Identification of an Acute Ethanol Response Quantitative Trait Locus on Mouse Chromosome 2

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A two-stage strategy was used to identify and confirm quantitative trait loci (QTLs) associated with the changes in locomotor activity induced by a 1.5 gm/kg ethanol challenge. For stage 1, putative QTLs were identified by analysis of the strain means for 25 strains of the BXD recombinant inbred (RI) series (males only). QTLs were identified on chromosomes 1, 2, 4, and 6. The activity response to chlordiazepoxide generated similar QTLs on chromosomes 2 and 6. None of the QTLs were similar to those generated from analysis of the saline response data. For stage 2, 900 male C57BL/6J (B6) $\times$ DBA/2J (D2) F_2 intercross animals were phenotyped for ethanol response, and the phenotypic extremes (those animals $>$ and $<$ 1 SD from the mean) were identified. These extremes differed by $>10,000$ cm/15 min

in their response to ethanol. The extreme progeny were used for a genome-wide scan both to confirm the putative RI-generated QTLs and to detect new QTLs. The F_2 analysis generated no new QTLs with logarithm of the likelihood for linkage (LOD) scores >3 . For RI-generated QTLs, only the QTL on chromosome 2 was confirmed (LOD = 5.3). The position of the peak LOD was estimated to be 47 cM with a 20 cM 1 LOD support interval; this QTL accounted for 6% of the phenotypic variance. The 1 LOD support interval overlaps with QTLs previously identified for alcohol preference and acute ethanol withdrawal (Melo et al., 1996; Buck et al., 1997; Phillips et al., 1999).

Key words: ethanol; mouse; genes; recombinant inbred; QTL; activity

The current study asks the question, “What genes contribute to the variability in the ethanol-induced locomotor response?” The study builds on previous observations of genetics and ethanol locomotor responses. (1) Among inbred strains of mice it is now clearly established that moderate doses of ethanol (1–2 gm/kg) markedly stimulate locomotor activity in some strains, e.g., DBA/2J (D2), but only mildly affect activity in other strains, e.g., C57BL/6J (B6) (Crabbe et al., 1982, 1983; Crabbe, 1986; Dudek and Phillips, 1990; Dudek et al., 1991; Dudek and Tritto, 1994). (2) Dudek and Tritto (1994) completed a full Mendelian analysis of the locomotor response for the B6D2 genotypes. These authors found an additive mode of inheritance for the total distance measure (of activity) and determined that there was no evidence of sex linkage or sex effects. Narrow sense heritability was estimated at 0.35. (3) The locomotor response to ethanol has been subject to three different quantitative trait locus (QTL) analyses involving the BXD recombinant inbred (RI) series. Crabbe et al. (1983) (but analyzed by Gora-Maslak et al., 1991) reported significant QTLs on chromosomes 2, 4, 9, and 13. Cunningham (1995) studied a different phenotype from that of Crabbe et al. (1983), in that the focus was on the initial ethanol response (first 5 min after injection) as opposed to beginning measurements 10 min after injection. Cunningham (1995) detected two QTLs at $p < 0.01$ on chromosomes 7 and 18. Phillips et al. (1995), using a yet different behavioral paradigm but still focusing on the initial ethanol response, confirmed the presence of a significant ($p <$

0.01) QTL on the distal part of chromosome 18 but also found a significant ($p < 0.01$) QTL on chromosome 3. For all QTLs significant at $p < 0.05$, Cunningham (1995) and Phillips et al. (1995) agreed on four (chromosomes 3, 12, 17, and 18).

Because the BXD RI series contains only 26 strains, the smallest QTL effect size that can be detected at $p < 0.01$ is associated with 25% of the genotypic variance. At this level of significance it is necessary to use an additional strategy for verification (Belknap et al., 1996). In the current study we have phenotyped the BXD RI strains for their locomotor response to a 1.5 gm/kg ethanol challenge; a B6D2 F_2 intercross genome-wide scan was used both to confirm the RI generated QTLs and to detect additional QTLs. Of the three RI studies noted above, the behavioral paradigm was closest to that of Crabbe et al. (1983) and was patterned after the testing procedure initially developed for the selection of the FAST and SLOW selected lines (Crabbe et al., 1987) and subsequently used in the biometric analysis of Dudek and Tritto (1994). To compare across RI studies (see above), data for ethanol response were collected over a broad time interval. A secondary goal of the current study was to use the RI strains to test the hypothesis that a genetic correlation exists between the ethanol- and benzodiazepine-induced effects on locomotor activity.

MATERIALS AND METHODS

Animals and sample sizes. Male and female C57BL/6 (B6), DBA/2 (D2), and B6D2 F_1 mice and male BXD RI mice (25 strains) were obtained from The Jackson Laboratory (Bar Harbor, ME). All of the RI strains were obtained over a 3 month period from December 1997 to March 1998. We attempted to obtain an N of 15 per strain, although this was not always possible. Mice were housed two to four per cage in a constant-temperature colony room with a 12 hr light/dark cycle. Food and water were provided *ad libitum* throughout the study. All testing was conducted during the light cycle and between 9 A.M. and 3:30 P.M. All mice were

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Table 1. Saline-induced locomotor response in the BXD RI series

