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Identification and validation of midbrain *Kcnq4* regulation of heavy alcohol consumption in rodents

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ABSTRACT

Currently available pharmacotherapies for treating alcohol use disorder (AUD) suffer from deleterious side effects and are not efficacious in diverse populations. Clinical and preclinical studies provide evidence that the *Kcnq* family of genes that encode K_V7 channels influence alcohol intake and dependence. K_V7 channels are a class of slowly activating voltage-dependent K^+ channels that regulate neuronal excitability. Studies indicate that the K_V7 channel positive modulator retigabine can decrease dopaminergic neuron firing, alter dopamine (DA) release, and reduce alcohol intake in heavy drinking rodents. Given the critical nature of ventral tegmental area (VTA) DA to the addiction process and predominant expression of *Kcnq4* in DA neurons, we investigated the role of midbrain *Kcnq* genes and K_V7 channels in the VTA of genetically diverse mice and long-term heavy drinking rats, respectively. Integrative bioinformatics analysis identified negative correlations between midbrain *Kcnq4* expression and alcohol intake and seeking behaviors. *Kcnq4* expression levels were also correlated with dopaminergic-related phenotypes in BXD strains, and *Kcnq4* was present in support intervals for alcohol sensitivity and alcohol withdrawal severity QTLs in rodents. Pharmacological validation studies revealed that VTA K_V7 channels regulate excessive alcohol intake in rats with a high-drinking phenotype. Administration of a novel and selective $K_V7.2/4$ channel positive modulator also reduced alcohol drinking in rats. Together, these findings indicate that midbrain *Kcnq4* expression regulates alcohol-related behaviors in genetically diverse mice and provide evidence that $K_V7.4$ channels are a critical mediator of excessive alcohol drinking.

1. Introduction

Current pharmacotherapies for the treatment of alcohol use disorder (AUD) suffer from deleterious side effects and prevent relapse in only a small subset of individuals. Anticonvulsants are a drug class that show promise for treating heavy alcohol (ethanol) drinking and severe alcohol withdrawal associated with AUD (Padula et al., 2013). Emerging evidence indicates that the anticonvulsant retigabine can reduce voluntary alcohol consumption in rodents, especially those with a high-drinking phenotype (McGuier et al., 2016; Rinker et al., 2017). Retigabine is unique among the anticonvulsant class in that it increases the open probability of K_V7 channels, which are encoded by the *KCNQ* family of genes and are responsible for generating the M-current in neurons (Blackburn-Munro et al., 2005). In *Drosophila*, loss of K_V7 channel function increased tolerance and sensitivity to alcohol's sedative effects (Cavaliere et al., 2012). In addition, genetic variance in *KCNQ* is associated with alcohol intake in rodents and alcohol dependence in individuals with AUD (Edenberg et al., 2010; Kendler et al., 2011; McGuier et al., 2016; Metten et al., 2014; Rinker et al., 2017). Acute alcohol exposure has been shown to reduce M-current in dopamine (DA) neurons in the ventral tegmental area (VTA) (Koyama et al., 2007), and chronic alcohol exposure alters surface trafficking and reduces function of K_V7 channels in the nucleus accumbens (NAc) and lateral habenula, respectively (Kang et al., 2017; McGuier et al., 2016). Thus, this converging evidence supports variations in K_V7 channels as a mechanism promoting heavy alcohol drinking and

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Table 1
Correlations of midbrain *Kcnq4* expression levels from male and female alcohol (EtOH)-naïve BXD recombinant inbred strains of mice with alcohol-related phenotypes. CPP, conditioned-place preference.

GeneNetwork Record ID	Phenotype	Sex	r	P value	Strain pairs	PubMed ID
10074	EtOH (10% v/v) intake	M	−0.5458	0.0435	14	6683363
12964	EtOH (15% v/v) intake	M, F	−0.5699	0.0420	13	7978106
12574	EtOH (15% v/v) intake	M, F	−0.5148	0.0413	16	27793543
10090	CPP - % time in EtOH-paired compartment	M	−0.6766	0.0020	18	7480533
10097	CPP - s/min in EtOH-paired compartment	M	−0.621	0.0059	18	7480533
10081	EtOH withdrawal severity	M	0.907	0.0048	7	6683363
10478	EtOH withdrawal severity	F	−0.5748	0.0198	16	6683363
11975	EtOH-induced ataxia (2.25 g/kg)	M, F	−0.4693	0.0059	33	19958391
11440	Locomotor activity after EtOH (2.25 g/kg)	M	−0.4084	0.0203	32	19958391
11697	Locomotor activity after EtOH (2.25 g/kg)	F	−0.3994	0.0213	33	19958391
12391	Anxiety assay after EtOH (1.8 g/kg)	M, F	−0.3689	0.0347	33	
10087	EtOH-induced hypothermia (2 g/kg)	F	0.4702	0.0364	20	8627539
10086	EtOH-induced hypothermia (3 g/kg)	F	0.5392	0.0142	20	8627539
10085	EtOH-induced hypothermia (4 g/kg)	F	0.4477	0.0478	20	8627539

alcohol-induced functional and behavioral adaptations.

