Confirmation of provisional quantitative trait loci for voluntary alcohol consumption: genetic analysis in chromosome substitution strains and F2 crosses derived from A/J and C57BL/6J progenitors

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Aims **Earlier research utilizing AXB/BXA recombinant inbred (RI) and AcB/BcA recombinant congenic (RC) strains of mice independently mapped provisional quantitative trait loci (QTL) for voluntary alcohol consumption (VAC) to common chromosomal regions. This study was designed to confirm QTL on chromosomes 2, 3, 5, 7, and 15 in an A/J (A) × C57Bl/6J (B6) F2 cross, and a panel of B6.A chromosome substitution strains (CSS).**

Methods and results **A × B6F2 mice, CSS, and A/J and C57BL/6J progenitors were tested for VAC. Previously identified QTL regions were targeted for genotyping in the A × B6F2 mice. Among the A × B6F2 mice, significant differences in VAC were associated with loci on chromosome 2 (peak marker D2Mit367) and chromosome 3 (D3Mit189). Additionally, a significant interaction was observed between loci on chromosome 15 (D15Mit245) and chromosome 2 (D2Mit367). A survey of the CSS panel provided further evidence for VAC QTLs on chromosomes 2 and 15. In the CSS panel, lower ethanol consumption was observed in those strains carrying the A/J 2 or 15 chromosome on a B6 background. This finding is consistent with the allelic influences observed in A × B6F2 mice in this study and those reported previously in the RI and RC strains of mice. Specifically, A/J alleles were associated with decreased ethanol consumption whereas C57BL/6J alleles were associated with increased ethanol consumption.**

Conclusion **The present results confirm previously reported QTL, on chromosomes 2 and 15 for VAC in RI and RC strains. Collectively, the regions on chromosomes 2 and 15 have now been replicated in at least three independent crosses derived from the A/J and C57BL/6J progenitors. The identification of potential candidate genes for the chromosome 15 QTL is discussed in the context of an in-silico analysis.** ***Pharmacogenetics and Genomics*** **18:1071–1082 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Alcoholism is a complex disorder that must be understood within the context of a vulnerability, which arises from the interaction between both genetic and environmental factors. The genetic contributions to the expression of vulnerability to alcoholism have been demonstrated across several lines of research [1–3]. Particularly compelling has been evidence from twin studies. It has been estimated that approximately 50–60% of the variability in alcoholism is associated with genetic factors [2,4,5]. However, the data fail to indicate a classic, or Mendelian, pattern of inheritance [6]. Rather, it is posited that the expression of alcoholism is a qualitative trait mediated by two or more genes and their interaction with the environment. However, the number of genes, their chromosome positions, and their roles in determining vulnerability to alcohol abuse remain to be clarified [7].

One approach for examining genetic factors contributing to differences in voluntary alcohol consumption (VAC) has been through the use of mouse models to identify quantitative trait loci (QTL). The genetic architecture of VAC (i.e. number of gene loci and dominance), as well as the chromosomal location of the genes underlying VAC have been studied primarily by cross breeding and recombinant techniques where a phenotype is followed through segregation and recombination of its genes across several types of matings and generations. A comparison of existing studies reveals that common QTL for VAC have been identified on a number of chromosomes [8–12]. For example, using B × D recombinant inbred (RI) strains derived from a C57BL/6J (B6) × DBA/2J (D2) cross [13] putative QTL for the consumption of sweetened and unsweetened 10% alcohol solutions were identified on Chrs 2, 4, 7, and 9. Reanalysis of these data using a larger

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marker set as well as bidirectional selective breeding confirmed QTL originally mapped to Chrs 2 (49 cM) and 9 (29 cM), with suggestive loci on Chrs 3 (77 cM), 4 (56 cM), 7 (58 cM), and 15 (43 cM) [11,14]. Using an F2 generated from a B6 × D2 cross, Tarantino et al. [12] found significant QTL on Chrs 1, 4, and 9. A backcross breeding protocol [(B6 × D2) × B6 or D2] was used to identify male-specific and female-specific QTL for alcohol preference [9,15]. A female-specific QTL (Alcp2) was found on Chr 11. Belknap and Atkins [16] used meta-analysis to examine the reliability of alcohol preference QTL across eight studies that had used crosses of the B6 and D2 progenitors. The most consistent evidence was for significant QTL on Chrs 2, 3, 4, and 9.

The present authors have phenotyped the C57BL/6J, A/J, AXB/BXA RI and AcB/BcA recombinant congenic (RC) strains of mice for sensitivity to the effects of ethanol. The differential sensitivity of the A/J and C57BL/6J strains of mice to the effects of ethanol has been demonstrated to generalize across several phenotypes, including ethanol-induced locomotor activation in an open field as well as VAC. Quantitative trait locus analyses of VAC in the AXB/BXA RI and AcB/BcA RC mice were performed on both alcohol preference and absolute alcohol intake (gm/kg) scores [10,17]. Analysis of VAC in the AXB/BXA RI strains found putative loci on QTLs on chromosomes 2, 7, 10, 12, 15, and 16. The results indicated that the Chr 2 QTL accounted for 30% of the genetic variance. Additional work in the AcB/BcA RC strains led to the identification of QTLs on chromosomes 2, 3, 5, 7, 10, and 15 [17]. Thus, to date the present authors have demonstrated good evidence to support the existence of significant QTLs regulating VAC.

The objective of this study was to confirm the provisional QTL for VAC, on chromosomes 2, 3, 5, 7, and 15, previously identified in the RI and RC strains of mice. This was accomplished by completing a targeted genetic analysis of VAC in an A × B6 F2 cross, derived from the A/J and C57BL/6J, and by conducting a phenotypic survey of VAC across a panel of B6.A chromosome substitution strains (CSS) mice. This study represents one component of a multistage strategy for mapping QTLs, in which provisional associations are confirmed in independently derived crosses. It has been suggested that a multistage approach for linkage reduces the probability of chance associations [18].

The B6.A CSS consists of a panel of 21 strains, each carrying a different A/J chromosome transferred intact onto the B6 background. The unique aspect of CSS (compared with RI or RC strains) is that the genetic background is homogeneous. Screening CCS strains has been proposed as a simple, rapid method of determining the chromosomal location of QTLs [19]. This method consists of phenotyping the entire panel of B6.A CCS strains; individual strains showing significant differences compared with the B6 progenitor are known to carry a QTL. The B6.A strains have been used to identify QTLs for several complex traits, including anxiety [20] and cancer [21]. The CSS model has been demonstrated to be more efficient than traditional crosses in that it requires the testing of fewer progeny to detect a specific effect, or allows smaller effects to be detected with a given number of subjects [22]. Furthermore, the ability to detect QTLs is enhanced as all, but the target chromosome, are fixed, eliminating variance owing to segregating QTLs on other chromosomes. Stylianou *et al.* [23] recently reported the detection of a QTL on chromosome 3 in the B6.A CSS panel that was not detectable in a traditional B6 × A/J F2 cross. The effect size of the QTL was sufficiently large to be detected in the F2; however, it appears likely that it was masked by epistatic loci segregating in the mixed background of the B × A intercross. These authors note that genetic background can have unpredictable effects, and conclude that mapping should be conducted with a combination of different cross designs, as utilized in the current series of experiments.