Strain	N	Time interval: distance traveled (cm)				
		0–5 min	5–10 min	10–15 min	15–20 min	5–20 min
1	15	4018 ± 428	2908 ± 266	2391 ± 183	1964 ± 123	7263 ± 572
5	15	1876 ± 235	1767 ± 243	1442 ± 250	1420 ± 226	4629 ± 718
6	15	2893 ± 263	2469 ± 243	2302 ± 157	2272 ± 196	7043 ± 596
8	15	1784 ± 156	1807 ± 158	1753 ± 162	1656 ± 158	5216 ± 477
9	15	3817 ± 249	3533 ± 220	2865 ± 137	2531 ± 175	8929 ± 531
11	11	1647 ± 198	1333 ± 147	1166 ± 166	1268 ± 209	3767 ± 522
12	4	3998 ± 847	2730 ± 662	2391 ± 448	2084 ± 584	7204 ± 1694
13	15	2876 ± 146	2356 ± 149	1806 ± 145	2148 ± 218	6311 ± 512
14	15	3481 ± 261	2640 ± 158	2433 ± 179	2658 ± 205	7731 ± 541
15	9	1086 ± 174	892 ± 106	865 ± 106	954 ± 80	2710 ± 293
16	15	1603 ± 225	1752 ± 159	1612 ± 146	1383 ± 171	4747 ± 477
18	15	3117 ± 356	3032 ± 222	2775 ± 211	2642 ± 227	8449 ± 660
19	15	2888 ± 241	2900 ± 204	2625 ± 185	2442 ± 165	7966 ± 553
20	15	2713 ± 237	2367 ± 223	1868 ± 217	1794 ± 187	6029 ± 627
21	15	1929 ± 99	2200 ± 91	1852 ± 147	1781 ± 182	5833 ± 419
22	15	1926 ± 226	1859 ± 247	1430 ± 168	1347 ± 115	4636 ± 530
23	15	2511 ± 305	1864 ± 244	1573 ± 217	1351 ± 154	4787 ± 615
24	14	2317 ± 245	1854 ± 214	1418 ± 195	1340 ± 233	4612 ± 641
25	15	2391 ± 199	2316 ± 220	1752 ± 214	1594 ± 169	5663 ± 603
27	15	2194 ± 233	1925 ± 184	1691 ± 188	1797 ± 254	5413 ± 615
28	13	1449 ± 177	1645 ± 140	1476 ± 173	1519 ± 129	4640 ± 443
29	15	4543 ± 298	4018 ± 343	3395 ± 286	3083 ± 280	10496 ± 909
30	15	2128 ± 184	2143 ± 171	1850 ± 226	1598 ± 204	5590 ± 601
31	15	2099 ± 251	2266 ± 186	2013 ± 185	1775 ± 161	6055 ± 531
32	15	1559 ± 131	1789 ± 149	1657 ± 127	1770 ± 162	5215 ± 438
B6	18	5131 ± 284	3595 ± 248	2783 ± 173	2580 ± 186	8958 ± 607
D2	18	2170 ± 173	1894 ± 136	1582 ± 138	1317 ± 109	4793 ± 383
F1	12	2811 ± 144	2046 ± 158	1845 ± 174	1554 ± 166	5445 ± 498
Avg. RI ^a		2514 ± 255	2255 ± 214	1936 ± 193	1847 ± 199	6037 ± 605

Animals were administered normal saline (20 mg/kg, i.p.) and placed in the testing arena (San Diego Instruments Flex Field System), and activity was monitored for 20 min; data were collected in four 5 min blocks, and the metric used for the measurement of locomotion was the distance traveled in centimeters. For each interval data are presented as the mean ± SE. Data are also presented for the collapsed 5–20 min interval. Correlation matrix among each of the 5 min activity intervals:

Time (min)	0–5	5–10	10–15	15–20
0–5	1.00			
5–10	0.91	1.00		
10–15	0.87	0.97	1.00	
15–20	0.82	0.91	0.95	1.00

^aAverage locomotor response in the BXD RI strains, calculated from the strain means.

allowed a minimum of 10 d to acclimatize to the colony conditions before testing. All animal care and testing protocols were approved by the Laboratory Animal Users Committee at the State University of New York at Stony Brook and conformed to National Institutes of Health Guidelines for Using Animals in Intramural Research.

For production of the F₂ mice, D2B6 F₁ mice were bred locally from the Jackson parental lines. Reciprocal F₁ crosses were used to generate a total of 900 male F₂ mice from June 1, 1996 to August 1, 1997. Sample size for the genome-wide scan was estimated as described by Solter et al. (1976) and Lander and Botstein (1989) for an F₂ population from $n = (Z\alpha + Z\beta)^2 / (s_{QTL}^2 / s_{RES}^2)$, where $Z\alpha$ and $Z\beta$ are the normal variates for the desired values of α and β , s_{QTL}^2 is the variance associated with or explained by the QTL, and s_{RES}^2 is the residual unexplained variance. The minimum h_{QTL}^2 was arbitrarily set at 0.05. It was recognized that the effect size for most QTLs is <0.05; however, the data from other behavioral phenotypes, e.g., Kanos et al. (1996), suggested that some QTLs of this size or larger were likely to be present. For $(s_{QTL}^2 / s_{RES}^2) = 0.053$ (from 0.05/0.95), $\alpha = 0.0001$ [$Z\alpha = 3.89$ (two-tailed)], and $\beta = 0.05$ ($Z\beta = 1.64$), the estimated sample size was 575. However, because only the extreme phenotypes (> and <1 SD from the mean) would be genotyped, to maintain power the sample size must be increased by 1/0.81 or 1.23 (Lander and Botstein, 1989). This correction is relatively

small because most of the genetic information is found in the extreme phenotypes. We also included an additional correction of 1.15 to account for nonadditive genetic effects. Sample size estimates were based on the assumption that all QTLs have equal and additive effects (Fisher, 1918; Barton and Turelli, 1989). Because this assumption most certainly was violated, some correction was necessary. Finally, a correction of 1.11 was included to account for the loss of animals from data censoring. Overall, these correction factors caused the sample size to increase from 575 to 900.

