Review

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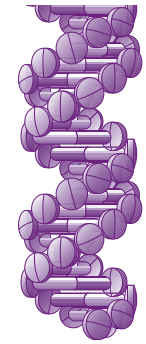
Pharmacogenomics



Promising pharmacogenetic targets for treating alcohol use disorder: evidence from preclinical models

Inherited genetic variants contribute to risk factors for developing an alcohol use disorder, and polymorphisms may inform precision medicine strategies for treating alcohol addiction. Targeting genetic mutations linked to alcohol phenotypes has provided promising initial evidence for reducing relapse rates in alcoholics. Although successful in some studies, there are conflicting findings and the reports of adverse effects may ultimately limit their clinical utility, suggesting that novel pharmacogenetic targets are necessary to advance precision medicine approaches. Here, we describe promising novel genetic variants derived from preclinical models of alcohol consumption and dependence that may uncover disease mechanisms that drive uncontrolled drinking and identify novel pharmacogenetic targets that facilitate therapeutic intervention for the treatment of alcohol use disorder.

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Alcohol use disorder (AUD) is a chronic neurological disorder characterized by an inability to regulate alcohol intake despite a host of serious and harmful health, societal and personal consequences [1]. The current pharmacotherapies available for AUD treatment have been met with variable outcomes that are modest at best and thus have not been widely embraced by the medical community. This gap in treatment options provides an opportunity to explore new avenues toward identifying novel pharmacotherapeutic targets. As with other addictive disorders, AUD is influenced, to a considerable degree, by genetics, with heritability estimates upward of 60% [2,3]. Despite the comparatively large influence of genetics, the exact etiology of AUD is by no means simple or straightforward, which underscores the difficulty in effective treatment of this disorder. In this review, we provide an evaluation of our understanding of the genetics of AUD in relation to current precision medicine approaches. Demonstrating the considerable heterogeneity that exists within the AUD population and understanding the contribution of genetic variation to treatment response provides a framework for promoting a pharmacogenetic approach. Moreover, understanding the genetic variation among individuals with AUD in treatment response will provide valuable information that will allow for personalized treatment plans.

A diagnosis of AUD is based strictly on a set of clinical diagnostic criteria, because as of yet, there are no reliable biomarkers to identify AUD or subpopulations that might be more or less responsive to certain medications. This means that, at best, we are treating AUD by trial and error on a, presumably, largely heterogeneous population. There are currently only three US FDA approved pharmacotherapies for treating AUD, naltrexone, acamprosate and disulfiram, all of

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which, however, have been met with limited success. This limited success is, in large part, attributable to the heterogeneity of the AUD patient population [4]. The interest in understanding the variable treatment responses in AUD patients has led to over a decade worth studies examining the pharmacogenetics of AUD (e.g., [5]). During this time, a number of SNPs have been identified and associate with various aspects of alcohol drinking and dependence. Because a number of recent reviews have detailed these SNPs [4,6–8], we will briefly highlight a few previously identified SNPs in the human AUD population with druggable targets and discuss how this has informed pharmacotherapeutic interventions. The remainder of the review will focus on newly identified pharmacogenetic targets from preclinical models that may provide novel treatment strategies for AUD. Finally, we incorporate transcriptome changes reported for these targets in postmortem brain tissue from alcoholics and provide initial supportive evidence for SNPs in these same genes that associate with alcohol consumption and dependence in humans.

Current precision medicine approaches for treating AUD

One of the earlier reported genetic variations associated with AUD was the $GABA_A$ receptor. Specifically, a decrease in the prevalence and frequency of the major allele (G1) of the *GABRB3* was associated with increased severity of alcoholism [9]. Subsequent to this, SNPs in genes coding for a number of additional $GABA_A$ receptor subunits were also found to be associated with alcohol dependence and subjective effects of alcohol, including: *GABRA1* [10], *GABRA2* [11–18], *GABRG1* [19,20], *GABRG3* [21]. There is a long history of GABAergic pharmacotherapies in treating AUD; unfortunately, drugs that target $GABA_A$ receptor subtypes have largely been abandoned due to the significant cross-tolerance with alcohol, the additive depressant effects on the CNS and the significant abuse potential. Thus, benzodiazepines have been relegated for use during the acute detoxification period [22].

In addition to the GABAergic receptor system, SNPs have been reported in numerous other neurotransmitter systems, producing a number of potential druggable targets, some with more promise than others. Polymorphisms in a number of cholinergic receptors have been implicated in the risk for AUD, associated with frequency of binge drinking, and subjective response to alcohol, including *CHRM2* [23,24], *CHRNA4* [25–27], *CHRNA5* [28], *CHRNB2* [26] and the *CHRNA5–CHRNA3–CHRNB4* cluster [29]. Recent advances in the treatment of nicotine dependence have given way to a number of potential pharmacotherapeutics to treat AUD. Mecamylamine, a nonselective nicotinic receptor antagonist, and varenicline, a partial agonist of the α4β2* nicotinic receptors (*indicates the possible presence of other nicotinic receptor subunits in the pentameric complex), have both shown potential for reducing alcohol consumption in individuals who smoke and have AUD [30–33], and recent evidence suggests that varenicline may be effective independent of smoking status [34]. Unfortunately, to our knowledge, none of the studies presently published have assessed efficacy by genotype interactions, information that could provide insight for enhanced treatment effectiveness in AUD subpopulations.

