al., 2018) and AUD (Hanlon et al., 2018; Padula et al., 2022). In treatment-seeking Veterans with AUD, we predicted:

1) *BDNF* rs6265 Met carriers show lower and greater age-related decline of NAA/Cr in the left DLPFC than Val homozygotes.
2) *BDNF* rs6265 Met carriers demonstrate a greater frequency of lifetime history of major depressive disorder (MDD) than Val homozygotes.

2. Methods

2.1. Participants

Veterans with AUD (n=95; 46±12 years of age, min = 25, max = 71) were recruited from the VA Palo Alto Health Care System (VAPAHCS) and were in residential treatment at the time of study. Treatment programs ranged from 28 to 90 days and participants had 34±39 days of abstinence from alcohol prior to participation. Participants in this study were obtained from projects examining neurobiological predictors of resumed alcohol consumption after treatment in AUD, and clinical trials investigating the efficacy of rTMS as an adjunct treatment for AUD (NCT03291431 and NCT03191266); all data used in this study were obtained prior to entry into the treatment phase of the clinical trials. Neuroimaging and *BDNF* rs6265 genotyping from healthy controls were not obtained in these projects. Participants met Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria for AUD (American Psychiatric Association, 2013) and 95% of the sample were in the severe range of AUD symptomatology. Participants provided written informed consent prior to the initiation of study procedures. All study procedures were approved by the Stanford University and VAPAHCS and institutional review boards and observed the ethical standards of the Declaration of Helsinki (see Table 1 for demographic and clinical characteristics for BDNF rs6265 groups; Val/Val n = 60, Val/Met n = 32, Met/Met n = 3).

Principal inclusion criteria were adults aged 18 and older, fluent and fully literate in English, and in residential treatment. Exclusion criteria were: 1) active suicidal ideations representing imminent risk for suicide, 2) biomedical diseases, conditions, or neurological disorders recognized to adversely affect brain neurobiology or neurocognition (i.e., space occupying cerebral lesion(s), cerebrovascular accident, multiple sclerosis, Parkinson disease, etc.), 3) traumatic brain injury producing a loss of consciousness > 10 min), 4) documented severe impairment of visual and/or auditory acuity or motor skills. 5) psychiatric exclusions: history of schizophrenia spectrum, bipolar, or other psychotic disorders. The

Table 1
Group demographic and clinical variables.

Variable	Val/Val (n = 65)	Val/Met (n = 30)	Group comparison
Age [years]	49 (13) min = 29 max = 71	43 (11) min = 25 max = 68	NS
Male (%)	88	77	NS
White (%)	69	90	Val/Val < Val/Met
Black (%)	17	0	Val/Val > Val/Met
Asian (%)	3	3	NS
Education [years]	14 (2)	14 (2)	NS
Duration of abstinence prior to study [days]	29 (31)	39 (46)	NS
Total drinks over 3 months prior to study	760 (73)	578 (108)	NS
Average drinks/day days over 3 month prior to study	14 (1)	11 (2)	NS
Total drinking days over 3 month prior to study	53 (4)	57 (5)	NS
CUDIT	10 (7)	12 (7)	NS
BDI-II	19 (12)	22 (10)	NS
BAI	14 (11)	14 (12)	NS
PTSD Checklist-5	57 (18)	58 (20)	NS
Current PTSD (%)	34	37	NS
History of Major Depressive Disorder (%)	40	60	Val/Val < Val/Met
Current Major Depressive Disorder (%)	11	20	NS
Current Anxiety Disorder (%)	23	37	NS
Antidepressant use (%)	30	42	NS
Any substance Use Disorder past 12 months (%)	12	33	Val/Val < Val/Met
Psychostimulant Use Disorder Past 12 months (%)	8	10	NS
Cannabis Use Disorder past 12 months (%)	8	33	Val/Val < Val/Met
Never tobacco consumption	30	20	NS
Former tobacco consumption	30	43	NS
Current tobacco consumption	40	37	NS
Chronic medical conditions	35	20	NS

Note: BAI: Beck Anxiety Inventory; BDI-II: Beck Depression Inventory-II; CUDIT: Cannabis Use Disorder Identification Test; NS: not significant.

