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Elevated transferrin saturation in individuals with alcohol use disorder: Association with HFE polymorphism and alcohol withdrawal severity

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Abstract

Iron loading has been consistently reported in those with alcohol use disorder (AUD), but its effect on the clinical course of the disease is not yet fully understood. Here we conducted a cohort study to examine whether peripheral iron measures, genetic variation in *HFE* rs1799945, and their interaction differed between 594 inpatient participants with alcohol use disorder (AUD) undergoing detoxification and 472 healthy controls (HC). We also assessed whether *HFE* rs1799945 was associated with elevated peripheral iron and can serve as a predictor of withdrawal severity. AUD patients showed significantly higher serum transferrin saturation than HC. Within the AUD group, transferrin saturation significantly predicted withdrawal symptoms (CIWA-Ar) and cumulative dose of benzodiazepine treatment during the first week of detoxification, which is an indicator of withdrawal severity. *HFE* rs1799945 minor allele carriers showed elevated transferrin saturation compared to non-carriers, both in AUD and healthy controls. Exploratory analyses indicated that, within the AUD cohort, *HFE* rs1799945 predicted CIWA withdrawal scores and this relationship was significantly mediated by transferrin saturation. We provide evidence that serum transferrin saturation predicts alcohol withdrawal severity in AUD. Moreover,

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Author Contributions

DK, PM, MS, ND, GJW, CEW, and NDV were responsible for study concept and design. MS and ND managed data acquisition. DK and CEW analyzed data and KM, PM, MS, PHC, DG assisted with data analysis. DK, KM, PM, and CEW drafted the manuscript. All authors critically reviewed content and approved the final version for publication.

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our findings replicated previous studies on elevated serum transferrin saturation in AUD and an involvement of *HFE* rs1799945 in serum transferrin saturation levels in both AUD and healthy controls. Future studies may use transferrin saturation measures as predictors for treatment; or potentially treat iron overload to ameliorate withdrawal symptoms.

Keywords

alcoholism; inflammation; iron; substance abuse; genetics

Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder composed of three cyclical stages: binge intoxication, preoccupation, and withdrawal.[1] The withdrawal stage poses several physical and psychological risks, including anxiety, tremors, insomnia, nausea, seizures, and delirium tremens.[2] The extent to which an individual experiences these symptoms can be quite variable: approximately 50% of individuals with a history of excessive alcohol use will experience some withdrawal symptoms while an estimated 2–8% will have delirium tremens, a more severe presentation of alcohol withdrawal with a high mortality rate when untreated.[3] Several risk factors for greater severity of withdrawal symptoms have been identified and include psychiatric history, biomarkers such as elevated blood urea nitrogen and plasma lipid levels, and genetic vulnerability.[3,4] However, none of these features are predictive of severity of withdrawal on their own.[3] Given the relevance of alcohol withdrawal for AUD clinical outcomes including relapse, understanding the factors contributing to heterogeneity of withdrawal severity would help guide treatment interventions.

Here, we examined whether the peripheral iron measure transferrin saturation can serve as a predictor of withdrawal severity. Iron overload has been consistently observed in patients with AUD.[5–9] and it generally normalizes with detoxification.[10,11] Chronic excessive alcohol intake leads to decreased expression of hepcidin, an hepatic protein that regulates the absorption of iron in duodenal enterocytes and macrophages, and increased expression of ferritin, an iron storage protein.[12,13] Together these processes contribute to increased absorption of dietary iron and iron from recycled red blood cells into the peripheral circulation.[12,13] Elevated peripheral iron levels affect the clinical course of AUD including an association with liver damage, likely through the production of reactive oxygen species.[14] Additionally, there is some preclinical evidence that iron loading affects withdrawal: One study in alcohol-dependent mice demonstrated that administration of iron chelators prior to detoxification, which normalized iron levels, lowered alcohol withdrawal scores and mortality.[15]

Moreover, the HFE protein, expressed in the liver and intestines, modulates hepcidin expression, and thus iron absorption.[16] There are several functional single nucleotide polymorphisms (SNP) in the *HFE* gene that lead to hereditary hemochromatosis.[16] One of these SNPs, rs1799945, results in a substitution from histidine to tyrosine at amino acid residue 63 (*HFE* H63D).[16] This SNP is thought to have arisen in the Basque region

and is thus particularly common among those of European descent, where its frequency is estimated to be 22%, compared to 5.4% and 2.8% in those with African and Asian ancestry, respectively.[17] Animal studies have shown that carriers of the rat analogue of the H63D allele are more likely to have mild iron loading than non-carriers.[18] This has been corroborated in humans, in which carriers were noted to have slightly higher levels of serum transferrin saturation than non-carriers, although it unclear if this is clinically significant.[19,20] While the frequency of the H63D SNP among those with AUD does not appear to be elevated relative to the general population, there is evidence that heavy alcohol use increases the penetrance of the associated phenotype.[21] Among those with the H63D SNP, those who drink heavily have more severe iron loading and present with symptoms of associated liver disease at an earlier age.[21] Further, a meta-analysis found that among AUD individuals, carriers of the H63D allele are marginally more likely to have alcoholic liver disease than non-carriers, suggesting a gene-environment interaction.[22]

