Novel molecular targets for treating alcohol use disorder and hepatotoxicity

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Abstract

Novel treatments for alcohol use disorder (AUD) and alcohol-related liver disease (ALD) are greatly needed. We utilized a recently developed Bayesian approach, Integrative Risk Gene Selector (iRIGS), to identify high-confidence risk genes (HRGs) for alcohol consumption using SNPs from the largest alcohol consumption GWAS to date (N = 941,280). We subsequently used the Target Central Resource Database (TCRD) to search for drug-protein interactions for these HRGs and previously identified risk genes for alcohol consumption. We identified several HRGs for alcohol consumption that are novel contributions to the previously published alcohol consumption GWAS. Namely, *ACVR2A*, codes for activin receptor type-2A, which is critical for liver function, and *PRKCE*, codes for protein kinase C epsilon, which has been linked to alcohol consumption. Furthermore, several previously identified risk genes for alcohol consumption code for proteins that are implicated in liver function and are targeted by drugs that are promising candidates for managing hepatotoxicity (e.g., metformin), highlighting potential candidates for drug repurposing. This study demonstrates the value of incorporating regulatory information and drug-protein interaction data to highlight promising molecular targets and drugs for treating AUD and ALD.

Introduction

Improving the treatment of patients with alcohol use disorder (AUD) and alcohol-related liver disease (ALD) is of vital importance from a clinical and public health standpoint (1). Only three medications have been approved by the FDA to treat AUD – the last approval took place almost 15 years ago. No medications are approved for ALD. Targeting disease mechanisms with genetic support can increase success in drug development (2). However, translating genome-wide association studies (GWASs) of complex diseases to target discovery and medication development remains challenging (3). AUD is exemplary of this challenge; numerous large GWASs have yielded many significant SNPs (4, 5), yet limited drug targets for treating AUD and alcohol-related consequences, such as ALD, have been identified.

To address this gap and attempt to accelerate medication discovery, we took a 2-step approach. In the first step, we applied a modified version of the Integrative Risk Gene Selector (iRIGS) (6) to 98 genome-wide significant SNPs from the largest alcohol consumption GWAS to date (N = 941,280) (4). In this study, iRIGS incorporated genomic features including distance from gene to SNP and four sets of regulatory connections derived from distal regulatory elements-promoter links in order to compute high-confidence risk genes (HRGs) for alcohol consumption (i.e., the gene within 2 megabases (Mb) of each SNP that has the highest posterior probability based on these genetic features; see Methods for more detail).

In the second step, we searched for drug-protein interactions for these HRGs and other previously identified alcohol consumption genes (4) with the Target Central Resource Database (TCRD), classifying gene druggability based on the Target Development/Druggability Level (TDL) classification system (3). The TDL classification system, part of the National Institutes of Health Illuminating the Druggable Genome initiative (3), was used to classify the genes into

groups in descending order of the degree to which each protein has been studied: T_{clin}, T_{chem}, T_{bio} and T_{dark}. The 4 TDLs are described in detail in the Supplemental Methods, but briefly, T_{clin} are targets of approved drugs, T_{chem} have drug activities above cutoffs, T_{bio} do not have activities above cutoffs but are annotated, T_{dark} have virtually nothing known.

Results and Discussion

Integrative Risk Gene Selector (iRIGS). Of the 98 HRGs identified with iRIGS, 14 (14.1%) were the closest gene to the corresponding SNP. This is consistent with prior work finding many HRGs are not the most proximal to the SNP (6) and supports the importance of incorporating regulatory information into risk gene identification. 17 (17.3%) of the HRGs exhibited high posterior probability ($\geq$.75), indicating support from multiple genetic features including distance to SNP and regulatory information. The HRGs that were both closest to the index SNP and exhibited high posterior probability were *CDH11* (cadherin 11), *CADM2* (cell adhesion molecule 2), *TCF4* (transcription factor 4), *TENM2* (teneurin transmembrane protein 2), *SORL1* (sortilin related receptor 1), and *PDE4B* (phosphodiesterase 4B).

Of the 17 HRGs with high posterior probability, only 5 (29.4%) overlapped with the 307 genes identified in the prior alcohol consumption GWAS. This indicates that 12 unique high-probability genes were identified via iRIGS (Table 1). The 98 HRGs and all protein-coding genes within 2Mb of each of the 98 SNPs are in the Supplemental Materials (Table S1 and Table S2, respectively).

