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Hepatic, lipid and genetic factors associated with obesity: crosstalk with alcohol dependence?

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Abstract

Objectives—Alcohol dependence represents a leading cause of mortality and morbidity.

Understanding the variables that contribute to this diagnosis and its severity is critical. An overlap between factors that may predispose people to become obese and those that may increase the risk of alcohol dependence may exist. However, data in the literature are not conclusive. Therefore, this study aimed to identify the association between alcohol dependence and obesity-related factors, including biochemical and genetic factors.

Methods—In a case-control study with 829 participants, factors involved with metabolism and obesity were assessed, including biochemical lipid and liver markers, and the fat mass and obesity-associated (FTO) single nucleotide polymorphism (SNP), rs8050136.

Results—Increased triglycerides, having one or two minor A alleles for rs8050136 and being a smoker were associated with increased risk of alcohol dependence, while, increased low-density lipoprotein (LDL) cholesterol was associated with decreased risk. Additionally, having abnormal gamma-glutamyl transferase (GGT) and being female were factors associated with an increased severity of alcohol dependence.

Conclusions—Our preliminary findings suggest a link between alcohol dependence and obesity-related biochemical and genetic factors. Future studies are needed to better understand if these factors may play a predictive role and/or may act as biomarkers for treatment response.

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Keywords

Obesity; alcohol dependence; FTO; genetics; metabolism

Introduction

A growing body of preclinical and clinical literature suggests that a variety of factors involved with appetite and food intake may play a role in alcohol-seeking behaviors (Leggio et al., 2011). On a neurobiological level, the neurocircuitries regulating food- and alcohol-seeking behaviors share common pathways such as the mesolimbic dopaminergic pathway (Kenny, 2011). Consistent with preclinical research, human neuroimaging studies on obesity and compulsive overeating have demonstrated similar disruptions in neural responses to rewards that are similar to those observed in individuals affected by alcohol dependence (AD) (Volkow, Wang, Tomasi, & Baler, 2013). Preclinical and clinical studies indicate that neuroendocrine regulators for hunger and satiety (*e.g.* leptin, ghrelin, glucagon-like peptide-1, oxytocin, and others) may also play a role in alcohol craving and consumption (Engel & Jerlhag, 2014; Haass-Koffler et al., 2015; Kenna, Swift, Hillemacher, & Leggio, 2012; Lee, Rohn, Tanda, & Leggio, 2016; Leggio et al., 2014). Additionally, a study investigating brain responses to palatable food, found that reductions in blood ghrelin and increases in blood triglycerides were associated with reduced responses in brain regions associated with reward and feeding (Sun et al., 2014). This is consistent with a body of literature indicating that triglycerides may affect reward processes associated with consumption of foods rich in fats and sugars (reviewed in: (Cansell & Luquet, 2015)).

Given the complexity of what contributes to addictive behaviors, revealing the relationship between factors involved with metabolism, obesity and AD can provide potentially substantive information. However, the data available to date remains conflicting. For example, a few studies have indicated that the prevalence of metabolic syndrome was higher in alcohol-dependent individuals (Kahl et al., 2010) and that excess alcohol consumption was associated with increased risk of metabolic syndrome (Fan et al., 2008). By contrast, other studies showed that alcohol consumption at and even above moderate levels was negatively associated with metabolic syndrome (Churilla et al., 2014); that the prevalence of metabolic syndrome was lower with mild to moderate alcohol consumption (Freiberg, Cabral, Heeren, Vasan, & Curtis Ellison, 2004); and that higher alcohol consumption was associated with lower risk of obesity (Rohrer, Rohland, Denison, & Way, 2005), lower body mass index (BMI) and decreased fat mass (de Timary et al., 2012). Notably, a systematic review looking at the effects of alcohol consumption on weight gain did not lead to any definitive conclusions (Sayon-Orea, Martinez-Gonzalez, & Bes-Rastrollo, 2011).

These discrepancies in the literature suggest that there may be other individual differences that must be considered, such as variation in genetic factors that are involved in both alcohol consumption and metabolism. In fact, the relationship between food and alcohol intake may be due to a shared genetic risk in addition to factors involved with metabolism and obesity. For instance, a family history of alcoholism is associated with increased risk of obesity (Grucza et al., 2010) and higher BMI (Lichenstein et al., 2014). In addition, genetic variants

of the fat mass and obesity-associated (FTO) gene have been associated with AD (Lichenstein et al., 2014; Wang et al., 2013). FTO gene variants have been examined through genome-wide association studies (GWAS) that have shown an increased risk for obesity (Frayling et al., 2007; Hinney et al., 2007), which may in part be due to food consumption and preferences (Loos & Yeo, 2014). However, another study showed that the FTO SNP variant, rs9939609 and alcohol consumption may be inversely related (Sobczyk-Kopciol et al., 2011), which warrants additional exploration on the relationship between obesity and AD.

In summary, despite the existing literature on biochemical and genetic factors involved with metabolism, obesity and alcohol consumption, the association between these variables and AD remains unclear. Thus, we aimed to investigate the association between obesity-related markers, including biochemical (hepatic and lipid) and genetic (*i.e.* FTO SNP, rs9939609) factors and their involvement with AD in a large and well-characterized cohort. Additionally, in the same cohort, we aimed to assess the relationship between these variables and the severity of AD. Specifically, we expected that biochemical factors such as lipid measures would have a positive association with AD due to their relationship with reward processes associated with addictive behaviors. Additionally, we *a priori* hypothesized an association between the FTO SNP variant, rs9939609 and AD. However, given the inconsistencies provided in the literature, we did not hypothesize the specific direction of such relationship.