Methods

Mice

Breeder pairs of the A/J, B6, and B6.A CSS (20 strains) were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Breeder pairs of A/J × B6 F2 and all CSS strains were set up in a climate-controlled colony in the Research Institute of the McGill University Health Centre, with strict adherence to protocols ensuring uniformity with regard to housing, lighting, handling, cage changing, noise, and exposure to stressors.

B6 and AJ progenitor mice were bred concurrently to assure a consistent exposure to environmental conditions. The mice were housed with same-sex littermates until 8 weeks of age when testing commenced. All breeder pairs and pups were housed in an animal colony controlled for temperature and humidity on a 12-hour day/night cycle (lights on between 6 am and 6 pm), in standard shoe-box solid bottom cages with β chip bedding and nestlet pads with Harland Teklab mouse chow and water *ad libitum*. All procedures were approved by the Facility Animal Care Committee, in compliance with the Canadian Council on Animal Care.

A/J × C57BL/6J F2 crosses

F1 and F2 mice were constructed using reciprocal crosses of the progenitors. For example, F1 crosses (A/J × B6F1, B6 × A/JF1) were mated in all possible combinations to produce F2 (AB6F1 × AB6F1, AB6F1 × B6AF1, etc), and sex and cross type were considered in all subsequent

analyses. F2 mice were phenotyped for VAC and subsequently genotyped using SSLP markers as described below. Before selection for genotyping, all mice were assessed for excessive variability in the expression of the ethanol preference phenotype (ethanol/total fluid). This was accomplished through the calculation of a coefficient of variation for each mouse. The mice with coefficient of variation greater than 1 (two mice) were excluded and not considered in further analyses. Similarly, mice with missing data (14 mice), due to factors such as spillage, were not genotyped or included in subsequent analyses. In addition to ethanol preference, measures for absolute ethanol intake (alcohol consumed/body weight, gm/kg) were obtained. A total of 355 F2 mice were included in the VAC database, and 201 F2 mice representing the top and bottom 28% from the extremes of the phenotypic distribution were subsequently genotyped. Approximately equal numbers of females (102) and males (99) were genotyped.

Chromosome substitution strain panel

Males and females for each of 20 CSS strains and both parental A/J and C57BL/6J strains were assessed for VAC. CSS breeders were purchased for 19 autosomes and the X chromosome. A total of 397 CSS male and female mice were evaluated, in addition to 79 C57BL/6J (43) and A/J (36) mice. Before conducting an analysis of variance (ANOVA), the strain means were assessed for outliers (>3 SDs) and subjected to a split plot analysis to determine the reliability of the strain means (phenotypic data from 10 CSS mice, distributed across strains were not included in the analysis). A total of 466 mice from the CSS and progenitor strains (209 female and 257 male mice), yielding an average of 19 mice/strain, were tested. Use of CSS strains for initial mapping of QTL does not require genotyping.

Voluntary ethanol consumption phenotype

Naive male and female mice were tested for alcohol consumption at 8 weeks of age. Mice were singly caged in stainless steel hanging cages containing nestlet pads. Standard mouse chow was available *ad libitum*, and fluids were presented in two glass drinking tubes mounted on the front of the cages. The mice were habituated to singleton housing conditions and drinking tubes for 1 week before testing. Following habituation, the mice were screened for their levels of alcohol intake by receiving 4 days of forced exposure to a 10% (w/v) solution of alcohol, followed by 3 weeks of free choice between water and 10% alcohol. Alcohol solutions were prepared from 95% ethyl alcohol in tap water. The drinking tubes were weighed every second day and each animal was weighed weekly. Alcohol and water bottles were switched from side to side at each measurement, to avoid position bias. Measures obtained from this experimental design included (i) alcohol preference calculated as the volume of alcohol consumed/total volume of fluid consumed and (ii) absolute alcohol consumption calculated as the grams alcohol consumed/body weight (kg)/day. Mean absolute ethanol intake and preference values were calculated for individual animals based upon the intake of ethanol observed during the third week of testing. Note that for the VAC testing, the A/J, B6, CSS, and F2 mice were tested together in batches of approximately 40–50 mice (mixed strain and sex), using housing conditions, procedures, and environmental conditions identical to past VAC studies on the RI and RC strains.

Genotyping

Genomic DNA samples were prepared from tail clips using standard extraction methods (tissue lysis, proteinase K, ethanol precipitation). MIT mouse MapPair primers were purchased from Invitrogen (Invitrogen Corporation, Carlsbad, California, USA) and PCR was conducted in 96-well microtiter plates using conditions recommended by the manufacturer [24]. PCR products were electrophoresed on agarose gels, stained, and scanned to a computer using a GeneGenius gel documentation system. Genotypes were scored independently by two individuals, entered into Microsoft Excel (Microsoft Corporation, 1 Microsoft Way, Redmond, Washington, USA), and exported to Map Manager QTX (Center of Excellence in Bioinformatics and Life Sciences, University of Buffalo, Buffalo, New York, USA) [25]. Previously identified QTL regions were targeted for genotyping in the A $\times$ B6F2 mice. For each provisional QTL identified in the RI and RC strains, at least three microsatellite markers were used for confirmation of the QTL (two markers only in the case of chromosome 5). One marker was selected nearest to the peak of the QTL, with two flanking markers within the 95% confidence interval [1 logarithm of odds (LOD) support interval] of the QTL. A total of 19 markers were genotyped as shown with their cM locations in Table 1.

Data analysis

Each mouse is assigned a unique identifier that is used to track and coordinate all aspects of the data collection (study ID, date, batch, phenotypic data, tail clip, DNA tube, genotypes). Data were coded and entered into a central tracking database using Microsoft Excel and Prism (Graphpad Software Inc., La Jolla, California, USA). Subsequent statistical analysis was conducted using the microcomputer version of SPSS (version 13.5 for Windows, SPSS Inc., Chicago, Illinois, USA). Data were tested for normality and outliers using the SPSS Explore function, and scores outside the 95% confidence interval for each inbred CSS strain were removed from subsequent analyses.

