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Differential Potassium Channel Gene Regulation in BXD Mice Reveals Novel Targets for Pharmacogenetic Therapies to Reduce Heavy Alcohol Drinking

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Abstract

Alcohol (ethanol) dependence is a chronic relapsing brain disorder partially influenced by genetics and characterized by an inability to regulate harmful levels of drinking. Emerging evidence has linked genes that encode K_V7, K_{IR}, and $K_{Ca}2$ K^+ channels with variation in alcohol-related behaviors in rodents and humans. This led us to experimentally test relations between K^+ channel genes and escalation of drinking in a chronic intermittent ethanol (CIE) exposure model of dependence in BXD recombinant inbred strains of mice. Transcript levels for K^+ channel genes in the prefrontal cortex (PFC) and nucleus accumbens (NAc) covary with voluntary ethanol drinking in a non-dependent cohort. Transcripts that encode K_V7 channels covary negatively with drinking in non-dependent BXD strains. Using a pharmacological approach to validate the genetic findings, C57BL/6J mice were allowed intermittent access to ethanol to establish baseline consumption before they were treated with retigabine, an FDA-approved K_V7 channel positive modulator. Systemic administration significantly reduced drinking, and consistent with previous evidence, retigabine was more effective at reducing voluntary consumption in high-drinking than low-drinking subjects. We evaluated the specific K^+ channel genes that were most sensitive to CIE

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exposure and identified a gene subset in the NAc and PFC dysregulated in the alcohol-dependent BXD cohort. CIE-induced modulation of nine genes in the NAc and six genes in the PFC covaried well with the changes in drinking induced by ethanol dependence. Here we identified novel candidate genes in the NAc and PFC that are regulated by ethanol dependence and correlate with voluntary drinking in non-dependent and dependent BXD mice. The findings that *Kcnq* expression correlate with drinking and that retigabine reduces consumption suggest that K_V7 channels could be pharmacogenetic targets to treat individuals with alcohol addiction.

Introduction

Alcohol use disorder (AUD) is a chronic, relapsing brain disease with comparatively high heritability of 40–60% that imposes an enormous social and economic burden, and a host of costly and serious health consequences (NIAAA, 2015). Despite the deleterious effects on individual health and society, there are currently only three FDA approved pharmacological treatments for AUD, all of which have limited success at reducing the high rates of relapse (Clapp, 2012; Williams, 2005). This clearly identified gap in treatment options highlights our limited understanding of the underling vulnerabilities for developing an AUD and demonstrates the need for more research to define neurobiological mechanisms of alcohol dependence in an effort to identify novel pharmacotherapeutics.

Recent evidence has shown that a number of well-tolerated anticonvulsants may prove useful in the prevention of relapse and reducing alcohol consumption (Book & Myrick, 2005; Clapp, 2012; De Sousa, 2010; Malcolm, Myrick, Brady, & Ballenger, 2001; Padula et al., 2013). Originally implicated for their role in epilepsy (Maljevic & Lerche, 2014; Smets et al., 2015), studies show that calcium-activated (K_{Ca}), voltage-dependent (K_V), and G protein-coupled inwardly-rectifying (K_{ir}) K^+ channels are targets for both acute and chronic effects of ethanol (Hopf et al., 2011; Mayfield, Blednov, & Harris, 2015; Mulholland, 2012; Mulholland, Hopf, et al., 2009). Additionally, preclinical studies have demonstrated that chronic ethanol reduces the function and trafficking of $K_{Ca}2$ (*Kcnn*), $K_V4.2$ (*Kcnd2*), and $K_V7.2$ (*Kcnq2*) channels in the nucleus accumbens (NAc) and hippocampus (Hopf et al., 2010; McGuier et al., 2015; Mulholland, Spencer, Hu, Kroener, & Chandler, 2015; Padula et al., 2015; Spencer, Mulholland, & Chandler, In Press), and pharmacologically enhancing $K_{Ca}2$ and K_V7 channel function attenuated voluntary drinking in rodents (Hopf et al., 2011; Knapp, O'Malley, Datta, & Ciraulo, 2014; McGuier et al., 2015; Padula et al., 2013). Ethanol actions on voltage- and calcium-dependent $K_{Ca}1.1$ (*Kcnma1*) channels are involved in acute ethanol tolerance, dependence, and heavy ethanol consumption (Bukiya et al., 2014; Ghezzi, Pohl, Wang, & Atkinson, 2010; Kreifeldt, Le, Treistman, Koob, & Contet, 2013; Treistman & Martin, 2009), and *Kcnma1* and *Kcnq5* were identified as major hub genes for the acute actions of ethanol (Wolen et al., 2012). Deletion of the gene that encodes $K_{ir}3.3$ channels (*Kcnj9*) enhanced ethanol conditioned place preference (Tipps, Raybuck, Kozell, Lattal, & Buck, 2016) and blunted ethanol-induced excitation of dopamine neurons and increased binge-like ethanol consumption in mice in both a limited-access 2-bottle choice (LA-2BC) model (15% v/v ethanol vs. water) and with limited-access to a single bottle of 20% v/v ethanol (Herman et al., 2015). In addition to ethanol actions on these K^+ channels, recent studies reveal that variation in genes that encode $K_{Ca}2$, $K_{ir}3$ and K_V7 channels may

modulate risk for susceptibility of developing an AUD (Clarke et al., 2011; Edenberg & Foroud, 2013; Kozell, Walter, Milner, Wickman, & Buck, 2009; McGuier et al., 2015; Padula et al., 2015). Thus, drugs that target K^+ channels may prove useful in the treatment of AUD.

To experimentally test associations between K^+ channel genes and ethanol drinking, we generated and analyzed data as part of an extensive collaborative behavioral and expression genetics study on a well-characterized model of dependence-induced escalation of voluntary LA-2BC drinking in a large cohort of genetically diverse BXD strains of mice. The BXDs are an effective genetic reference population to study both causes and correlates of complex behaviors, such as ethanol consumption in dependent and non-dependent mice (Philip et al., 2010). A full description of the chronic intermittent ethanol (CIE) exposure model in BXD RI mice and the analysis of variation in drinking and gene expression are reported elsewhere in this issue (Lopez, Miles, Williams, & Becker, This issue; van der Vaart et al., This issue). We utilized the GeneNetwork database (www.genenetwork.org) to generate and analyze correlations in K^+ channel transcript levels in two key regions of the addiction circuitry, the prefrontal cortex (PFC) and NAc, with voluntary drinking in non-dependent and dependent BXD mice. We then used a computational modeling approach to determine how CIE-induced adaptations in K^+ channel gene expression impacts function of deep-layer PFC projection neurons. Finally, we validated one identified target using a pharmacological approach in C57BL/6J mice.

Methods

Animals

As described in Lopez et al., (*this issue*), adult male and female C57BL/6J (B6) mice (10 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME), and BXD RI strains (12–16 weeks old) and DBA/2(UT) mice were obtained from the University of Tennessee Health Science Center or Jackson Laboratory. The sex, treatment groups, strains and the numbers of mice/strain are shown in Supplemental Table 1 (75 total mice from 28 unique strains; 54 males, 21 females; 36 control and 39 CIE-exposed mice). While littermates were used for some of the BXD strains, males and females from the same strain were not used in the present experiment. After 1–3 weeks acclimation in group housing conditions on a 12 hr light/dark cycle, mice were individually housed in temperature and humidity controlled environments for 72 hr prior to the start of alcohol access in their home cages. Food and water were available ad libitum during all procedures. The Medical University of South Carolina Institutional Animal Care and Use Committee approved all procedures in accordance with NIH guidelines for the humane care and use of laboratory animals.

CIE exposure and limited-access 2-bottle choice drinking

To establish baseline drinking, mice consumed ethanol in their home cage using a standard limited-access (2 hr) 2-bottle choice (15% ethanol (v/v) vs. water) protocol (LA-2BC) for 6 weeks following routine procedures (Becker & Lopez, 2004; Lopez et al., This issue) (Figure 1A). The reader is referred to Lopez et al., (*this issue*) for a full description of the drinking data in these BXD strains. At 30 min before the beginning of the dark cycle, water

bottles were removed from the home cage and replaced with 15 ml drinking bottles that contained water or a 15% ethanol solution. After a 2-hr access period, the drinking bottles were removed from the home cage and replaced with water bottles. Mice then underwent 4 cycles of chronic intermittent ethanol (CIE or dependent) exposure in vapor inhalation chambers. CIE treated mice underwent sixteen hours of vapor exposure followed by eight hours of withdrawal, and weekly CIE exposure in vapor inhalation chambers was alternated weekly with home cage ethanol (15% v/v) or water drinking sessions (Becker & Lopez, 2004; Lopez et al., This issue). Chamber ethanol concentrations were monitored daily and air flow was adjusted to maintain ethanol concentrations within a range that yields stable blood ethanol levels (~175 mg/dl) throughout exposure for the C57BL/6J strain (see Lopez et al. (*this issue*) for explanation and potential confounds of this experimental design). Prior to entry into the ethanol chambers, EtOH mice were administered ethanol (1.6 g/kg; 8% w/v) and the alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg; ip, dose volume 20 ml/kg). Control mice were handled similarly, but received injections of saline and pyrazole and were not exposed to CIE (Air or non-dependent) and, thus, had a total of 10 weeks of LA-2BC drinking. Mice were put through one final cycle of CIE exposure after a week of drinking, and tissue was collected for microarray analysis after a 72-hr withdrawal period. As described in Lopez et al. (*this issue*), all mice were exposed to the same experimental conditions in ethanol vapor inhalation chambers, and BXD RI strains of mice display considerable amounts of variability in their blood ethanol concentrations during the CIE exposure cycles. As a consequence of the study design, 24 mice died during exposure in the inhalation chambers or during withdrawal.