Measurement of ethanol and chlordiazepoxide effects on locomotor activity. Mice were removed from the home cage and placed individually in the testing arena; the arena floor was covered with standard laboratory bedding. Thirty minutes later, the mice were administered saline and returned to the testing arena, and activity was monitored for 20 min. On the next day, the procedure was repeated, except that the animals were administered 1.5 gm/kg ethanol dissolved in saline (20% v/v). One week later the saline/ethanol days were repeated for the F₂ mice only. For the RI strains, the second week of testing included saline on day 1 and chlordiazepoxide (10 mg/kg) on day 2. Locomotor activity was assessed in a San Diego Instruments Flex Field locomotor system. The apparatus comprised a four-by-eight array of photo cells mounted in a 25 × 47 cm metal frame, situated 1 cm off the floor, and surrounding a 22 × 42 ×

Table 2. Ethanol-induced locomotor response (difference score) in the BXD RI series

Strain	N	Time interval: distance traveled (cm)				
		0–5 min	5–10 min	10–15 min	15–20 min	5–20 min
1	15	1274 ± 516	533 ± 283	182 ± 265	639 ± 336	1354 ± 884
5	15	323 ± 413	–144 ± 293	–824 ± 280	–936 ± 270	–1905 ± 843
6	15	–208 ± 364	–504 ± 420	–1115 ± 348	–1376 ± 316	–2995 ± 1083
8	15	1182 ± 324	1387 ± 322	1344 ± 318	1147 ± 309	3879 ± 950
9	15	289 ± 370	–2024 ± 460	–2458 ± 169	–2107 ± 151	–6588 ± 779
11	11	308 ± 265	459 ± 213	599 ± 249	578 ± 280	1636 ± 743
12	4	1819 ± 347	2298 ± 279	1244 ± 141	1495 ± 188	5037 ± 608
13	15	925 ± 291	392 ± 306	282 ± 199	–308 ± 296	366 ± 800
14	15	146 ± 375	288 ± 224	–354 ± 416	–857 ± 304	–923 ± 944
15	9	943 ± 341	–119 ± 132	–324 ± 158	–420 ± 133	–864 ± 423
16	12	1845 ± 410	1884 ± 351	1555 ± 427	1416 ± 422	4855 ± 1200
18	15	2042 ± 447	2243 ± 436	2235 ± 367	1799 ± 299	6277 ± 1102
19	15	1786 ± 338	264 ± 402	–357 ± 444	–606 ± 424	–717 ± 1271
20	15	1423 ± 389	2291 ± 437	1742 ± 498	1234 ± 241	5267 ± 1175
21	15	1228 ± 250	1904 ± 361	1693 ± 339	1406 ± 323	5003 ± 1023
22	15	481 ± 269	–1116 ± 257	–591 ± 233	–528 ± 233	–2235 ± 724
23	15	109 ± 420	–138 ± 318	–450 ± 260	–173 ± 248	–761 ± 826
24	12	683 ± 560	–281 ± 444	–319 ± 336	–380 ± 293	–979 ± 1073
25	15	698 ± 403	–378 ± 558	–634 ± 355	–711 ± 375	–1722 ± 1288
27	15	447 ± 266	1689 ± 528	800 ± 368	319 ± 396	2808 ± 1293
28	13	486 ± 126	566 ± 142	749 ± 203	515 ± 269	1829 ± 614
29	13	155 ± 600	1038 ± 253	799 ± 325	142 ± 369	1980 ± 947
30	15	983 ± 495	902 ± 424	758 ± 315	1065 ± 211	2725 ± 951
31	15	414 ± 325	671 ± 216	908 ± 301	1225 ± 200	2805 ± 716
32	15	1073 ± 289	515 ± 296	766 ± 391	851 ± 238	2132 ± 924
B6	18	208 ± 321	536 ± 330	356 ± 331	215 ± 232	1107 ± 893
D2	18	2284 ± 261	1689 ± 353	1094 ± 233	739 ± 207	3522 ± 793
F1	12	2227 ± 410	721 ± 325	271 ± 296	117 ± 256	1109 ± 877
Avg. RI ^a		834 ± 368	585 ± 334	336 ± 309	217 ± 285	1137 ± 928

Twenty-four hours after the saline trial (Table 1), animals were administered 1.5 gm/kg ethanol dissolved in normal saline (20% v/v, i.p.) and placed in the testing arena (San Diego Instruments Flex Field System), and activity was monitored for 20 min; data were collected in four 5 min blocks, and the metric used for the measurement of locomotion was the distance traveled in centimeters. The ethanol response was calculated as the difference in activity between day₂ (ethanol treatment) and day₁ (saline treatment). For each interval data are presented as the mean ± SE. Data are also presented for the collapsed 5–20 min interval. Correlation matrix among each of the 5 min activity intervals:

Time (min)	0–5	5–10	10–15	15–20
0–5	1.00			
5–10	0.63	1.00		
10–15	0.61	0.94	1.00	
15–20	0.63	0.88	0.95	1.00.

The values in bold are significantly different ($p < 0.01$) from the relative values in the 0–5 min column.

^aAverage locomotor response in the BXD RI strains, calculated from the strain means.

20-cm-high plastic arena. Activity was recorded over four 5 min blocks. The distance traveled during each block was used as the measure of activity.

For the F₂ sample, ethanol response (day₂ – day₁) data were obtained from two trials, and these data were used to identify the 10% of individuals showing the poorest test–retest reliability. These individuals, with a test–retest reliability of < 0.1, were censored from the data set. The remaining individuals were ranked from least to greatest ethanol-induced activation, and the phenotypic extremes (those individuals > and < 1 SD from the mean) were identified. The extremes were denoted for historical reasons (Demarest et al., 1997) as very responsive (RR) and very nonresponsive (NN), although the NN group actually showed a marked inhibition of locomotor activity.

The phenotypic data generated from the RI strains and the F₂ sample were analyzed using standard ANOVA techniques; the Student–Neuman–Keuls test was used for the *post hoc* analysis. Because of the multiple tests being performed for each phenotype, the threshold for significance was set at $p < 0.01$.

