Fine Mapping a Quantitative Trait Locus for the Anxiolytic-like Response to Acute Ethanol in BXD Recombinant Inbred Strains

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Abstract

Initial responses to acute ethanol have proven to be heritable predictors of an individual's proclivity for ethanol consumption and long-term risk for alcohol dependence, and provide a convenient model for investigating the genetic correlates of susceptibility to alcoholism. Anxiety attenuation is an acute ethanol response of particular importance because anxiolysis is considered a primary motivator for habitual drinkers and substantial comorbidity exists between anxiety disorders and alcohol dependence. Our lab has used the BXD panel of recombinant inbred mice and light-dark box model of anxiety to dissect the genetic variation of the anxiolytic-like response to acute ethanol. All strains received IP injections of ethanol (1.8g/kg) or physiological saline 5 minutes prior to entering the light-dark box. Global gene expression levels in prefrontal cortex (PFC) and nucleus accumbens (NA) were measured from both treatment groups using Affymetrix Mouse 430 2.0 microarrays. As presented at a previous RSA conference, we have identified a quantitative trait locus (QTL) significantly associated with acute ethanol-induced anxiolysis (*Etanq1*) on chromosome 12. The calculated support interval for *Etanq1* initially spanned nearly 18 megabases (Mb's) and encapsulated 132 genes. Here, we present the results of our attempt to fine map *Etanq1* by adding novel BXD strains from an advanced intercross that carry informative recombinations within the QTL's critical region, as part of an ongoing effort to identify the quantitative trait gene(s) (QTG) underlying the QTL. The additional strains have increased the LOD score of *Etanq1* to 5.6 from 5.1 and reduced the length of the QTL's support interval by over 14 Mb's. The updated interval now spans the chromosome from 68.9 to 72.3 Mb's and contains 87 fewer genes, greatly reducing the number of potential candidate genes. We used the large microarray datasets to facilitate the process of screening for candidate genes by conducting expression QTL (eQTL) analysis. Genes showing cis linkage within the *Etanq1* region are considered strong candidates, since the local sequence polymorphisms driving the allele-specific differences in expression may be modulating the degree of anxiolysis induced by acute ethanol. We have identified 14 cis eQTL's with LOD scores ≥ 3 in the support interval representing 6 unique genes. The expression patterns for 2 of these 6 genes were highly associated with our behavioral measures; Ninein and Trim9.

Anxiety-Related Behavior Protocol

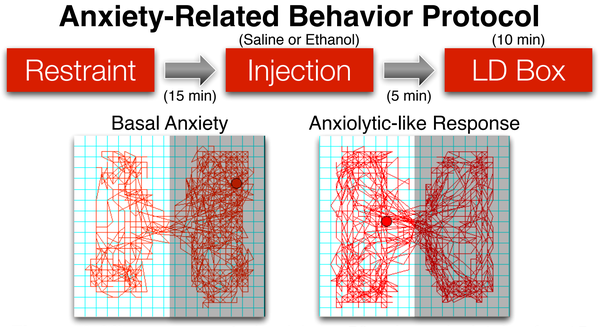

Figure 1 Mice were restrained in a 50 mL conical tube for 15 minutes to increase baseline anxiety. Following an IP injection of saline or ethanol, mice were put back in their home cage for 5 minutes then placed in the light-dark box apparatus. An increase in the distance traveled in the light side of the box was interpreted as a reduction in anxiety.

% Distance Traveled in the Light

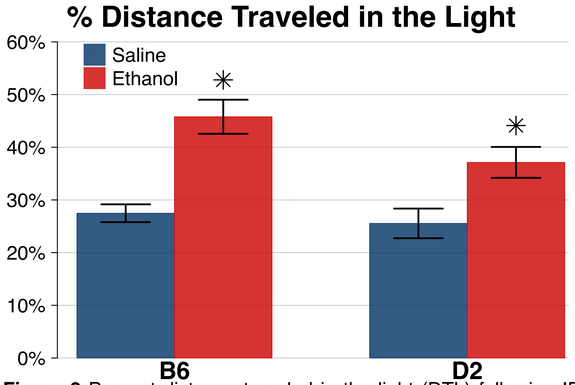

Figure 2 Percent distance traveled in the light (DTL) following IP injection of saline or 1.8g/kg of ethanol across the BXD RI progenitors, C57BL/6 (B6) and DBA/2 (D2) mice. In both strains ethanol significantly increased the distance mice traveled in the light side of the box, demonstrating the anxiolytic-like response to acute ethanol. Significance was determined by ANOVA with Tukey *post-hoc* analysis (✳ $p < 0.01$ versus within strain saline).