Microarray data generation and analysis

The reader is referred to van der Vaart et al., (*this issue*) for a full description of the tissue collection and microarray data generation and analysis. Briefly, tissue punch dissections, RNA isolation, and hybridization/scanning procedures with Affymetrix Mouse Genome 430 2.0 arrays (Affymetrix, Cat #900497) were performed as described previously (van der Vaart et al., This issue; Wolen et al., 2012). To minimize the risk of technical variation confounding the detection of an alcohol response, an Air- and CIE-exposed mouse from each strain were processed simultaneously. Annotation data for Mouse Genome 430 2.0 probe-sets were obtained from the GeneNetwork Data Sharing Zone (genenetwork.org/share/annotations). Expression data from the Air and CIE treatment groups were background corrected, quantile normalized and summarized using the robust multi-array average (RMA) expression measure. To directly assess CIE-responsive expression changes across BXD strains, the S-score algorithm (Kennedy, Kerns, Kong, Archer, & Miles, 2006; Kerns, Zhang, & Miles, 2003; Zhang, Wang, Ravindranathan, & Miles, 2002) was used for probe-level comparison of CIE vs. Air samples. S-scores represent a significance score in a similar manner to Z-scores, with a mean of 0 and a standard deviation of 1 across an entire array. Positive S-scores denote an increase in expression with CIE. S-scores were generated using the S-score R package from each BXD RI strain, C57BL/6J, DBA/2(UT), and C57BL/6J +DBA/2J F1 mice separately. Because only 1 CIE and 1 control sample was available for most BXD RI strains, significance of gene expression response across the BXD cohort was determined using Fisher's Combined Probability Test with S-scores as previously described

(Wolen et al., 2012). All datasets generated for this paper are accessible on GeneNetwork (www.genenetwork.org). All data are MIAME compliant.

Bioinformatics analyses

We performed three sets of analyses to determine the relationship between K^+ channel gene expression, voluntary drinking, and ethanol dependence in the LA-2BC paradigm (Figure 1A): 1) correlation of ethanol consumption in non-dependent (Air exposed) controls (Supplemental Figure 1) and K^+ channel gene expression in the PFC and NAc; 2) dependence-induced changes in K^+ channel gene expression (difference in gene expression between CIE and Air, i.e., S-scores); and 3) correlation of dependence-induced change in ethanol consumption (calculated across all strains as a percent change between intake during Test 4 and intake during week 6 of baseline drinking) and S-scores. Specifically, using standard linear regression analysis, we conducted a phenotype correlation analysis of K^+ channel RMA levels (196 probe sets for 79 K^+ channel genes) in the PFC (GeneNetwork data set: *VCU BXD PFC CIE Air M430 2.0 (Jan11) RMA*) and NAc (GeneNetwork data set: *VCU BXD NAc CIE Air M430 2.0 (Jan13) RMA*) and ethanol intake values after 10 weeks of 2-bottle choice drinking (GeneNetwork record ID 12961: Ethanol intake (5 day average in g/kg/2 hr) after cycle 4 of Air exposure) obtained from non-dependent BXD RI mice. Next, to determine which K^+ channel genes were responsive (i.e., differentially regulated) to CIE exposure in drinking mice, we measured the impact of CIE exposure on K^+ channel transcript abundance using the Significance-score (S-score) algorithm, as described previously (Wolen et al., 2012; Zhang et al., 2002). This analysis utilized the S-score algorithm to compare K^+ channel expression levels across BXD strains and between the two treatment groups to generate an Air vs CIE S-score for each K^+ channel probe set in the PFC (GeneNetwork data set: *VCU BXD PFC EtOH vs CIE Air M430 2.0 (Jan11) Sscore*) and NAc (GeneNetwork data set: *VCU BXD NAc EtOH vs CIE Air M430 2.0 (Jan13) Sscore*). A positive S-score indicates up-regulation by CIE exposure and vice versa. Statistical significance of the K^+ channel changes following CIE exposure across strains were assessed using Fisher's combined probability test. To correct for multiple testing, *q* values were generated from the empirical *p* values, and probe-sets with q values <.05 were considered significantly CIE-responsive. As a final step, we determine how K^+ channel S-scores in the PFC and NAc correlated with CIE-induced changes in alcohol consumption (GeneNetwork Record ID: 12741). CIE-induced changes in drinking were calculated across all strains as a percent change between intake at Test 4 and intake at week 6 of baseline drinking (Figure 1A), and the percent change highly correlated with the difference (in g/kg) in intake between Test 4 and baseline week 6 ($F_{1,27} = 169.2$, $R^2 = 0.862$, $p < 0.0001$, Supplemental Figure 2). Standard linear regression analysis was used, and *p* values <.05 for the slope were considered significant. Data from male and female subjects were collapsed for all analyses. The hypergeometric functional enrichment analysis to determine if K^+ channel genes that correlated with drinking were over-represented was performed using the 'phyper' function in R software (https://www.R-project.org).

Computational modeling

The NEURON 7.1 simulation environment (Carnevale & Hines, 2006) was used to generate a computational model containing 10 compartments (soma with proximal and apical

dendrites) to determine how coordinated changes in CIE-responsive K^+ channel genes in the PFC affect firing of pyramidal neurons. Computer simulations were performed on a modified deep-layer PFC pyramidal neuron model taken from ModelDb accession number 82849 (Durstewitz, Seamans, & Sejnowski, 2000). This model incorporated passive leak conductance, incorporated Hodgkin-Huxley fast sodium channel currents and delayed-rectifier K^+ channel currents. Calcium-activated K^+ channel currents were modeled according to the parameters used in ModelDb accession number 123453 (Akemann, Lundby, Mutoh, & Knopfel, 2009), delayed rectifier K^+ channel currents were derived using the criteria in ModelDb accession number 74298 (Gillies & Willshaw, 2006), and inwardly-rectifying K^+ channel kinetics were based on ModelDb accession number 147514 (van der Velden, van Hooft, & Chameau, 2012). The reversal potential of the passive leak conductance was −70 mV. Passive parameters included Cm (ranging from 0.9 to 1.2 $\mu F*cm^{-2}$), Rm (ranging from 36.4 to 44.9 $k\Omega*cm^2$), Ri (ranging from 70 to 150 Ω*cm), and temperature (32–36°C). Our past studies t hat have measured functional changes in K^+ channels in neurons revealed that chronic ethanol exposure (both *in vitro* and *in vivo*) reduced currents by 56.4 ± 5.3% (Mulholland, Becker, Woodward, & Chandler, 2011; Mulholland et al., 2015; Nimitvilai, Lopez, Mulholland, & Woodward, 2016; Padula et al., 2015). To model the coordinated gene expression changes in the PFC of ethanol dependent BXD mice, currents mediated by $K_V3.1$, K_V7, $K_{Ca}1.1$, and $K_V2.1$ channels were either simultaneously decreased by 0.5-fold or were enhanced 1.5-fold in the pyramidal neuron model to mimic published functional changes in K+ channel currents. Current clamp simulations examined evoked firing in response to current steps of increasing amplitude (100 pA steps; 1 sec duration) injected at the soma starting with −1.0 nA and ending with +1.0 nA. Resting membrane potential was held at −65 mV, and active and passive membrane properties were analyzed. One-way ANOVA was used to analyze afterhyperpolarization (AHP) duration and interspike interval (ISI) with a Newman-Keuls post-hoc test and a significance level of $p < 0.05$.

Intermittent 2-bottle choice drinking

To pharmacologically validate and demonstrate the potential utility of using this database to identify novel pharmacotherapeutic targets for reducing ethanol consumption, we chose to utilize the intermittent-access 2-bottle choice (IA-2BC) model of voluntary ethanol drinking (Figure 1B). In a separate cohort of male C57BL/6J mice (n = 19), approximately 8 weeks old at the start of the study, were given chronic, intermittent access to 20% ethanol (v/v) and water in a 2-bottle choice design on Mondays, Wednesdays and Fridays for 24 hr, with two bottles of water on alternate days and weekends. Fluid access began 3 h into the dark cycle. Mice were allowed to drink for 7 weeks to establish stable baseline ethanol consumption prior to testing. On test days, mice were injected with retigabine (RTG, dihydrochloride, Alomone Labs; 0, 5, 10 mg/kg – doses reflect the base, IP, 10 ml/kg in sterile saline) approximately 30 min prior to bottle access in a repeated measures fashion (i.e., each animal received each dose across a 3 week testing period in a counterbalanced order). Ethanol consumption was measured at 2, 4 and 24 hr after bottles were placed on the cages. Prior data indicate that RTG is most effective in rats with a high-drinking phenotype (McGuier et al., 2015), and despite the fact they are inbred and nearly genetically identical, C57BL/6 mice exhibit dramatic intra-strain variability in drinking behavior (Wolstenholme et al.,