Dopamine release from the VTA to the NAc is a central aspect of the addiction process (Koob and Le Moal, 2005). Although activation of K_V7 channels can attenuate DA synthesis and efflux in the striatum and reduce VTA dopaminergic neurotransmission (Hansen et al., 2006; Jensen et al., 2011; Martire et al., 2007; Sotty et al., 2009), the role that VTA Kv7 channels play in regulating drinking behaviors is unknown. We previously reported that *Kcnq2* and *Kcnq3* lie within multiple alcohol-related QTLs in rodents (McGuier et al., 2016), and *Kcnq* transcript levels in the NAc and prefrontal cortex are negatively correlated with voluntary alcohol drinking in BXD recombinant inbred (RI) strains of mice (Rinker et al., 2017). Whereas *Kcnq2/3* expression is relatively low in DA neurons, *Kcnq4* expression is predominant in DA neurons in the VTA and substantia nigra pars compacta (SNc) (Hansen et al., 2007; Kharkovets et al., 2000). Thus, it is possible that midbrain *Kcnq4* is a critical mediator of alcohol-related behaviors and heavy alcohol drinking.

Here, we performed an integrative functional genomic analysis on midbrain *Kcnq4* expression and alcohol- and DA-related phenotypes in BXD RI strains. We also used pharmacological approaches targeting K_V7 channels in the VTA using a rat intermittent access to alcohol (IAA) model of drinking to validate our genetic findings. Despite its efficacy as adjunct therapy to treat partial onset seizures, production of retigabine was discontinued because of retina and skin pigment changes caused by accumulation of low solubility retigabine dimers after prolonged use (Clark et al., 2015). Retigabine dimers likely form because resonance structures of retigabine are Brandowski's bases that are self-reactive (Dousa et al., 2014). Because K_V7 channels remain a promising target for treating AUD, we explored a novel and selective analog of retigabine (i.e., ML213) with a simplified chemical scaffold that lacks a critical amine group promoting formation of insoluble dimers. Unlike the broad actions and modest potency of retigabine on $K_V7.2$–7.5 channels, ML213 is an opener of K_V7 channels with selectivity for $K_V7.2$ ($EC_{50} = 230$ nM) and $K_V7.4$ ($EC_{50} = 510$ nM) over other K_V7 channel subtypes and a panel of 68 other ion channels, transporters, and G-protein coupled receptors (Yu et al., 2010). Results from our studies provide evidence that midbrain *Kcnq4* regulates alcohol drinking in genetically diverse mice and pharmacologically targeting these channels reduces excessive alcohol consumption in rats.

2. Materials and methods

2.1. Bioinformatics

To provide evidence for genetic links between *Kcnq4* and alcohol-related behaviors, we performed two integrative bioinformatics analyses using open source databases (www.geneweaver.org and www.genenetwork.org) following our previously reported methods (McGuier et al., 2016; Padula et al., 2015; Rinker et al., 2017). First, we identified QTLs related to alcohol that contain *Kcnq4*. Next, we correlated *Kcnq4* midbrain robust multi-array average (RMA) expression levels in alcohol-naïve male BXD RI strains of mice ($N = 37$ strains and 129 mice) with alcohol-related phenotypes and phenotypes related to monoaminergic signaling in male and female BXD RI strains. When available, PubMed identification numbers for the original manuscripts describing the phenotypes are reported in Tables 1 and 2.

2.2. Animals and housing

Male Wistar rats were purchased from Harlan (Indianapolis, IN) and housed individually in standard home cages in temperature

Table 2
Correlations of midbrain *Kcnq4* expression levels from alcohol-naïve BXD recombinant inbred strains of mice with components of the monoaminergic system. 5-HIAA, 5-Hydroxyindoleacetic acid; DAT1, dopamine transporter; DOPAC, 3,4-Dihydroxyphenylacetic acid; DRD2, dopamine receptor D2; DRD3, dopamine receptor D3; HVA, Homovanillic acid; NE, norepinephrine; TH, tyrosine hydroxylase; PFC, prefrontal cortex, NAc, nucleus accumbens.

GeneNetwork Record ID	Phenotype	Sex	r	P value	Strain pairs	PubMed ID
12800	5-HIAA level in the medial septal nucleus	M, F	0.7349	0.0378	8	
10234	DAT1 (*SLC6A3*) binding maximum in PFC	M	−0.5351	0.0182	19	11454925
10280	DAT1 (*SLC6A3*) binding maximum in PFC	F	0.5719	0.0259	15	10591541
10281	DAT1 (*SLC6A3*) binding maximum in PFC	M, F	0.5563	0.0252	16	10591541
12798	DOPAC level in the medial septal nucleus in EtOH-dependent mice	M, F	0.8304	0.0107	8	
15146	DOPAC/DA ratio in striatum	M	−0.8495	0.0323	6	23558233
10262	DRD2/DRD3 binding maximum in NAc	F	0.5362	0.0394	15	10591541
12801	HVA level in the medial septal nucleus	M, F	0.8149	0.0137	8	
15161	TH protein level in striatum	M	0.9372	0.0058	6	