Target Development/Druggability Level (TDL). Applying the TDL classification system to the 98 HRGs found that 4 HRGs were T_{clin}, targeted by 14 drugs (Figure 1); 20 were T_{chem}; 71 were T_{bio}; and 3 were T_{dark}. Of the 17 HRGs with high posterior probability, *PDE4B* was T_{clin}, targeted by 8 approved drugs, and *ACVR2A* (activin receptor type-2A), *PRKCE* (protein kinase C epsilon), and *PPP3CA* (protein phosphatase 3 catalytic subunit alpha) were T_{chem} designation (i.e., small molecules bind to them with high potency). Notably, *ACVR2A*, *PRKCE*, *PPP3CA* were all unique genes not identified by the alcohol consumption GWAS (4). Details on the 17 HRGs are summarized in Table 1.

Of the 307 protein-coding genes identified in the alcohol consumption GWAS (4), 17 were T_{clin}, targeted by 104 unique drugs (65 of which target *DRD2*), 29 were T_{chem}, 198 were T_{bio}, and 63 were T_{dark}. 16 of the 17 T_{clin} genes were distinct from the HRGs identified (*PDE4B* overlapped). The 17 T_{clin} genes and their approved drugs are depicted in Figure 2. The TDLs for all 307 genes are provided in the Supplemental Materials (Table S3).

Promising molecular targets. This study identified several HRGs for alcohol consumption that are novel contributions to the previously published alcohol consumption GWAS and highlight putative targets for the treatment of AUD and/or ALD. In particular, *ACVR2A* codes for activin receptor type-2A, which has been linked to liver function (7), cocaine self-administration (8, 9), anxiety (10), and memory (11). With regard to liver function, one study using *in vitro* models found activin A, a ligand that binds with high affinity to activin type 2 receptors, is critical to normal liver function and suggested inhibition of activin A or its downstream signaling could be a new approach for treating liver disease (7). Furthermore, activin A serum levels have been found to be elevated in patients with ALD compared to patients with non-alcohol related liver disease at various stages including: alcoholic fatty liver disease vs. hepatitis C and primary biliary cholangitis (PBC) stage I-II; alcohol-related cirrhosis vs PBC stage IV and viral cirrhosis; and alcohol-related vs. viral hepatocellular carcinoma (12). Rodent studies have identified increases in activin type-2A mRNA and protein levels in the nucleus accumbens following withdrawal from extended-access cocaine self-administration and activin A levels in the nucleus accumbens after a cocaine binge following withdrawal (8, 9). Relatedly, two studies using transgenic mice expressing a dominant-negative activin receptor type-1B (also recruited in the activin A signaling pathway (13)) in forebrain neurons found they displayed

reduced alterations in GABAergic inhibition, exhibiting hypersensitivity to the sedating effects of alcohol (14) and low anxiety (15).

With regard to *PRKCE*, rodent studies have found a robust link between PRKCE expression and alcohol consumption phenotypes (16). *PRKCE* encodes for protein kinase C epsilon (PKCε) and ethanol exposure causes changes to PKCε expression and localization in various brain regions that are implicated in addiction leading to increased ethanol tolerance and consumption (for a review see (17)). Given evidence for the role of PKCε in AUD, a recent study tested several novel molecules that act as PKCε inhibitors. They found that two compounds inhibited PKCε with *K*i <20 nM, were highly selective of PKCε, crossed the blood-brain barrier, prevented alcohol-stimulated GABA release in the central amygdala, and reduced alcohol consumption in an intermittent 24-hour access, two-bottle choice drinking paradigm in wild-type but not in *Prkce*$^{-/-}$ mice (18).

PPP3CA codes for protein phosphatase 3 catalytic subunit alpha, also known as calcineurin, which has been found to be a key regulator of $GABA_A$ receptor synaptic retention and plasticity (19, 20) and linked to diazepam response *in vitro* (21) and *in vivo* (22) in rodents. Furthermore, *PPP3CA* has been linked to opioid dependence in a GWAS (23). Given $GABA_A$ receptors are a primary target responsible for the effects of alcohol (24, 25), calcineurin is likely linked with drinking via this mechanism.