We investigated the relationship between transferrin saturation, liver function, the *HFE* rs1799945 genotype, and withdrawal severity in participants with AUD. We hypothesized that (1) transferrin saturation would be elevated in AUD relative to healthy controls. Since there is evidence of iron loading in individuals with AUD in acute withdrawal and that iron chelators reduced withdrawal symptoms in preclinical studies, we also hypothesized that (2) in AUD participants' serum transferrin saturation would predict withdrawal symptoms. We also aimed to replicate previous findings that the H63D allele was associated with elevated iron stores in those with AUD, but not in healthy controls.[18–20,22] Thus, we further hypothesized (3) a significant interaction of AUD diagnosis x HFE genotype, and (4) that there would be a gene-environment interaction such that H63D allele carriers, with AUD would have higher transferrin saturation than healthy control carriers. Finally, we conducted exploratory analyses to assess whether withdrawal symptoms were elevated in H63D carriers relative to non-carriers and whether that effect was mediated by transferrin saturation, and whether the H63D allele and transferrin saturation were associated with liver function in AUD.

Materials and Methods

Participants

Participants (total $n = 1066$; 594 AUD; 472 healthy non-dependent controls) completed a natural history protocol at the National Institutes of Health (NIH) Clinical Center (Unit and Clinic Evaluation, Screening, Assessment, and Management", ClinicalTrials.gov Identifier: NCT02231840). All participants provided written informed consent to participate in this study as approved by the NIH. The assessments were completed between September 2011 and March 2020. Table 1 describes the clinical and demographic characteristics of the samples.

Clinical Assessments and Behavioral Measures

All participants were assessed for AUD and other psychopathology per the Structured Clinical Interview for DSM (SCID), version -IV or -5.[23,24] Healthy volunteers had no current or past diagnosis of AUD. Participants completed the Timeline Followback[25]

interview to assess daily drinking patterns for 90 days prior to the study date. the Alcohol Dependence Scale[26], Lifetime Drinking History[27], and the Fagerström test for nicotine dependence[28]. AUD participants were inpatients at the NIH Clinical Center and withdrawal symptoms were monitored using the clinical institute withdrawal assessment for alcoholism – revised (CIWA-Ar).[29] AUD participants with moderate to severe withdrawal were treated with benzodiazepines (oxazepam and diazepam) based on their CIWA scores during detoxification.[29] Diazepam doses were converted to oxazepam equivalent doses using a 2:1 ratio, a suggested conversion equivalency.[30]

Iron Measures and HFE Genotype

Fasting bloodwork was drawn on initial screening day for HC between 8 AM and 11 AM and for AUD immediately upon admission. Serum transferrin saturation, plasma CRP, and liver function enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were acquired using standard NIH Department of Laboratory Medicine procedures (Abbott Diagnostics Core laboratory equipment, reagents and machinery). Other iron markers included serum ferritin and iron and group differences are presented in Supplementary Fig. S1. In individuals with alcoholic liver disease, serum iron levels may be elevated without actual iron overload. For our analyses we chose fasting transferrin saturation since it is considered a reliable screening method for hemochromatosis.[31] *HFE* SNP rs1799945 and ancestry informative markers (AIMs) were determined using the Illumina human OmniExpressExome array (Illumina, San Diego, CA, USA). The AIMs for African, European, Asian, Far East Asian, Oceanian, and American ancestry were used to determine ethnicity of participants. Additionally, a principal component analysis (PCA) was performed based on 133,486 SNPs using the -CPA option in PLINK 1.9 https://www.cog-genomics.org/plink/1.9/strat#pca (cut-offs: minor allele frequency>=0.01, Hardy Weinberg Equilibrium p>= 1×10^{-6}, pairwise SNPs r^2 <=0.2 in a 50kb window). The first 3 ancestry principal components (PCs) were used to correct for ancestry in the statistical models. Supplementary Table 1 shows correlations between the 3 PCAs and AIMs.

Statistical Analyses

All statistical analyses were completed using SPSS (IBM, Armonk, NY). Two-sample t-tests were used to compare groups on demographic variables; Chi-square tests to compare groups on categorical variables (sex, self-reported race, *HFE* SNP); and zero-order Pearson's Correlations to explore relationships between study variables for both AUD and controls are listed in Supplementary Table 2 (age, sex, BMI, transferrin saturation, albumin, CRP, European/African ancestry, smoking status, ADS score, TLFB total drinks in past 90 days, average daily max CIWA, overall Max CIWA and total benzodiazepine use).