following comorbidities were permitted due to their high prevalence in alcohol use disorders, particularly in Veterans: hepatitis C, type-2 diabetes, hypertension, unipolar mood disorders (major depression, substance-induced mood disorder), anxiety disorders (generalized anxiety disorder, panic disorder) and post-traumatic stress disorder (PTSD) (Batki et al., 2011; Durazzo et al., 2020; Gilman and Abraham, 2001; Stinson et al., 2005). Participants who met DSM-5 criteria for current or past substance use disorder were included, given the high co-occurrence of substance abusemisuse in AUD (Mannes et al., 2021; Stinson et al., 2005), however AUD was the primary reported reason for seeking treatment. Participants were urine-tested for illicit substances and breathalyzed for recent alcohol consumption prior to assessments. Participants had zero blood alcohol concentration (BAC) and all tested negative for illicit or non-prescribed substances.

2.2. Medical, psychiatric, substance, and alcohol consumption history

Medical history for participants was obtained via self-report and confirmed and amended, if required via available electronic medical records. Psychiatric symptomatology and diagnoses were assessed by the Mini-International Neuropsychiatric Interview for DSM-5 (MINI). Participants also completed the Clinical Interview for DSM-5 Alcohol Use Disorder and in-house questionnaires assessing demographics, history of biomedical and other illicit and prescribed substance use. The Timeline Follow-back (TLFB) was used to obtain alcohol consumption (average drinks/day, maximum drinks/day, total number of drinks) over the 3 months prior to study participation. PTSD symptomatology was evaluated with the PTSD Checklist for DSM-5 (PCL-5). Major depressive symptomatology was assessed with the Beck Depression Inventory-II (BDI-II), and anxiety symptomatology with the Beck Anxiety Inventory (BAI). Nicotine dependence level was evaluated via the Fagerstrom Test for Nicotine Dependence (FTND). Please see (Nguyen et al., 2020) for cititions for the above measures.

2.3. BDNF rs6265 genotyping

Salivary DNA was collected using the Oragene collection kit from DNA Genotek (ORG-500) and DNA was isolated in accordance with the manufacturer's (prepIT●L2P) instructions. Briefly, samples were incubated in a lysis buffer and DNA was precipitated from the supernatant via ethanol. DNA quantification and purity were assessed via nanodrop and reisolated if the sample had low purity or low yield (Desjardins and Conklin, 2010). *BDNF* rs6265 genotyping followed procedures by Sheikh and colleagues (Sheikh et al., 2010). Twenty-five (25) uL of master mix was prepared containing 4 specific primers, 3 mM MgSO4 (ThermoFisher), 1.5 U taq polymerase (New England BioSciences), 10x PCR Enhancer (Invitrogen), and genomic DNA. The PCR reaction was run in the following phases: denature (94 C for 5 min), anneal (30 cycles of 94 C for 45 s, 62.5 C for 60 s, 72 C for 60 s) and extension (76 C for 5 min). Amplicons were resolved in a 2% agarose gel containing 1:10, 000 Syber Safe DNA stain (Invitrogen). The location of the Val (G) allele band was at approximately 253 bp and the Met (A) allele at approximately 201 bp for rs6265. There were three Met/Met participants; the Met homozygotes were combined in the Met carrier group because there was an insufficient number of these individuals to compare to Val/Val and Val/Met. The *BDNF* rs6265 allelic frequency was within Hardy-Weinberg equilibrium ($\chi^2 = 3.39$. p =.20).

2.4. Magnetic resonance imaging (MRI) and MRS acquisition and processing

MRI and MRS data were acquired on a 3 T GE system (General Electric Healthcare, Milwaukee, WI, USA) equipped with a 32-channel head coil (Nova Medical, Wilmington, MA, USA) at the Stanford University Center for Cognitive and Neurobiological Imaging.

T1-weighted images were acquired with the following parameters: repetition time (TR): 8.692 ms, echo time (TE): 3.44 ms, inversion time: 500 ms, 11-degree flip angle, 256×256 matrix, 1 mm^3 resolution.