Associations between genotypes and phenotypes were examined using a number of techniques including

Table 1 Markers selected for genotyping in the A/J × C57BL/6J F2 strains

Chromosome 2	cM
D2Mit367	26.2
D2Mit241	30.0
D2Mit90	37.0
D2Mit37	45.0
D2Mit229	99.0
D2Mit113	103.0
D2Mit265	105.0
D2Mit266	109.0
Chromosome 3	
D3Mit141	45.2
D3Mit189	49.7
D3Mit106	55.0
Chromosome 5	
D5Mit148	18.0
D5Mit182	21.0
Chromosome 7	
D7Mit159	27.8
D7Mit222	52.6
D7Mit66	57.5
D7Mit105	63.5
Chromosome 15	
D15Mit156	39.1
D15Mit158	46.9
D15Mit42	55.5
D15Mit245	58.9
D15Mit161	69.2

ANOVA, single-locus association analysis, and composite interval mapping. ANOVA was used to examine phenotypic differences among genotypes (homozygous A/J or B6, heterozygous) in F2 mice at each marker tested (including factors related to sex and cross type where appropriate). Each significant locus was retested for association using multiple regression. Corrections were made for multiple comparisons, on the basis of the number of markers and phenotypes assessed.

Linkage analysis was performed to confirm previously identified QTLs using Windows QTL Cartographer 2.5 [26]. Confirmatory QTL mapping in this study proceeded in two steps. Initially, an analysis at defined loci (simple marker associations) was performed. Confirmation in the F2 mice of previously identified QTLs on chromosomes 2, 3, 5, 7, and 15 was based upon a nominal P value of 0.01, as proposed by Lander and Kruglyak [27]. The α levels were adjusted for multiple tests using the false discovery rate. Second, an analysis of positions inferred between loci with statistical control for other loci known to affect the trait (composite interval mapping) was conducted. (Significant and suggestive QTL regions were selected as controls.) Permutation tests were performed on the data to empirically estimate the threshold for suggestive and significant loci (1000 permutations at 1-cM intervals).

The statistical analysis of the phenotypic differences between the C57BL/6J and CSS strains was accomplished using a two factor (strain × sex) ANOVA. The identification of informative CSS strains (i.e. those strains carrying a donor A/J chromosome that produced a significantly different phenotype compared with the C57BL/6J) was conducted using simple main effects contrasts (planned comparisons). A nominal P value of 0.01 was used as a threshold for establishing confirmation of loci *a priori* hypothesized to mediate the expression of VAC in the CSS strains. Novel QTL, identified in the CSS, will be considered tentative and require further study for confirmation.

In the cases of both the A/J (A) × C57Bl/6J (B6) F2 cross, and the panel of B6.A CSS, confirmation of identified QTL will be dependent upon the expression of concordant directionality of the allelic influences upon VAC.

Identification of potential candidate genes within QTL intervals was done using the in-silico strategy described in the discussion. This strategy takes advantage of public sequence, genotype, and expression databases that have characterized single nucleotide polymorphisms (SNPs) in multiple inbred strains of mice, including the A/J and B6.

Results

A/J × C57BL/6J F2

Mean VAC values for the F2 mice were assessed as a function of sex and F2 cross type (AB6, B6A). A two-way ANOVA (sex × cross type) indicated that overall there was no significant main effects for sex [$F(1,201) = 0.150$, $P = 0.699$] or cross type [$F(1,201) = 2.216$, $P = 0.138$] or sex × cross interactions [$F(1,201) = 0.074$, $P = 0.786$].

Allelic influences on the expression of voluntary alcohol consumption

Two-way ANOVAs (sex × marker genotype) yielded significant main effects for sex and sex by marker interactions. Therefore, analyses were conducted individually for the males and females and for the combined group. The results indicated that in the female subjects significant differences in ethanol preference were observed as a function of differences in allelic status (A, B6, heterozygous) for markers D2Mit367 [$F(2,99) = 6.31$, $P = 0.003$], D2Mit241 [$F(2,99) = 6.949$, $P = 0.002$], D2Mit290 [$F(2,99) = 5.775$, $P = 0.004$], and D2Mit37 [$F(2,99) = 6.054$, $P = 0.003$] on chromosome 2, and D3Mit189 [$F(2,99) = 5.37$, $P = 0.006$] on chromosome 3. Linear regression indicated that these loci accounted for 15.4% of the variance in ethanol preference. The confirmed loci are presented in Table 2.

Utilizing the statistical criteria established by Lander and Kruglyak [27], for a confirmatory analysis, the present results confirmed the previously identified loci on chromosomes 2 and 3. The results indicated that for loci on chromosomes 2 and 3, the C57BL/6J allele was associated with a higher ethanol preference relative to

Table 2 Analysis of previously identified QTLs in A × B6 F2 mice

Markers	Loci (cM)	Genome coordinates	Sex	Chromosome	*P* value[a]
D2Mit367	26.2	33455019–33455169	F	2	$P<0.01$
D2Mit90	37.0	65466716–65466799	F	2	$P<0.01$
D2Mit37	45.0	74494612–74494783	F	2	$P<0.01$
D3Mit189	49.7	101104657–101104789	F	3	$P<0.01$
Interaction					
D2Mit367 ×	(26.2)	33455019–33455169	F+M	2 × 15	$P<0.01$
D15Mit245	(58.9)	94106731–94106849			

Confirmed QTLs and interactions are presented.
QTL, quantitative trait loci.
[a]For confirmation, a nominal *P* value of 0.01 (corrected for multiple comparisons) was required, as described in Lander and Kruglyak [27].

the A/J allele. In contrast to the analysis of females, no significant associations were observed with the male F2 mice.

An analysis of the differences in ethanol preference, within the combined group (males and females), was observed with D2Mit367 [$F(2,197) = 3.287$, $P = 0.039$], D2Mit241 [$F(2,194) = 3.334$, $P = 0.038$], D2Mit290 [$F(2,198) = 4.1$, $P = 0.018$], and D2Mit37 [$F(2,187) = 3.969$, $P = 0.020$] on chromosome 2, and, D3Mit141 [$F(2,192) = 0.4.359$, $P = 0.014$] and D3Mit189 [$F(2,197) = 3.523$, $P = 0.031$] on chromosome 3.

Previously published findings [17] indicated that the expression of VAC might be a function of the interactions between QTLs on chromosomes 2 and 15. Therefore, the present analysis examined the extent to which the phenotypic level of alcohol preference exhibited by the F2 mice are dependent upon the particular combination of QTLs inherited on chromosomes 2 and 15. The findings of an interaction between QTL on chromosomes 2 and 15 were confirmed. A significant interaction was observed between D15Mit245 and D2Mit367 [$F(2,183) = 3.640$, $P < 0.007$] in the combined male and female sample (Table 2). A graphical representation of the interaction between QTL on chromosomes 2 (D2Mit367) and 15 (D15Mit245) is presented in Fig. 1.

Additionally, an analysis of ethanol preference was examined as a function of cross type and allelic status at each marker. In this particular analysis, the two crosses [AB6F2 (AB6 F1 female × AB6F1 male) and B6AF2 (B6AF1 female × B6AF1 male)] were compared using two-way ANOVA with cross type (AB6 vs. B6A) and marker genotype as factors. The results of two-way ANOVAs failed to indicate any effects of cross on chromosomes identified with QTLs.