The following main analyses were performed (1) A linear regression model with transferrin saturation as dependent variable with AUD as predictor and age, BMI, sex, albumin levels, smoking status, CRP, and ancestry PCs as covariates. (2) Linear regression was also performed with average daily maximum CIWA score as dependent variable with transferrin saturation as predictor, and age, BMI, sex albumin levels, ADS, total drinks in last 90 days, sex, CRP, and ancestry PCs as covariates. The maximum Variance Inflation Factor

for these two regression models was 2.256, which indicates no serious multicollinearity [32]. Albumin was added as a covariate to the regression analyses because it has been shown to be depressed in AUD[33], and could potentially affect withdrawal symptoms and benzodiazepine pharmacokinetics [34,35]. CRP was added as a covariate to explore potential effects of inflammation on transferrin saturation and CIWA scores, as transferrin is a negative acute-phase reactant[36] and inflammation has been implicated in iron metabolism in substance use disorders[37]. Smoking status was included as a covariate as per self-report: current smoker versus non-smoker. (3) We performed a Chi square test to investigate the association of AUD diagnosis with *HFE* rs1799945 (i.e., H63D-carriers vs wild-type). (4) We ran a 2-way ANOVA to test for an AUD x *HFE* interaction on transferrin status, with age, sex, BMI, and ancestry PCs as covariates. Effects of HFE on transferrin status were calculated post-hoc for each diagnostic group. The outcomes of these four main analyses were Bonferroni-corrected for multiple comparisons ($\alpha < 0.0125$). Effect sizes were calculated using partial η^2.

We then performed exploratory testing to determine whether transferrin saturation mediated the relationship between *HFE* and average daily maximum CIWA scores using the Sobel test for mediation[38,39], and PROCESS bootstrapping with bias-corrected 95% confidence intervals (CIs) for SPSS[40]. Further exploratory analyses were performed to assess the relationship between iron loading and liver function using zero-order Pearson's correlations in AUD and HC and mediation analyses in AUD to assess whether the effect of iron loading on withdrawal were mediated by elevated liver function. We ran two-sample t-tests to compare AST, ALT, and ALT:AST ratio in AUD and HC and in H63D carriers and wildtypes in both cohorts.

Results

Demographics and Clinical Characteristics

Demographic and clinical characteristics of the AUD and control cohorts are summarized in table 1. The AUD cohort was significantly older (45.56 vs 37.49, p<0.001), had lower IQ scores (97.34 vs. 108.56, p<0.001), fewer years of education (13.44 vs 15.58, p<0.001), and a greater proportion of male participants (69% vs 50%, p<0.001) and smokers (65% vs 14%, p<0.001) than the control cohort. As anticipated, AUD participants compared to healthy controls reported greater alcohol consumption across various drinking behavior metrics, including standard drinks per month (363.49 vs 17.37, p<0.001), heavy drinking years (16.1 vs. 0.9, p<0.001), total lifetime alcohol intake (1,085,247 g vs 58,530 g, p<0.001), and ADS scores (20.9 vs 1.4, p<0.001).

1. Higher transferrin saturation in AUD compared to controls—Sample t-tests between AUD and controls, without controlling for covariates, indicated elevated transferrin saturation (38.96 vs. 23.91, t_{1064}=13.3, p<0.0001), elevated CRP (4.37 vs. 2.39, t_{1012}=3.1, p=0.002), and lower albumin levels (3.92 vs. 4.46, t_{1064}=24.8, p<0.0001) in AUD compared to controls.

The linear regression model indicated that AUD status significantly predicted serum transferrin saturation (β=0.365, p<0.0001) when correcting for age, BMI sex, albumin

levels, smoking status, CRP and the three main ancestry PCs (Fig. 1; Table 2). The effect remained significant after Bonferroni correction for 4 multiple comparisons ($p<0.0125$). Other significant predictors of transferrin saturation were BMI ($\beta=-0.116$, $p<0.001$), CRP ($\beta=-0.099$, $p=0.002$), and the first ancestry PC ($\beta=0.188$, $p<0.0001$) (Table 2). The effect of AUD status on transferrin saturation was significant for those who self-identified as African American/ Black ($\beta=0.273$, $p<0.0001$), White ($\beta=0.480$, $p<0.0001$), and Asian ($\beta=0.648$, $p=0.003$), while correcting for age, BMI sex, albumin levels, smoking status, and CRP.

Exploratory analyses indicated no main effect of sex on transferrin saturation in the entire cohort, but there was an interaction of sex-by-AUD diagnosis ($F_{1,1062}=10.170$, $p=0.001$, $\eta^2=0.009$). In healthy controls, but not in AUD, men had higher serum transferrin saturation than women (26.54% vs. 21.55%, $t=5.281$, $p<0.001$).