An improved MEGA-SPECIAL single voxel spectroscopy editing sequence was used to obtain gamma-aminobutyric-acid (GABA) and other neurometabolite levels in the left DLPFC [editing on/off = 1.9/7.5 ppm, TR/TE = 2000/80 ms, 256 transients, 10.6 min acquisition (Gu et al., 2018)]. The 40×22×22 mm^3 (19.4 mL) single voxel was prescribed in the left dorsolateral prefrontal cortex localized from the 3D T1-weighted anatomical image using a semi-automated voxel placement procedure to place the voxel. This was accomplished by applying non-linear normalization to identify subject-specific MRI coordinates from a previously selected target coordinate located in DLPFC in the Montreal Neurological Institute (MNI) template. During the semi-automated voxel placement procedure, after initial automated coordinate identification, the MRS voxel (see Fig. 1) was then aligned to the angle of the skull in the sagittal plane, and then the left DLPFC mask in MNI standard space was co-registered to the subject's T1-weighted image (Bishop et al., 2021; Gozdas et al., 2022). In this study, we report on NAA (combined concentrations of NAA and N-acetylaspartylglutamate), Cho (combined concentrations of phosphocholine and glycerophosphocholine) an Cr (combined concentrations of creatine and phosphocreatine) and the editing-off spectra were fit with LC Model [v 6.3–1 J (Provencher, 2001)]. See Fig. 2 for representative fit of spectra. Z-scores were formed for each metabolite ratio, based on the entire participant sample. The results for GABA and glutamate in this sample were fit with a customized procedure and will be presented in a separate report.

Across genotypes, left DLPFC line width (Hz, FWHM) was 9.06 ± 1.45. Cramer-Rao lower bounds were 2.32 ± 1.14 for NAA, 3.45 ± 1.83 for Cho and 3.16 ± 1.51 for Cr, which are well within recommended quality control standards for LC Model spectral fits for these metabolites (Provencher, 2001). There were no significant differences between Val/Val and Val/Met for line width or Cramer-Rao bounds for NAA, Cho or Cr.

2.5. Data analyses

Comparisons between *BDNF* rs6265 genotypes on clinical and demographic variables were conducted with univariate analysis of variance, Fisher's Exact Test or Mann-Whitney Test, where appropriate (see Table 1). Group differences on these variables were considered statistically significant at $p < 0.05$.

For NAA/Cr, Cho/Cr and NAA/Cho, generalized linear model (GENLIN) was used to test for main effects of genotype (Val/Val vs. Val/Met) and the interaction of genotype and age. Any substance use

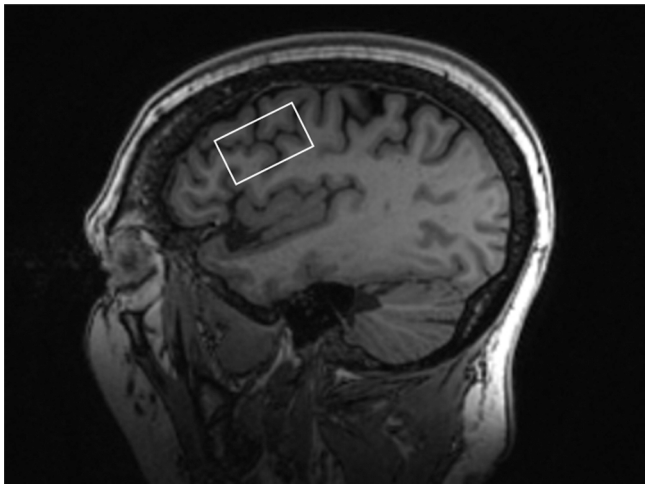

Fig. 1. Representative voxel placement.