Fig. 1

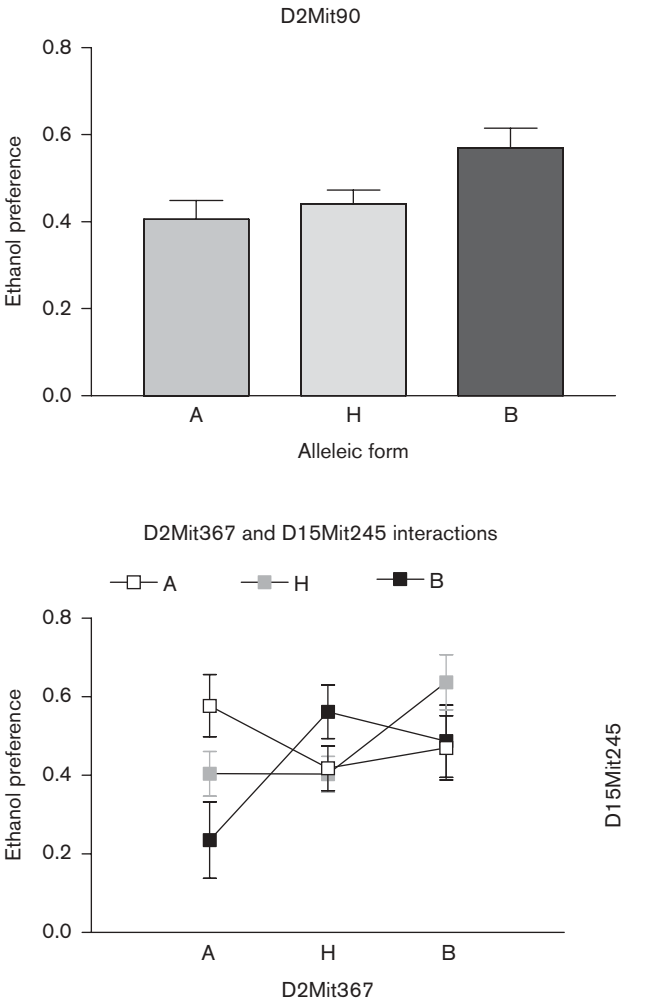


Illustration of ethanol preference (combined females and males) presented as a function of allelic status for the D2Mit367 quantitative trait loci (top panel) and as a function of the interaction between D2Mit367 and D15Mit245 (lower panel).

Quantitative trait loci analysis

A test of single locus associations was conducted to identify associations between the genotyped markers and the two measures of VAC (preference and absolute ethanol intake). Markers that were associated with ethanol preference, in female mice, are presented in Table 3. Loci in the range of 26–45 cM (D2Mit 367, D2Mit 90, D2Mit 37) were associated with ethanol

preference, and the peak likelihood ratio statistic (LRS) values were observed for D2Mit367 at 26.2 cM and D2Mit90 at 33 cM. Loci between 45.2 and 55 cM, on chromosome 3, were additionally associated with ethanol preference. A peak LRS value was obtained with D3MIt189 (49.7 cM). Similarly, associations were obtained with the analysis of absolute ethanol intake. No significant associations were identified in males.

Table 3 QTL Analysis (simple associations) derived from the output of WinQTL Cartographer using the A × B6 female F2 mice

Chromosome	Marker	cM	Genome coordinates	*P* value
2	D2Mit367	26.2	33455019–33455169	0.001*
2	D2Mit241	30	45160125–45160260	0.008
2	D2Mit90	37	65466716–65466799	0.001*
2	D2Mit37	45	74494612–74494783	0.005*
2	D2Mit229	99	168472818–168472963	0.946
2	D2Mit113	103	172997610–172997756	0.898
2	D2Mit265	105	174125905–174126009	0.919
2	D2Mit266	109	181802767–181802893	0.837
3	D3Mit141	45.2	–	0.017
3	D3Mit189	49.7	101104657–101104789	0.001*
3	D3Mit106	55	112166357–112166520	0.092
5	D5Mit148	18	32252471–32252619	0.116
5	D5Mit182	24	37817898–37818039	0.037
7	D7Mit159	27.8	57107058–57107205	0.574
7	D7Mit222	52.6	112725647–112725793	0.889
7	D7Mit66	57.5	119677145–119677306	0.794
7	D7Mit105	63.5	128355546–128355803	0.504
15	D15Mit156	39.1	71155215–71155358	0.500
15	D15Mit158	46.9	–	0.649
15	D15Mit42	55.5	98884619–98884804	0.666
15	D15Mit245	58.9	94106731–94106849	0.991
15	D15Mit161	69.2	–	0.923

QTL, quantitative trait loci.
*Confirmed QTL (based upon the criteria established by Lander and Kruglyak [27]). Nominal *P* values are below the 0.01 threshold. [The α levels were adjusted for multiple tests using the false discovery rate (FDR)].

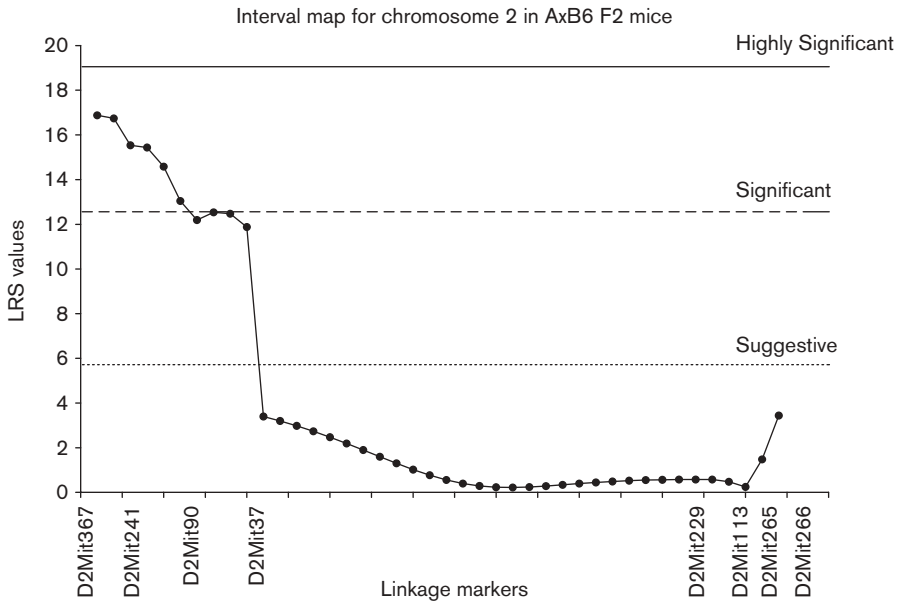
Composite interval mapping analysis was subsequently used to control for the influence of unlinked QTLs. Target loci associated with ethanol preference scores, in females, were mapped while controlling for the influence of other QTLs. The results indicated that a peak LRS value of 16.59 (LOD = 3.6, 1.5 LOD support interval = 17.3 cM) was obtained at 26.2 cM on chromosome 2, and a peak LRS value of 12.4 (LOD = 2.7, 1.5 LOD support interval = 8.5 cM) at 49.6 cMs on chromosome 3. The interval map illustrating the putative QTL on chromosome 2 (females) is presented in Fig. 2.