There have been several SNPs that show some potential in predicting pharmacotherapy treatment response. The most well known of these, perhaps, is the polymorphism in *OPRM1* gene. Several studies suggest that the adenine to guanine substitution at position 118 (A118G), which results in a likely loss of function and expression of the μ-opioid receptor in the *G* allele carriers [35,36], is predictive of naltrexone response in individuals with AUD [37–40]. Additionally, this predictive relationship is further supported by a similar relationship between the analogous SNP (C77G in the *OPRM1* gene) in rhesus macaques and alcohol consumption and naltrexone response [41]. As well, a reverse translational approach examining the A118G SNP in a humanized mouse model show similar enhancements in alcohol consumption and response to naltrexone in the mice homozygous for the *G* allele [42,43]. While encouraging, these studies are tempered by a number of conflicting reports showing inconsistencies or no predictive relationship between the A118G polymorphism and naltrexone response [44–47]. The relationship between the *OPRM1* gene and naltrexone response in individuals with AUD is further complicated by the possible influence of other concomitantly expressed SNPs, for example, *DAT1* [48–50].

A number of SNPs in the serotonergic system have also been associated with AUD, including *HTR1B* [51–53], *HTR2A* [54–57], and more recently, *HTR3A* and *HTR3B* [58] as well as *SLC6A4* [58,59]. In an initial study, genotypic combinations of polymorphisms in the *HTR3A*, *HTR3B* and *SLC6A4* genes predicted treatment outcome with the 5-HT3 antagonist, ondansetron, among alcohol-dependent individuals [60]. These results, while promising, should be taken with caution, as more data are needed to validate the predictive abilities of these genotypic variations.

And while genotypic variations can be useful in predicting responsiveness to pharmacotherapeutics interventions, they can also be used to predict likelihood of adverse effects of the pharmacological intervention which may limit its utility, as is the case with topiramate. Topiramate is an anticonvulsant that works through a number of different mechanisms, including

antagonism at AMPA and Kainate receptors, allosteric modulation of $GABA_A$ receptors, inhibition of voltage-gated Na^+ and Ca^{2+} channels, and enhancement of K^+ conductance. Topiramate has shown great promise as a potential pharmacotherapeutic in the treatment of AUD, reducing alcohol consumption and increasing abstinent periods [61,62] and reducing alcohol craving [63]. However, it is commonly associated adverse drug effects including, fatigue, sleepiness and nervousness, among others [64], thus as with many AUD treatments, overall response to treatment with topiramate varied. Thus, using a reverse pharmacogenetics approach, interrogation of SNPs in the genes encoding the Kainate receptor subunits (*GRIK1* and *GRIK2*), which topiramate most potently and selectively inhibits, was initiated to determine potential variants that might predict better treatment outcomes. An SNP in the *GRIK1* gene was found to be significantly associated with adverse effects of topiramate [65], which, in turn, moderates the therapeutic response in individuals with AUD [64,66].

These examples highlight the importance of examining the genotypic variation among individuals with AUD, but even with all this potential, we are still met with limited treatment efficacy and only minor progress over the course of decades of study. While the ultimate goal is to determine pharmacogenetic targets for use in treating the clinical population, identifying and understanding the influence of individual SNPs, alone and in combination, poses a number of difficulties and current studies vary in power, size, subject demographics, SNP examined and outcome. Additionally, these issues can be somewhat mitigated by utilizing preclinical models to facilitate a higher throughput strategy of identifying SNPs and testing potential druggable targets for the treatment of AUD prior to testing in clinical populations, rather than concurrently or *post hoc* to garner clarity. The remainder of this review will focus on several more recently identified genetic variations identified in preclinical models of AUD. Specifically, we will discuss promising preclinical evidence linking alcohol drinking with SNPs in genes encoding K^+ channels, neuroimmune signaling proteins, neurokinin receptors and the RAS superfamily of proteins (Table 1). In a number of the preclinical studies, there is evidence showing reductions in alcohol consumption when these targets are pharmacologically manipulated, providing initial validation that these genes are promising pharmacogenetic targets for treating AUD.

Preclinical pharamcogenetic targets for treating alcohol addiction

K^+ channels

Although topiramate appears quite effective at reducing alcohol craving and consumption in dependent individuals, its utility as a pharmacotherapeutic for AUD is tempered by a host of adverse side effects. Recent interest has been directed toward other well-tolerated anticonvulsants that target potassium (K^+) channels to potentially fill this gap in treatment options. Potassium channels represent one of the most diverse groups of ion channels with 79 genes encoding K^+ channel subunits (*KCN**), and functional channels formed in a monomeric or heteromeric fashion giving rise to many more functional channels than genes [67,68]. Potassium channels generally fall into one of four major categories based on their physiological, pharmacological and structural characteristics: voltage-gated, calcium-activated, inwardly rectifying and two-pore domain [68]. All K^+ channels are responsible, in some respect, for stabilizing the membrane potential, stimulating repolarization and regulating neuronal excitability (see Figure 1) [67,68]. While better known for their role in epileptic disorders [68,69], a number of clinical and preclinical studies have identified a potential role for genetic variants in K^+ channel genes as mediators of alcohol consumption and dependence [70–78].