2. Association between withdrawal and transferrin saturation in AUD—Within AUD, transferrin saturation significantly predicted the average daily max CIWA withdrawal scores ($\beta=0.236$, $p<0.0001$), when correcting for age, BMI, sex, albumin levels, ADS scores, TLFB total drinks in past 90 days, CRP, and ancestry PCs, which remained significant after correcting for 4 comparisons ($p<0.0125$). Moreover, the ADS score ($\beta=0.237$, $p<0.0001$), total drinks in past 90 days ($\beta=0.119$, $p=0.005$), CRP ($\beta=0.103$, $p=0.012$), and ancestry PC 1($\beta=0.209$, $p<0.0001$) predicted average daily maximum CIWA score (Table 3).

The effect of transferrin saturation on CIWA withdrawal scores was significant for those who self-identified as White ($\beta=0.303$, $p<0.0001$), but not for African American/ Black ($\beta=0.095$, $p=0.16$), or Asian ($\beta=5.10$, $p=0.15$), while correcting for age, BMI sex, albumin levels, smoking status, and CRP.

3. Association of *HFE* rs1799945 with AUD diagnosis—Contrary to our hypothesis, there was no significant difference in percentage of *HFE* rs1799945 SNP carriers versus wildtype between AUD and healthy controls ($\chi^2= 2.23$, $p=0.33$).

4. Association of *HFE* rs1799945 with transferrin saturation in AUD and controls—In line with our hypothesis, there was a significant interaction effect of rs1799945 genotype x AUD status on transferrin saturation levels ($F_{1,925}=6.356$, $p=0.013$, $\eta^2=0.007$), which remained significant after correcting for multiple comparisons ($p<0.0125$). Effects of rs1799945 were stronger in the AUD group (49.05 vs. 37.80, $t_{503}=4.1$, $p<0.001$; Fig. 3A) than in the control group (26.87 vs 23.25, $t_{430}=2.5$, $p=0.012$, Fig. 3B). The main effect of rs1799945 genotype on transferrin saturation was significant ($F_{1,925}=7.389$, $p=0.007$, $\eta^2=0.008$) (controlled for BMI, sex, age, and ancestry PCs), with higher transferrin saturation for G allele carriers than non-carriers in both groups pooled together (39.8 vs 31.0, $t_{935}=4.9$, $p<0.0001$).

The frequency of the G allele differed among racial groups ($\chi^2=70.5$, $p<0.001$), and those who self-identified as Black/African American had a lower G allele frequency (2.9%; n=408) than those who identified as White (14.3%; n=411), or Asian (6.8%, n=44). The effect of rs1799945 on transferrin saturation was significant in Whites; both for AUD and

control groups combined (t_{409}=2.6, p=0.008) and in the AUD group (t_{240}=2.6, p=0.009), but not in African Americans or Asians.

Exploratory analyses indicated no differences in drinking behaviors (ADS and total drinks per 90 days), age, or sex between carriers and non-carriers in AUD or healthy controls (all p>0.05). While there was a significant group difference in BMI between carriers and non-carriers in the AUD cohort, but not the HC cohort, the interaction between the two factors was not significant (AUD: 25.472 vs. 26.984, p=0.012; HC: 26.862 vs. 26.442, p=0.529; AUD x rs1799945: $F_{1,925}$=1.479, p=0.224).

Exploratory results: Association of *HFE* rs1799945 and transferrin saturation with withdrawal severity

Among AUD participants, G allele carriers compared to non-carriers had higher average daily maximum CIWA score than non-carriers (5.26 vs 4.41, t=2.091, p=0.037). However, group differences for overall maximum CIWA scores (8.93 vs 7.72, t=1.867, p=0.063) and total benzodiazepine consumption (92.15 mg vs 70.70 mg, t=1.633, p=0.103) did not reach significance.

The exploratory mediation test demonstrated that transferrin saturation mediated the relationship between *HFE* rs1799945 genotype and average daily maximum CIWA score (Sobel t=3.69, p<0.001; Bootstrapping indirect effect size: k^2=0.54, 95% CI [0.2409, 0.9001]) (Supplemental Fig. S2).

Exploratory results: Association of *HFE* rs1799945 and transferrin saturation with liver function

As expected, those with AUD compared to controls had elevated levels of both AST (56.51 U/L vs. 22.62 U/L, t=12.816, p<0.001) and ALT (50.03 U/L vs. 20.13 U/L, t=13.102, p<0.001), but did not differ in AST:ALT ratio, a marker of alcohol-associated liver disease. Among AUD, but not controls, AST:ALT ratio correlated positively with transferrin saturation (r=0.333, p<0.001) and average daily maximum CIWA score (r=0.201, p<0.001). Further, AST:ALT ratio partially mediated the relationship between transferrin saturation and CIWA (Sobel t=2.56, p=0.01; Bootstrapping indirect effect size: k^2=0.006, 95% BCa CI [0.0013, 0.0103]) (Supplemental Fig. S3). Also, in AUD, but not in controls, the G allele carriers had higher ratios of AST:ALT than non-carriers (1.38 vs 1.21, t=2.254, p=0.024).