Participants and Methods

Participants

Participants included in the study were selected among those enrolled in screening protocols at the National Institute on Alcohol Abuse and Alcoholism (NIAAA) from 2008 – 2015 at the National Institutes of Health (NIH), Clinical Center in Bethesda, MD, USA. These screening protocols were approved by the appropriate NIH Institutional Review Boards. Participants provided written informed consent. Exclusion criteria included a positive test for hepatitis C virus, liver cirrhosis and end-stage liver disease. Data from 829 participants were used for the analysis. Cases ($n = 596$) included individuals with current AD and controls ($n = 233$) included individuals with no history of past and/or current diagnosis of AD.

Methods

Blood sample and blood pressure measurements—Blood tests included: 1) a chemistry blood panel with glucose, and liver enzyme tests, *i.e.* gamma-glutamyl transferase (GGT), aspartate transaminase (AST) and alanine transaminase (ALT); 2) lipid panel with triglycerides, HDL cholesterol and LDL cholesterol. Liver enzyme tests were split into binary variables of normal and abnormal based on standard ranges determined at the Department of Laboratory Medicine of the NIH Clinical Center (GGT: abnormal > 60 U/L, normal 59 U/L; AST: abnormal > 41 U/L, normal 40 U/L; ALT: abnormal > 42 U/L, normal 41 U/L). Blood pressure (systolic and diastolic, mmHg) was also assessed.

Genotyping—Genotyping was performed at the NIAAA Laboratory of Neurogenetics on the Illumina OmniExpress BeadChip (Illumina Inc., San Diego, CA). The FTO SNP variant, rs9939609 has been shown to be involved with obesity, AD and alcohol consumption (Sobczyk-Kopciol et al., 2011; Wang et al., 2013). Based on the strong Linkage Disequilibrium (LD), or the non-random association of alleles at different loci, of rs8050136 to rs9939609 ($R^2 = 0.843$) in both European and African ancestry, rs8050136 was selected as a proxy SNP for the genetic analysis since our array did not include rs9939609. A dominant genetic model was implemented for the analyses due to the low frequency of individuals who were homozygous for the minor A allele for rs8050136. For the dominant genetic model, AA and AC participants were combined (see Table 2 for frequencies). The SNP selected for analysis was in Hardy-Weinberg equilibrium (HWE) ($\chi^2 = 0.57$, $p = 0.45$) and had minor allele frequency (MAF = 0.393) > 5%. To control for population stratification, ancestry informative markers (AIMs, $n = 2500$) were extracted from the Illumina array and ancestral proportions were calculated for all participants.

AD diagnosis and symptom counts—The Structured Clinical Interview (SCID) for DSM-IV Axis I Disorders (First, Spitzer, Gibbon, & Williams, 1995) was utilized for the diagnosis of AD and for AD symptom counts, the latter used to measure severity of AD.

Demographic and anthropometric measurements—Demographic and anthropometric measures included education level (years), age, BMI, gender (male, female), ethnicity [Caucasian (non-Hispanic or Latino), African American, Hispanic or Latino, Other] and income level [Below Average: \$19,999, Average: \$20,000 – \$49,999, Above Average: \$50,000, Unknown (income not reported)]. Female, Average Income and Unknown Income were used as reference categories.

Alcohol and tobacco consumption—Average drinks per drinking day during the three months prior to the assessment was assessed using the Alcohol Timeline Followback (TLFB) questionnaire (Sobell, Brown, Leo, & Sobell, 1996). Given the high comorbidity between alcohol and tobacco misuse (Gruza & Bierut, 2005), smoking status (yes, no) was also assessed.

Data Analysis

To investigate the association between factors involved with metabolism and AD, we first implemented logistic regression analyses as determined by the odds ratios (OR) (95% confidence interval (CI)) for AD diagnosis, and linear regression analyses to model the severity of the diagnosis in alcohol-dependent patients. Due to missing data for AD symptoms, the linear regression sample size ($n = 577$) was reduced. For the logistic regression, AD classification (yes, no) was chosen as the outcome variable, and for the linear regression, the symptom count range for the diagnosis as indicated by the SCID was chosen as the outcome variable. A diagnosis of AD included 3 out of 7 symptoms (each symptom consisted of one answer: 1 = absent or false, 2 = subthreshold, 3 = threshold or true). The severity of the diagnosis was assessed by the symptom count criteria with a minimum score of 13 (3 threshold/true symptoms) and a maximum score of 21. The independent variables for all of the models included demographic measures, anthropometric measurements, blood

pressure, glucose, lipid profiles, liver enzymes and tobacco and alcohol consumption (Table 1). Having no minor alleles versus having one or two minor alleles for rs8050136 was added as a predictor variable as well as the AIMS scores (European Americans, African Americans) (Table 2). Planned additional analyses for AD participants and significant biochemical variables included bivariate Pearson correlations with average drinks per drinking day as well as linear regressions with length of AD as an additional predictor variable. Data were analyzed with the Statistical Package for Social Sciences 20.0 (SPSS 20.0, IBM Corp.) and the alpha was set to $p < .05$ (two-tailed).