Mutations in human *KCNQ2* and *KCNQ3*, which encode the $K_V7.2$ and $K_V7.3$ subunits of the voltage-gated K^+ channels responsible for M-current, result in reduced function of K_V7 channels and seizures associated with epilepsy suggesting that these channels could be key targets in regulating neuronal hyperexcitability associated with a host of disorders, including alcohol dependence [80]. Recent preclinical evidence showed that acute alcohol directly inhibits K_V7 channel activity resulting in reduced M-current and a corresponding increase in the firing rate of hippocampal pyramidal neurons and dopaminergic VTA neurons [81,82]. In addition, a recent study reported that 10 mM alcohol blocked *Drosophila KCNQ* currents and flies with *KCNQ* loss-of-function in dopaminergic neurons showed increased tolerance and sensitivity to the sedating effects of alcohol exposure [83]. Interestingly, several studies have implicated the *Kcnq* family of genes in preclinical rodent alcohol drinking models. *Kcnq2* expression was increased in the ventral striatum of mice selectively bred for high alcohol consumption/low withdrawal severity compared with those bred for low alcohol consumption/high withdrawal severity (Table 2) [84]. Additionally, these authors found that *Kcnq2* was a positional candidate within the *cis*-eQTL for alcohol consumption and withdrawal. *Kcnq2* and *Kcnq3* were reported to lie within multiple alcohol-related QTLs and two candidate SNPs in *Kcnq2* were identified that differentiated between high drinking and low drinking mouse strains [74]. Another study examining alcohol consumption in the BXD recombinant inbred strains of mice showed that dif-

Table 1. Genes with polymorphisms related to alcohol consumption, dependence and other alcohol-related behaviors.

Gene	Protein	Function	SNP	PMID
RAS signaling family				
RASGRF2	Ras-specific guanine nucleotide-releasing factor 2	Ras and RAC1 activator	rs26907	21471458
NF1	Neurofibromin	Negative regulator of Ras GAP	Numerous	25483400
KALRN	Kalirin	Rho GTPase activator	rs6438839, rs4634050	27092175
RSU1	Ras suppressor protein 1	Rac1 suppressor	Numerous	26170296
Rab42	Ras-related protein Rab-42	Putative Ras activator	NR	25833023
Rap1gap	Rap1 GTPase-activating protein 1	Rap1 suppressor	NR	25833023
PPAR family				
PPARA	PPAR-α	Nuclear receptor that binds peroxisome proliferators	NR	25516156
PPARG	PPAR-γ	Nuclear receptor that binds peroxisome proliferators	NR	25516156
PPARGC1A	PPAR-γ coactivator 1-α	Transcriptional coactivator	NR	25516156
K^+ channel family				
KCNB2	$K_V2.2$	Voltage-gated delayed-rectifier	rs2128158, rs2929567 & *cis*-eQTL	23953852
KCND2	$K_V4.2$	Voltage-gated A-type	rs728115, rs17142876	21956439, 22488850, 25603899
KCNMA1	$K_{Ca}1.1$	Calcium- and voltage-activated, Large conductance	rs717207, rs12219105	21314694, 20201924
KCNQ1	$K_V7.1$	Voltage-gated, M-current delayed rectifier	rs12574151	20201924
KCNQ5	$K_V7.5$	Voltage-gated, M-current delayed rectifier	rs3799285	21314694
Kcnq2	$K_V7.2$	Voltage-gated, M-current delayed rectifier	rs27642425, rs2971971 & *cis*-QTL	26104325, 25581648
Kcnj10	$K_{IR}4.1$	Inwardly rectifying	rs46006714	19053975
Neurokinin family				
TACR1	NK_1R	G_q-GPCR activated by substance P	rs6715729–rs735668–rs6741029	19553914
Tacr1	NK_1R	G_q-GPCR activated by substance P	NR	23419547

NR: Not reported; PMID: PubMed identification number.