Discussion

In the present study, we replicated findings that serum transferrin saturation was elevated in AUD relative to non-dependent healthy controls.[6–9,41] While it has been suggested that iron loading in AUD is independent of the amount of alcohol consumed, we found that among those with AUD, alcohol consumption history positively correlated with transferrin saturation.[5] We further demonstrated that there was an interaction effect of AUD and *HFE* rs1799945 genotype on transferrin saturation. This is consistent with previous studies and supports the clinical recommendation that those carrying functional *HFE* SNPs, including rs1799945, should limit alcohol use.[22,42] We only found evidence of this interaction in those who self-identified as White, consistent with the higher prevalence of hemochromatosis in

those with European ancestry.[17] However, this may also reflect the small sample size of *HFE* rs1799945 carriers of other ancestries.

We also report, for the first time to our knowledge, that transferrin saturation predicted withdrawal symptoms in AUD. Transferrin saturation was as strong of a predictor as alcohol dependence severity, which itself, in part, assesses withdrawal symptoms.[26] One possible mechanism for this relationship may be attributed to iron stores in the liver. Our exploratory analyses support previous findings that iron loading contributes to liver damage in AUD.[14,22] We further found that liver function partially mediated the relationship between transferrin saturation and withdrawal severity, consistent with the underlying pathophysiology of alcohol-related liver damage. Liver iron stores contribute to oxidative stress and thus necrosis of hepatocytes and elevation of serum aminotransferase levels.[43] Liver damage, as indexed by elevated serum AST, was previously associated with withdrawal severity.[44] Given the cognitive impairments and physical symptoms seen in people with alcoholic liver damage, it follows that those with a greater iron burden would have more severe withdrawal.[43] In addition to iron and liver enzymes, elevated cholesterol, blood urea nitrogen, thrombocytopenia, and hypokalemia have been found to be predictive of more severe withdrawal.[3,45,46] Together, these biomarkers could help clinicians identify patients who may need more support in the detoxification and withdrawal process.

The relationship between elevated peripheral iron and withdrawal severity may also be attributed to the effects of iron stores in the brain. Elevated peripheral iron measures (i.e. transferrin saturation >50%) tend to associate with subcortical brain iron stores.[47] A recent study in a population with cocaine use disorder found that serum transferrin saturation positively correlated with brain iron stores, as assessed by quantitative susceptibility mapping magnetic resonance imaging (QSM-MRI).[37] The transport of excess iron from the blood to the brain is likely attributable to a more permeable or “leaky” blood brain barrier (BBB) from chronic inflammation in AUD and other substance use disorders.[37,48,49] Increased BBB permeability allows for more iron to enter the brain than normal, as transferrin (which shuttles iron) typically does not diffuse across the healthy BBB.[50] Indeed, a QSM-MRI study revealed elevated iron stores in subcortical dopamine-rich brain structures in AUD patients during acute withdrawal.[48] Iron stores in the brain can lead to cell death via production of reactive oxygen species and have been linked to neurodegenerative diseases, including Parkinson’s and Alzheimer’s disease.[51,52] This pathway may too have implications for alcohol withdrawal, as there is evidence of elevated peripheral oxidative stress in recently detoxified patients with AUD that correlated with withdrawal severity.[53] Future neuroimaging studies should examine the interplay of iron loading, oxidative stress, neurodegeneration, and alcohol withdrawal to elucidate the mechanism behind these findings.

Interestingly, even though we found that H63D carriers had significantly elevated transferrin saturation relative to non-carriers in AUD participants, the SNP did not strongly predict withdrawal symptoms. There are several possible explanations for this, which include the relatively small sample size of carriers (n=86) relative to non-carriers (n=419), or the strong main effect of AUD diagnosis relative to H63D on transferrin saturation. This could also reflect increases in $GABA_A$ receptors in H63D carriers; a recent study reported elevated

$GABA_A$ receptor expression and reduced anxiety in non-alcohol dependent mice with a murine analog of the H63D allele compared to wildtype mice.[54] The authors posit that this may be a unique gain of function feature of the H63D SNP, as the *HFE* knock-out hemochromatosis model had elevated anxiety relative to wildtype.[54,55] $GABA_A$ receptors are typically downregulated during alcohol withdrawal in AUD, but future studies are needed to evaluate if the downregulation is modulated by *HFE* rs1799945 genotype.[4]

Additionally, we found evidence suggesting that inflammation in AUD may impact iron metabolism and alcohol withdrawal. As expected, systemic inflammation, as indexed by plasma CRP, was elevated in AUD relative to Controls, but had a modest negative association with transferrin saturation in both groups. It has been established that systemic inflammation disrupts iron homeostasis via the release of pro-inflammatory cytokines.[56] These cytokines act in opposition to alcohol upregulating hepcidin and reducing iron plasma levels.[56] Even though CRP and transferrin saturation were negatively associated with each other, both positively predicted alcohol withdrawal in our sample. Leclercq and colleagues (2012) similarly found that adults in an alcohol detoxification program had elevated pro-inflammatory markers, including CRP, relative to healthy comparisons in both acute and protracted withdrawal.[57] These peripheral pro-inflammatory markers positively correlated with anxiety and depression symptoms and with alcohol craving.[57] The downstream effects of cytokines appear to parallel that of iron loading: there is evidence that peripheral pro-inflammatory cytokines activate microglial cells, which in turn produce reactive oxygen species and thus lead to neuronal death.[58] However, how the direct actions of alcohol and inflammation interact to affect hepcidin expression, iron metabolism, and withdrawal in AUD is unclear.