2011), thus mice were divided into “high-drinking” and “low-drinking” groups. To identify “high-drinking” mice, we initially split animals around the median (12.1 g/kg) of average consumption during week 7 of baseline drinking. But, because C57BL/6J mice tend to front-load in most drinking paradigms (Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Wilcox et al., 2014) and generally drink in excess of 1.5 g/kg in the first 2 hr of two-bottle choice access (Linsenbardt & Boehm, 2014), we set the additional criteria that mice also had to drink a minimum of 1.5 g/kg in the first 2 hr after vehicle injections. A mixed-model analysis of variance accounting for within-subjects effects was used in the statistical software language SAS (SAS Institute, Cary, NC) to analyze all drinking data. On occasions where intake data were not obtainable, these individual data points were removed from analyses entirely, as opposed to mean substitution or listwise deletion (a total of 12 individual data points were removed from analysis, 9 in the 5 mg/kg dose and 3 in the 10 mg/kg dose). To follow up significant treatment effects, Tukey’s post-hoc tests were used. To determine the variability in drinking across the 7 weeks of baseline drinking, the coefficient of variation (CV = SD/mean) was calculated each week for the individual mice, and the weekly averages were analyzed by repeated measures one-way ANOVA with a Tukey’s post-hoc test (GraphPad Prism, version 6.04). All data are reported as mean ± SEM and statistical significance was established with $p < .05$, and effect sizes were calculated according to corrected Hedges’s *g*. Additionally, the dependence of RTG effects on baseline drinking was assessed in a 3-level hierarchical linear model (HLM 7.0) with subject at level 3, dose at level 2, and vehicle drinking and time and their interaction at level 1. The response to K_V7 activation by RTG was indicated by the difference between ethanol consumption after vehicle injection and consumption after RTG injections.

Results

K+ channel genes correlate with voluntary drinking

We have previously reported that differences in *Kcnn3* and *Kcnq2/3* gene expression correlates with voluntary alcohol drinking and that these genes are positional candidates for ethanol consumption in the BXD family of strains (McGuier et al., 2015; Padula et al., 2015). To identify novel K^+ channel genes that correlate with drinking in non-dependent and dependent mice, we conducted an extensive analysis of K^+ channel genes in the NAc and PFC from C57BL/6J and DBA/2 mice and BXD strains that experienced the LA-2BC and CIE (or Air) paradigm, which results in dependence-induced escalation in 2BC drinking. We first determined significant correlations between K^+ channel transcript expression levels and voluntary drinking in non-dependent (Air controls) BXD cases. Thirty-five probe sets corresponding to 16 unique genes in the NAc (Table 1) and 8 probe sets and 7 unique genes in the PFC (Table 2) correlate with voluntary ethanol (15%, v/v) consumption in the non-dependent mice that had 10 weeks of LA-2BC home cage drinking. Although we identified multiple K^+ channel genes that correlated with drinking in non-dependent BXD strains, functional enrichment analysis revealed that K^+ channel genes were not over-represented in the total gene set that correlated with LA-2BC drinking (NAc: $p = 0.196$; PFC: $p = 0.141$; Supplemental Table 2). Tables 1–6 lists the functional class and predominant subcellular localization in brain for each K^+ channel gene (Goldstein et al., 2005; Gutman et al., 2005; Kubo et al., 2005; Sun, Zaydman, & Cui, 2012; Trimmer, 2015; Wei et al., 2005). As shown

in Tables 1 and 2, the majority of genes that covary with voluntary LA-2BC drinking in non-dependent BXDs encode voltage- and calcium-activated and delayed rectifier K^+ channels that localize to dendrites, axons, or presynaptic terminals.

Complementary to our previous findings (McGuier et al., 2015; Padula et al., 2015), *Kcnn* and *Kcnq* transcript levels in the NAc and PFC significantly correlate with voluntary LA-2BC drinking in non-dependent BXDs. In the current study, all of the 13 *Kcnq* probe sets showed moderate to strong negative covariation with voluntary consumption ($r = -0.55 \pm 0.02$). Low *Kcnq* transcript levels are associated with high levels of drinking. This is consistent with pharmacological evidence showing that positive modulation of K_V7 channel function reduces drinking in rats (Knapp et al., 2014; McGuier et al., 2015). To confirm the previous rat studies, we examined the ability of retigabine, an FDA-approved K_V7 channel positive modulator, to reduce drinking in C57BL/6J mice that were allowed intermittent access (IA) to ethanol (20%, v/v). Although not considered a traditional model to induce ethanol dependence, mice and rats consume large amounts of ethanol in the IA-2BC model and show mild-to-moderate signs of physical dependence during acute withdrawal (Hwa et al., 2011; Li, Bian, Dave, & Ye, 2011; Steensland et al., 2012). Thus, we chose to use the IA-2BC model (Figure 1B) in a separate cohort of C57BL/6J mice as an initial screen to validate *Kcnq* as a target for reducing ethanol consumption in high-drinking mice. After 7 weeks of drinking in the IA-2BC model (Figure 2A), C57BL/6J mice displayed a large degree of intra-strain variability in ethanol consumption on week 7, ranging from 8.4 ± 0.21 to 18.4 ± 0.37 g/kg/24 hr (Figure 2B). However, analysis of the intra-individual coefficient of variation of drinking showed significant decreases from 0.30 ± 0.02 in week 1 to 0.11 ± 0.02 in week 7 ($F_{4.216, 75.88} = 8.797$, $p < 0.0001$, n = 19 mice; Hedges's $g = 2.34$; Figure 2C), demonstrating that drinking in each mouse became more consistent across time. After reaching this stable baseline of intake, mice were injected with RTG (5 or 10 mg/kg, IP) or vehicle approximately 30 min before the start of the 24 hr drinking session, and consumption was measured at 2, 4 and 24 hr time points. There was a significant main effect of RTG treatment on ethanol drinking ($F_{2,32} = 4.76$, $p = 0.0154$, n = 19; Figure 2D), and post-hoc analysis revealed that 10 mg/kg RTG significantly reduced voluntary consumption (Hedges's $g = 0.57$). Our previous studies demonstrated that RTG was more effective at reducing intake in Wistar rats with a high-drinking phenotype (McGuier et al., 2015). Consistent with this, both doses of RTG significantly reduced ethanol consumption in high-drinking C57BL/6J mice ($F_{2,13} = 16.74$, $p = 0.0003$; n = 8 mice; 5 mg/kg: Hedges's $g = 0.82$, 10 mg/kg: Hedges's $g = 1.08$; Figure 2E), whereas neither dose affected intake in low-drinking mice ($F_{2,17} = 0.06$, $p = 0.9451$; n = 11 mice; Figure 2F). To further examine responsivity of RTG, we assessed the relationship between baseline drinking after vehicle injection and RTG-induced changes in ethanol consumption using HLM. There was a strong negative linear relationship between vehicle drinking and RTG-induced alteration of intake across time ($\beta = -0.99$, SE = 0.26, $t_{47} = 2.32$, $p = 0.025$), that is, the greater the vehicle drinking, the more K_V7 channel activation reduced ethanol consumption (Figure 2G). The two doses of RTG did not differ ($\beta = 0.432$, SE = 0.56, $t_{47} = 0.444$, $p > 0.05$) and similar effects were seen at each time point (all Dose X Time interactions, $p > 0.5$). Finally, treatment with RTG did not alter water consumption overall ($F_{2,32} = 0.38$, $p = 0.6843$; n = 19) or across time ($F_{2,64} = 0.64$, $p = 0.6358$; n = 19) (Supplemental Figure 3), and this

remained true when mice were divided into high versus low drinkers ($p = 0.2302$ and 0.5548, respectively).

Chronic intermittent ethanol-responsive K^+ channel genes

We previously reported that chronic ethanol exposure reduces expression and function of $K_{Ca}2$ (*Kcnn*) and $K_V4.2$ (*Kcnd2*) channels in the NAc and hippocampus (Mulholland et al., 2015; Padula et al., 2015; Spencer et al., In Press). To identify novel K^+ channel genes in the NAc and PFC that are responsive to ethanol dependence, we utilized the Significance-score (S-score) algorithm (Wolen et al., 2012; Zhang et al., 2002) for probe-level analysis of each strain's K^+ channel transcriptional response to CIE exposure. This analysis was followed by Fisher's combined probability test to identify K^+ channel genes that consistently respond to CIE exposure across BXD strains of mice, regardless of direction. In the NAc, CIE exposure significantly altered 13 probe sets and 9 unique K^+ channel genes, including genes that encode $K_{Ca}1.1$ (*Kcnma1*) and $K_V4.2$ channels (Figure 3 and Table 3). Our previous study demonstrated that high *Kcnn3* expression levels in the NAc of BXD mice were protective against escalation of drinking in ethanol dependent BXD mice (Padula et al., 2015). Interestingly, BXD strains that showed a general increase in S-scores for these 13 probe sets (BXD12, BXD71, BXD74, and BXD103) did not escalate their LA-2BC drinking after CIE exposure ($\Delta = -5.9 \pm 10.28\%$; Δ calculated as percent change between Intake Test 4 and Week 6 of baseline drinking). However, there wasn't a clear relationship with LA-2BC drinking ($\Delta = 15.1 \pm 41.81\%$) in BXD strains that showed a general down-regulation of these K^+ channel genes.