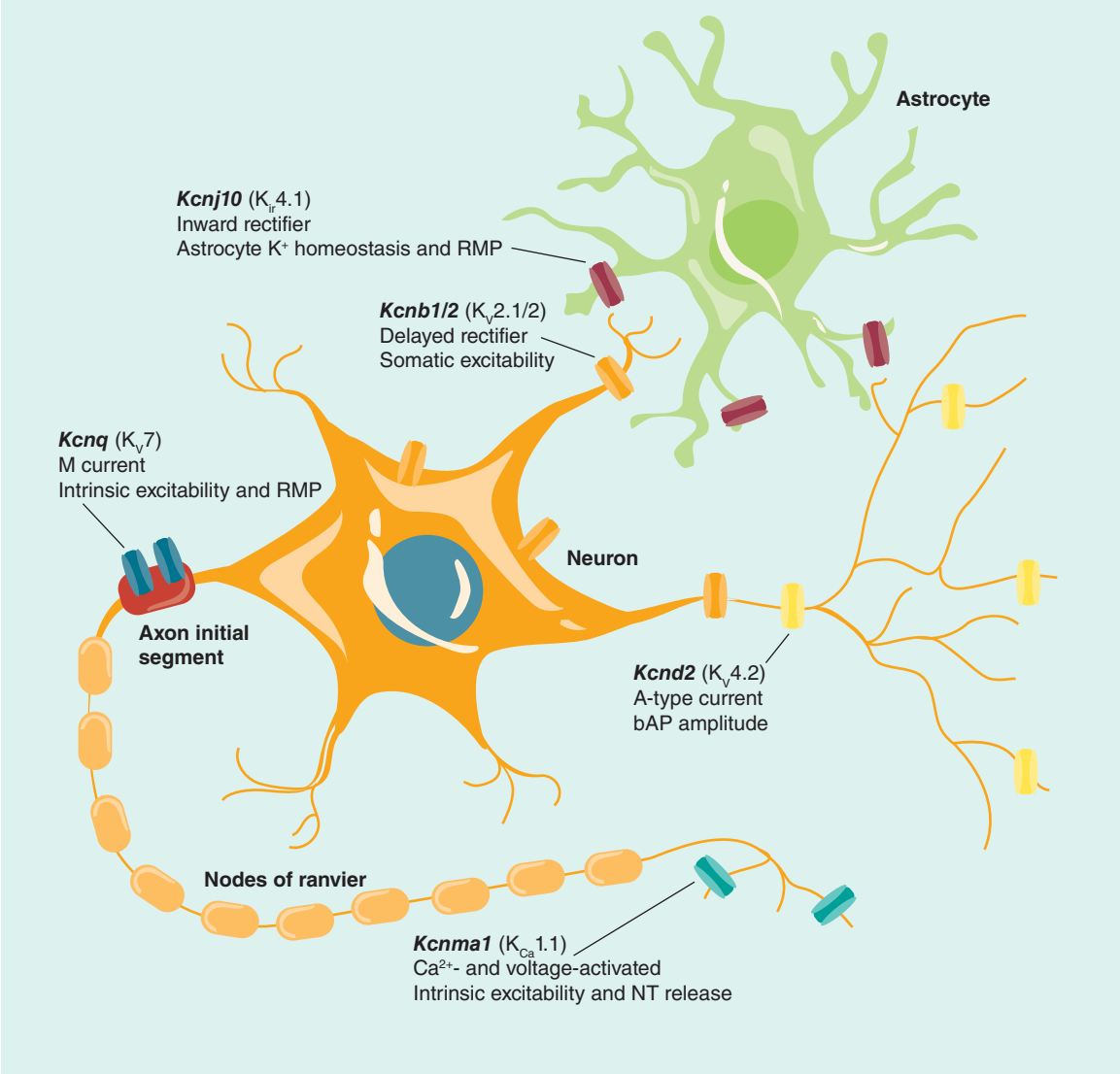

Figure 1. Schematic identifying prominent subcellular localization of K$^+$ channels in neurons and astroglia with SNPs and correlations related to alcohol consumption.
bAP: Back-propagating action potential; NT: Neurotransmitter; RMP: Resting membrane potential.
Images were acquired with permission from [79] and subsequently modified.

ferential expression of *Kcnq5* across strains negatively correlated with drinking, further implicating this family of K$^+$ channels in regulating alcohol drinking [76]. Using whole genome sequencing, a recent study reported that *Kcnq5* was differentially expressed between low and high alcohol drinking rat lines [85], and multiple reports identify transcript changes in the *KCNQ* family of genes in alcoholic brain and preclinical models (Table 2). It should be noted that transcriptome changes in expression of *KCNQ* and other alcohol-sensitive K$^+$ channel genes are not reported consistently in preclinical and human postmortem studies [86–88]. However, in support of the evidence linking *KCNQ* and alcoholism, SNPs associated with human alcohol consumption have also been reported in *KCNQ1* and *KCNQ5* [89,90]. To test the viability of the *Kcnq* family of genes as a pharmacogenetics target, several preclinical studies have demonstrated that systemic administration of the K$_V$7 channel opener, retigabine, significantly reduces alcohol consumption in both rats and mice [74,76,91], and is most efficacious in those that display a heavy drinking phenotype [74,76]. What is particularly encouraging about these studies is that retigabine is already US FDA approved to treat epileptic disorders and is well-tolerated in the clinical population, and while there is some evidence that alcohol alters pharmacokinetics of retigabine in moderate drinkers, it did not alter the pharmacodynamics measures or the adverse effects of retigabine [92]. Additionally, retigabine shows minimal off

Table 2. K^+ channel genes that are altered by chronic alcohol drinking or alcohol dependence in brain from human alcoholics or animal models.

Gene	Species	Diagnosis or preclinical model	Region	Direction of change	PubMed ID
KCNB1	Human	Alcohol abusers	Hippocampus	↑	26041984
Kcnb1	Mouse	Chronic intermittent alcohol in BXD recombinant inbred strains	Prefrontal cortex, nucleus accumbens	NR	27838001, 27432260
KCNB1/2	Human	DSM-IV for alcohol abuse	Superior frontal gyrus	NR	25450227
KCND2	Human	DSM-IV for alcohol abuse	Amygdala, frontal cortex	↓	22302827
Kcnd2	Mouse	Chronic intermittent alcohol in BXD recombinant inbred strains	Prefrontal cortex, nucleus accumbens	NR	27838001, 27432260
KCNJ10	Human	Alcoholics (>80 g alcohol/day)	Superior frontal cortex	↓	11141048
KCNJ10	Human	Alcoholics (>0.50 g alcohol/day)	Hippocampus	↓	23981442
KCNMA1	Human	DSM-IV for alcohol abuse	Frontal cortex	↓	22302827
Kcnma1	Mouse	Chronic intermittent alcohol in BXD recombinant inbred strains	Prefrontal cortex, nucleus accumbens	NR	27838001, 27432260
KCNQ2	Human	Alcoholics (>100 g alcohol/day)	Amygdala	↑	20153402
Kcnq2	Mouse	Continuous alcohol access	Amygdala (synaptoneurosomes)	↓	25135349
Kcnq2	Mouse	Chronic intermittent alcohol	Prefrontal cortex	↑	25803291
Kcnq2	Mouse	High drinking/low withdrawal and low drinking/high withdrawal mouse lines	Ventral striatum	NR	25581648
Kcnq2	Mouse	High and low alcohol drinking mouse strains	Whole brain	↑	16618939
KCNQ2/3	Human	DSM-IV for alcohol abuse	Frontal cortex	↑	22302827
Kcnq3	Rat	Continuous alcohol access & alcohol self-administration	Nucleus accumbens core	↓	19666046, 18405950
Kcnq5	Mouse	Chronic intermittent alcohol in BXD recombinant inbred strains	Prefrontal cortex	NR	27838001, 27432260
Kcnq5	Rat	Continuous alcohol access	Dorsal hippocampus	↑	12462420
Kcnq5	Rat	High and low alcohol drinking rat lines	Whole genome	NR	27490364