Chromosome substitution strain survey

Males and females for each of the 20 strains of the CSS panel and both parental strains A/J and C57BL/6J were assessed for VAC. A two-way ANOVA, with sex and strain (all CSS and progenitors), was used to examine both ethanol intake phenotypes (preference and gm/kg). Planned comparisons (simple main contrasts) were used to evaluate the significance of main effect differences between each CSS strain and the C57BL/6J parental strain.

The results of the two-way ANOVA indicated a significant main effect for both strain [$F(21,422) = 6.979$, $P < 0.01$] and sex [$F(1,422) = 28.099$, $P < 0.001$]. The results failed

Fig. 2



An interval map for the quantitative trait loci on chr 2 was derived from the output of WinQTL Cartographer using the A/J × C57BL/6J F2 female mice. The interval map data indicate that a peak LRS was obtained for the region bounded by the markers D2Mit3667and D2Mit241.

to indicate any significant sex × strain interaction effects [$F(21,422) = 1.098$, $P = 0.347$]. Overall, female mice exhibited significantly greater ethanol preference relative to the males. A post-hoc analysis indicated that, when compared with the parental C57BL/6J strain, the CSS shows evidence of ethanol preference QTLs on chromosomes 2, 13, 15, and 18. The CSS-2, CSS-15, and CSS18 strains exhibited significantly ($P < 0.01$) lower ethanol preference than the C57BL/6J. In contrast, the CSS-13 exhibited greater ($P < 0.01$) ethanol preference than the C57BL/6J. The strain distribution pattern is presented in Fig. 3. The informative CSS-2 and CSS-15 strains provide confirmation of QTL previously identified in RI and RC strains, based upon their identification as informative strains (a nominal P value of 0.01) and in concordance with allelic influences observed in F2 mice in this study and those reported previously in the RI and RC strains of mice (Fig. 3).

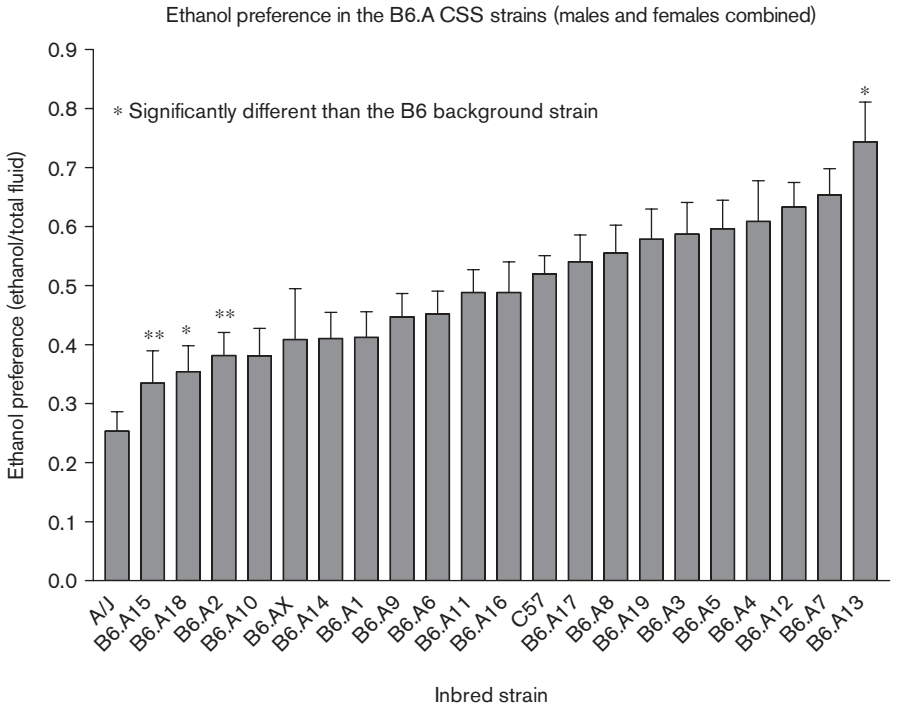
A two-way analysis of the absolute ethanol intake data revealed a similar pattern of results. A significant main effect for both CSS strain [$F(21,422) = 3.967$, $P < 0.01$] and sex [$F(1,422) = 108.671$, $P < 0.001$] was observed. The results failed to indicate any significant strain by sex interaction effects [$F(21,422) = 1.517$, $P < 0.07$].

Discussion

The present results confirm previously reported QTL for voluntary ethanol intake on chromosomes 2, 3, and 15 in A × B/B × A RI [10,17] and AcB/BcA RC [17] strains. QTL on these chromosomes exhibited consistent directionality of effects across multiple independently derived crosses. Collectively, the identification of QTLs on chromosomes 2 and 15 has now been replicated in multiple crosses (three and four times respectively), derived from the A/J and C57BL/6J progenitors. The replication of QTLs mapped for VAC, in the AXB/BXA RIS, AcB/BcA RCS, an A/J × B6 F2 cross, and the B6.A CSS panel are presented in Table 4. The confirmation of the QTL in multiple independent crosses suggests that they are unlikely to simply represent chance associations.

The QTLs identified on chromosomes 2 and 15 are in concordance with those reported in the literature (Table 4). The region identified on chromosome 2 overlapped with those, independently, in other crosses [9,11,12,16,28]. Similarly, the QTLs identified on chromosome 15 are close to regions identified in the literature [11,12,14,28,30]. In this study the CSS-2 and CSS-15 strains exhibited significantly lower ethanol consumption relative to the C57BL/6J progenitor strain. These results

Fig. 3



Mean ethanol preference (+SEM) for male and female chromosome substitution strains (CSS) mice. * denotes CSS strains, which are significantly different from the C57BL/6J at a nominal P value of 0.01 (corrected for multiple comparisons). ** denotes CSS strains (2 and 15) that confirm quantitative trait loci previously identified in recombinant inbred and recombinant congenic strains. The A/J and C57BL/6J progenitor strains are included in the figure as reference.