Analysis of array datasets in the PFC identified five probe sets and four unique K^+ channel genes that encode $K_V2.1$ (*Kcnb1*), $K_V3.1$ (*Kcnc1*), $K_{Ca}1.1$ (*Kcnma1*), and $K_V7.5$ (*Kcnq5*) channels that were responsive to CIE exposure (Figure 4A and Table 4). Interestingly, BXD strains (BXD14, BXD45, BXD71, and BXD100) that generally showed negative S-scores across these 5 probe sets were resistant to CIE-induced escalations in LA-2BC drinking. The average CIE-induced percent change from baseline drinking values in these strains was $-13.4 \pm 11.6\%$. In contrast, strains that generally showed positive S-scores across these 5 probe sets (BXD55, BXD66, BXD75, and BXD80) elevated their LA-2BC drinking after CIE exposure ($\Delta = 59.9 \pm 31.74\%$). This suggests that coordinated adaptations in this set of four K^+ channel genes may influence output neurons of the PFC that control drinking behavior in dependent BXDs.

To begin to understand how coordinated changes in $K_V3.1$, $K_V7.5$, $K_{Ca}1.1$, and $K_V2.1$ channels affect PFC physiology, we modeled the passive and active membrane properties of a deep-layer PFC pyramidal neuron. The conductances of these four K^+ channels of interest were then simultaneously decreased by 0.5-fold to model gene changes in ethanol-dependent BXD14, BXD45, BXD71, and BXD100 strains or were increased by 1.5-fold to model ethanol-dependent BXD55, BXD66, BXD75, and BXD80 strains (Figure 4B). The biophysical stimulations suggest minimal changes in the resting membrane potential (in mV; *CTL*: −63.9; *0.5-fold*: −63.6; *1.5-fold*: −62.9), action potential (AP) threshold (in mV: *CTL*: 48; *0.5-fold*: 48; *1.5-fold*: 49), AP amplitude (in mV: *CTL*: 91; *0.5-fold*: 89; *1.5-fold*: 90), or AHP duration measured at 500–1000 pA steps (in ms: *CTL*: 59.7 ± 4.6; *0.5-fold*: 50.3 ± 6.7;

1.5-fold: 66.7 ± 5.3; one-way ANOVA, $F_{2,15} = 0.55$, $p = 0.22$) by coordinated up- or down-regulation of these four K^+ channels. However, other measures of intrinsic excitability that could directly affect output of the PFC were affected. The number of evoked APs in response to depolarizing current steps delivered to the soma were reduced by 23.0 ± 2.5% in the 1.5-fold model simulation compared the 0.5-fold simulation, which resulted in 27.6 ± 6.5% increase in evoked firing (500–1000 pA steps; Figure 4B and C). Consistent with this, the rheobase current (lowest amount of current needed to produce an AP) was increased in the 1.5-fold simulation by 40 pA (Figure 4D), suggesting that the neuron is less likely to fire when depolarized. The ISI (Figure 4E; one-way ANOVA, $F_{2,15} = 1.82$, $p = 0.019$, post-hoc test, $^*p < 0.05$) was markedly increased in the simulations with a 1.5-fold increase in channel conductance (500–1000 pA steps), suggesting that when the neurons reach threshold, fewer action potentials are produced due to changes in mechanisms that control AP generation. These modeling data demonstrate that coordinated regulation of $K_V3.1$, $K_V7.5$, $K_{Ca}1.1$, and $K_V2.1$ channels by CIE exposure may affect intrinsic excitability of deep layer PFC pyramidal neurons.

CIE-responsive K^+ channel genes that correlate with drinking in dependent mice

As a final analysis, we correlated S-scores (CIE-induced change in transcript levels) in the PFC and NAc with the percent change from baseline drinking (CIE-induced change in LA-2BC ethanol consumption) to further explore the relationship between dependence-induced regulation of K^+ channel transcripts and drinking in CIE exposed BXD mice. As shown in Supplemental Figure 4 and Table 5, in the NAc S-scores for 13 probe sets and 12 unique K^+ channel genes significantly correlated the change in drinking induced by chronic exposure to ethanol vapor in inhalation chambers, with S-scores for 7 of the probe sets demonstrating moderate-to-strong positive relationships. In the PFC, S-scores for 9 probe sets and 8 unique K^+ channel genes significantly correlated the CIE-induced change in LA-2BC drinking (Supplemental Figure 5 and Table 6). Moderate-to-strong negative relationships were observed for 5 of the 9 S-scores for K^+ channel genes, with the remaining S-scores for 4 genes showing moderate-to-strong positive relationships. Functional enrichment analysis showed that K^+ channel genes in the NAc, but not PFC, were over-represented when compared with all of the genes that significantly correlated with the CIE-induced change in LA-2BC drinking in dependent BXD mice (NAc: $p = 0.00063$; PFC: $p = 0.597$; Supplemental Table 3).

Discussion

The purpose of this study was to use existing BXD RI mRNA data in GeneNetwork as an experimental resource to evaluate potential critical K^+ channels genes that correlate with drinking in non-dependent and dependent subjects. We identified a number of novel K^+ channel genes (e.g., *Kcnd2*) in the PFC and NAc that covary well with voluntary ethanol drinking in non-dependent BXD cases. We also identified a smaller subset of K^+ channels genes in the PFC (*Kcnc1*) and NAc (*Kcnv* family) that are dysregulated by CIE exposure and that correlate with the CIE-induced changes in drinking. While this analysis fulfilled the goal of identifying new relations between K^+ channels genes and dependence-induced drinking, this study also validated existing genetic findings on K^+ channels genes—*KCNN*,

KCNQ, and *KCNJ*—previously linked to AUD. As discussed below, many of these genes have been reported in human genetic studies of AUD risk, further validating the use of BXD cohort for experimentally testing the genetic underpinnings of complex behaviors, such as voluntary drinking associated with ethanol dependence. Finally, the strong overlap between the present findings and previous evidence validates this experimental genetic approach to investigate the link between dependence-induced drinking and other classes of ion channels, receptors, or signaling cascades.

Voltage-dependent K^+ channels and risk for alcohol dependence

In non-dependent BXD RI mice, genes encoding voltage-dependent K_V7 channels in the PFC (*Kcnq5*) and NAc (*Kcnq1*, *Kcnq2*, and *Kcnq5*) significantly correlate with voluntary LA-2BC drinking. This confirmed our previous findings linking *Kcnq2 and Kcnq3* genes and alcohol-related behaviors, including ethanol preference and consumption in an IA-2BC model (McGuier et al., 2015). Using a pharmacological approach to validate a role for *Kcnq* in regulating drinking, systemic administration of the K_V7 channel positive modulator retigabine reduced drinking in C57BL/6J mice given access to ethanol in the IA-2BC model. Consistent with existing findings in rats (McGuier et al., 2015), retigabine was most effective at reducing drinking in mice with a high-drinking, but not low-drinking phenotype. This functional genomic analysis also showed that *Kcnq5* was differentially expressed in the PFC of ethanol-dependent BXD RI mice, and the dependence-induced regulation of *Kcnq1* and *Kcnq5* transcript levels in the NAc correlated with the change in LA-2BC drinking induced by CIE exposure. Interestingly, single nucleotide polymorphisms (SNPs) in *KCNQ1* (rs12574151) and *KCNQ5* (rs3799285) are associated with early onset and symptoms associated with alcohol dependence (Edenberg et al., 2010; Kendler et al., 2011). Together with our previous study, these data provide additional evidence that *KCNQ* genes are candidates for genetic diversity in alcohol-related behaviors, and suggest that differential expression or altered function of *KCNQ* produced by dependence in these populations may underlie the phenotypic differences that are risk factors for developing an AUD.

It is important to note that, while overlap in the genes that correlated with non-dependent and dependence-induced ethanol consumption was minimal, these differences could reflect compensatory neuroadaptations in different circuits as a mechanism to counteract changes in neural activity during repeated bouts of ethanol exposure and withdrawal. It is possible that correlations seen prior to dependence represent genes that are markers of a predisposition to heavy ethanol consumption, whereas changes seen after dependence are more reflective of the consequences of heavy alcohol exposure. For example, other voltage-dependent K^+ channels (i.e., *Kcnd2*) correlated with LA-2BC drinking in non-dependent and dependent BXD RI strains of mice and were also differentially regulated following induction of ethanol dependence. Of particular interest are voltage-dependent K^+ channels composed of $K_V4.2$ α subunits that are encoded by *Kcnd2*. $K_V4.2$ channels underlie the transient A-type K^+ current in cortical and hippocampal dendrites where they shape postsynaptic responses, constrain coincidence detection, and control the amplitude of bAPs (Chen et al., 2006; Hoffman, Magee, Colbert, & Johnston, 1997), and recent evidence has implicated an important role of $K_V4.2$ channels in both synaptic plasticity and cognition (Jung, Kim, & Hoffman, 2008; Lockridge & Yuan, 2011; Lugo, Brewster, Spencer, & Anderson, 2012;

Truchet et al., 2012). Consistent with emerging data on chronic ethanol and $K_V4.2$ channels, *Kcnd2* transcript levels were significantly regulated by CIE exposure in the NAc. Prolonged withdrawal from intermittent ethanol exposure enhanced A-type K^+ currents in medium spiny neurons in the NAc (Marty & Spigelman, 2012). Our previous studies showed that chronic ethanol reduced A-type K^+ currents and $K_V4.2$ channel surface expression in hippocampus, (Mulholland et al., 2015; Spencer et al., In Press), demonstrating differential effects of chronic ethanol on $K_V4.2$ channels in across brain structures. Interestingly, two SNPs in *KCND2* (rs728115 and rs17142876) were recently identified as a risk factor for developing alcohol and nicotine co-dependence (Buhler et al., 2015; Zuo et al., 2012), and decreased expression of *KCND2* was reported in the frontal cortex and amygdala (central and basolateral nuclei) of alcoholics (Ponomarev, Wang, Zhang, Harris, & Mayfield, 2012). However, our evidence that *Kcnd2* correlates with drinking in BXD RI mice is the first study to suggest that Kv4.2 channels in the PFC and NAc regulate voluntary drinking in non-dependent and dependent mice. Thus, future studies are necessary to determine if ethanol dependence alters $K_V4.2$ channel expression in the PFC or NAc and if $K_V4.2$ channels are a novel therapeutic target for reducing escalated drinking in dependence models.