Results

Logistic regression comparing alcohol-dependent participants to controls—

The logistic regression revealed that increased triglycerides (OR (95% CI)): (1.009 (1.002 – 1.016), $p = 0.015$), having one or two A alleles compared to no A alleles (1.991 (1.010 – 3.942), $p = 0.047$), being older (1.110 (1.072 – 1.150), $p < 0.0001$), having higher average drinks per drinking day, (1.885 (1.636 – 2.172), $p < 0.0001$) and being a smoker (4.381 (2.001 – 9.590), $p < 0.0001$) significantly increased the odds for a diagnosis of AD. Increased LDL cholesterol (0.989 (0.978 – 1.000), $p = 0.048$) and participants with above average income (0.375 (0.175 – 0.801), $p = 0.011$) significantly decreased the odds for a diagnosis of AD (Table 3). For comparison purposes, an additional logistic regression with an additive genetic model with all the same factors (*i.e.*, biochemical, genetic, anthropometric, demographics, behavioral) as the dominant genetic model logistic regression was implemented (Supplementary Table 1).

Additional analyses were performed for AD patients only with significant biochemical variables (triglycerides and LDL cholesterol) from the logistic regression. First, bivariate Pearson correlations were performed to identify associations between average drinks per drinking day and biochemical variables. There were no significant correlations found between average drinks per drinking day and triglycerides ($r(596) = .038$, $p = .358$) or LDL cholesterol ($r(596) = .029$, $p = .479$), indicating that amount of drinking was not associated with any deviations from the norm of these biological variable levels. Furthermore, regression analyses were performed with triglycerides and LDL cholesterol as the outcome variables to provide evidence that certain biochemical factors may be predictors of AD, rather than being AD as predictor of those factors. To investigate if having AD may cause deviations from the norm of the biological variables or *vice versa*, the length of time that participants were AD was added in as an additional predictor (current age *minus* age of AD onset). The linear regressions revealed that length of time that participants were AD was not significantly associated with triglycerides or LDL cholesterol (Supplementary Tables 2 and 3).

Furthermore, to ensure that the findings of our study were not due merely to BMI status, an additional *post hoc* logistic regression analysis was performed to assess any interactions between BMI and LDL cholesterol, triglycerides and/or rs8050136. Results of this analysis indicated that BMI did not interact with LDL cholesterol, triglycerides, or rs8050136 (Supplementary Table 4).

Finally, while the alcohol drinking variable in this study, average drinks per drinking day, was specifically selected based on recently published work (Schwandt, Cortes, Kwako, George, & Momenan, 2016), this variable highly correlated with other alcohol drinking variables (*e.g.*, average drinks per day) as well as the Alcohol Use Disorders Identification Test and Alcohol Dependence Scale scores (*data not shown*).

Linear regression with severity of AD diagnosis—The linear regression revealed that having abnormal GGT (0.746 (0.270 – 1.222), $p = 0.002$), being female (β coefficient (95% CI): (0.874 (0.426 – 1.321), $p < 0.0001$) and having a higher average drinks per drinking day (0.086 (0.015 – 0.227), $p < 0.0001$) were significant factors associated with increased symptom counts. Conversely, having more years of education (–0.101 (–0.181 – –0.020), $p = 0.014$) was significantly associated with decreased symptom counts (Table 4). For comparison purposes, an additional linear regression with an additive genetic model with all the same factors (*i.e.*, biochemical, genetic, anthropometric, demographics, behavioral) as the dominant genetic model linear regression was implemented (Supplementary Table 5).

Discussion

This study examined the relationship between the diagnosis and severity of AD and factors involved with metabolism and obesity, including biochemical markers (*i.e.*, liver and lipid profiles) and genetic markers (*i.e.*, the FTO SNP variant, rs8050136, used as a proxy for rs9939609), as well as demographic and anthropometric measures. We first aimed to identify the variables that are associated with AD and we showed that increased triglycerides, having one or two A alleles for rs8050136, being older, higher average drinks per drinking day and being a smoker significantly increased the odds of being diagnosed with AD. Conversely, increased LDL cholesterol and having above average income significantly decreased the odds of being diagnosed with AD. Having abnormal GGT, being a female and higher average drinks per drinking day were factors significantly associated with increased severity of AD (*i.e.*, SCID symptom counts) within the sample of alcohol-dependent patients, while higher education led to lower severity of AD.

It has been shown that alcohol-dependent patients have increased blood pressure, impaired glucose and HDL cholesterol metabolism (Kahl et al., 2010) and low-to-moderate alcohol consumption is associated with increased levels of HDL cholesterol and decreased levels of LDL cholesterol (Agarwal, 2002; Brien, Ronksley, Turner, Mukamal, & Ghali, 2011). We found that increased LDL cholesterol was associated with decreased risk of AD. While the mechanisms for this association are not fully understood, excessive alcohol consumption, as in individuals with current AD, may influence cholesterol metabolism, including transport rate of lipoproteins and lipoprotein lipase activities (Brien et al., 2011). For example, increased LDL cholesterol may be caused by a decrease in very-low-density lipoprotein (VLDL) conversion to LDL and apolipoprotein B resulting from alcohol drinking (Nakanishi, Yoshida, Nakamura, Kawashimo, & Tatara, 2001; Naruszewicz, Mirkiewicz, & Wehr, 1990), which may explain our inverse finding. Additionally, we found that increased triglycerides increased the odds of being diagnosed with AD. Triglycerides may directly affect processes involved with drug-seeking and rewarding behaviors through the

involvement of the mesolimbic pathway (Cansell & Luquet, 2015) and attenuated responses in brain regions that regulate feeding (Sun et al., 2014), which may be a potential explanation for the positive relationship between triglycerides and AD. In summary, our study suggests, albeit preliminary and speculative, that LDL cholesterol- and triglyceride-related pathways may be involved in central mechanisms related to AD. Notably, preclinical studies have indicated that fibrate medications can help with smoking cessation (Panlilio et al., 2012) and brain-penetrating statins can reduce drug-seeking behaviors (Chauvet et al., 2016), thus supporting the hypothesis that acting on pathways related to lipid profiles can possibly reduce addiction relapse.