DNA isolation. High molecular weight genomic DNA was isolated from liver samples as follows: 250–500 mg of liver tissue was minced with a

sterile razor blade, transferred to a 15 ml polypropylene Falcon tube with 5 ml of lysis buffer (100 mM Tris-HCl, pH 8.0, 5 mM EDTA, 100 µg/ml proteinase K, and 200 mM NaCl), and incubated with rocking at 55°C overnight. After incubation, 20 µl/ml 5 M NaCl was added with gentle inversion. The tissue digest was extracted twice with equilibrated phenol, once with equal volumes of phenol and chloroform/isoamyl alcohol (chisam, 24:1) and once with chisam alone. DNA was precipitated with 0.5 vol of 7.5 M ammonium acetate and 2 vol of ice cold ethanol. Dried DNA pellets were resuspended in double distilled water. Purity and concentration of the final samples were evaluated by UV spectroscopy, and only samples with a 260:280 ratio > 1.4 were used for genotyping.

Genotyping the microsatellite polymorphisms. All of the genotyping involved the -(CA)_n- repeating microsatellites first described by Dietrich et al. (1992). The PCR primer sets were obtained from the Massachusetts Institute of Technology–Whitehead catalog (Research Genetics). One to 5 ng of genomic DNA was amplified with an 18 pM concentration of each primer, 0.5 U of *Taq* polymerase (Ampli[®]Taq, Perkin-Elmer, Emeryville, CA; or *Taq* DNA polymerase, Boehringer Mannheim, Indianapolis, IN), and 100 nM dNTPs in a 20 µl reaction under the standard conditions recommended by the manufacturer. All reactions were amplified in a

Figure 1. Strain means for the activity response to ethanol (1.5 gm/kg) among the BXD RI series ($n = 25$ strains). On the day after a saline challenge, animals were administered 1.5 gm/kg ethanol intraperitoneally, and activity was monitored for the next 20 min. Data in this figure are expressed as the difference score ($\text{day}_2 - \text{day}_1$) and reported for the 0–5 and 5–20 min intervals after ethanol administration. In addition to data for the RI strains, data are also reported for the B6 and D2 inbred strains and the B6D2 F₁ hybrids. Black bars illustrate the range of significant ($p < 0.01$) steps (of difference) for the RI strains as determined by the Student–Neuman–Keuls test. Thus for the 5–20 min data, strains 24 and 8 are significantly different, but strains 14 and 27 are not. All data are reported as the mean $\pm$ SE for the distance traveled.

Perkin-Elmer thermal cycler. Products were visualized by electrophoresis in 1 $\times$ Tris borate-EDTA buffer on a 3% agarose gel (3:1 Metaphor agarose; FMC Bioproducts, Rockland, ME). Bands were visualized by ethidium bromide staining.

Detection and mapping of QTLs. Candidate QTLs were generated from the BXD RI data essentially as described elsewhere (Kanes et al., 1996). Briefly, the analysis was performed by correlating the strain means with the strain distribution patterns of 1500 marker gene loci, each mapped to a chromosome region and showing allelic differences between the B6 and D2 strains. By convention, the B6 allele is scored as 0, and the D2 allele is scored as 1; the correlation coefficient has the same meaning as a t test for significant differences between strains with the B6 and D2 alleles. Because of linkage disequilibrium, the actual number of independent tests is <1500 but >100 (Belknap, 1992). Thus, although the threshold for acceptance of a candidate QTL was set at $p < 0.01$, some of these QTLs will be significant because of chance. Belknap et al. (1996) have estimated the false-positive rate to be 50%. We recognize that it is common, even in our own work (Kanes et al., 1996), to report QTL data generated from RI analyses that are significant at $p < 0.05$ but not $p < 0.01$. However, because no behavioral QTL detected in this range has been independently confirmed using a non-RI strategy, we feel there is little value in reporting these data. For the interested reader, the strain means reported in Tables 1–3 are sufficient to conduct such analyses.

For analysis of the F₂ intercross, 25 each of the extreme phenotypes (approximately one-fifth of each extreme sample) were randomly se-

lected for the genome-wide scan, except in the regions of the RI-generated QTLs, where a minimum N of 100 per extreme was used. For QTL detection, markers were spaced at ~ 20 cM (Darvasi et al., 1993). The screening threshold for a significant segregation of the alleles was set at $p < 0.05$. For markers meeting the screening threshold, 25 each of the extreme phenotypes were again randomly selected and genotyped to confirm a significant effect. For markers meeting the threshold of $p < 0.01$, the entire sample of phenotypic extremes was genotyped, and additional markers were added in the region of interest. The data from this more dense map were then subjected to analysis using the MAPMAKER-QTL program (Lander and Botstein, 1989) to determine the position of the peak logarithm of the likelihood for linkage (LOD) and 1 LOD support intervals.

RESULTS

RI strains: saline response

The saline-induced locomotor response among 25 BXD RI strains is summarized in Table 1 for the four 5 min intervals and the collapsed 5–20 min interval. Data are also provided for the B6 and D2 inbred strains and the B6D2 F₁ hybrids (but not the reciprocal D2B6 cross). The correlation matrix for the activity intervals is presented in the legend to Table 1. All of the intervals