In the PFC and NAc, *Kncb1* ($K_V2.1$) and the genes that encode K_V2 channels auxiliary subunits (*Kcns1/2* and *Kcnv1/2*) that act as K_V2 channel modifier/silencers correlate with voluntary ethanol consumption and dependence-induced regulation of drinking in BXD mice. In addition, *Kcnb1* and *Kcnv1* were differentially regulated by CIE exposure in the PFC or NAc. $K_V2.1$ channels are delayed-rectifiers that localize to somatodendritic domains and the axon initial segment of neocortical and hippocampal pyramidal neurons where they regulate intrinsic excitability during periods of repetitive, high-frequency activity. $K_V2.1$ channel phosphorylation and surface clustering are regulated by NMDA receptor and glutamate transporter activity (Mulholland et al., 2008), and we previously demonstrated that acute and chronic ethanol exposure or ethanol withdrawal do not affect $K_V2.1$ channel expression or phosphorylation in organotypic hippocampal cultures (Mulholland et al., 2008; Mulholland, Carpenter-Hyland, Woodward, & Chandler, 2009). It is unknown how $K_V2.1$ channels and its modifiers influence excitability of accumbens neurons or if ethanol dependence affects their function or expression in the PFC or NAc. However, intriguing evidence from a meta-analysis using samples from the Collaborative Study on the Genetics of Alcoholism (COGA) and the Study of Addiction: Genetics and Environment (SAGE) revealed that two SNPs in *KCNB1* (rs2128158 and rs2929567) showed significant associations with maximum number of alcoholic drinks consumed in a 24 hr period (Pan et al., 2013). Given these converging lines of evidence, future studies should determine if ethanol-induced dysregulation of or genetic polymorphisms in $K_V2.1$ channels and their modifiers/silencers impact risk for developing an alcohol use disorder.

Calcium-activated K^+ channels and ethanol dependence

Similar to *KCNB1*, recent studies identified two SNPs in *KCNMA1* (rs717207 and rs12219105) as a genetic risk variant that associated with symptoms of alcohol dependence and early onset alcohol dependence in European- and African-Americans (Edenberg et al., 2010; Kendler et al., 2011). In our study, transcript levels in the PFC and NAc for *Kcnma1* (large-conductance, voltage and calcium-activated $K_{Ca}1.1$ channels) significantly correlated

with voluntary drinking in non-dependent BXD RI strains of mice. *Kcnma1* in both regions was also dysregulated by ethanol dependence. In addition, dependence-induced regulation of the *Kcnmb2* β2 auxiliary subunit of $K_{Ca}1.1$ channels correlated with the change in LA-2BC drinking in ethanol dependent BXD RI mice. Auxiliary β subunits confer resistance to acute ethanol-induced potentiation of $K_{Ca}1.1$ channel-mediated currents (Feinberg-Zadek & Treistman, 2007; Martin et al., 2004), and a recent report demonstrated that $K_{Ca}1$ β1 or β4 subunits did not influence voluntary consumption of ethanol in either a continuous access 2BC or the LA-2BC model in non-dependent C57BL/6J mice (Kreifeldt et al., 2013). However, genetic deletion of the $K_{Ca}1$ β4 subunit attenuated, whereas deletion of the $K_{Ca}1$ β1 subunit enhanced the escalation of ethanol drinking ethanol-dependent C57BL/6J mice in the LA-2BC model (Kreifeldt et al., 2013). In addition to $K_{Ca}1$ channels, *Kcnn1* and *Kcnn3* (small-conductance, calcium-activated $K_{Ca}2.1/3$ channels) transcript levels in the NAc significantly correlated with voluntary drinking in BXD RI strains of mice. Multiple studies in rats and mice provide strong evidence that $K_{Ca}2$ channel positive modulators are promising drugs for reducing drinking (Hopf et al., 2010; Hopf et al., 2011; Mulholland, 2012; Padula et al., 2013), and our previous study demonstrated that elevated NAc *Kcnn3* expression levels protected against dependence-induced escalation of drinking in BXD RI mice (Padula et al., 2015). Thus, future studies are necessary to determine if calcium-activated $K_{Ca}1$ and $K_{Ca}2$ channels are effective pharmacogenetic targets for treating alcohol use disorder.

Computational modeling of PFC neurons

In the PFC, coordinated decreases in transcript expression levels of *Kcnb1*, *Kcnc1*, *Kcnma1*, and *Kcnq5* protected against dependence-induced escalation of drinking, whereas enhanced expression of these same four genes was associated with general increases in consumption induced by dependence. Over-expression of these four K^+ channels in a computational model of a deep-layer PFC pyramidal neuron reduced intrinsic excitability largely driven by an increase in rheobase and ISI duration. Recent studies have demonstrated that CIE exposure of C57BL/6J mice produces plasticity changes in passive and active membrane properties of layer V PFC pyramidal neurons (Hu, Morris, Carrasco, & Kroener, 2015; Nimitvilai, Lopez, Mulholland, & Woodward, 2015; Pleil et al., 2015). K_V7 and $K_V2.1$ channels are known regulators of intrinsic excitability and ISI in cortical neurons (Guan, Armstrong, & Foehring, 2013; Santini & Porter, 2010). Our bioinformatics and computational modeling analyses identified possible molecular substrates that may drive aberrant plasticity of intrinsic excitability of PFC projection neurons. We hypothesize that ethanol dependence produces functional adaptations in expression of these K^+ channels in the PFC neurons that project to subcortical structures that regulate consummatory behaviors. Future studies are necessary to determine if CIE-induced regulation of K^+ channels in the PFC or NAc work independently or in conjunction to protect against or promote escalation in drinking in dependent mice.

Summary and future directions

The major finding of this multifaceted approach was the identification of novel ethanol-sensitive K^+ channel candidate genes that regulate long-term and escalated drinking in the PFC and NAc of BXD RI strains of mice. In addition, this targeted bioinformatics approach

also confirmed previous studies identifying relationships between select K^+ channel genes and alcoholism, providing further evidence that BXD strains of mice are a powerful model for studying complex phenotypes. We also demonstrated that these bioinformatics approaches are useful for identifying novel pharmacological targets by validating one of the implicated K^+ channel genes using an *in vivo* pharmacological approach. And lastly, we established that these approaches can reveal coordinated changes in gene expression that, when modeled together, can reveal important cellular signaling patterns that explicate their role in behavior.

This study shows that select K^+ channel genes in the PFC and NAc covary with voluntary drinking only in non-dependent BXD mice, while a smaller subset of K^+ channel genes were sensitive to chronic ethanol exposure and correlated with drinking in ethanol dependent mice. Some of these K^+ channel genes (e.g., *KCND2*, *KCNK3*, *KCNMA1*, *KCNQ2/3*) were also differentially expressed in postmortem human frontal cortex, amygdala, or NAc from alcohol dependent individuals (Flatscher-Bader, Harrison, Matsumoto, & Wilce, 2010; Liu et al., 2006; Ponomarev et al., 2012). Recent studies show that activity-dependent processes regulate K^+ channel gene expression, including *Kcnmb2*, *Kcnn1*, *Kcnq3*, and *Kcnv1* (Lee et al., 2015), and NMDA receptor activity can modulate surface trafficking and function of $K_{Ca}2$, $K_V2.1$, and $K_V4.2$ K^+ channels (Kim, Jung, Clemens, Petralia, & Hoffman, 2007; Mulholland et al., 2008; Ngo-Anh et al., 2005). Because chronic ethanol exposure results in imbalances in NMDA receptor expression and synaptic transmission, we speculate that CIE-induced adaptations in some K^+ channel genes are compensatory mechanisms to counterbalance chronic ethanol inhibition of NMDA receptor activity, whereas adaptations in other K^+ channel genes may be protective against or facilitate escalation of drinking in dependent BXD mice.