Obviously, an alternative hypothesis is that the results presented here are merely due to the effects of chronic excessive alcohol consumption on LDL cholesterol and triglycerides. However, when we examined the influence of alcohol consumption on deviations from the norm of the biochemical factors in the alcohol-dependent participants, we found no significant relationship between average drinks per drinking day and either triglycerides or LDL cholesterol. This substantiates our findings that triglycerides and LDL cholesterol may be viable predictors of AD. Additionally, our analyses revealed that the number of years an individual is alcohol-dependent has no influence on triglyceride or LDL cholesterol levels. If the state of being alcohol-dependent causes differences in biological variables, then someone who is alcohol-dependent for a longer period of time should have a bigger change associated with these factors (*e.g.*, higher triglycerides with more alcohol consumption). However, since we did not find any significant associations with these variables it can further inferred that they are sustainable predictors to assess susceptibility of an AD diagnosis.

We hypothesized that the FTO SNP, rs8050136 would be associated with AD and our findings illustrate that individuals with one or two A alleles for rs8050136 had increased odds of being diagnosed with AD. This suggests that biochemical pathways and obesity-related genes may contribute to AD as indicated by the link between genetic predispositions and phenotypic variables. When assessing biochemical variables in alcohol-dependent participants, the linear regressions with triglycerides and LDL cholesterol indicated no significant direct relationship with rs8050136. This indicates that any overlap that may exist between these variables is specific when comparing alcohol-dependent individuals to controls. An inverse relationship between rs9939609 and AD has been previously reported (Sobczyk-Kopciol et al., 2011). However, a study attempting to replicate the results by Sobczyk-Kopciol et al. (2011) was not able to find a significant association between the same FTO SNP and alcohol consumption (Hubacek et al., 2012). The aforementioned studies may not be comparable to our study for two reasons. First, both studies were based on Caucasian populations and thus may not be translatable to a sample with mixed ethnicities like ours was. Secondly, the study by Sobczyk-Kopciol et al. (2011) did not control for any other variables (*e.g.*, BMI, age, etc.) when comparing alcohol-dependent subjects to controls. Notably, we assessed interactions between BMI with those factors known to be involved with obesity that were related to AD in our cohort (*i.e.*, triglycerides, LDL cholesterol and rs8050136). The logistic regression analysis revealed that there were no significant interactions with BMI and triglycerides, LDL cholesterol, or rs8050136, which provides evidence that these factors are significant predictors of AD, independent from BMI. It should be noted that these factors can contribute to BMI independently, and also the

findings of this study do not necessarily imply that genetic components are not associated with obesity. However, in our cohort of AD patients, when accounting for all the variables that may characterize an alcohol-dependent patient, interactions between obesity-related traits were not found to be significant.

The comorbidity between smoking and alcoholism is well known (Grucza & Bierut, 2005), thus our findings are consistent with previous literature. Furthermore, prevalence of alcohol use disorders has been associated with socioeconomic variables such as low income and lower education levels (Grant et al., 2015) and our findings are consistent with this. Interestingly, while being male (Grant et al., 2015) has been associated with higher alcohol consumption and a higher prevalence of AD, our findings suggest that being female is associated with higher symptom counts. It may be that when a female is diagnosed with AD, there may be more serious implications for them compared to their male counterparts. For instance, it has been indicated that alcohol affects women differently in terms of metabolism (Roche, Gilvarry, & Day, 2010) and development of dependence from initial abuse is more rapid for women compared to men (Randall et al., 1999).

GGT has been shown to be a useful biomarker of alcohol drinking and metabolic syndrome (Jacobs et al., 2011; Niemela & Alatalo, 2010) and our analyses showing an association between GGT and symptom counts replicates this finding. The fact that this effect was only found for severity of the diagnosis, and not in the case-control analysis, may be accounted for by the fact that the controls, while not being AD, were also drinkers. Altogether, the results indicate that the factors contributing to a simple diagnosis of AD, and the factors associated with the severity of the disorder, are not necessarily the same. Although speculative, we theorize that while factors associated with symptom counts only reflect peripheral effects of alcohol consumption (e.g., increased GGT as a proxy of alcohol-related liver enzymatic induction), factors associated with AD diagnosis (e.g. triglycerides, LDL cholesterol and rs8050136) may interact more on central factors. The latter hypothesis is consistent with previously work discussed above (Cansell & Luquet, 2015; Chauvet et al., 2016; Panlilio et al., 2012; Sun et al., 2014). However, additional studies, including bench-to-bedside research, are needed to further investigate these questions.