QTL for % Distance Traveled in the Light

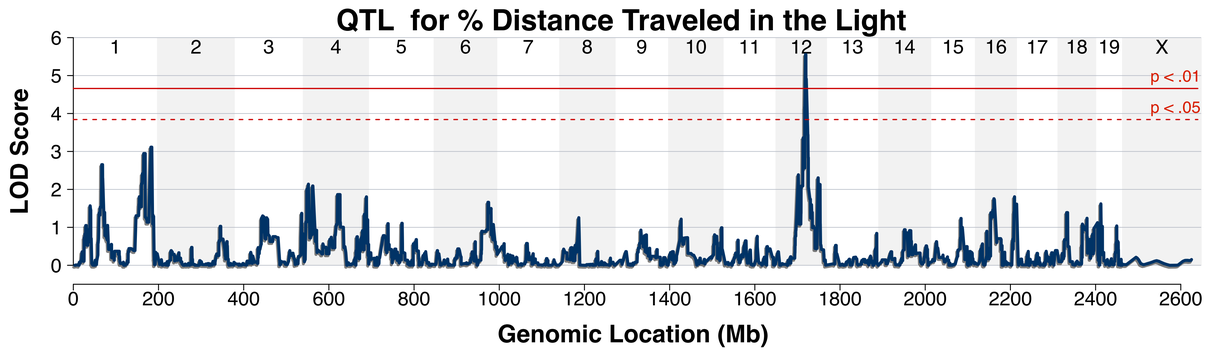

Figure 3 Genome-wide interval mapping results for DTL following ethanol treatment for 37 BXD RI strains. As we have previously reported, there is a significant QTL for ethanol-induced anxiolysis (*Etanq1*) on chromosome 12. The novel BXD RI strains we have since added further increased the significance of this QTL. 10,000 permutations were used to determine genome-wide significance thresholds.

Etanq1 Fine Mapping

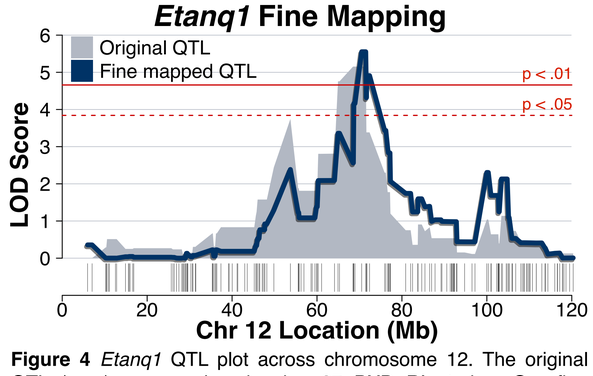

Figure 4 *Etanq1* QTL plot across chromosome 12. The original QTL (grey) was produced using 27 BXD RI strains. Our fine mapping effort has increased the QTL's LOD score, narrowed its peak and shifted the region of peak association slightly distally. Vertical lines indicate location of genetic makers used for mapping.

Etanq1 98% Support Intervals

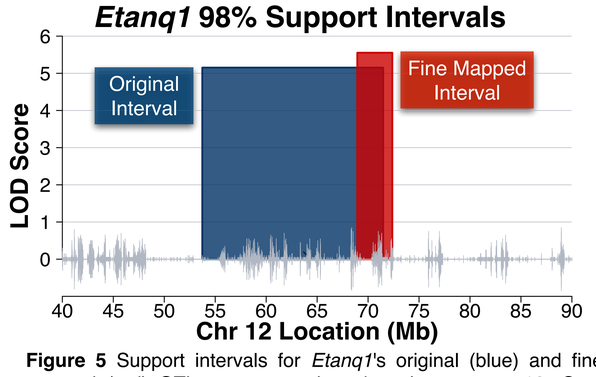

Figure 5 Support intervals for *Etanq1*'s original (blue) and fine mapped (red) QTL across a subsection chromosome 12. Grey seismograph indicates number of polymorphisms per one kb (1×20^{-1}) between B6 and D2. The fine mapped support interval is just under 1/5th the length of the original and is centered around a chromosomal region that is highly polymorphic.

Table 1: Support Interval Comparison

Support Interval Features	Original	Fine Mapped
Length	17.75 Mb	3.44 Mb
Peak LOD	5.1	5.6
Peak location	70.52 Mb	70.52 Mb
Upstream border	53.73 Mb	68.94 Mb
Downstream border	71.48 Mb	72.37 Mb
Number of genes	132	45

Etanq1 Linkage Disequilibrium Map

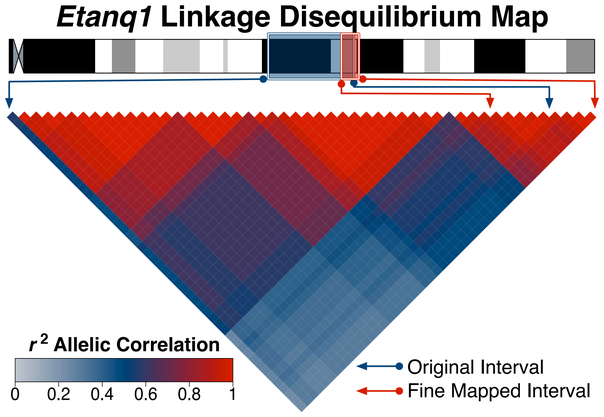

Figure 6 Linkage disequilibrium across original and fine mapped support intervals for *Etanq1*.