NR: Not reported; PMID: PubMed identification number.

target effects in rodents at doses that alter alcohol consumption, giving K_V7 channels substantial potential as a pharmacogenetic target for treating AUD.

In addition to *Kcnq* genes, a number of other K^+ channel SNPs (Table 1) and transcriptome changes (Table 2) in human alcoholics and preclinical models have been reported that present unique opportunities for the development of small molecules to target these channels. One such target is the inwardly rectifying K^+ channel 4.1 protein ($K_{IR}4.1$), encoded by the *Kcnj10* gene. $K_{IR}4.1$ is largely expressed on glial cells and is involved in K^+ buffering action of astrocytes [93], and mutations in *Kcnj10* result in increased seizure susceptibility [94,95]. The genomic location of *Kcnj10* also maps onto QTLs for both alcohol preference [96] and withdrawal [97,98], making *Kcnj10* an attractive candidate gene. Interestingly, C57BL/6J mice show a downregulation in brain levels of *Kcnj10* transcript in comparison with DBA/2J mice [99], and DBA/2J mice express a SNP in *Kcnj10* (C → G), which generates a missense mutation in $K_{IR}4.1$, resulting in greater susceptibility to seizures. In an elegant demonstration, Zou and colleagues showed that when C allele carriers (*C57BL/6J* allele) were crossed with G allele carriers (*DBA/2J* allele), the F2 offspring showed a genotype-dependent preference for alcohol, with animals carrying the C allele having the highest preference [99]. In support of these findings, analogous SNPs in *KCNJ10* have been reported to alter seizure activity in humans [100], and *KCNJ10* transcripts are reduced in postmortem brain tissue from the superior frontal cortex of human alcoholics [101]. Progress is limited by the lack of pharmacological tools to manipulate $K_{IR}4.1$, but these results are encouraging and represents a promising opportunity for drug development.

A number of other voltage-gated K^+ channel genes have been associated with AUD as genome wide association studies (GWAS) become more prevalent. The gene encoding the $K_V2.2$ channel protein, *KCNB2*, was found in a *cis*-eQTL for alcohol consumption, and two SNPs in the *KCNB2* gene are associated with maximum number of drinks consumed in 24 h [102]. Additionally, transcript levels of the gene encoding the $K_V2.1$ channel protein, *KCNB1*, was increased in hippocampus from alcoholic postmortem brain [103], and expression of both *KCNB1* and *KCNB2* in the superior frontal gyrus were strongly associated with lifetime drinking in alcoholics [78]. In a preclinical model, expression of *Kcnb1* in the nucleus accumbens correlated negatively with alcohol intake in BXD mice [76], indicating a potential role of the *KCNB* family in regulating alcohol consumption across species. Another voltage-gated K^+ channel identified through GWAS exploration was *KCND2* that encodes for the $K_V4.2$ channel that regulate rapidly inactivating A-type K^+ current (I_A). A SNP in *KCND2* was found in the top ten SNPs associated with alcohol and nicotine dependence and alcohol dependence alone [104–106]. And finally, an SNP in the *KCNMA1* gene, which encodes the α1 subunit of $K_{Ca}1.1$ was significantly associated with the development of alcohol dependence [89,90] and subjective response to alcohol [107]. These associations are supported by experimental evidence showing a negative correlation between *Kcnma1* and alcohol consumption [76] and a role for this gene in the development of tolerance [108–110]. Given that many of these K^+ channel genes are altered in postmortem brain tissue from alcoholics (Table 2) [78,103,111], further preclinical research into the potential involvement of $K_V2.1/2$, $K_V4.1$ and $K_{Ca}1.1$ in regulating alcohol consumption is warranted, but will require better pharmacological tools.

Ras signaling

The RAS superfamily of GTPase signaling proteins function as binary molecular switches by cycling between active GTP-bound and inactive GDP-bound states to modulate a diverse range of intracellular processes. In the mature brain, RAS GTPases control synaptic and morphological plasticity that are important for memory formation [112] and, as described below, are implicated in alcohol addiction. Indeed, converging evidence has demonstrated that SNPs in RAS signaling proteins are prominent in preclinical and clinical genetic studies on alcohol drinking and alcohol dependence (Figure 2). An elegant cross-species study linked *Rsu1*/β-integrin/Rac1 signaling with actin cycling and alcohol consumption in *Drosophila* [113]. These authors also reported that polymorphisms in *RSU1* are associated with alcohol dependence in adults and increased NAc activation during reward processing in adolescents. *Kras*$^{+/-}$ mice do not escalate their drinking following induction of alcohol dependence [114], and knockout of *Nf1*, a negative regulator of Ras, does not affect drinking in multiple models of heavy drinking but prevents escalation of intake in alcohol-dependent mice [115]. SNPs in *NF1* associate with alcohol dependence and alcoholism severity in African and European–Americans [115], whereas *Rab42* (putative Ras activator) and *Rap1gap* (a negative regulator of Rap-1A) SNPs associate with acute functional tolerance in LXS recombinant inbred strains of mice [116]. Interestingly, a GWAS meta-analysis reported that an SNP in *RASGRF2* (rs26907), another Ras activator, was associated with alcohol intake [117]. A comprehensive follow-up functional characterization study targeting Ras–GRF2 demonstrated reduced alcohol consumption and preference and attenuated alcohol-induced dopamine release in ventral striatum in mice with genetic depletion of the *Rasgrf2* gene [118]. Knockdown of *Rasgrf2* reduced intrinsic excitability of VTA dopamine neurons likely mediated by a change I_A. Interestingly, K_V4 channels control I_A current amplitude and firing of dopamine neurons [119], and, as discussed above, SNPs in genes that encode K_V4 channels associate risk for developing alcohol and nicotine co-dependence [105,106].