Our study has several limitations. First, the research design was cross-sectional, which does not allow us to infer a direct casual role between the factors we examined and AD. In fact, our results suggest that the variables investigated increased the odds for a diagnosis of AD, but do not necessarily cause AD. Furthermore, the selection of the rs8050136 SNP was based on previously published work (Sobczyk-Kopciol et al., 2011; Wang et al., 2013) and not from independent criteria. Although this SNP was chosen based on an *a priori* specific hypothesis, there is the potential for selection bias since other SNPs were not analyzed. Furthermore, since our research questions were hypothesis-driven and considering the small sample size, we did not attempt to conduct a GWAS analysis (Hong & Park, 2012). Moreover, although there are findings that support the association with rs9939609 and preference for energy-dense foods (Cecil, Tavendale, Watt, Hetherington, & Palmer, 2008), as well as evidence for a potential pathway involving noncoding variants in FTO and their influence on adipocyte thermogenesis regulation (Claussnitzer et al., 2015), the exact mechanistic role of these SNPs (rs8050136 and rs9939609) remains unknown. In addition,

while we examined BMI, we did not have any data on dietary or exercise habits that could have been potential contributors to differences in metabolism. Future studies could include caloric intake and exercise regimens to assess any influence that these variables have on metabolism in relationship to AD. Moreover, future studies could investigate type of drink, time of drinking and drinking with or without a meal to assess how metabolism factors like triglycerides and LDL cholesterol are affected by such variables. Finally, it is important to keep in mind the preliminary nature of this study; as an exploratory analysis, no corrections were made for multiple inferential tests. Our approach is consistent with recommendations by independent groups (Bender & Lange, 2001; Perneger, 1998; Rothman, 1990), suggesting that exploratory analyses be done without multiplicity adjustment, but confirmed in follow-up studies. As such, a separate sample will be necessary to confirm the present findings.

In conclusion, the results of this study provide insight into the different factors that may contribute to AD and the severity of the disorder by expanding on existing research with a large and well-characterized cohort. Our study contributes to the existing knowledgebase by replicating past research in conjunction with introducing novel findings. Specifically, our study provides preliminary evidence that there is a shared genetic and biochemical link between factors related to metabolism and obesity, and AD. Overall, these findings suggest that factors related to metabolism and obesity can reveal pertinent information about the underlying physiological mechanisms involved with the development of AD. Having a greater understanding of the numerous components involved with AD can provide a foundation for the development of novel treatments for AD and for the alcohol-related medical consequences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Agarwal DP. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. *Alcohol Alcohol*. 2002; 37(5):409–415. [PubMed: 12217928]
- Bender R, Lange S. Adjusting for multiple testing—when and how? *J Clin Epidemiol*. 2001; 54(4):343–349. [PubMed: 11297884]
- Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. *Bmj*. 2011; 342doi: 10.1136/bmj.d636

- Cansell C, Luquet S. Triglyceride sensing in the reward circuitry: A new insight in feeding behaviour regulation. *Biochimie*. 2015; 120:75–80. [PubMed: 26159487]
- Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med*. 2008; 359(24):2558–2566. DOI: 10.1056/NEJMoa0803839 [PubMed: 19073975]
- Chauvet C, Nicolas C, Lafay-Chebassier C, Jaber M, Thiriet N, Solinas M. Statins Reduce the Risks of Relapse to Addiction in Rats. *Neuropsychopharmacology*. 2016; 41:1588–1597. [PubMed: 26466819]
- Churilla JR, Johnson TM, Curls R, Richardson MR, Boyer WR, Devore SR, Alnojeidi AH. Association between alcohol consumption patterns and metabolic syndrome. *Diabetes Metab Syndr*. 2014; 8(2):119–123. DOI: 10.1016/j.dsx.2014.04.001 [PubMed: 24907178]
- Claussnitzer M, Dankel SN, Kim KH, Quon G, Meuleman W, Haugen C, Kellis M. FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. *N Engl J Med*. 2015; 373(10):895–907. DOI: 10.1056/NEJMoa1502214 [PubMed: 26287746]
- de Timary P, Cani PD, Duchemin J, Neyrinck AM, Gihousse D, Laterre PF, Stärkel P. The Loss of Metabolic Control on Alcohol Drinking in Heavy Drinking Alcohol-Dependent Subjects. *PLoS ONE*. 2012; 7(7):e38682.doi: 10.1371/journal.pone.0038682 [PubMed: 22808013]
- Engel JA, Jerlhag E. Role of appetite-regulating peptides in the pathophysiology of addiction: implications for pharmacotherapy. *CNS Drugs*. 2014; 28(10):875–886. DOI: 10.1007/s40263-014-0178-y [PubMed: 24958205]
- Fan AZ, Russell M, Naimi T, Li Y, Liao Y, Jiles R, Mokdad AH. Patterns of alcohol consumption and the metabolic syndrome. *J Clin Endocrinol Metab*. 2008; 93(10):3833–3838. DOI: 10.1210/jc.2007-2788 [PubMed: 18628524]
- First, M., Spitzer, R., Gibbon, M., Williams, JB. Structured Clinical Interview for DSM-IV Axis I Disorders (SCID). New York: Biometrics Research: New York State Psychiatric Institute; 1995.
- Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. 2007; 316(5826):889–894. DOI: 10.1126/science.1141634 [PubMed: 17434869]
- Freiberg MS, Cabral HJ, Heeren TC, Vasani RS, Curtis Ellison R. Alcohol consumption and the prevalence of the Metabolic Syndrome in the US.: a cross-sectional analysis of data from the Third National Health and Nutrition Examination Survey. *Diabetes Care*. 2004; 27(12):2954–2959. [PubMed: 15562213]
- Grant BF, Saha TD, Ruan WJ, Goldstein RB, Chou SP, Jung J, Hasin DS. Epidemiology of DSM-5 Drug Use Disorder: Results from the National Epidemiologic Survey on Alcohol and Related Conditions-III. *JAMA Psychiatry*. 2015; 72(8):757–766. DOI: 10.1001/jamapsychiatry.2015.0584 [PubMed: 26039070]
- Gruza RA, Bierut LJ. Co-occurring risk factors for alcohol dependence and habitual smoking: update on findings from the Collaborative Study on the Genetics of Alcoholism. *Alcohol Res Health*. 2005; 29(3):172–178.
- Gruza RA, Krueger RF, Racette SB, Norberg KE, Hipp PR, Bierut LJ. The emerging link between alcoholism risk and obesity in the United States. *Arch Gen Psychiatry*. 2010; 67(12):1301–1308. DOI: 10.1001/archgenpsychiatry.2010.155 [PubMed: 21135330]
- Haass-Koffler CL, Aoun EG, Swift RM, de la Monte SM, Kenna GA, Leggio L. Leptin levels are reduced by intravenous ghrelin administration and correlated with cue-induced alcohol craving. *Transl Psychiatry*. 2015; 5:e646.doi: 10.1038/tp.2015.140 [PubMed: 26418274]
- Hinney A, Nguyen TT, Scherag A, Friedel S, Brönnert G, Müller TD, Rief W. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. *PloS one*. 2007; 2(12):e1361. [PubMed: 18159244]
- Hong EP, Park JW. Sample size and statistical power calculation in genetic association studies. *Genomics Inform*. 2012; 10(2):117–122. DOI: 10.5808/gi.2012.10.2.117 [PubMed: 23105939]
- Hubacek JA, Adamkova V, Dlouha D, Jirsa M, Sperl J, Tonjes A, Bobak M. Fat mass and obesity-associated (FTO) gene and alcohol intake. *Addiction*. 2012; 107(6):1185–1186. DOI: 10.1111/j.1360-0443.2012.03825.x [PubMed: 22509951]