Additionally, in an effort to pharmacologically validate one of these genetic targets, *Kcnq*, which has been implicated in a number of studies to influence ethanol consumption, we utilized a mouse model of ethanol consumption, the IA-2BC, which represents an intermediate stage of the transition to dependence. Using RTG, a nonselective *Kcnq* channel opener, we demonstrated that activation of this family of voltage-gated K^+ channels can reduce heavy ethanol consumption in “high-drinking” mice. While future studies should test the remaining hypotheses generated from this bioinformatics approach, it is also necessary to determine which subregion in the PFC (e.g., prelimbic or infralimbic medial, orbitofrontal, or anterior cingulate cortex) and NAc (e.g., core or shell) these adaptations are occurring with an additional emphasis on identifying the specific subcellular compartment (e.g., axon vs dendrite) or cell type (e.g., interneuron, projection neuron). Finally, functional studies should measure how CIE exposure affects K^+ channel influence on intrinsic excitability, action potential characteristics, and synaptic communication within the corticostriatal circuitry.

In recent years, personalized medicine approaches have proven useful for treating alcohol use disorders (Litten et al., 2015). For example, individuals with SNPs in *OPRM1*, *HTR3A/B*, or *GRIK1* genes moderate the reduction in heavy drinking by naltrexone, ondansetron, and topiramate treatment, respectively (Anton et al., 2008; Johnson, Seneviratne, Wang, Ait-Daoud, & Li, 2013; Kranzler, Armeli, et al., 2014; Kranzler,

Covault, et al., 2014; Oslin et al., 2003). There is evidence that SNPs in *KCNB1*, *KCND2*, *KCNJ6*, *KCNMA1*, and *KCNQ1/5* are associated with alcohol dependence or increased risk for developing an alcohol use disorder (Buhler et al., 2015; Clarke et al., 2011; Edenberg et al., 2010; Kendler et al., 2011; Namkung, Kim, & Park, 2005; Pan et al., 2013; Zuo et al., 2012). This emerging evidence that K^+ channel transcript levels correlate with drinking and polymorphisms in K^+ channels are related to alcohol dependence implicate select K^+ channels that may cause or maintain heavy alcohol intake and may represent a new relevant set of biomarkers for alcohol dependence. Thus, our preclinical data suggest that clinical laboratory studies are necessary to determine if these K^+ channels are relevant pharmacogenetic targets that may advance personalized medicine approaches for treating alcohol use disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Bioinformatic analyses of genetic regulation by ethanol dependence identified novel candidate K^+ channel genes.
- Analyses also confirmed the known genetic relationship between *Kcnq, Kcnj, and Kcnn* genes and alcohol drinking.
- Pharmacological validation of *Kcnq* as a pharmacogenetic target with retigabine-induced reduction in alcohol consumption in “high-drinking” mice demonstrates the potential utility of the personalized medicine approach.

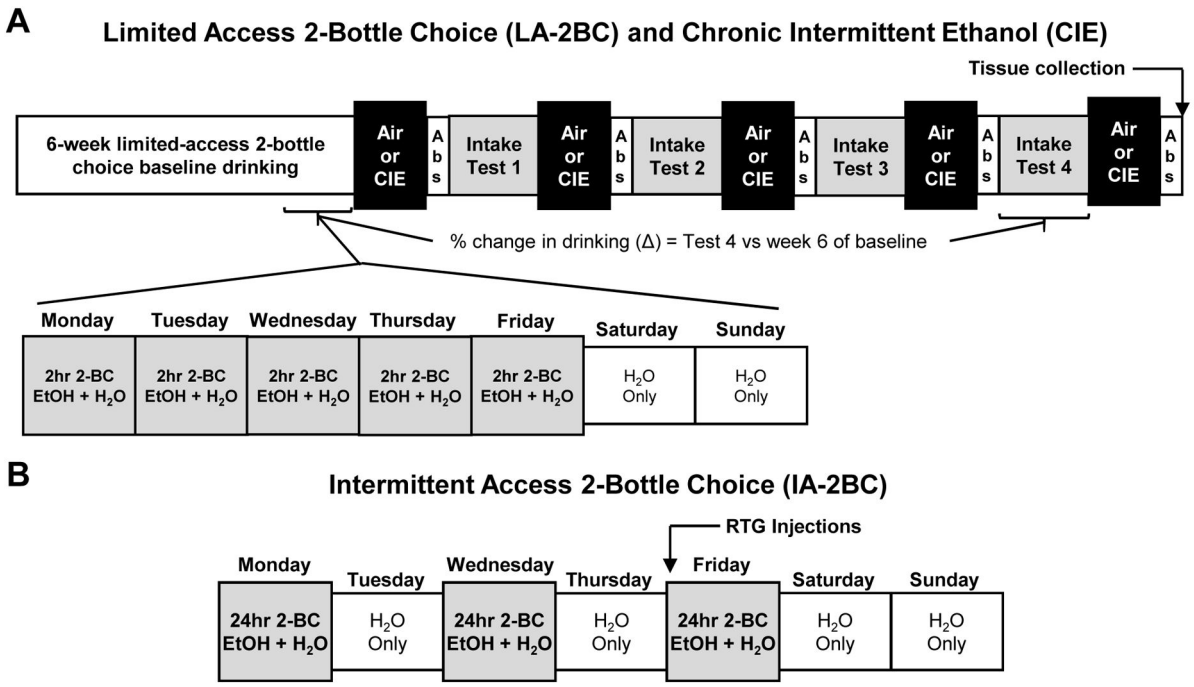

Figure 1. Schematic of the drinking models used in BXD RI strains of mice and the C57BL/6J parental strain

(A) The two-bottle choice limited-access voluntary drinking and chronic intermittent ethanol (CIE) exposure paradigm used in BXD RI strains of mice. Mice were given access to ethanol (15% v/v) for 2 hr using a limited-access 2-bottle choice (LA-2BC) procedure for 6 weeks to establish baseline ethanol intake. Mice then received either CIE vapor in inhalation chambers or air in control chambers. After chamber exposure, mice entered a 72 hr abstinence (Abs) period and were then tested for ethanol drinking for 5 days. This pattern of chamber exposure, abstinence, and voluntary drinking was repeated for 4 cycles. Brain tissue was collected 72 hr after the final chamber exposure. The percent change in drinking (Δ) was calculated as the percent change between Intake Test 4 and the 6th week of baseline drinking. (B) Schematic of the intermittent access 2-bottle choice (IA-2BC) paradigm used in C57BL/6J mice. In this model, mice had access to 20% ethanol (v/v) for 24 hr three days a week with a 24 hr forced abstinence period in between drinking sessions. Mice were then treated with retigabine after 7 weeks of baseline drinking.

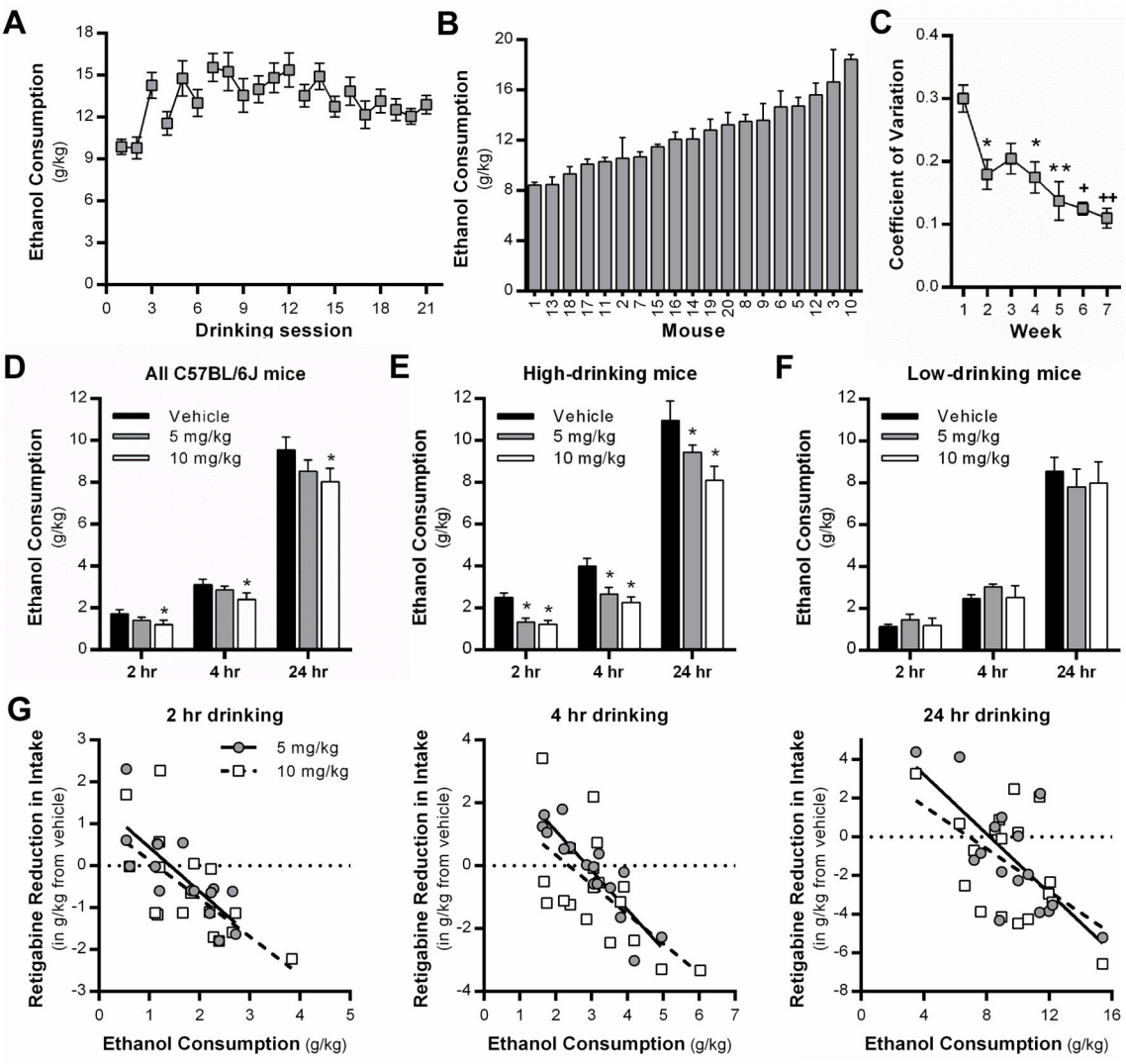

Figure 2. The Kv7 channel positive modulator, retigabine, reduced ethanol consumption in high-drinking, but not low-drinking male C57BL/6J mice