The present study further supported the role of *PDE4B* as it was identified in the novel HRG analysis and via the methods in the prior alcohol GWAS (4). Additionally, it has been associated with multiple psychiatric phenotypes (e.g., tobacco use (4), schizophrenia (26)) in prior GWASs, and linked with alcohol preference in rodent studies (27, 28). Phosphodiesterase 4 (PDE4) inhibitors have been found to reduce alcohol self-administration, preference, relapse, and

acute functional tolerance in multiple rodent studies (27–33), have been found to be safe and tolerable in a Phase I trial in individuals with AUD (34), and a Phase II clinical trial is ongoing (NCT03489850).

Interactions with approved medications. This study's analysis of the 307 genes from the prior GWAS (4) using the TDL system highlighted 17 genes that code for proteins that are targeted by at least one approved medication (Figure 2). Many of the drugs are promising candidates for managing liver toxicity. Fomepizole (*ADH1A*, *ADH1B*, *ADH1C*) blocks alcohol dehydrogenase and is already approved to treat methanol and ethylene glycol toxicity (35). Metformin (*NDUFS3*) has been highlighted as a promising hepatoprotective agent including for ALD (36). Likewise, beraprost and misoprostol (*PTGER3*), have shown promise for managing acute liver injury and liver disease (37–39). Pentoxifylline has been often used to treat severe alcoholic hepatitis and a recent clinical practice update indicates that patients with a contraindication to glucocorticoids may be treated with pentoxifylline (40). However, recent data question its clinical utility, thus further research is needed (41). Iloprost (*PTGER3*) has shown promise for managing bone marrow oedema and early stages osteonecrosis, of which excessive alcohol use is a risk factor for (42).

Numerous genes and associated drugs were also identified for the Cytochromes P450 (CYPs) family of enzymes. The majority of the drugs interacted with CYP3A4, a major metabolic enzyme in the liver and intestine (43) that is implicated in the metabolism of numerous approved drugs including many antiretroviral drugs identified here (e.g., (44)). Nefazodone (*CYP3A4*), the only drug identified with an approved nervous system indication, is an atypical antidepressant. Three small studies of nefazodone have been conducted for AUD, with one finding no effect on drinking (45) and one of two studies in individuals with co-occurring major

depressive disorder and AUD finding support for it reducing drinking (46, 47). However, interest in this medication has waned given risk for severe liver toxicity in a minority of patients and an associated FDA black box warning in 2002 (48). Verapamil (*CYP3A4*) has been found to prevent cue-induced reinstatement of alcohol seeking in rats, suggesting it may be a promising compound for relapse prevention (49). Methyrapone (*CYP3A4*) has been found to prevent alcohol withdrawal associated working memory deficits and reestablish prefrontal cortex activity in withdrawn mice (50). In summary, the *CYP3A4* findings suggest the TCRD may be a novel method for not only identifying putative treatment targets, but also flagging potential deleterious drug-alcohol interactions. This latter aspect is of paramount importance in medication development for AUD, as it was also highlighted in recent guidelines from the FDA (51) and European Medicines Agency (52).

Conclusion. In summary, we have shown the value of incorporating regulatory information to and drug-protein interaction data to discover new promising molecular targets and identify preexisting drugs for potential repurposing. Our findings represent a step forward in understanding the genetic basis of alcohol consumption and putative candidates for novel drug discovery and drug repurposing to treat patients with AUD and ALD. Furthermore, we have demonstrated a genetic-driven method to identify potential harmful drug-alcohol interactions, an aspect of crucial importance in the medication development process. Finally, the methods utilized within this manuscript are readily available (https://github.com/CNPsyLab/Alcohol-Genetics-iRIGS-TCRD) and applicable to other complex traits for which there remains a gap between genome-wide discovery and the clarification of promising targets and drugs.

Methods

A detailed description of the TDL classification system is provided in the Supplemental Methods.

Statistics. For the first set of analyses (iRIGS), we utilized 98 genome-wide significant SNPs ($p < 5 \times 10^{-8}$) from the GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN) study (4) (one SNP, rs7074871, was excluded because it had no nearby protein-coding genes). We applied a modified version of iRIGS to the 98 SNPs. Briefly, iRIGS incorporates several genomic features (i.e., distance from gene to SNP and four sets of regulatory connections derived from distal regulatory elements-promoter links from Hi-C, capture Hi-C, and FANTOM5 data) to compute a posterior probability for genes within 2 megabases (Mb) of each SNP. The notable difference in our modified version of the iRIGS method is that we did not include de novo mutation enrichment or differential expression in the model, as the original study did, because the datasets used for the latter were specific to schizophrenia and there are no equivalent datasets to our knowledge for alcohol consumption. We defined high posterior probability to be $\geq$.75, indicating the gene is 75% likely to be related to the SNP (the probabilities of all genes within 2Mb a given SNP add up to 100%).