Table 4 Significant QTLs for VAC independently mapped in the A × B/B × A RIS, AcB/BcA RCS, A/J × B6 F2 cross, and the B6.A CSS panel

	A × B/B × A RI strains			AcB/BcA RC strains				A × B6 F2			B6.A CSS		Literature	
Chr	Peak marker	cM	Allelic influence	Peak marker	cM	Range cM	Allelic influence	Peak marker	cM	Allelic influence		Allelic influence	Range cM	Alcohol consumption – includes studies using RI panels or crosses with B6 or A/J progenitors
2	D2Mit74	107	A↓	D2Mit359	5	5–31.7	(AcB) B↑	D2Mit90	37	B↑	+ +	A↓	28–49	Melo *et al.*, 1996 [9]; Gehle and Irwin, 1998 [28]; Phillips *et al.*, 1998 [11]; Tarantino *et al.*, 1998 [12]; Bice *et al.*, 2006 [29]
				D2Mit241	30	30–37	(BcA) A↓							
3				D3Mit189	49.7	47–55	(BcA) A↓	D3Mit189	49.7	A↓	NS		49	Tarantino *et al.*, 1998 [12]
15	Pdgfb	46.8	A↓	D15Mit28	43.7	43.7–47.9	(AcB) B↑	Chr 2 × 15 interaction			+ +	A↓	39–57	Belknap *et al.*, 1997 [14]; Gehle and Irwin, 1998 [28]; Phillips *et al.*, 1998 [11]; Tarantino *et al.*, 1998 [12]; Vadasz *et al.*, 2000 [30]
				D15Mit42	59.2	54.5–69.2								

There was concordance in the directionality of allelic influences for QTLs on chromosomes 2 and 15, suggesting that these chromosomal regions harbor genes that influence VAC in mice.
1. Results for A × B/B × A RIS published by Gill *et al.* [10]; results from AcB/BcA RCS published by Gill and Boyle [17].
2. Peak marker identified by Map Manager QTX, and position provided in recombinant distance in centimorgans from centromere.
3. The directionality of allelic influences upon the expression of VAC across strains is presented: A(A/J); B(C57BL6J).
4. Significance of QTLs determined through permutation tests in Map Manager QTX, WinQTL Cartographer following guidelines of Lander and Kruglyak [27].
5. Genotyping of the AxB6 F2 was conducted with SSLP markers on chromosomes 2,3,5,7,15.
6. B6.A CSS + + indicates that the B6.A CSS carrying the designated A/J chromosome displayed a significantly different VAC phenotype compared with the B6 progenitor, indicating the presence of a QTL on the chromosome. NS indicates non-significant loci.
CSS, chromosome substitution strains; QTL, quantitative trait loci; RCS, recombinant congenic strain; RIS, recombinant inbred strain; VAC, voluntary alcohol consumption.

indicate that in the CSS panel, the transfer of chromosome 2 or 15 derived from the inbred A/J strain onto a B6 background is associated with lower ethanol consumption. This is consistent with the allelic influences observed in F2 mice in this study and those reported previously in the RI and RC strains of mice. Specifically, C57BL/6J alleles for QTLs on chromosomes 2 and 15 (AcB RC, F2) were associated with increased ethanol consumption and conversely, A/J alleles (RI, BcA RC) were associated with decreased consumption (presented in Table 4).

Although this study confirmed a QTL on chromosome 15, the region is too broad to effectively identify relevant candidate genes. However, the recent availability of bioinformatic tools and SNP maps for divergent strains of mice, including the AXB/BXA RI strains, allows for the narrowing of QTL regions through in-silico analysis [31]. Therefore, we demonstrate here how haplotype mapping was used to narrow the chromosome 15 QTL region, previously identified in the AXB/BXA RI strains mice (10), and significantly reduce the number of potential candidate genes. The premise was to identify genetic regions of identity by descent between strains that are unlikely to contain polymorphisms. The identity by descent regions are described as consisting of genetic variation among inbred strains of an ancestral nature.

Initially, the Ensembl database was used to identify the base pair boundaries for the markers flanking the chromosome 15 QTL (Table 4) identified in the RI strains, and confirmed in this study (the region contained the 95% confidence interval calculated from a bootstrap analysis of the RI data). An interval from 74 742 609–84 214 412 bp was identified. A search of the database, consisting of the NCBI-build 37, identified 274 genes within this region. Subsequently, the mouse phenome database was accessed to conduct a haplotype analysis (AXB/BXA RIs strains and phenotypes) and potentially reduce the size of the region of interest. A bivariate analysis was conducted to identify significant ($P < 0.01$) associations between ethanol preference and haplotype structure. The haplotype maps with the AXB/BXA RI strains, arranged as a function of ascending ethanol preference, revealed that only the region between 76 524 736 and 83 225 646 bp showed consistent significant correlations between the haplotype structure and ethanol preference (Fig. 4). The significant correlations are presented and identified in Fig. 4. This region was found to have 189 genes. The narrowed interval was used to analyze the coding regions in the A/J and C57BL/6J progenitor strains. Within this region, a survey of polymorphic sites produced a list of 33 unique genes producing nonsynonymous substitutions in amino acids of relevant proteins. *A priori*, the filtering of strong candidate genes is based upon the potential for changes in DNA sequence altering the amino acid makeup of the translated