(**A**) Average weekly ethanol consumption and (**B**) intra-strain variability of 24 hr ethanol consumption in C57BL/6J mice during week 7 of the intermittent access model. (**C**) Despite the large between-subjects variation, the coefficient of variance during 7 weeks of access to ethanol in the intermittent model indicates increased within-subject stability (n = 19 mice, * $p < .05$ vs week 1, ** $p < .05$ vs week 1, $^{+}$ $p < .0001$ vs week 1, $^{++}$ $p < 0.001$ vs week 3). (**D**) Overall, retigabine (10 mg/kg, IP) reduced drinking when measured at 2, 4 and 24 hr in C57BL/6J mice (*$p < .005$ vs vehicle, main effect of treatment). (**E, F**) Retigabine (5 and 10 mg/kg) significantly reduced drinking in high-drinking, but not low-drinking C57BL/6J mice (*$p < .01$ vs vehicle, main effect of treatment). (**G**) HLM analyses revealed a strong negative linear relationship ($p = 0.025$) between vehicle drinking and RTG-induced alteration of ethanol intake across time, in that the greater the vehicle drinking, the more K_V7 channel activation reduced ethanol consumption.

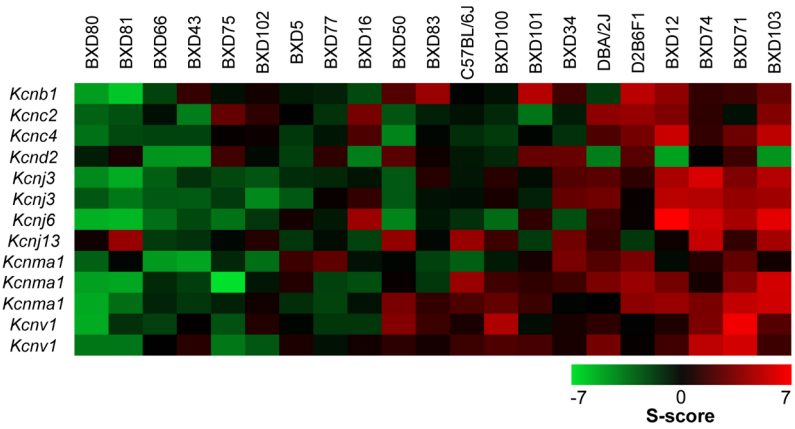

Figure 3. K^+ channel gene expression changes in the nucleus accumbens induced by chronic intermittent ethanol exposure of BXD strains of mice

Strain heat maps of significantly different gene expression responses based on S-score analysis in the nucleus accumbens (n = 21 strains). Positive S-scores (shown in red) indicate up-regulation by CIE exposure and negative S-scores (shown in green) indicate down-regulation by CIE exposure.

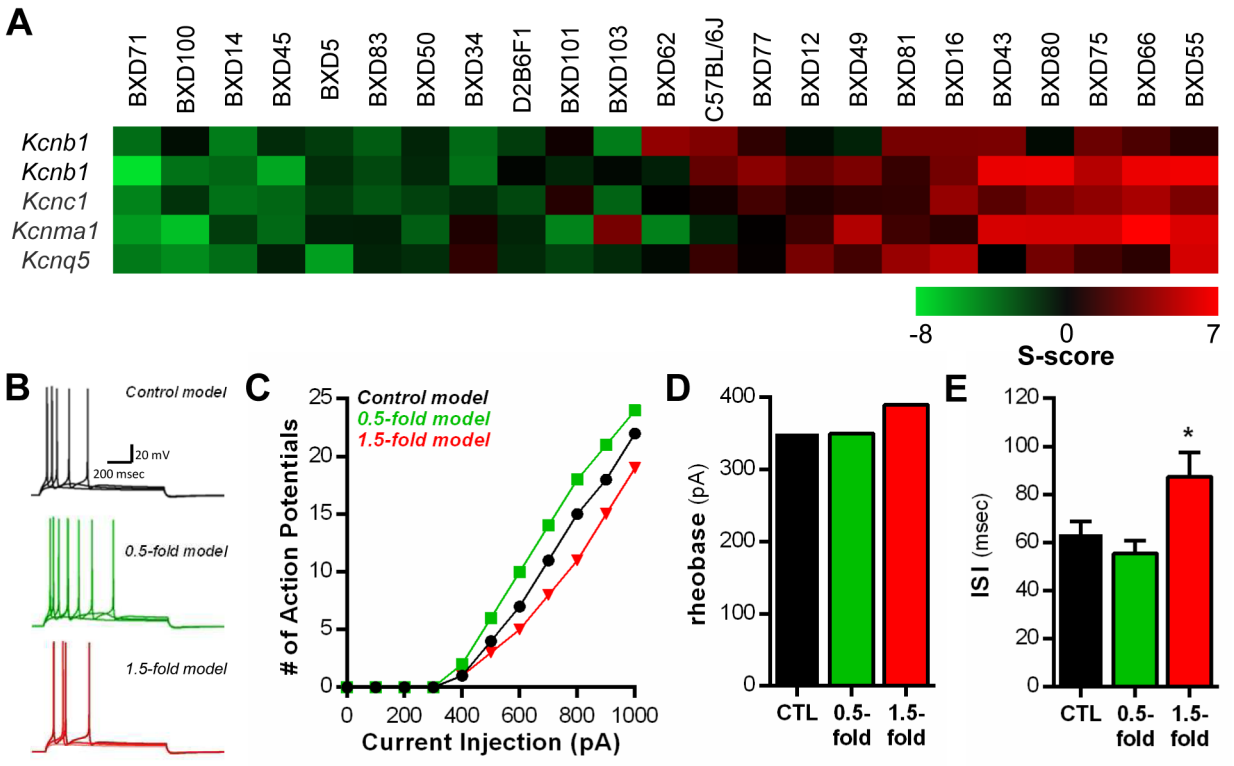

Figure 4. Intrinsic excitability changes revealed by computational modeling of K^+ channel gene expression adaptations in the prefrontal cortex of ethanol dependent BXD mice

(**A**) Strain heat maps of significantly different gene expression responses based on S-score analysis in the prefrontal cortex (n = 23 strains). Positive S-scores (shown in red) indicate up-regulation by CIE exposure and negative S-scores (shown in green) indicate down-regulation by CIE exposure. (**B**) Traces showing simulated evoked action potentials in response to depolarizing current steps (300, 400, 500 pA) under control conditions (black traces), 0.5-fold down-regulation of channel activity (green traces), and 1.5-fold increase in channel conductance (red traces). **(C)** Input-output relationship for each simulation. The number of action potentials produced by each current step is compared across the simulations. The 1.5-fold model revealed a reduction in the number of action potentials elicited by current injection at the soma. **(D, E)** The rheobase current and ISI are increased in the 1.5-fold model simulations, resulting in fewer action potentials evoked by current injection (*$p < 0.05$ vs. CTL stimulations).

Table 1

Genes for potassium channels in the nucleus accumbens that significantly correlated with voluntary consumption in non-dependent BXD strains of mice (n = 24 strains).