Table 3. CDP-induced locomotor response (difference score) in the BXD RI series

Strain	N	Time interval: distance traveled (cm)				
		0–5 min	5–10 min	10–15 min	15–20 min	5–20 min
1	15	−716 ± 282	−248 ± 283	−462 ± 245	−817 ± 248	−1526 ± 776
5	15	−40 ± 182	−487 ± 209	−846 ± 231	−705 ± 170	−2039 ± 610
6	15	−9 ± 448	−36 ± 410	−137 ± 413	−358 ± 318	−531 ± 1141
8	15	−284 ± 391	−18 ± 446	−285 ± 269	−425 ± 269	−727 ± 985
9	15	197 ± 163	−1623 ± 286	−2600 ± 262	−2564 ± 356	−6786 ± 903
11	9	919 ± 668	1166 ± 246	518 ± 381	319 ± 284	2002 ± 911
12	3	112 ± 156	1306 ± 119	477 ± 773	−383 ± 707	1401 ± 1600
13	15	−509 ± 310	−451 ± 365	−642 ± 239	−888 ± 242	−1981 ± 846
14	15	−594 ± 250	132 ± 370	443 ± 414	268 ± 390	843 ± 1174
15	7	216 ± 233	−1104 ± 304	−1036 ± 171	−835 ± 359	−2974 ± 833
16	12	7 ± 239	1377 ± 407	1013 ± 337	814 ± 387	3204 ± 1131
18	15	668 ± 276	10 ± 431	266 ± 372	367 ± 347	643 ± 1149
19	15	335 ± 299	−39 ± 228	−403 ± 306	−828 ± 309	−1270 ± 843
20	15	744 ± 295	510 ± 505	453 ± 487	−40 ± 420	923 ± 1412
21	15	76 ± 115	−246 ± 299	−520 ± 255	−354 ± 319	−1119 ± 873
22	15	787 ± 282	−448 ± 295	−743 ± 297	−622 ± 335	−1812 ± 926
23	8	−113 ± 208	256 ± 545	104 ± 378	825 ± 552	1185 ± 1475
24	7	739 ± 317	174 ± 393	−390 ± 370	−351 ± 206	−567 ± 969
25	14	−39 ± 304	−86 ± 294	−110 ± 345	−132 ± 365	−327 ± 1005
27	6	−413 ± 337	−497 ± 574	−555 ± 658	−1058 ± 181	−2110 ± 1413
28	10	387 ± 280	409 ± 278	9 ± 170	−340 ± 206	78 ± 653
29	8	742 ± 291	1909 ± 316	903 ± 362	1059 ± 399	3870 ± 1077
30	15	204 ± 328	−590 ± 283	−28 ± 281	−312 ± 160	−930 ± 724
31	15	328 ± 268	527 ± 331	774 ± 305	233 ± 371	1534 ± 1007
32	15	299 ± 200	500 ± 370	419 ± 345	504 ± 314	1423 ± 1030
B6	6	−696 ± 292	−459 ± 253	−69 ± 352	297 ± 156	−231 ± 761
D2	6	−81 ± 123	863 ± 307	875 ± 219	857 ± 293	2595 ± 819
Avg. RI ^a		162 ± 285	96 ± 344	−135 ± 347	−265 ± 329	−304 ± 1019

Twenty-four hours after a saline trial, animals were administered 10 mg/kg CDP, i.p., and placed in the testing arena (San Diego Instruments Flex Field System), and activity was monitored for 20 min; data were collected in four 5 min blocks, and the metric used for the measurement of locomotion was the distance traveled in centimeters. The data for the saline trial are not presented, but the correlation between the ethanol saline trial and the CDP saline trial was at 0.80 or better for all intervals. The CDP response was calculated as the difference in activity between day₂ (CDP treatment) and day₁ (saline treatment). For each interval data are presented as the mean ± SE. Data are also presented for the collapsed 5–20 min interval. Correlation matrix among each of the 5 min activity intervals:

Time (min)	0–5	5–10	10–15	15–20
0–5	1.00			
5–10	0.32	1.00		
10–15	0.19	0.88	1.00	
15–20	0.24	0.79	0.91	1.00

The values in bold are significantly different ($p < 0.001$) from the relative values in the 0–5 min column.

^aAverage locomotor response in the BXD RI strains, calculated from the strain means.

were highly correlated; the correlation coefficients ranged from 0.82 to 0.97. The ANOVA for both the 0–5 and 5–20 min intervals revealed highly significant ($p < 0.00001$) strain effects ($F_{(24,295)} = 10$ and 8.4, respectively). The split-halves reliabilities (Blizard, 1992) for the RI strain means were 0.94 (0–5 min) and 0.93 (5–20 min). The narrow-sense heritability [DeFries et al., 1989; $h^2 = 0.5V_A/(0.5V_A + V_W)$] was 0.59 for the 0–5 min interval and 0.38 for the 5–20 min interval. The data in Table 1 illustrate that the average activity in the RI strains was similar to that seen in the D2 strain and markedly less than the activity of the B6 strain. Activity in the F₁ cross was similar to that of the D2 strain.

RI strains: ethanol response (difference score)

The ethanol response was calculated as the difference in activity between day₂ [ethanol (1.5 gm/kg) challenge] and day₁ (saline challenge). The ethanol locomotor responses among the 25 BXD

RI strains, the B6 and D2 inbred strains, and the B6D2 F₁ hybrids are summarized in Table 2 and presented graphically in Figure 1 for the 0–5 min interval and the collapsed 5–20 min interval. The correlation matrix for the activity intervals is presented in the legend to Table 2. All of the intervals were highly correlated; the correlation coefficients ranged from 0.61 to 0.95. However, the correlations among the 5–10, 10–15, and 15–20 min intervals were stronger and in two cases significantly ($p < 0.01$) so when compared with the correlations between the 0–5 min interval and the subsequent intervals. The ANOVA for both the 0–5 and 5–20 min intervals revealed highly significant strain effects: 0–5 min ($F_{(23,288)} = 2.4$; $p < 0.0003$) and 5–20 min ($F_{(24,288)} = 9.0$; $p < 0.00001$). *Post hoc* analysis revealed numerous significant differences among the strain means, some of which are illustrated in Figure 1.

The split-halves reliabilities for the RI strain means were 0.65

Figure 2. Strain means for the activity response to chlordiazepoxide (10 mg/kg) among the BXD RI series ($n = 25$ strains). Seven to 14 d after the ethanol challenge, animals were challenged with saline (day_1) and chlordiazepoxide (day_2). The data presented are for the difference score ($\text{day}_2 - \text{day}_1$). All other details are the same as in the legend to Figure 1.