Impaired impulsivity and altered brain responsivity in areas responsible for executive functioning and reward processing are risk factors for developing an AUD [120,121]. A recent preclinical study examined expression patterns of cortical genes and impulsive behavior in BXD strains of mice [122]. PFC transcript levels of *Kalrn*, a gene encoding the Rho activating GTPase kalirin, significantly correlated with premature responding in BXDs tested on the 5-choice serial reaction time task. Of the human homologs of genes that correlated with premature responding in mice, SNPs in *KALRN* were associated with enhanced activation of the ventral striatum during anticipation of rewards and an increased frequency of binge drinking in adolescents [122]. The basolateral amygdala sends excitatory projections to the PFC, and a recent paper reported reduced *KALRN* expression in the basolateral amygdala from human alcoholic postmortem tissue [111]. An exploratory bioinformatics study identified Di-Ras2, a Rho activator, as a key synaptic protein that discriminated between low and heavy drinking cynomolgus macaques [123]. In addition, PFC expression levels of *Diras2* negatively correlated with drinking in BXDs, and *DIRAS2* is found within multiple published QTLs for alcohol drinking and dependence in human and rodent studies [123]. Similar to *KALRN*, *DIRAS2* expression is significantly reduced in the

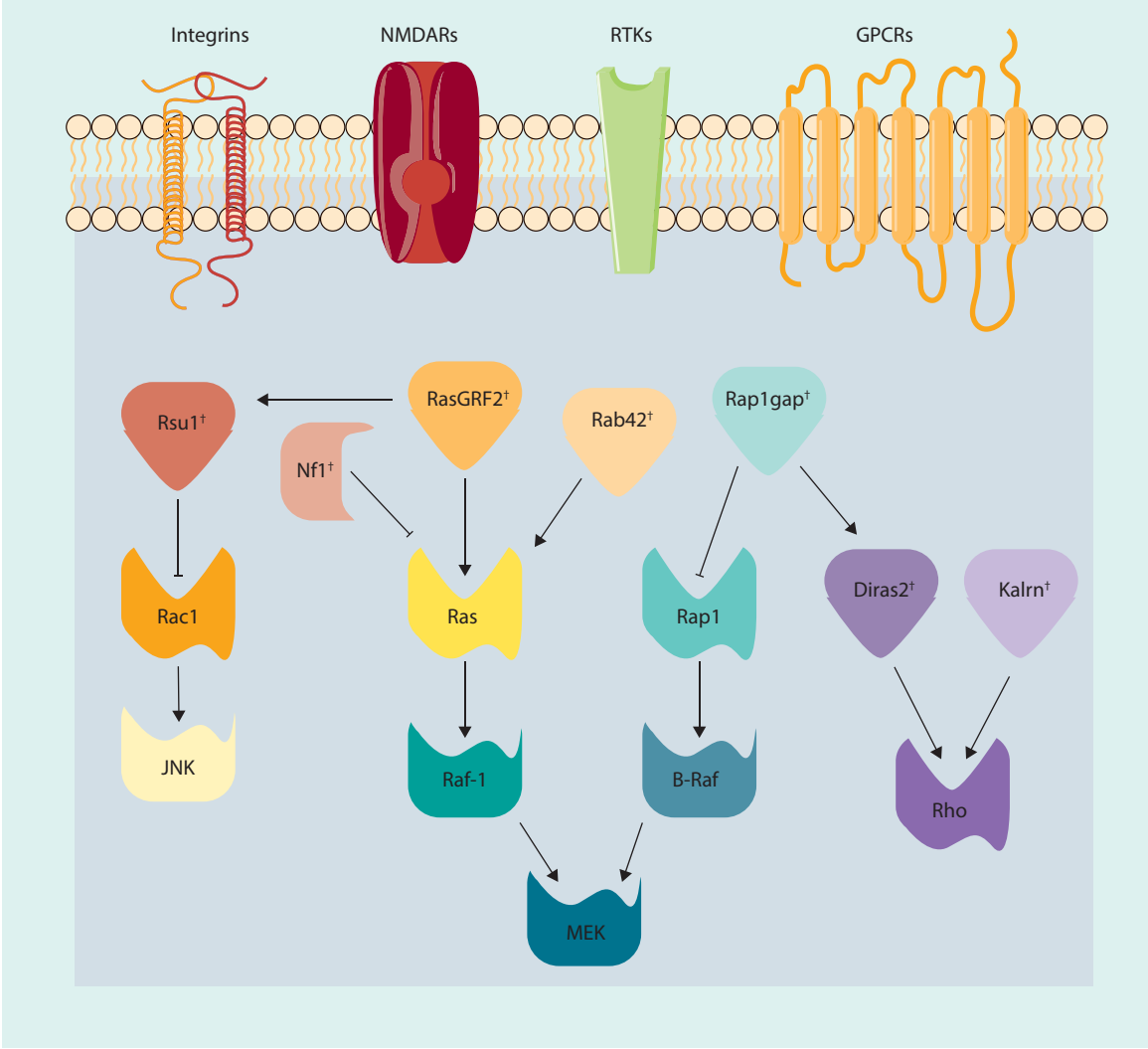

Figure 2. SNPs in RAS superfamily of GTP hydrolysis-coupled signal transduction relay proteins related to alcohol intake and alcohol-related behaviors. Plasma-membrane bound integrins, NMDARs, RTKs, and GPCRs can provide an activation trigger for RAS signal transduction. Functional SNPs in proteins in the Ras, Rac, and Rho pathways may impact cellular proliferation and adhesion, membrane trafficking, and cytoskeletal morphology.
†RAS superfamily-related proteins with SNPs or genetic variations correlated with alcohol intake/behaviors.
GPCR: G-protein coupled receptor; NMDAR: NMDA-type glutamate receptor; RTK: Receptor tyrosine-kinase.
Images were acquired from [79] and subsequently modified.

basolateral amygdala of alcoholics [111]. Importantly, data from Ron and colleagues showing that pharmacological inhibition of H- and K-Ras signaling with the farnesyltransferase, FTI-276, in the nucleus accumbens reduces voluntary alcohol drinking and alcohol self-administration in rats [124] validates the RAS superfamily as pharmacogenetic targets for treatment of AUD. The high rate of K-Ras mutations in multiple forms of cancer has led to the development of anticancer drugs that target the RAS signaling system that are now under clinical trials [125]. Progress in anticancer drug development may identify novel pharmacogenetic therapies that could be tested in treatment-seeking alcoholics with SNPs in RAS genes.

Neurokinin receptor 1