In our study we also showed a positive association between the first ancestry PC and CIWA-Ar withdrawal, indicative of lower severity of alcohol withdrawal among AUD patients who self-identified as African American/Black (mean CIWA-Ar=6.50, SD=4.4) compared to those who identified as White (mean=9.31, SD=5.9) or Asian (mean=4.17, SD=9.7) ($F_{2,539}$=19.3, $p<0.0001$). These findings replicate prior studies reported lower severity in alcohol withdrawal symptoms among African Americans with AUD than among Caucasians and it is possible that H63D might contribute to these racial differences.[59]

There were several limitations in our study. First, though we demonstrated an association between peripheral iron measures and alcohol withdrawal severity, we cannot prove a causal relationship. Differences in nutritional status between cohorts may be a potential confounder. People who drink heavily might have altered micronutrient intake and absorption.[60] While this would exacerbate withdrawal symptoms, a nutrient-poor diet in conjunction with impaired absorption would be expected to negatively impact iron levels, which is not what was seen in the present study. Additionally, albumin levels are typically depressed in those with AUD.[33] This could affect the pharmacokinetics of oxazepam and diazepam as they are both bound to albumin in serum.[34,35] Thus, lower albumin levels in the AUD cohort could have impacted the trajectory of withdrawal symptoms and benzodiazepine dosing. Future studies should examine the role of nutrition, albumin, and withdrawal in the context of AUD to better understand these relationships.

Additionally, in our study, we used peripheral measures of iron and while others have found they are positively associated with brain iron stores, the exact relationship between peripheral and brain iron is unclear.[37,47] Future studies using QSM-MRI are needed to investigate the role of brain iron stores in alcohol withdrawal and to assess the effects of *HFE* rs1799945 genotype. Uncovering the distribution of iron stores in the brain in detoxification and their relationship to withdrawal symptoms may further elucidate the neural underpinnings of alcohol withdrawal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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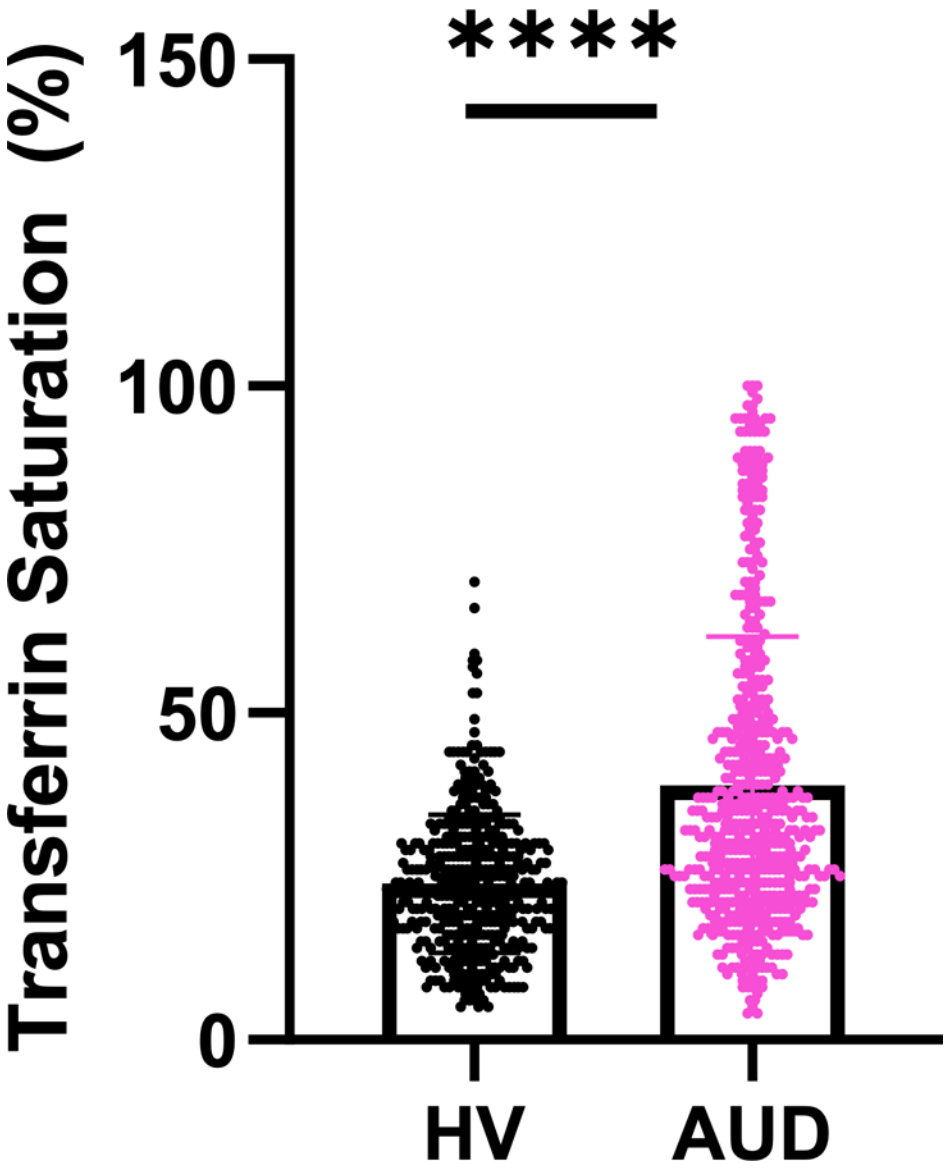