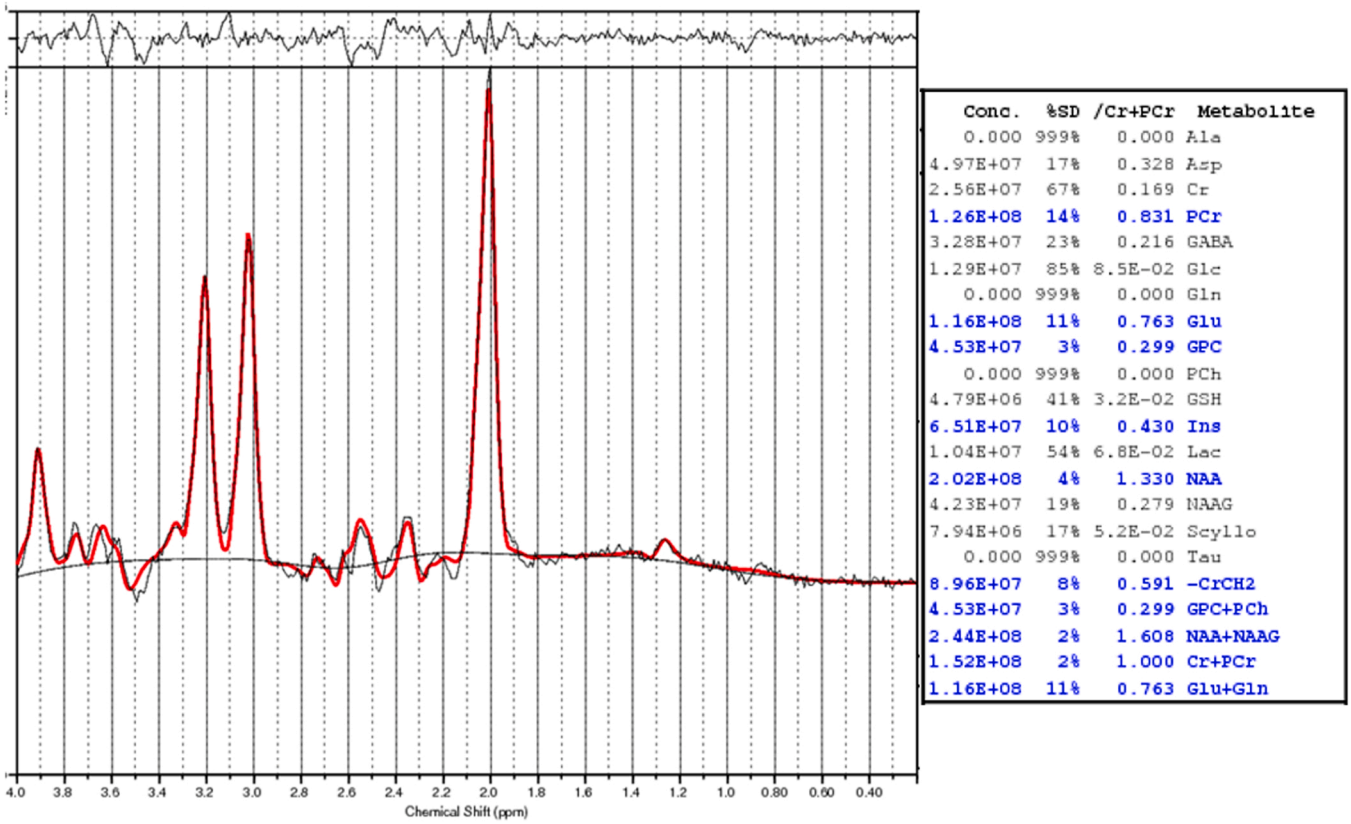

Fig. 2. Representative spectral fit for left DLPFC.

disorder and cannabis use disorder in the past 12 months and history of MDD were individually entered as covariates, given the higher frequency of these factors in Val/Met. Race (White vs. Non-white), sex, education, duration of abstinence prior to study and alcohol consumption variables were also individually considered as covariates in final models to determine their potential association with left DLPFC NAA and Cho levels. Significant main effects for genotype were followed up with two-tailed t-tests. Main effects and interactions were considered statistically significant at $p < 0.017$, with traditional Bonferroni correction. Effect sizes (ES) for significant mean differences between genotypes in neurometabolite ratios were calculated with Cohen's *d* (Cohen, 1988). All analyses were completed with SPSS v24.