- Jacobs M, van Greevenbroek MM, van der Kallen CJ, Ferreira I, Feskens EJ, Jansen EH, Stehouwer C. The association between the metabolic syndrome and alanine amino transferase is mediated by insulin resistance via related metabolic intermediates (the Cohort on Diabetes and Atherosclerosis Maastricht [CODAM] study). *Metabolism*. 2011; 60(7):969–975. DOI: 10.1016/j.metabol.2010.09.006 [PubMed: 21040936]
- Kahl KG, Greggersen W, Schweiger U, Cordes J, Correll CU, Ristow J, Moebus S. Prevalence of the metabolic syndrome in men and women with alcohol dependence: results from a cross-sectional study during behavioural treatment in a controlled environment. *Addiction*. 2010; 105(11):1921–1927. DOI: 10.1111/j.1360-0443.2010.03059.x [PubMed: 20735365]
- Kenna GA, Swift RM, Hillemacher T, Leggio L. The relationship of appetitive, reproductive and posterior pituitary hormones to alcoholism and craving in humans. *Neuropsychol Rev*. 2012; 22(3):211–228. DOI: 10.1007/s11065-012-9209-y [PubMed: 22772772]
- Kenny PJ. Common cellular and molecular mechanisms in obesity and drug addiction. *Nature Reviews Neuroscience*. 2011; 12(11):638–651. [PubMed: 22011680]
- Lee MR, Rohn MC, Tanda G, Leggio L. Targeting the Oxytocin System to Treat Addictive Disorders: Rationale and Progress to Date. *CNS Drugs*. 2016; 30(2):109–123. DOI: 10.1007/s40263-016-0313-z [PubMed: 26932552]
- Leggio L, Addolorato G, Cippitelli A, Jerlhag E, Kampov-Polevoy AB, Swift RM. Role of feeding-related pathways in alcohol dependence: A focus on sweet preference, NPY, and ghrelin. *Alcohol Clin Exp Res*. 2011; 35(2):194–202. DOI: 10.1111/j.1530-0277.2010.01334.x [PubMed: 21058960]
- Leggio L, Zywiak WH, Fricchione SR, Edwards SM, de la Monte SM, Swift RM, Kenna GA. Intravenous ghrelin administration increases alcohol craving in alcohol-dependent heavy drinkers: a preliminary investigation. *Biol Psychiatry*. 2014; 76(9):734–741. DOI: 10.1016/j.biopsych.2014.03.019 [PubMed: 24775991]
- Lichenstein SD, Jones BL, O'Brien JW, Zezza N, Stiffler S, Holmes B, Hill SY. Familial risk for alcohol dependence and developmental changes in BMI: the moderating influence of addiction and obesity genes. *Pharmacogenomics*. 2014; 15(10):1311–1321. DOI: 10.2217/pgs.14.86 [PubMed: 25155933]
- Loos RJ, Yeo GS. The bigger picture of FTO: the first GWAS-identified obesity gene. *Nat Rev Endocrinol*. 2014; 10(1):51–61. DOI: 10.1038/nrendo.2013.227 [PubMed: 24247219]
- Nakanishi N, Yoshida H, Nakamura K, Kawashimo H, Tatara K. Influence of Alcohol Intake on Risk for Increased Low-Density Lipoprotein Cholesterol in Middle-Aged Japanese Men. *Alcohol Clin Exp Res*. 2001; 25(7):1046–1050. [PubMed: 11505031]
- Naruszewicz M, Mirkiewicz E, Wehr H. Abnormal low density lipoproteins composition in some chronic alcoholics: a possible mechanism. *Alcohol Alcohol*. 1990; 25(5):533–538. [PubMed: 2088351]
- Niemela O, Alatalo P. Biomarkers of alcohol consumption and related liver disease. *Scand J Clin Lab Invest*. 2010; 70(5):305–312. DOI: 10.3109/00365513.2010.486442 [PubMed: 20470213]
- Panlilio LV, Justinova Z, Mascia P, Pistis M, Luchicchi A, Lecca S, Heishman SJ. Novel use of a lipid-lowering fibrate medication to prevent nicotine reward and relapse: preclinical findings. *Neuropsychopharmacology*. 2012; 37(8):1838–1847. [PubMed: 22453137]
- Perneger TV. What's wrong with Bonferroni adjustments. *Bmj*. 1998; 316(7139):1236–1238. [PubMed: 9553006]
- Randall CL, Roberts JS, Del Boca FK, Carroll KM, Connors GJ, Mattson ME. Telescoping of landmark events associated with drinking: a gender comparison. *J Stud Alcohol Drugs*. 1999; 60(2):252–260.
- Roche J, Gilvarry E, Day E. Women and alcohol. In: Kohen D, editor. *Oxford Textbook of Women and Mental Health*. Oxford: Oxford University Press; 2010. p. 201–207.
- Rohrer JE, Rohland BM, Denison A, Way A. Frequency of alcohol use and obesity in community medicine patients. *BMC family practice*. 2005; 6(1):17. [PubMed: 15845150]
- Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990; 1(1):43–46. [PubMed: 2081237]

- Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: a systematic review. *Nutrition reviews*. 2011; 69(8):419–431. [PubMed: 21790610]
- Schwandt ML, Cortes CR, Kwako LE, George DT, Momenan R. The CRF1 Antagonist Verucerfont in Anxious Alcohol-Dependent Women: Translation of Neuroendocrine, But not of Anti-Craving Effects. 2016; doi: 10.1038/npp.2016.61
- Sobczyk-Kopciol A, Broda G, Wojnar M, Kurjata P, Jakubczyk A, Klimkiewicz A, Ploski R. Inverse association of the obesity predisposing FTO rs9939609 genotype with alcohol consumption and risk for alcohol dependence. *Addiction*. 2011; 106(4):739–748. DOI: 10.1111/j.1360-0443.2010.03248.x [PubMed: 21182554]
- Sobell LC, Brown J, Leo GI, Sobell MB. The reliability of the Alcohol Timeline Followback when administered by telephone and by computer. *Drug Alcohol Depend*. 1996; 42(1):49–54. [PubMed: 8889403]
- Sun X, Veldhuizen MG, Wray AE, de Araujo IE, Sherwin RS, Sinha R, Small DM. The neural signature of satiation is associated with ghrelin response and triglyceride metabolism. *Physiol Behav*. 2014; 136:63–73. DOI: 10.1016/j.physbeh.2014.04.017 [PubMed: 24732416]
- Volkow ND, Wang GJ, Tomasi D, Baler RD. The addictive dimensionality of obesity. *Biol Psychiatry*. 2013; 73(9):811–818. DOI: 10.1016/j.biopsych.2012.12.020 [PubMed: 23374642]
- Wang L, Liu X, Luo X, Zeng M, Zuo L, Wang KS. Genetic variants in the fat mass-and obesity-associated (FTO) gene are associated with alcohol dependence. *J Mol Neurosci*. 2013; 51(2):416–424. [PubMed: 23771786]

Table 1
Demographic and biochemical characteristics for alcohol-dependent and control patients

BMI = body mass index, HDL = high-density lipoprotein, LDL = high-density lipoprotein GGT = gamma-glutamyl transferase, AST = aspartate transaminase, ALT = alanine transaminase.

Variables	Cases (n = 596)	Controls (n = 233)
Female	178 (30%)	103 (44%)
Male	418 (70%)	130 (56%)
African American	274 (46%)	77 (33%)
Hispanic or Latino	20 (3%)	11 (5%)
Caucasian (Non-Hispanic or Latino)	271 (45%)	124 (53%)
Other ^I	31 (5%)	21 (9%)
Smoker	373 (63%)	15 (6%)
Non-Smoker	223 (37%)	218 (94%)
Above Average Income	117 (20%)	95 (41%)
Average Income	144 (24%)	90 (39%)
Below Average Income	204 (34%)	46 (20%)
Unknown Income	131 (22%)	2 (0.9%)
GGT (Abnormal)	279 (47%)	19 (8%)
GGT (Normal)	317 (53%)	214 (92%)
AST (Abnormal)	168 (28%)	12 (5%)
AST (Normal)	428 (72%)	221 (95%)
ALT (Abnormal)	208 (35%)	28 (12%)
ALT (Normal)	388 (65%)	205 (88%)
Age (years) (mean ± SD)	42.5 ± 10.6	30.8 ± 9.4
BMI (mean ± SD)	27.1 ± 5.2	25.7 ± 4.9
HDL Cholesterol (mean ± SD)	68.9 ± 27.1	62.1 ± 17.2
LDL Cholesterol (mean ± SD)	103.2 ± 38.9	96.4 ± 30.6
Triglycerides (mean ± SD)	118.9 ± 76.7	86.1 ± 46.6
Glucose (mean ± SD)	99.5 ± 22.2	89.3 ± 8.2
Diastolic Blood Pressure (mean ± SD)	82.0 ± 13.0	71.4 ± 10.6
Systolic Blood Pressure (mean ± SD)	133.2 ± 17.4	118.0 ± 14.1
Education (mean ± SD)	13.8 ± 2.7	16.0 ± 3.0
Average Drinks (mean ± SD)	12.4 ± 6.9	3.0 ± 2.1

^I American Indian or Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, multiracial and unknown race.