Figure 1.
Elevated transferrin saturation among AUD relative to HC. ****p < 0.0001

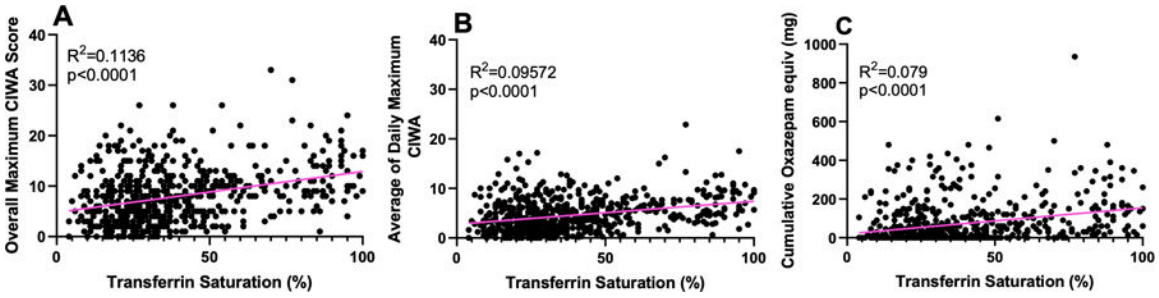

Figure 2.

Association between transferrin saturation and withdrawal measures among AUD participants. (A) Transferrin saturation and overall maximum CIWA score. (B) Transferrin saturation and average of daily maximum CIWA score. (C) Transferrin saturation and cumulative oxazepam equivalent doses (mg)