Gene	Probe set	Protein	r	R^2	P value	Functional Class	Subcellular Localization
Kcna1	1455785_at	K_V1.1	0.514	0.264	0.009	Delayed rectifier voltage-gated	Axon/Terminals
Kcnab3	1436019_a_at	$K_V\beta$3	0.461	0.213	0.022	β subunit, K_V1.5 modifier	Axon/Terminals
Kcnb1	1440838_at	K_V2.1	−0.404	0.163	0.050	Delayed rectifier voltage-gated	Somatodendritic
Kcnd2	1433015_at	K_V4.2	−0.684	0.468	1.25^{-04}	A-type voltage-gated	Dendrites
	1446065_at		−0.665	0.442	2.42^{-04}		
	1458167_at		−0.599	0.359	0.002		
	1459288_at		−0.580	0.337	0.002		
Kcne1l	1416640_at	minK	−0.610	0.372	0.001	β subunit modulator of K_V7.1 and K_V12.2	Axon/Terminals
Kcnj15	1435354_at	K_{ir}4.2	0.417	0.174	0.042	Inwardly rectifying	Unknown
Kcnj3	1444025_at	K_{ir}3.1	0.438	0.192	0.031	G protein-gated, inwardly rectifying	Dendrites
	1455374_at		0.430	0.185	0.035		
Kcnk4	1421419_at	K_{2P}4.1	0.453	0.205	0.025	Two-pore domain open rectifier	Intracellular
Kcnma1	1440728_at	K_{Ca}1.1	−0.502	0.252	0.011	Large conductance, calcium- and voltage-activated	Axon/Terminals
	1444973_at		−0.466	0.217	0.021		
	1457018_at		−0.426	0.182	0.037		
	1445774_at		−0.415	0.173	0.043		
	1430276_at		0.522	0.272	0.008		
Kcnn1	1419617_at	K_{Ca}2.1	0.469	0.220	0.020	Small-conductance, calcium-activated	Dendrites
Kcnn3	1459308_at	K_{Ca}2.3	−0.551	0.304	0.005	Small-conductance, calcium-activated	Dendrites
Kcnq1	1458161_at	K_V7.1	−0.734	0.538	1.78^{-05}	M-current delayed rectifier	Axon/Terminals
	1457781_at		−0.644	0.415	4.52^{-04}		
	1441588_at		−0.545	0.297	0.005		
	1442029_at		−0.476	0.226	0.018		
	1446255_at		−0.406	0.165	0.048		
Kcnq2	1440258_at	K_V7.2	−0.573	0.328	0.003	M-current delayed rectifier	Axon/Terminals
	1451595_a_at		−0.537	0.289	0.006		

Gene	Probe set	Protein	r	R^2	P value	Functional Class	Subcellular Localization
	1438260_at		−0.515	0.265	0.009		
Kcnq5	1446537_at	$K_V7.5$	−0.581	0.338	0.002	M-current delayed rectifier	Axon/Terminals
	1445837_at		−0.572	0.328	0.003		
	1457898_at		−0.561	0.315	0.004		
	1433110_at		−0.523	0.274	0.008		
Kcnt1	1458428_at	$K_{Ca}4.1$	−0.477	0.227	0.017	Sodium-activated, voltage-gated	Somatodendritic
	1439486_at		−0.470	0.220	0.020		
Kcnv1	1453273_at	$K_V8.1$	0.446	0.199	0.028	Modifier/silencer of K_V2 currents	Unknown
	1439333_at		0.421	0.177	0.040		

Table 2

Potassium channel genes in the prefrontal cortex that significantly correlated with voluntary consumption in non-dependent BXD strains of mice (n = 24 strains).

Gene	Probe set	Protein	r	R^2	P value	Functional Class	Subcellular Localization
Kcna5	1417680_at	K_V1.5	0.559	0.312	0.005	Delayed rectifier voltage-gated	Axon/Terminals
Kcnc3	1421980_at	K_V3.3	−0.448	0.200	0.031	Delayed rectifier voltage-gated	Axon/Terminals
Kcnd2	1447764_at	K_V4.2	0.630	0.397	0.001	A-type voltage-gated	Dendrites
Kcnj6	1425044_at	Kir3.2	−0.448	0.201	0.031	G-protein gated, inwardly rectifying	Dendrites
Kcnma1	1445774_at	K_{Ca}1.1	−0.456	0.208	0.028	Large conductance, calcium- and voltage- activated	Axon/Terminals
	1425987_a_at		−0.442	0.196	0.033		
Kcnq5	1445837_at	K_V7.5	−0.469	0.220	0.023	M-current delayed rectifier	Axon/Terminals
Kcns1	1421518_at	K_V9.1	0.560	0.314	0.005	Modifier/silencer	Unknown

Table 3

Potassium channel genes in the nucleus accumbens that are significantly altered by chronic intermittent ethanol exposure of BXD strains of mice (n = 21 strains).

Gene	Probe set	Protein	Fisher q-value	Empiric q-value	Functional Class	Subcellular Localization
Kcnb1	1423180_at	Kv2.1	2.93^{-19}	0.0067	Delayed rectifier voltage-gated	Somatodendritic
Kcnc2	1455258_at	Kv3.2	1.52^{-14}	0.0242	Delayed rectifier voltage-gated	Axons/terminals
Kcnc4	1425090_s_at	Kv3.4	2.65^{-14}	0.0257	Delayed rectifier voltage-gated	Axons/terminals
Kcnd2	1459288_at	Kv4.2	5.99^{-16}	0.0171	A-type voltage-gated	Dendrites
Kcnj3	1444025_at	$K_{ir}3.1$	1.93^{-23}	0.0015	G protein-gated, inwardly rectifying	Dendrites
	1455374_at		7.79^{-22}	0.0026		
Kcnj6	1441983_at	$K_{ir}3.2$	6.71^{-40}	1.30^{-5}	G-protein gated, inwardly rectifying	Dendrites
Kcnj13	1456418_at	$K_{ir}7.1$	6.03^{-13}	0.0346	Inwardly rectifying	Unknown
Kcnma1	1424848_at	$K_{Ca}1.1$	1.71^{-27}	4.62^{-4}	Large conductance, calcium- and voltage-activated	Axons/terminals
	1425987_a_at		6.57^{-24}	0.0013		
	1457018_at		1.83^{-11}	0.0492		
Kcnv1	1429741_at	Kv8.1	2.87^{-15}	0.0203	Modifier/silencer of Kv2 currents	Unknown
	1453273_at		2.00^{-13}	0.0312		

Table 4

Potassium channel genes in the prefrontal cortex that are responsive to chronic intermittent ethanol exposure of BXD strains of mice (n = 23 strains).

Gene	Probe set	Protein	Fisher q-value	Empiric q-value	Functional Class	Subcellular Localization
Kcnb1	1423179_at	Kv2.1	1.07^{-20}	.0382	Delayed rectifier voltage-gated	Somatodendritic
	1423180_at		8.77^{-62}	5.33^{-05}		
Kcnc1	1423559_at	Kv3.1	1.94^{-23}	0.0274	Delayed rectifier voltage-gated	Axons/terminals
Kcnma1	1424848_at	$K_{Ca}1.1$	1.75^{-57}	1.31^{-04}	Large conductance, calcium- and voltage-activated	Axons/terminals
Kcnq5	1433110_at	Kv7.5	1.18^{-27}	0.0181	M-current delayed rectifier	Axons/terminals

Table 5

Potassium channel gene S-scores in the nucleus accumbens that significantly correlated with the change in voluntary drinking after chronic intermittent ethanol exposure of BXD mice.

Gene	Probe set	Protein	r	R^2	P value	Functional Class	Subcellular Localization
Kcna4	1422255_at	Kv1.4	0.477	0.227	0.029	A-type voltage-gated	Axon/Terminals
Kcnh2	1449544_a_at	Kv11.1	0.527	0.277	0.014	Inwardly rectifying voltage-gated	Unknown
Kcnh7	1427634_at	Kv11.3	0.467	0.218	0.033	Voltage-gated	Unknown
Kcnj1	1418613_at	$K_{ir}1.1$	−0.538	0.289	0.012	Inwardly rectifying	Unknown
Kcnj8	1418142_at	$K_{ir}6.1$	−0.451	0.203	0.04	ATP-sensitive	Dendrites
Kcnj11	1455417_at	$K_{ir}6.2$	0.484	0.235	0.026	ATP-sensitive	Dendrites
Kcnj16	1435094_at	$K_{ir}5.1$	−0.455	0.207	0.038	Inwardly rectifying	Unknown
Kcnq1	1459443_at	Kv7.1	−0.507	0.257	0.019	M-current delayed rectifier	Axon/Terminals
	1449464_at		−0.451	0.204	0.04		
Kcnq5	1457587_at	Kv7.5	0.625	0.391	0.002	M-current delayed rectifier	Axon/Terminals
Kcns1	1421518_at	Kv9.1	−0.545	0.297	0.011	Modifier/silencer of Kv2 currents	Unknown
Kcns2	1457325_at	Kv9.2	0.472	0.223	0.031	Modifier/silencer of Kv2 currents	Unknown
Kcnv2	1440537_at	Kv8.2	0.538	0.29	0.012	Modifier/silencer of Kv2 currents	Unknown

Table 6

Potassium channel gene S-scores in the prefrontal cortex of BXD mice that significantly correlated with the change in voluntary drinking induced by ethanol dependence.

Gene	Probe set	Protein	r	R^2	P value	Functional Class	Subcellular Localization
Kcna7	1450490_at	Kv1.7	−0.558	0.311	0.006	Delayed rectifier voltage-gated	Axon/Terminals
Kcnc1	1456965_at	Kv3.1	−0.459	0.211	0.028	Delayed rectifier voltage-gated	Axon/Terminals
Kcnd2	1447764_at	Kv4.2	−0.446	0.199	0.033	A-type voltage-gated	Dendrites
Kcne1	1420672_at	minK	−0.460	0.211	0.027	β subunit modulator of Kv7.1 and Kv12.2	Axon/Terminals
Kcnh3	1459107_at	Kv12.2	0.482	0.233	0.020	Voltage-gated	Unknown
	1445805_x_at		0.477	0.228	0.021		
Kcnj9	1450712_at	Kir3.3	0.449	0.202	0.032	G-protein gated, inwardly rectifying	Dendrites
Kcnk3	1425341_at	$K_{2P}3.1$	0.640	0.409	0.001	Two-pore domain open rectifier	Intracellular
Kcnmb2	1431844_at	BK-β2 subunit	−0.420	0.176	0.046	Auxillary subunit of $K_{Ca}1$ channels	Axon/Terminals