(0–5 min) and 0.88 (5–20 min). The narrow-sense heritabilities were 0.19 (0–5 min) and 0.32 (5–20 min). The data in Table 2 and Figure 1 illustrate that the average ethanol response in the RI strains for the 0–5 min interval was intermediate to that seen in the D2 and B6 strains; in contrast, the response in the F_1 cross was identical to that found in the D2 strain (marked activation). A different pattern of ethanol responses was observed for the 5–20 min interval. Over this time frame, ethanol responses in the RI strains (average response), in the B6 strain and in the F_1 cross were identical and markedly less than the response found in the D2 strain. The 5–20 min interval was characterized by several RI strains that showed a marked decrease in activity after the ethanol challenge.

RI strains: chlordiazepoxide response

In a series of preliminary studies, B6 and D2 mice ($n = 6$ –8 per strain per dose) were challenged with saline (day_1) and 3, 10, or 30 mg/kg of chlordiazepoxide (CDP) (day_2) and the difference scores calculated. The 10 mg/kg dose produced the maximum activation in the D2 strain (Table 3), which was observed over the 5–20 min interval. In contrast, no significant effect was observed in the B6 strain. At 30 mg/kg, CDP significantly decreased activ-

ity in both the B6 and D2 strains (data not shown). Thus, the 10 mg/kg CDP dose was used to challenge the RI strains.

The CDP locomotor responses among the 25 BXD RI strains and the B6 and D2 inbred strains are summarized in Table 3 for the four 5 min blocks and presented graphically in Figure 2 for the 0–5 min interval and the collapsed 5–20 min interval. The correlation matrix for the activity blocks is presented in the legend to Table 3. There were no significant correlations between the 0–5 min interval and the subsequent intervals. However, the 5–10, 10–15, and 15–20 min intervals were all highly correlated (range of r values, 0.79–0.91). The ANOVA for the 0–5 min interval did not meet the study criterion ($p < 0.01$) for a significant strain effect ($F_{(24,283)} = 1.7$; $p < 0.028$) and was not considered for further analysis. In contrast, the ANOVA for the 5–20 min interval was highly significant ($F_{(24,283)} = 3.8$; $p < 0.0001$); the *post hoc* analysis detected strain mean differences significant at $p < 0.01$ or better (see Fig. 2). The split-halves reliability for the RI strain means (5–20 min) was 0.91, and the narrow-sense heritability was 0.14. The data in Table 3 and Figure 2 illustrate that the average CDP response in the RI strains for 5–20 min interval was similar to that seen in the B6 strain. Overall, the RI

Table 4. Saline and ethanol (difference score) locomotor responses in male B6D2 F₂ intercross animals (N = 900)

Treatment	Trial/analysis	0–5 min	5–10 min	10–15 min	15–20 min	5–20 min
Saline	1	2052 ± 930	1772 ± 810	1645 ± 750	1602 ± 827	5017 ± 2141
	2	2095 ± 1013	1855 ± 931	1718 ± 876	1655 ± 910	5212 ± 2487
	Average ^a	2068 ± 854	1809 ± 749	1679 ± 695	1626 ± 738	5115 ± 2033
	Average-C	2009 ± 823				4985 ± 1968
	F test	1.3	1.36	1.38	1.39	1.3
	Test–retest	0.54	0.47	0.45	0.44	0.54
	Test–retest-C	0.71				0.71
Ethanol	1	970 ± 1460	410 ± 1610	48 ± 1430	–4 ± 1340	450 ± 4050
	2	1180 ± 1490	910 ± 1580	480 ± 1430	370 ± 1330	1760 ± 3990
	Average ^b	1080 ± 1120	660 ± 1240	270 ± 1140	180 ± 1030	1110 ± 3170
	Average-C	1090 ± 1100				1090 ± 3160
	F test	1.73	1.65	1.57	1.68	1.6
	Test/retest	0.15	0.21	0.22	0.22	0.25*
	Test/retest-C	0.38				0.51*

The saline and ethanol responses were determined as described in the legends to Tables 1 and 2. The saline–ethanol challenge was repeated in all animals 7–10 d after the first trial. For each interval data are also presented for the average of trial₁ and trial₂. The *F* test compared the average of the variances from trials 1 and 2 to the variance associated with the average activity. Test–retest data were calculated as the correlation between trial₁ and trial₂. The absolute value of the difference between trial₁ and trial₂ was calculated and used to rank order the animals. The 10% of the animals showing the poorest test–retest reliability were censored from the data set. The censored (C) values for the average response and the test–retest reliability are also presented (0–5 and 5–20 min intervals, only). All data are presented as the mean ± SD.

*Significantly different from the 0–5 min value; *p* < 0.01.

^aCorrelation matrix for the average saline response:

Time (min)	0–5	5–10	10–15	15–20
0–5	1.00			
5–10	0.78	1.00		
10–15	0.71	0.84	1.00	
15–20	0.63	0.75	0.81	1.00.

^bCorrelation matrix for average ethanol response:

Time (min)	0–5	5–20	10–15	15–20
0–5	1.00			
5–10	0.63	1.00		
10–15	0.50	0.84	1.00	
15–20	0.41	0.73	0.81	1.00.

The values in bold are significantly different (*p* < 0.01) from the relative value in the 0–5 min column.

strain means showed a trend toward expansion in the direction of an inhibitory effect on activity.

RI strains: correlations among phenotypes

For the 5–20 min data, baseline (saline response) locomotor activity was not significantly (*p* > 0.2) correlated with either the ethanol or the CDP response. However, it was observed that there was a significant correlation between the ethanol and CDP response for this interval (*r* = 0.60; *p* < 0.01).