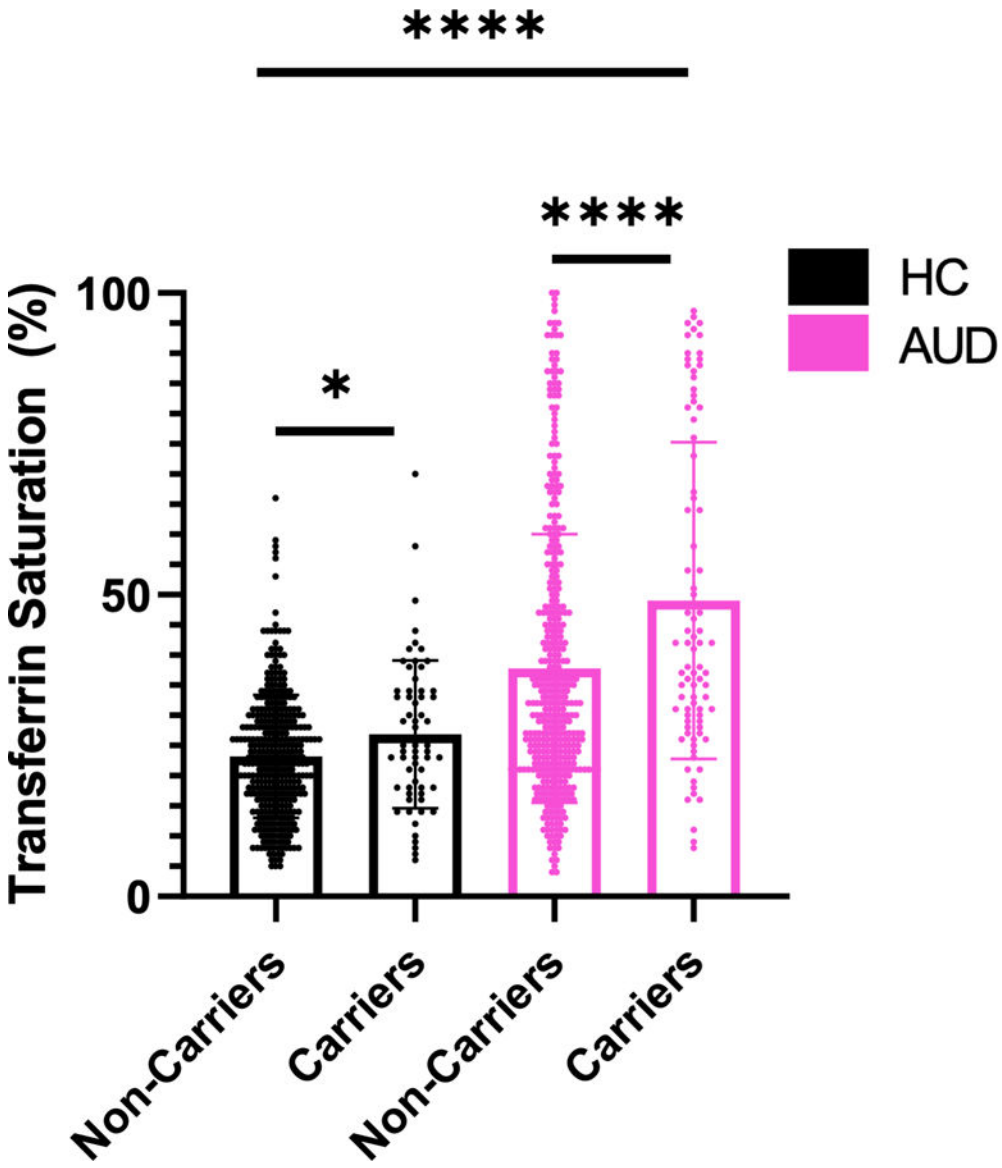

Figure 3.

Elevated transferrin saturation among AUD relative to HC and in G allele carriers of rs1799945 in HC and AUD relative to non-carriers of each cohort. *p < 0.05; ****p < 0.0001

Table 1.

Demographic and clinical characteristics of the alcohol use disorder (AUD) and healthy control (HC) cohorts.

Characteristics	AUD (n=594)	Healthy Controls (n=472)	Statistics	p value
Age, years	45.56 (11.06)	37.47 (13.76)	t=10.6	p<0.001
BMI	26.64 (5.06)	26.74 (4.86)	t=0.3	p=0.73
Years of education	13.44 (3.05)	15.83 (3.44)	t=11.5	p<0.001
IQ score	97.34 (16.43)	110.39 (17.16)	t=11.6	p<0.001
Sex			χ^2= 53.4	p<0.001
Female	182	249		
Male	412	223		
Self-reported Race			χ^2= 34.7	p<0.001
Black/AA	252	214		
White	279	182		
Asian	10	41		
Multiracial	17	14		
American Indian/ Alaska native	4	2		
Unknown Race	32	18		
HFE Genotype			χ^2= 2.23	p=0.328
CC	419	370		
GC	80	60		
GG	6	2		
No data	89	40		
Smoking status			χ^2=346.1	p<0.001
Smoker	351	31		
Non-Smoker	192	413		
No data	51	28		
Drinks per month	363.49 (287.72)	15.78 (47.43)	t=25.1	p<0.001

Characteristics	AUD (n=594)	Healthy Controls (n=472)	Statistics	p value
Heavy drinking years	16.10 (10.79)	0.37(2.61)	t=27.8	p<0.001
Total Lifetime Drink kg)	1085247.1 (1067572.5)	38848.12 (156250.77)	t=19.1	p<0.001
ADS score	20.9 (8.12)	1.3 (3.2)	t=47.4	p<0.001

Table 2.

Summary of regression analyses for transferrin saturation and covariates predicting transferrin saturation in both HC and AUD group combined.

	Transferrin Saturation		
Variable	**B**	**SE B**	**β**
AUD Status	14.456	1.806	**0.365** ***
Age	−0.042	0.051	−0.028
BMI	−0.459	0.125	−**0.116** ***
Sex	−2.075	1.277	−0.052
Albumin levels	1.656	1.862	0.036
Smoking Status	2.355	1.566	0.058
CRP	−0.184	0.058	−**0.099** **
PC1	114.085	18.997	**0.188** ***
PC2	−21.483	18.893	−0.036
PC3	8.410	18.327	0.014
R^2	0.228		
F	**24.737** ***		

** p<0.01

*** p<0.001

Table 3.

Summary of regression analyses for average daily max CIWA and covariates predicting CIWA in the AUD group, covarying for systemic inflammation.

	Average Daily Max CIWA		
Variable	**B**	**SE B**	**β**
Transferrin Saturation	0.034	.0.006	**0.236** ***
Age	0.001	0.013	0.004
BMI	−0.015	0.027	−0.024
Sex	1.022	0.299	0.142
Albumin levels	−0.793	0.364	−0.092
ADS Score	0.094	0.018	**0.237** ***
Total Drinks (past 90 days)	0.000	0.000	**0.119** **
CRP	0.025	0.010	**0.103** **
PC1	21.82	4.58	**0.209** ***
PC2	5.05	7.20	0.034
PC3	4.98	5.62	0.042
R^2	0.287		
F	**16.236** ***		

** p<0.01

*** p<0.001.