3. Results

3.1. Participant demographics and clinical variables

Val/Met had a higher percent of White race, history of MDD, any substance use disorder and cannabis use disorder in the past 12 months (all $p < 0.05$) than Val/Val. Val/Val had a higher percentage of Black race than Val/Met ($p < 0.05$). No significant group differences were observed on other demographic and clinical variables (see Table 1).

3.2. Genotype comparisons on Left DLPFC metabolite ratios

A genotype x age interaction was observed for NAA/Cr level [$\chi^2(1) = 8.48$, $p = .004$], where Val/Met demonstrated greater age-related decline in NAA/Cr than Val/Val (see Fig. 3 for semi-partial correlation coefficients for age effects for each group, adjusted for education). Across genotypes, greater age was inversely related to NAA/Cr concentration [$\chi^2(1) = 46.00$, $p < 0.001$], and higher education showed a trend for association with NAA/Cr level [$\chi^2(1) = 5.35$, $p = .021$]. Val homozygotes showed numerically higher NAA/Cr, but the main effect for genotype was not significant and the associated effect size was weak ($p = .19$; ES $= 0.30$). Any substance use disorder and cannabis use disorder in past 12 months, history of MDD, race (White vs. Non-white), sex, duration of abstinence prior to study, and alcohol consumption variables were not significant predictors of NAA/Cr level (all $p > 0.20$).

For Cho/Cr, there was no main effect for genotype or genotype x age interaction (all $p > 0.50$). Age, any substance use disorder and cannabis use disorder in past 12 months, history of MDD, race, sex, education, duration of abstinence prior to study and alcohol consumption variables were not significant predictors of Cho concentration (all $p > 0.35$).

For NAA/Cho level, no main effect for genotype or genotype x age interaction were observed (both $p < 0.40$). Higher age was inversely related to NAA/Cho [$\chi^2(1) = 5.70$, $p = .016$]). Any substance use disorder and cannabis use disorder in past 12 months, history of MDD, race, sex, education, duration of abstinence prior to study and alcohol consumption variables were not related to NAA/Cho concentration (all $p > 0.19$).

4. Discussion

In this study of predominately male Veterans seeking treatment for AUD, Val/Met showed greater age-related decline in left DLPFC NAA/Cr level than Val/Val. Val/Met also showed greater frequency of history of MDD and higher frequency of any substance use disorder in the 12 months prior to study, which was driven by greater frequency of cannabis use disorder 12 months prior to study. Val/Val and Val/Met genotypes were not significantly different on mean left DLPFC NAA/Cr, Cho/Cr or NAA/Cho level. Age was not associated with Cho/Cr level, but negatively associated with NAA/Cho level, indicating the genotype x age interaction and significant age effect across groups for NAA/Cr were likely not driven by age-related changes in Cr. Co-occurring substance use disorders, history of MDD, race (White vs. Non-white), sex, duration of abstinence prior to study, and alcohol consumption variables were not