Phenotypic characterization of the F₂ sample

A total of 900 male B6D2 F₂ mice were phenotyped for their response to saline (day₁) and ethanol (1.5 gm/kg; day₂). As with the RI strains, the ethanol response was defined as the difference in activity between day₂ and day₁. The entire procedure was repeated one week later (trial₂). The results obtained are summarized in Table 4. For the saline response it is useful to compare the results in Table 4 with those in Table 1. The data illustrate that the average activity in the RI strains and the F₂ sample were quite similar: the saline response phenotype is similar to that seen in the D2 strain. As expected (see Markel et al., 1995), the average activity obtained from the test–retest design significantly reduced the sample variance without affecting activity. For both the 0–5 and 5–20 min intervals, the *F* test was 1.3 (*p* < 0.01). The test–retest reliability from trial₁ to trial₂ was 0.54 (*p* < 0.0001) for both the 0–5 and 5–20 min intervals. The correlation matrix for each of the 5 min intervals (average data) is presented in Table 4;

all of the intervals were highly correlated (range of *r* values, 0.63–0.84). Animals were rank-ordered on the basis of the test–retest reliability. The effect of censoring the data set of the bottom 10% (those individuals showing the poorest reliability) is also illustrated in Table 4. Censoring significantly improved the test–retest reliability of the remaining sample for both the 0–5 and 5–20 min intervals from 0.54 to 0.71 (*p* < 0.01) without affecting the sample means or variances.

The ethanol response data for the F₂ sample are also found in Table 4. The average response in the F₂ sample was similar to the average response in the RI strains (Table 2). A potential confound in using the test–retest design was the sensitization effect from trial₁ to trial₂; i.e., on average there was a greater activation response in trial₂ compared with trial₁. However, sensitization and locomotor activity appear to be phenotypically independent. No significant relationship was detected between the average ethanol effect and the difference in the ethanol effect from trial₁ to trial₂; for the 0–5 min interval, *r* was 0.017 and for the 5–20 min interval, *r* was 0.08. For all intervals, the test–retest design significantly reduced the sample variance (*F* > 1.6; *p* < 0.0001). The test–retest reliability of the uncensored data were poor and ranged from 0.15 for the 0–5 min interval to 0.25 for the 5–20 min interval; this difference was significant (*p* < 0.01). The correlation matrix for each of the 5 min intervals (average data) is presented in Table 4; all of the intervals were significantly correlated (range of *r* values, 0.41–0.84). However, the correlations

Figure 3. Distribution of the average saline response in the B6D2 F₂ intercross. Data are presented for the average from trials 1 and 2 (censored data set). In addition, the mean activity in the parental B6 and D2 strains ($n = 18$ per strain) are also illustrated. For both the 0–5 and 5–20 min intervals, the average intercross activity was similar to that seen in the D2 strain. Both distributions were not significantly different from normal. $n = 810$.

among the 5–10, 10–15, and 15–20 min intervals were significantly ($p < 0.01$) stronger when compared with the correlations between the 0–5 min interval and the subsequent intervals. The effect of censoring the data set of the bottom 10% is also illustrated in Table 4. Censoring significantly improved the test–retest reliability of the remaining sample for the 0–5 min sample from 0.15 to 0.38 ($p < 0.001$) and for 5–20 min intervals from 0.25 to 0.51 ($p < 0.01$) without affecting the sample means or variances. For the censored data, the reliability of the 5–20 min interval remained significantly ($p < 0.01$) better than the reliability of the 0–5 min data.

The distributions of the censored data sets for the 0–5 min and 5–20 min intervals are shown in Figures 3 (saline response) and 4 (ethanol response). The data in Figure 3 illustrate that the saline responses were generally normally distributed, despite the floor effect (activity cannot be < 0). For the parental strains, there was no significant change in activity from trial₁ to trial₂; the average (of trial₁ and trial₂) D2 and B6 data are shown in Figure 3. For the F₂ sample, only a few animals showed a saline response that exceeded that seen in the B6 strain, but numerous animals were

Figure 4. Distribution of the average ethanol response in the B6D2 F₂ intercross. Difference score data are presented for the average from trials 1 and 2 (censored data set). In addition, the mean activities in the parental B6 and D2 strains ($n = 18$ per strain) are also illustrated. For both the 0–5 and 5–20 min intervals, a significant proportion of the animals showed an ethanol response below that seen in the B6 strain; for the 5–20 min interval, significant inhibition of activity was observed. Both distributions were not significantly different from normal. $n = 810$.

less active than the D2 strain. The data in Figure 4 illustrate that the F₂ sample ethanol responses were also approximately normally distributed. For the parental D2 strain, there was no significant change in the ethanol response from trial₁ to trial₂; however, there was a significant sensitization in the B6 strain (activity increased from 1107 to 1940 cm/15 min). The average (of trial₁ and trial₂) D2 and B6 data are shown in Figure 4 for comparison with the F₂ sample. The F₂ extreme phenotypes were defined as those individuals > 1 SD from the mean and were denoted RR and NN. For the 0–5 min interval, activity in the RR and NN extremes was 3100 ± 60 and -400 ± 60 cm, respectively. For the 5–20 min interval, activity in the RR and NN extremes was 7310 ± 190 and -2910 ± 110 cm, respectively. A comparison of these data for the 5–20 min interval with the mean $\pm$ SE values for the D2 and B6 strains (3850 ± 225 and 1940 ± 175 cm, respectively) illustrates the expansion in the F₂ sample of the ethanol response phenotype beyond the parental boundaries. For both the 0–5 min interval ($r = 0.09$) and the 5–20 min interval ($r = 0.01$), there was no significant relationship

Table 5. QTL analysis of the BXD RI strain means for the saline-, ethanol-, and CDP-induced responses