For the second set of analyses (Target Development/Druggability Level (TDL)), we incorporated genes identified in the analyses conducted in the prior GWAS (in addition to the HRGs we identified with iRIGS). The methods for identifying these alcohol consumption genes are discussed in detail in (4). Briefly, they defined a gene as implicated if it harbored variation of LD $r^2 > .3$ with a genome-wide significant SNP or if it was located within the 500kb of the SNP and was significant by the PASCAL gene-based test. This yielded 307 unique genes for our analyses.

***Study approval*.** This study was a secondary data analysis incorporating results from a prior GWAS (4) which obtained institutional review board approval and informed consent.

Supplemental Methods

Target Development/Druggability Level (TDL). In order to identify the overlap of HRGs and previously identified risk genes for alcohol consumption with preexisting drugs, we integrated drug-protein interaction information from the Target Central Resource Database (TCRD). The TCRD defines target development/druggability levels (TDLs) according to 4 levels of confidence in descending order: T_{clin}, targets have approved drug(s) with known mechanism(s) of action; T_{chem} targets have activities in ChEMBL or DrugCentral that satisfy activity thresholds or have been manually migrated by human curation based on data from other sources; T_{bio} targets have no known drug or small molecule activities that satisfy activity thresholds, but have Gene Ontology (GO) leaf term annotations, confirmed Online Mendelian Inheritance in Man (OMIM) phenotypes, or meet two of the three conditions: a fractional PubMed count > 5, > 3 National Center for Biotechnology (NCBI) Gene Reference Intro Function (RIF) annotations, or > 50 commercial antibodies; T_{dark} refers to proteins that have been manually curated at the primary sequence level in UniProt, but do not meet the criteria for the above categories.

Author contributions

JCG conceptualized the study, analyzed the data, and wrote the manuscript. MM analyzed the data and generated the figures. LL revised the manuscript.

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Table 1. Genetic and target development/druggability level (TDL) information for the high-probability (≥.75) high-confidence risk gene (HRGs)

SNP	HRG	Posterior probability	TDL	Nearest Gene	Overlap of HRG with Liu et al. (2019)
rs62044525	*CDH11*	1	T_{bio}	--	N
rs62250685	*CADM2*	1	T_{bio}	*CADM2*	Y
rs74664784	*CADM2*	1	T_{bio}	*CADM2*	Y
rs4916723	*MEF2C*	0.99	T_{bio}	--	N
rs9950000	*TCF4*	0.98	T_{bio}	*TCF4*	Y
rs10085696	*GALNT17*	0.98	T_{bio}	*AUTS2*	N
rs12907323	*AKAP13*	0.91	T_{bio}	*AGBL1*	N
rs10004020	*FBXW7*	0.90	T_{bio}	--	N
rs72859280	*ACVR2A*	0.88	T_{chem}	--	N
rs4699791	*PPP3CA*	0.87	T_{chem}	*EMCN*	N
rs4842786	*BTG1*	0.86	T_{bio}	--	N
rs10978550	*KLF4*	0.85	T_{bio}	--	N
rs11739827	*TENM2*	0.84	T_{bio}	*TENM2*	Y
rs1004787	*PRKCE*	0.83	T_{chem}	--	N
rs13024996	*ZEB2*	0.82	T_{bio}	*ARHGAP15*	N
rs13383034	*PRKCE*	0.81	T_{chem}	--	N
rs682011	*SORL1*	0.75	T_{bio}	--	N
rs12088813	*PDE4B*	0.75	T_{clin}	*PDE4B*	Y

Note. Nearest genes to each SNP were identified using SNPnexus (53); -- = non-protein coding gene (e.g., non-coding RNA). The 4 TDLs are described in the Supplemental Methods.

Fig 1.
Approved drugs that interact with proteins produced by the high-confidence risk genes (HRGs). Anatomical Therapeutic Chemical (ATC) Classification System codes are color coded as indicated in the key. Amlexanox has two ATC codes: (1) alimentary tract and metabolism and 2) respiratory system. Posterior probabilities for the HRGs are as follows: *PDE4B* (.75), *TPO* (.63), *ITGAL* (.09), and *CRHR1* (.30) (Table S1).