Fig. 4

Loci	Observed	Etoh pref	0.186	0.154	0.162	0.210	0.218	0.231	0.233	0.235	0.235	0.239	0.249	0.274	0.282	0.292	0.305	0.348	0.354	0.366	0.368	0.377	0.433	0.459	0.468	0.477	0.595	0.614	0.541		
		n	13	13	13	7	8	15	9	12	12	11	8	11	13	15	14	9	9	12	10	13	10	13	13	6	15	12	16		
			A/J	A × B24/PgnJ	B×A17/PgnJ	B×A8/PgnJ	A×B5/PgnJ	A×B19/PgnJ	B×A7/PgnJ	B×A25/PgnJ	B×A11/PgnJ	A×B11/Pgn	A×B2/PgnJ	A×B8/PgnJ	A×B4/PgnJ	B×A12/PgnJ	B×A14/PgnJ	A×B6/PgnJ	A×B15/PgnJ	A×B12/PgnJ	A×B10/PgnJ	A×B1/PgnJ	A×B13/PgnJ	B×A24/PgnJ	B×A4/PgnJ	B×A16/PgnJ	B×A2/PgnJ	B×A13/PgnJ	C57BL/6J	*r*	*P*<0.01
74.082232	C/T		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	
74.420468	A/G		G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	.	
74.546985	A/C		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	
74.680379	A/G		A	A	A	A	G	G	G	G	G	G	A	G	G	G	A	A	G	G	G	A	G	G	G	G	G	G	G	−0.41	
74.802457	A/T		T	T	T	T	A	A	A	A	A	A	T	A	A	A	T	T	A	A	A	T	A	A	A	A	A	A	A	0.41	
74.98893	C/T		C	C	C	C	T	T	T	T	T	T	C	T	T	T	C	C	T	T	T	C	T	T	T	T	T	T	T	0.41	
75.136229	C/G		G	G	G	G	C	C	C	C	C	C	G	C	C	C	G	G	C	C	C	G	C	C	C	C	C	C	C	−0.41	
75.279271	A/G		G	G	G	G	A	A	A	A	A	A	G	A	A	A	G	G	A	A	A	G	A	A	A	A	A	A	A	0.41	
75.435472	A/T		T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	.	
75.739827	C/G		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	
75.893609	G/T		T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	.	
76.524736	C/T		T	T	T	T	T	T	C	C	C	C	T	C	C	C	T	T	T	C	C	T	C	C	C	C	C	C	C	−0.50	0.009
76.753842	A/C		C	C	C	C	C	C	A	A	A	A	C	A	A	A	C	C	C	A	A	C	A	A	A	A	A	A	A	0.50	0.009
76.886663	A/C		C	C	C	C	C	C	A	A	A	A	C	A	A	A	C	C	C	A	A	C	A	A	A	A	A	A	A	0.50	0.009
77.037767	C/T		C	C	C	C	C	C	T	T	T	T	C	T	T	T	C	C	C	T	T	C	T	T	T	T	T	T	T	0.50	0.009
77.135459	A/G		A	A	A	A	A	A	G	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.50	0.009
77.361647	C/G		C	C	C	C	C	C	G	G	G	G	C	G	G	G	C	C	C	G	G	C	G	G	G	G	G	G	G	0.50	0.009
77.655021	A/G		G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	.	.
77.990182	C/T		C	C	C	C	C	C	T	T	T	T	C	T	T	T	C	C	C	T	T	C	T	T	T	T	T	T	T	0.50	0.009
78.020723	A/G		G	G	G	G	G	G	A	A	A	A	G	A	A	A	G	G	G	A	A	G	A	A	A	A	A	A	A	0.50	0.009
78.182168	C/T		C	C	C	C	C	C	T	T	T	T	C	T	T	T	C	C	C	T	T	C	T	T	T	T	T	T	T	0.50	0.009
78.589641	A/G		A	A	A	A	A	A	G	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	=	G	G	G	G	−0.48	
78.741348	A/C		C	C	C	C	C	C	A	A	A	A	C	A	A	A	C	C	C	A	A	C	A	A	A	A	A	A	A	0.50	0.009
78.989787	G/T		G	G	G	G	G	G	T	T	T	T	G	T	T	T	G	G	G	T	T	G	T	T	T	T	T	T	T	−0.50	0.009
79.003501	A/G		A	A	A	A	A	A	G	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.50	0.009
79.221676	A/G		G	G	G	G	G	G	A	A	A	A	G	A	A	A	G	G	G	A	A	G	A	A	A	A	A	A	A	0.50	0.009
79.22365	A/G		G	G	G	G	G	G	A	A	A	A	G	A	A	A	G	G	G	A	A	G	A	A	A	A	A	A	A	0.50	0.009
79.503136	C/G		G	G	G	G	G	G	C	C	C	C	G	C	C	C	G	G	G	C	C	G	C	C	C	C	C	C	C	−0.55	0.003
79.746551	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
79.87882	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
80.236059	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
80.255973	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
80.754004	A/C		C	C	C	C	C	C	C	A	A	A	C	A	A	A	C	C	C	A	A	C	A	A	A	A	A	A	A	0.55	0.003
80.921237	C/T		T	T	T	T	T	T	T	C	C	C	T	C	C	C	T	T	T	C	C	T	C	C	C	C	C	C	C	−0.55	0.003
81.061616	G/T		T	T	T	T	T	T	T	G	G	G	T	G	G	G	T	T	T	G	G	T	G	G	G	G	G	G	G	0.55	0.003
81.179714	A/T		T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	.	.
81.657104	G/T		G	G	G	G	G	G	G	T	T	T	G	T	T	T	G	G	G	T	T	G	T	T	T	T	T	T	T	−0.55	0.003
81.745078	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
81.745214	A/T		T	T	T	T	T	T	T	A	A	A	T	A	A	A	T	T	T	A	A	T	A	A	A	A	A	A	A	0.55	0.003
82.049731	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
82.176449	A/C		C	C	C	C	C	C	C	A	A	A	C	A	A	A	C	C	C	A	A	C	A	A	A	A	A	A	A	0.55	0.003
82.755972	A/G		G	G	G	G	G	G	G	A	A	A	G	A	A	A	G	G	G	A	A	G	A	A	A	A	A	A	A	0.55	0.003
82.944825	C/T		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	.
82.956832	A/G		G	G	G	G	G	G	G	A	A	A	G	A	A	A	G	G	G	A	A	G	A	A	A	A	A	A	A	0.55	0.003
83.225646	A/T		T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	.	.
83.409617	C/G		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	.
83.491049	A/C		A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	.	.
83.609704	C/T		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	.
83.867917	G/T		G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	.	.
83.91749	A/G		A	A	A	A	A	A	A	G	G	G	A	G	G	G	A	A	A	G	G	A	G	G	G	G	G	G	G	−0.55	0.003
84.205526	C/T		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	.	.

The haplotype structure of the chromosome 15 quantitative trait loci interval in the A/J, C57BL/6J, and A × B/B × A recombinant inbred strains. The strains are ordered as a function of ascending levels of ethanol preference (EtOH pref). The significant correlations are presented in the far right column. The results indicate the absence of consistent correlations between the haplotype structure and phenotype outside of the region between 76.52 and 82.95.

protein. In part, this approach is based upon the literature, which suggests that the value in identifying regulatory polymorphisms is diminished by a limited knowledge of functional regulatory elements (DiPetrillo *et al.* 2005). Additional research will be required to identify and assess the functional impact of polymorphisms in noncoding regulatory DNA sequences. The list of these genes with SNP differences in coding regions is presented in Table 5. Of the 33 genes with nonsynonymous mutations, 12 were found to be expressed in brain tissue (Table 6).

To assess the relevance of the candidate genes, additional research will be required to examine the relationship between gene expression patterns, in A/J and C57BL/6J strains, and the expression of VAC. However, of the candidate genes identified in the chromosome 15 region, only the *PICK1* gene has been currently associated in substance abuse. It is suggested that the interaction of *PICK1* with the dopamine transporter results in a clustering of transporters on the cell surface and a subsequent enhancement of dopamine uptake activity as

Table 5 Genes within the narrowed chromosome 15 QTL region, with SNP differences between the A/J and C57BL/6J inbred strains of mice that lead to nonsynonymous coding differences in amino acids