Table 2

Fat mass and obesity-associated (FTO) genotype information (rs8050136 SNP) and ancestry informative markers (AIMs) scores for alcohol-dependent and control patients.

Variables	Cases (<i>n</i> = 596)	Controls (<i>n</i> = 233)
CC	212 (36%)	99 (43%)
AC	284 (48%)	101 (43%)
AA	100 (17%)	33 (14%)
European Americans	0.56 ± 0.38	0.61 ± 0.39
African Americans	0.37 ± 0.39	0.28 ± 0.38

Table 3
Logistic regression results for factors associated with alcohol dependence

OR = odds ratio, CI = confidence interval. $R^2 = .573$. HDL = high-density lipoprotein, LDL = high-density lipoprotein, GGT = gamma-glutamyl transferase, AST = aspartate transaminase, ALT = alanine transaminase, BMI = body mass index.

Factors	Variables	β coefficient	OR (95% CI)	Significance
<i>Biochemical</i>	HDL Cholesterol	0.005	1.005 (0.986 – 1.024)	$p = 0.599$
	LDL Cholesterol	-0.011	0.989 (0.978 – 1.000)	$p = 0.048$
	Triglycerides	0.009	1.009 (1.002 – 1.016)	$p = 0.015$
	Glucose	0.022	1.023 (0.993 – 1.053)	$p = 0.129$
	Diastolic Blood Pressure	-0.003	0.997 (0.958 – 1.038)	$p = 0.894$
	Systolic Blood Pressure	-0.002	0.998 (0.967 – 1.031)	$p = 0.916$
	Abnormal GGT	0.53	1.700 (0.646 – 4.468)	$p = 0.282$
	Abnormal AST	-0.112	0.894 (0.206 – 3.881)	$p = 0.881$
	Abnormal ALT	0.103	1.108 (0.356 – 3.452)	$p = 0.860$
<i>Genetics</i>	AA/AC	0.689	1.991 (1.010 – 3.924)	$p = 0.047$
<i>Anthropometric</i>	BMI	-0.002	0.998 (0.932 – 1.069)	$p = 0.956$
<i>Demographics</i>	Age	0.104	1.110 (1.072 – 1.150)	$p < 0.0001$
	Female	0.518	1.679 (0.822 – 3.428)	$p = 0.155$
	African American	0.298	1.347 (0.218 – 8.329)	$p = 0.749$
	European American	-0.09	0.914 (0.170 – 4.908)	$p = 0.916$
	Below Average Income	-0.251	0.778 (0.341 – 1.775)	$p = 0.551$
	Above Average Income	-0.982	0.375 (0.175 – 0.801)	$p = 0.011$
	Education	-0.053	0.948 (0.848 – 1.059)	$p = 0.345$
<i>Behavioral</i>	Average Drinks	0.634	1.885 (1.636 – 2.172)	$p < 0.0001$
	Smoker	1.477	4.381 (2.001 – 9.590)	$p < 0.0001$

Table 4
Linear regression results for factors associated with the severity of the diagnosis in alcohol-dependent patients

CI = confidence interval. Adjusted $R^2 = .189$. HDL = high-density lipoprotein, LDL = high-density lipoprotein, GGT = gamma-glutamyl transferase, AST = aspartate transaminase, ALT = alanine transaminase, BMI = body mass index.

Factors	Variables	β coefficient (95% CI)	Significance
<i>Biochemical</i>	HDL Cholesterol	0.001 (−0.007 – 0.010)	$p = 0.748$
	LDL Cholesterol	−0.003 (−0.009 – 0.002)	$p = 0.275$
	Triglycerides	0.001 (−0.002 – 0.004)	$p = 0.540$
	Glucose	0.003 (−0.006 – 0.012)	$p = 0.531$
	Diastolic Blood Pressure	0.022 (−0.001 – 0.045)	$p = 0.059$
	Systolic Blood Pressure	−0.002 (−0.020 – 0.015)	$p = 0.790$
	Abnormal GGT	0.746 (0.270 – 1.222)	$p = 0.002$
	Abnormal AST	−0.109 (−0.767 – 0.548)	$p = 0.745$
	Abnormal ALT	0.530 (−0.087 – 1.147)	$p = 0.092$
<i>Genetics</i>	AA/AC	−0.318 (−0.738 – 0.092)	$p = 0.128$
<i>Anthropometric</i>	BMI	−0.030 (−0.072 – 0.012)	$p = 0.164$
<i>Demographics</i>	Age	0.014 (−0.006 – 0.034)	$p = 0.185$
	Female	0.874 (0.426 – 1.321)	$p < 0.0001$
	African American	−1.241 (−2.633 – 0.150)	$p = 0.080$
	European American	−0.048 (−1.445 – 1.348)	$p = 0.946$
	Below Average Income	−0.119 (−0.580 – 0.342)	$p = 0.612$
	Above Average Income	−0.353 (−0.897 – 0.181)	$p = 0.195$
	Education	−0.101 (−0.181 – −0.020)	$p = 0.014$
	Average Drinks	0.086 (0.015 – 0.227)	$p < 0.0001$
<i>Behavioral</i>	Smoker	0.331 (−0.094 – 0.755)	$p = 0.127$