Table 2: *Etanq1* Candidate QTG's

Candidate Gene	Description	Mb	Coding SNP	cis eQTL Source	Peak LOD
D130076G13Rik	Riken cDNA D130076G13	70.54	No	NA Salline	9.1
Atp5s	Atp synthase H+ transporting, mitochondrial F0 complex	70.65	Yes	NA Saline	5.2
Map4k5	Mitogen-activated protein kinase 5	70.80	Yes	NA Saline	5.8
Nin	Ninein (GSK3β Interacting Protein)	70.94	Yes	NA Ethanol	6.1
Pygl	Liver glycogen phosphorylase	71.11	Yes	PFC Saline	3.6
Trim9	Tripartite motif protein 9	71.17	No	NA Saline	9.1

Table 2 Candidate genes within the fine mapped *Etanq1* region were identified by selecting for probesets with a significant QTL within 5 Mb's of the gene's physical location. The affyGG package for R (Alberts, 2007) was used to screen for cis eQTL's that are likely the product of probe sequence polymorphisms affecting hybridization, rather than genuine allele specific differences in expression. Non-synonymous coding SNP's were identified using GeneNetwork (http://www.genenetwork.org) and Ensembl's BioMart (http://www.biomart.org).

Materials & Methods

BXD RI Mice: Our study includes 37 male BXD strains and their progenitor strains C57BL/6J (B6) and DBA/2J (D2) strains. Each RI strain consisted of 5-8 mice per treatment and and each progenitor strain consisted of 16 mice per treatment. The novel BXD RI strains were derived from two independent advanced intercrosses between the B6 and D2 progenitor strains (Peirce *et al.*, 2004) and were imported from Oak Ridge National Laboratory.
Microarray Analysis: Mice were euthanized 4 hours post IP injection by cervical dislocation and dissected immediately thereafter. Medial prefrontal cortex and nucleus accumens tissue was isolated and subjected to RNA extraction as previously described (Kerns, 2005). Pooled samples of 4-5 mice were processed according to the Affymetrix GeneChip standard protocol and hybridized to Mouse Genome 430 2.0 arrays. RMA (Irizarry, 2003) was used to normalize the data and calculate expression summaries.
QTL Analysis: QTL mapping was conducted using GeneNetwork (Chesler, 2004) and the qtl package for R (Broman, 2009). Confidence intervals for QTLs were calculated using 98% Bayes credible intervals as recommended by Manichaikul (2006).

NA Expression Correlation Analysis with Anxiolytic-like Response to Ethanol

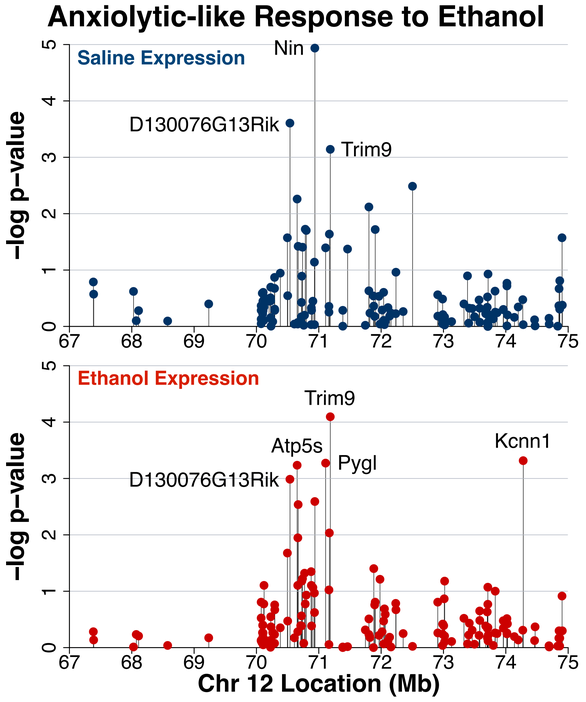

Figure 7 Correlation between DTL following ethanol treatment and expression level was measured for all gene's physically located within the above subsection of chromosome 12 and plotted against each gene's physical location. The effects of linkage disequilibrium cause many genes in the region to correlate somewhat with DTL. However, in NA tissue from saline treated (blue) and ethanol treated (red) mice ninein (Nin) and tripartite motif-containing 9 (Trim9) are stand out genes.

Results Summary

- **There is a significant QTL on chromosome 12 for ethanol associated anxiolytic-like behaviors (*Etanq1*).**
- **Fine mapping the QTL using novel BXD strains derived from an advanced intercross has reduced the QTL's 98% support interval to a 3.5 Mb region and increased its peak LOD score to 5.6.**
- ***Etanq1*'s fine mapped support interval encapsulates a haplotype block that is highly polymorphic between the B6 an D2 progenitor strains.**
- **We have identified 6 genes in this interval with significant cis eQTL's as candidates for the QTG underlying the anxiolytic-like response to acute ethanol.**
- **Our top priority candidate QTG's are Nin and Trim9. Future directions include validation studies using viral vector mediated gene expression.**

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