The tachykinins are a family of neuromodulatory peptides, including substance P (SP), neurokinin-A and neurokinin-B, which bind with varying affinities to the family of neurokinin receptors, NK_1R, NK_2R or NK_3R [126]. For the purposes of this review, emphasis will be placed on the NK_1R and its preferential endogenous ligand, SP, due to its recently discovered role in drug addiction. Activation of NK_1R, a Gq-coupled GPCR, by SP results in a host of downstream consequences, including: endocrine secretion, transmission of pain signals, vasodilation and neuroimmune signaling [127], and more recently modulating stress and anxiety [128]. NK_1R are located throughout

the brain, including regions involved in mediating the reinforcing properties of alcohol, like the ventral tegmental area [129], ventral striatum [130–132] and extended amygdala [133,134]. Preclinical data implicate a role for NK_1R signaling in opiate reward and reinforcement [133,135–138]. Given the known overlap in mechanisms between opiates and alcohol [139], it is not surprising that more recently NK_1R have also been implicated in modulating alcohol consumption and reward [140–142]. Additionally, the NK_1R seems to be particularly involved in the stress-related aspects of alcohol consumption, as NK_1R antagonism reduced stress-induced, but not cue-induced, reinstatement of alcohol seeking [143,144] and escalation of alcohol consumption [140].

Interestingly, it has recently been shown that the alcohol-preferring (P) rats, which are selectively bred for high alcohol preference, display increased sensitivity to the effects of NK_1R antagonism on alcohol self-administration and motivation to work for alcohol compared with their Wistar parent strain [145]. What is more intriguing about the genetically selected P rats is that they show an upregulated NK_1R system, that is, increased transcript levels of *Tacr1* and *Tac1* [145]. Furthermore, Schank and colleagues also showed the presence of a G–C SNP in the transcriptional start sequence for *Tacr1* and an increased transcription factor binding in the presence of the C allele, indicative of increased transcriptional activation of *Tacr1*. The importance of this finding is underscored by the fact that 100% of the P rats expressed the C allele, whereas only 18% of the Wistar controls did, suggesting that the SNP in *Tacr1*, and thereby signaling at the NK_1R, contributes to a predisposition toward alcohol preference, and predicts the response to drugs that target NK_1R. These findings are in line with the human literature that has found several SNPs in the *TACR1* gene that are associated with a risk for alcohol dependence [146]. Additionally, there is some evidence that NK_1R antagonists, like aprepitant which is US FDA approved for the treatment of nausea and emesis, can be utilized off-label for the treatment of stress, anxiety and depression [147–149] as well as reducing alcohol craving and stress-induced craving [141]. Together with the preclinical evidence suggesting that a SNP in the *Tacr1* gene can result in increased alcohol consumption and increased responsiveness to NK_1R antagonism, these studies strongly support increased efforts in identifying the functional consequences of polymorphisms in *Tacr1* and *Tac1* on a molecular and cellular level, as well as clinical studies incorporating prospective genotyping to determine explicitly the relationship between genotypic variation and efficacy of NK_1R inhibition.