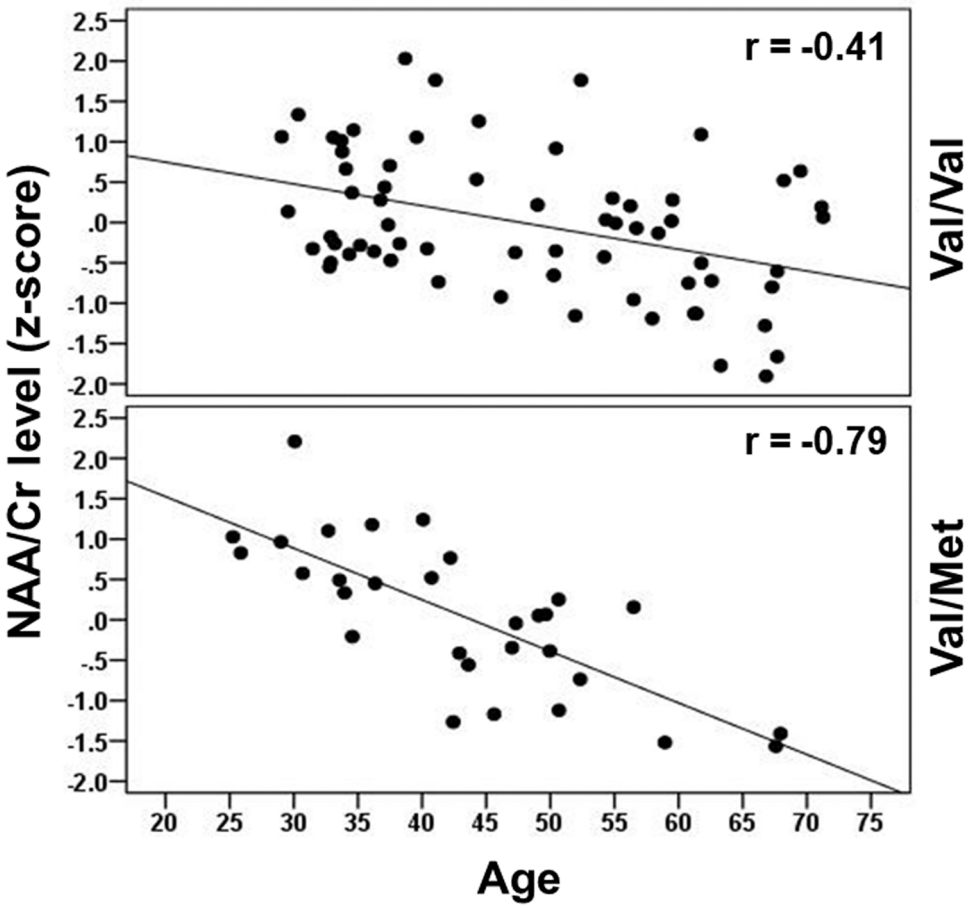

Fig. 3. Associations of NAA/Cr with age for Val/Val and Val/Met.

significant predictors of any metabolite ratio.

In neuronal mitochondria, NAA is synthesized through a bioenergetically demanding condensation reaction from L-aspartate and acetyl coenzyme A, catalyzed by aspartate N-acetyltransferase [see (Meyerhoff et al., 2013) for review]. Diminished efficiency of mitochondrial-based cellular respiration is associated with decreased neuronal NAA levels (Baslow and Guilfoyle, 2007; Pan and Takahashi, 2005). Activity dependent BDNF release is purportedly compromised in rs6265 Met carriers [see (Szarowicz et al., 2022) for review], which, in turn, may alter high affinity BDNF tropomyosin receptor kinase-B (TrkB) triggered signaling pathways involved in the regulation of cellular bioenergetics (Martin and Finsterwald, 2011). Additionally, both brain NAA and BDNF levels are inversely related to age, likely via decreased mitochondrial respiratory chain efficiency (Markham et al., 2014). Therefore, the greater age-related decline of NAA in Met carriers may be related to the above putative mechanisms affecting cellular respiration. Previous studies in healthy White younger adults reported increased NAA levels in the anterior cingulate cortex rs6265 Met carriers, compared to Val homozygotes (Gallinat et al., 2010). Similarly, male, but not female, rs6265 Met carriers showed higher NAA levels than Val homozygotes in the perigenual anterior cingulate cortex (Martens et al., 2021). No genotype x age interaction was reported in either study, possibly due to the limited age range of participants. Martens and colleagues suggested that the greater anterior cingulate NAA concentration in Met carriers may be region specific, and additional investigation of Met carrier molecular pathways, or potential gene–gene interactions, should be considered in larger samples. In the present study, we did not observe significant mean differences in left DLPFC NAA/Cr, Cho/Cr or NAA/Cho and the effect sizes for the direct comparisons between genotypes were weak, indicating the lack of group differences were not attributable to power.

In our previous studies, with a different sample of predominately male Veterans seeking treatment for AUD, over approximately 5 weeks of abstinence, we observed rs6265 Val homozygotes exhibited significantly greater frontal gray matter volume recovery than Met carriers, while Met carriers showed greater frontal white matter volume increases than Val homozygotes (Mon et al., 2013). We also found that *BDNF* rs6265 Val homozygotes demonstrated significantly larger bilateral hippocampi than Met carriers after 7 months of sustained abstinence from alcohol (Hoefer et al., 2014). Taken together, makers of cortical neuronal integrity and brain structure in our treatment-seeking AUD samples appear to be adversely affected by the rs6265 Met allele, possibly via compromised cellular bioenergetics.