Mbp location (build 37) (β)	NCBI gene annotation	A/J	C57BL/6J	dbSNP rs	Observed
SNPs data downloaded from Mouse Phenome database (*http://www.jax.org/phenome*)					
15 76.537559	Recql4 exon10	T	G	rs31610005	G/T
15 76.545859	Lrrc24 exon5	C	A	rs32254830	A/C
15 76.718746	Zfp7 exon4	T	G	rs31575067	G/T
15 76.731499	Commd5 exon2	G	T	rs47080875	G/T
15 77.219562	Apol3 exon6	A	C	rs31472996	A/C
15 77.319471	9030421J09Rik exon4	A	C	rs31476660	A/C
15 77.347702	EG626615 exon4	A	G	rs31475019	A/G
15 77.359262	2210421G13Rik exon4	T	G	rs31475401	G/T
15 77.456420	LOC666661 exon5	G	A	rs31487264	A/G
15 78.115276	Csf2rb2 exon14	T	C	rs31577888	C/T
15 78.169263	Csf2rb1 exon4	A	G	rs31529514	A/G
15 78.240804	Mpst exon2	G	A	rs31781085	A/G
15 78.384992	EG626952 exon1	T	C	rs31552817	C/T
15 78.617900	Card10 exon12	C	G	rs31566172	C/G
15 78.734976	Sh3bp1 exon9	T	C	rs33865795	C/T
15 78.768271	Nol12 exon4	T	C	rs13466090	C/T
15 78.788726	Triobp exon5	G	A	rs31576014	A/G
15 78.995632	Sox10 exon2	C	A	rs32166492	A/C
15 79.079229	Pick1 exon13	G	A	rs31929691	A/G
15 79.728323	Apobec3 exon3	A	G	rs36471871	A/G
15 79.830813	Pdgfb exon5	T	C	rs37435882	C/T
15 79.910233	Rpl3 exon6	A	G	rs46950095	A/G
15 80.042941	Mgat3 exon2	T	A	rs32082120	A/T
15 80.754004	Tnrc6b exon23	C	A	rs13482668	A/C
15 81.517415	L3mbtl2 exon17	C	T	rs31664902	C/T
15 81.540819	Rangap1 exon12	A	G	rs52167303	A/G
15 81.607518	Zc3h7b exon10	A	C	rs31656888	A/C
15 81.803096	EG545121 exon1	C	G	rs31663910	C/G
15 81.811104	1700029P11Rik exon1	A	G	rs31667378	A/G
15 81.860040	Xrcc6 exon9	A	G	rs31670390	A/G
15 81.937526	Mei1 exon22	C	T	rs31675296	C/T
15 82.174175	C920005C14Rik exon2	G	A	rs32081036	A/G
15 82.220002	LOC545123 exon8	G	T	rs32047531	G/T

QTL, quantitative trait loci; SNP, single nucleotide polymorphism.

Table 6 Genes within the narrowed chromosome 15 QTL region for which there is current evidence of CNS expression

Mbp location (build 37) (β)	NCBI gene annotation	Description
SNPs data downloaded from Mouse Phenome database (*http://www.jax.org/phenome*)		
15 76.731499	Commd5 exon2	COMM domain containing 5
15 78.768271	Nol12 exon4	Nucleolar protein 12
15 78.788726	Triobp exon5	Triobp TRIO and F-actin binding protein
15 78.995632	Sox10 exon2	SRY-box containing gene 10
15 79.079229	Pick1 exon13	Pick1 protein interacting with C kinase 1
15 80.042941	Mgat3 exon2	Mannoside acetylglucosaminyltransferase 3
15 79.728323	Apobec3 exon3	Apolipoprotein B editing complex 3
15 81.607518	Zc3h7b exon10	Zinc finger CCCH type containing 7B
15 81.517415	L3mbtl2 exon17	l(3)mbt-like 2 (Drosophila)
15 81.540819	Rangap1 exon12	RAN GTPase activating protein 1
15 81.860040	Xrcc6 exon9	X-ray repair complementing defective repair in Chinese hamster cells 6
15 81.937526	Mei1 exon22	Meiosis defective 1

CNS, central nervous system; QTL, quantitative trait loci; SNPs, single nucleotide polymorphisms.

measured in mammalian cells and dopamine neurons in culture [32]. The potential association of the *PICK1* gene with the functioning of the dopamine system is significant. The dopamine system, and the DAT in particular, has been hypothesized to contribute to the development of drug addiction [33,34]. In regard to ethanol, research has indicated that genetic modifications of the DAT and dopamine D1, D2 receptor functioning alter the expression of ethanol self-administration [35].

Research directly examining the influence of the *PICK1* gene on the expression of drug-induced behavioral effects is extremely limited to date. However, a review of the literature indicates that the gene has been associated with the effects of amphetamine [36]. Specifically, an association between *PICK1* gene polymorphisms and the expression of psychosis in methamphetamine abusers was identified. Ultimately, the relevance of the *PICK1* gene, or any other candidate genes in general, in the expression of ethanol consumption will require future proteomics research. Specifically, an analysis of protein expression across brain regions and developmental stages will be required.

It is of interest that the survey of the CSS resulted in the identification of putative QTL associated with ethanol preference on chromosomes 13 and 18 ($P < 0.01$). These loci were not identified in the A × B6F2 cross in this study. It should be noted that Stylianou *et al.* [23] similarly reported the detection of QTLs in the B6.A CSS panel that were not detectable in a B6 × A/J F2 cross. It seems likely that the QTLs were masked by epistatic loci segregating in the mixed background of the B6 × A/J

intercross. In the case of chromosomes 13 and 18 QTL, this will be resolved by testing a CSS (13 and 18) × B6 F2, for confirmation. Additionally, the QTL region identified on chromosome 13 in the CSS, in this study, was consistent with a region identified previously in the AcB RCS mice (17). However, an examination of the association between A/J and C57BL/6J alleles on VAC, in these independent lines, indicate discordant allelic influences. Specifically, the A/J allele in the CSS was associated with increased VAC, whereas in the AcB RC the C57BL/6J allele was associated with enhanced ethanol intake. Thus, the informative CSS-13 strain cannot provide confirmation of the previously identified QTL on chromosome 13.

The use of multiple genetic constructs based on the A/J and B6 progenitors (such as the A × B/B × A RI, AcB/BcA RC, A × B6F2, and B6.A CSS) is a potentially powerful approach for the detection, mapping, and fine mapping of QTL. Through the use of multiple crosses, the influence of an identified QTL can be observed in crosses with different levels of segregating variation. This is significant given the notion of a potential effect of sex on the segregation of QTL influencing complex traits [37]. In this study, potential sex-dependent interactions were observed for the confirmed QTLs. Illustrating this point, the association between the chromosome 2 QTL and ethanol preference was identified solely in female A × B6F2 mice, whereas the association was significant in the combined samples in the B6.A CSS. Farber *et al.* [38] describes how a CAST/EiJ (CAST) × C57BL/6J-hg/hg (HG) F2 cross and a subsequently developed HG2D congenic strain (CAST donor regions on HG background) differentially exhibited QTL × sex interactions. Similarly, Stylianou *et al.* [23] reported that a number of high-density lipoprotein cholesterol QTL common to both A × B6 F2 and B6.A CSS were sex specific.

The results of this study confirm previously reported QTL for voluntary ethanol intake in AXB/BXA RI and AcB/BcA RC strains. Collectively, these loci on chromosomes 2 and 15 have now been replicated in at least three independent crosses derived from the A/J and C57BL/6J progenitors. The use of in-silico analyses was discussed as a means to effectively reduce the number of candidate genes within these QTL regions. Through the use of in-silico analysis, the pool of potential candidate genes, in the chromosome QTL 15 region, was reduced from 274 initially identified genes to 33 nonsynonymous coding genes, only 12 of which have been shown to be expressed in brain tissue.

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