Neuroimmune signaling

Preclinical studies provide emerging evidence that neuroimmune genes are promising targets for treating AUD [150]. Meta-analyses of gene expression studies in alcohol preferring strains of mice and postmortem tissue from human alcoholics revealed dysregulation of neuroimmune and neuroinflammatory signaling pathways [151]. Null mutant mice for six of these dysregulated immune candidate genes (*B2m*, *Ctsf*, *Ctss*, *Il1rn*, *Cd14* and *Il6*) showed reduced voluntary alcohol intake and preference [151]. The findings from this study validate the use of genomic analysis for identifying functional groups of genes that regulate alcohol consumption and, in doing so, indicated a novel role for neuroimmune signaling in controlling drinking. PPARs are nuclear proteins that regulate gene expression and control neuroimmune responses and inflammation [152]. Expression of *PPARD* (PPARδ subunit) and *PPARGC1A* (PPAR-γ coactivator) are altered in the basolateral and central amygdala of alcoholics [111], and PPAR agonists show promise in preclinical models of alcohol drinking, stress-induced relapse and withdrawal [153–157]. Because of the strong preclinical pharmacology and genetic data linking PPAR signaling with alcohol intake, a clinical trial is currently underway to determine if the PPAR agonist fenofibrate will decrease craving for alcohol following cue-exposure and reduce the number of drinks consumed in alcohol-dependent subjects (ClinicalTrial.gov trial number: NCT02158273). A recent study demonstrated that SNPs in *PPARG* and *PPARGC1A* were associated with alcohol dependence in European–American alcoholics [155]. Moreover, these authors also reported that *PPARA* and *PPARG* were associated with a DSM-IV criterion for an alcohol withdrawal phenotype. Future studies are necessary to determine if polymorphisms in *PPARG* and *PPARGC1A* moderate behavioral responses to treatment with PPAR agonists in alcoholics.

Future perspective

In this review, we have demonstrated that the complex nature of the genetic basis of AUD can influence precision medicine approaches for treating alcohol addiction. The diverse, heterogeneous population of individuals diagnosed with AUD makes determining pharmacogenetic targets from human GWAS difficult in that the inherently qualitative nature of defining the AUD phenotype could lead to false positives within a study or the masking of relevant results due to influence and interaction of, and often times lack of control for, other factors such as comorbid disease states (e.g., impulsivity, anxiety, depression, co-dependence on other drugs of abuse, among others). Addition-

ally, the stringent nature of GWAS in general means that, unless GWAS sample sizes are increased significantly, SNPs that may be related to the disease state could be masked in an effort to avoid false positives. In some studies, pharmacogenetic targets previously identified through clinical studies have failed to reveal viable targets and resulted in inconsistent success in predicting treatment response. In an effort to gain a better understanding and produce higher throughput results, we highlight a number of translational findings from rodent genetic analyses and preclinical pharmacology studies where there is supporting genetic evidence in clinical studies. Although these SNPs do not always achieve genome wide significance for alcohol use disorder [158], compelling preclinical evidence identified potential candidate genes and polymorphisms to pharmacologically target in future clinical investigations.

How can the addiction biology field use these preclinical findings to advance precision medicine approaches for the treatment of AUD? First, additional preclinical studies are necessary to determine what, if any, functional adaptations are produced by the SNPs in genes linked to alcohol drinking in rodents. In doing so, this functional characterization may elucidate the neurobiological impact of these genetic variants on alcohol dependence and heavy alcohol drinking. Targeted studies to validate these SNPs in human alcoholics and identification of suitable drugs with US FDA-approval will be crucial prior to embarking on large-scale clinical trials in treatment-seeking alcoholics. As discussed in this review, there are a number of promising preclinical candidate targets raising the question of how to prioritize future preclinical and clinical studies. While it is clear that preclinical models of alcohol consumption provide us with a means to determine functionally relevant SNPs and genetic targets, an additional challenge for future studies will be for medicinal chemists to develop small molecules that preferentially target proteins with these polymorphisms to reverse any gain or loss of function. Regardless of these practical and theoretical challenges, preclinical genetic variation studies will inform precision medicine approaches that will advance treatment of AUD. Further validation of these candidate SNPs is necessary in preclinical models and clinical studies.

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Executive summary

Precision medicine & alcohol use disorder

- While there are promising initial clinical studies demonstrating the utility of precision medicine approaches for treating alcohol use disorder (AUD), there are conflicting findings and reports of adverse side effects that suggest novel pharmacogenetic targets are necessary.

Potassium channels

- There are mutations in *KCNQ*, *KCNJ10*, *KCNB*, *KCND2* and *KCNMA1* that associate with heavy alcohol drinking and alcohol dependence in preclinical and clinical studies.
- Preclinical pharmacological evidence demonstrating the ability to reduce alcohol drinking by targeting these K^+ channels supports future pharmacogenetic studies for treating alcohol addiction.

RAS signaling

- Many genes and SNPs in the RAS superfamily signaling pathways are implicated in alcohol intake and dependence in rodents and humans.
- Pharmacologically inhibiting RAS signaling reduces drinking in rodents thus initially validating the RAS superfamily as pharmacogenetic targets for the treatment of AUD.

Tachykinins

- Neurokinin 1 receptors are particularly involved in the stress-related aspects of alcohol consumption.
- SNPs in the *Tacr1* gene contribute to a predisposition toward alcohol preference in rodents and associate with a risk for alcohol dependence in humans.

Neuroimmune signaling

- Neuroimmune genes are promising targets for treating AUD.
- Expression of peroxisome proliferator-activated receptor genes is altered in preclinical models of alcohol addiction and SNPs in this gene family associate with alcohol dependence and an alcohol withdrawal phenotype.

Conclusion

- Preclinical genetic variation studies provide strong support for future precision medicine approaches that will advance treatment of AUD.

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