Figure 1.

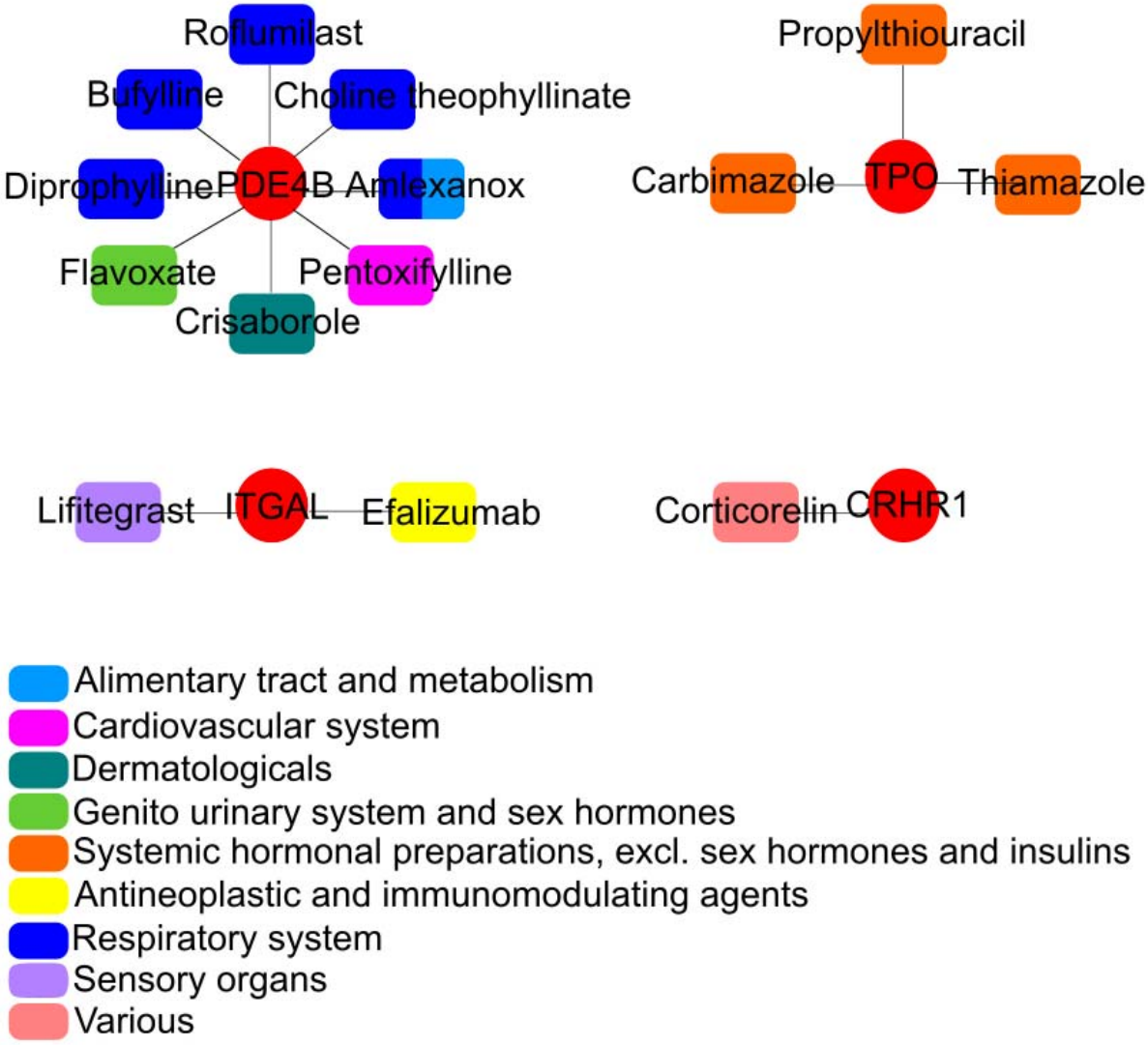

Fig 2.

Approved drugs that interact with proteins produced by the genes identified in Liu et al. (2019). Anatomical Therapeutic Chemical (ATC) Classification System codes are color coded as indicated in the key. Medications with multiple ATC codes are assigned all of the corresponding colors (e.g., miconazole).

Figure 2.