As predicted, Met carriers showed higher frequency of lifetime MDD history, but not current MDD diagnosis. Additionally, there were no significant differences between genotypes on self-reported depressive symptomatology on the BDI-II. To our knowledge, there are no previous published studies reporting a higher frequency of MDD history in *BDNF* rs6265 Met carriers in those with AUD. The association of *BDNF* rs6265 genotype and MDD is not consistent across multiple non-AUD populations (Faris et al., 2020; Hartig and Nemeş, 2022). The greater frequency of MDD history in Met carriers may represent a genetic risk factor for recurrent MDD, given MDD and high self-reported MDD symptomatology is frequently observed in those seeking treatment for AUD (Durazzo and Meyerhoff, 2017; Nguyen et al., 2020). The greater frequency of Cannabis Use Disorder (CUD) in Met carriers was not anticipated and must be considered preliminary, given the low numbers of participants with this diagnosis in the current study (Val/Val n = 5, Val/Met n = 10). The mechanism(s) contributing to the higher frequency of MDD history and CUD in Met carriers in this AUD sample

remain to be determined.

The significantly greater age-related decrease in left DLPFC NAA/Cr observed in rs6265 Met carriers may have implications for the efficacy of rTMS stimulation targeting this brain region. More specifically, since NAA is a recognized surrogate marker of neuronal integrity/viability, the decreasing NAA/Cr observed with increasing age in Met carriers may be associated with differential responsivity to rTMS inhibitory or excitatory stimulation protocols delivered to the left DLPFC across the adult lifespan. To our knowledge, there are no published studies that specifically investigated the effects of rTMS on left DLPFC NAA or Cho levels in those with AUD; however, four sessions of active 10 Hz (80% of resting rTMS to the right DLPFC of middle-aged treatment-seeking AUD was associated with significant increases in NAA/Cr and Cho/Cr in the bilateral hippocampi (Qiao et al., 2016).

This study has limitations that may affect the generalizability of the findings. The study sample was composed of primarily males, therefore, the effects of biological sex in this sample could not be meaningfully interrogated. We did not obtain a normal control reference group to determine if the age-related effects of BDNF genotype on left DLPFC NAA/Cr, observed in this AUD cohort, were apparent in a healthy group of adults. The percentage of gray and white matter and cerebrospinal fluid was not calculated for the left DLPFC voxel, which may have influenced the reported findings, if there were significant differences between Val/Val and Val/Met on these tissue contributions. Metabolites are often scaled to Cr, because this metabolite has been reported to be stable across many, but, not all, brain regions and populations (Kirkland et al., 2022; Meyerhoff et al., 2013). Finally, we did not include other SNPs (e.g., COMT, DRD4), which may have mediated or moderated the findings reported in this study.

In conclusion, the greater age-related decline in left DLPFC NAA/Cr and the higher frequency of MDD history and CUD in *BDNF* rs6265 Met carriers with AUD are novel and may have implications for non-invasive brain stimulation that target the left DLPFC and other psychosocial interventions typically utilized in the treatment of AUD. Determination of the potential effects of the *BDNF* rs6265 Met allele on brain metabolite recovery with abstinence, across adulthood, in those seeking treatment for AUD is warranted.

Role of funding sources

The study sponsors (Department of Veteran Affairs and Stanford University) had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

CRediT authorship contribution statement

Drs. Durazzo, McNerney and Padula were responsible for study concept and design. Dr. Durazzo executed all statistical analyses and wrote the manuscript. Dr. McNerney and Ms. Hansen completed all BDNF rs6265 genotyping. Dr. Gu was the primary developer of the MRS sequence used in this study and created the MRS spectral fitting pipeline. Dr. Sacchet was involved in the development and implementation of the MRS voxel placement method. All authors were involved in data interpretation and editing and contributed significant intellectual content to the manuscript.

Declaration of Competing Interest

The Authors have no actual or potential conflicts of interest to report.

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