Chr	Marker	cM	Saline		Ethanol		CDP 5–20 min
			0–5 min	5–20 min	0–5 min	5–20 min	
1	<i>Ugt1</i>	52	–0.51				
1	<i>D1Mit87</i>	62		–0.55			
1	<i>DiRik88</i>	80–90			–0.66	–0.56	
2	<i>D2Mit17</i>	53–72				0.69	
2	<i>Iapls2.4</i>	86				0.53	0.55
2	<i>Pmv33</i>	98			0.56		
3	<i>D3Byu3</i>	12		0.53			
3	<i>D3Mit12</i>	49		–0.57			
4	<i>D4Mc2</i>	35–40					–0.67
4	<i>Ly31</i>	66–78			0.56	0.52	
5	<i>D5Byu1</i>	24–38	–0.55				
6	<i>D6Rik59</i>	36–48					–0.59
6	<i>Ly49c</i>	62				–0.55	
9	<i>D9Lws1</i>	48			0.58		
10	<i>D10Bir1</i>	55		–0.51			
13	<i>Tcrg</i>	10		0.52			
15	<i>D15Mit2</i>	43–56					–0.63
18	<i>D18Mit111</i>	2–11	–0.53	–0.57			

The data from Tables 1–3 were subjected to a QTL analysis as described in Materials and Methods. Candidate QTLs reported are those that are significant at $p < 0.01$ or better. The markers reported are those that showed the most significant effect. The range of the QTL effect was estimated from correlations significant at $p < 0.05$.

between the average saline response and the average ethanol response.

QTL analysis of the RI strain means

Table 5 lists the QTLs detected from analysis of the RI strain means (Tables 1–3) and found to be significant at $p < 0.01$ or better. For the saline response, a total of four QTLs were identified on chromosomes 1, 3, 5, 10, and 18. With the possible exception of the QTL on chromosome 1, these QTLs appear to be distinct from those detected for the ethanol or CDP responses. The QTL on chromosome 5 appears to be the same as a baseline activity QTL identified by Phillips et al. (1995, 1998) for the BXD RI series and also by K. Buck, T. Lisehka, J. Dorow, and J. C. Crabbe (unpublished observations).

Significant QTLs for ethanol response were identified on chromosomes 1, 2, 4, and 6; the relationships between RI strain genotype and phenotype (5–20 min data) are illustrated in Figure 5. These data demonstrate both the genetic homogeneity of the extreme phenotypes and for some QTLs the reversal of genotype based on the parental phenotype. Thus, on chromosomes 1 and 6, it is the B6 allele that contributes to increased locomotor activity and the D2 allele that contributes to decreased activity.

The QTLs on chromosomes 1 and 2 were detected for both the 0–5 min and the 5–20 min intervals. The data for chromosome 2 suggest that the QTL interval stretches from ~50 to 100 cM. Four significant QTLs were detected for the CDP response (chromosomes 2, 4, 6, and 15). The QTLs on chromosomes 2 and 6 appear to be similar to those found for the ethanol response.

QTL confirmation and detection of additional QTLs

Approximately 100 each of the F_2 ethanol response phenotypic extremes from the 0–5 and 5–20 min intervals were used to confirm the RI-generated ethanol response QTLs described in Table 5. Given the expansion of the genetic map in the RI strains and the associated difficulty in directly comparing map distances in the RI and F_2 samples, summary data are provided for chro-

mosomes 1, 2, 4, and 6 (Fig. 6). The QTLs on chromosomes 1, 4, and 6 were not confirmed in this analysis, although there was a trend (LOD = 1.6) for confirmation of the 0–5 min QTL on chromosome 1. On chromosome 2, the data illustrate that the QTLs for the 0–5 and 5–20 min data were probably identical, but the effect sizes for the 5–20 min data were greater. For the 0–5 min data, the peak LOD was 3.0, which is below the suggested criteria for acceptance (Lander and Kruglyak, 1995); however, combining the RI and F_2 data would yield an LOD > 4. For the 5–20 min data, additional markers were added for fine mapping, and the data were analyzed using the MAPMAKER/QTL program. Because there was generally good agreement between the primary linkage map and the consensus map for chromosome 2 (Peters et al., 1998), the consensus map was used to place the markers in Figure 7. This figure illustrates that a peak LOD of 5.3 was obtained at ~48 cM with either the free or additive QTL model. The 1 LOD support interval was 20 cM, and the peak LOD was associated with 6% of the phenotypic variance.

The remainder of the genome in the F_2 sample was screened for additional QTLs using the strategy outlined in Materials and Methods. The distribution of the markers used is given in Figure 8. No QTLs with LOD scores > 3 were detected. However, it is important to note that the screening strategy used was biased to detecting additive genetic effects. To detect dominant genetic effects, which are common for quantitative traits (Lynch and Walsh, 1998), would require that a substantially greater proportion of the total F_2 sample be used for screening.

DISCUSSION

It is estimated that 40–60% of the risk for developing alcoholism is explained by genetic factors (Schuckit, 1994). However, neither the genes involved nor the mode of inheritance have been characterized. There are several reasons why progress has been slow. (1) Although the diagnosis of alcoholism is highly reliable, it is quite clear that one is dealing with a heterogeneous phenotype,

Figure 5. Relationship between phenotype and genotype for the activity response QTL (5–20 min) data. Data are presented for the four QTLs described in Table 5. For each marker the graph illustrates whether a particular RI strain carried the B6 or D2 genotype.

which markedly reduces the power to detect specific gene effects. (2) Alcoholism is clearly a polygenic disorder and, thus, will require large samples to detect relatively small gene effects. For complex phenotypes under the control of multiple genes (or QTLs), gene effect sizes of 5–10% are generally considered large (Paterson et al., 1991). (3) Although recent reports show promise (Camp and Bansal, 1997; Gu and Rao, 1997; Hoeschele et al., 1997; Iyengar et al., 1997; Martin et al., 1997), there has been a lag in the execution of clinical QTL studies (in which one must deal with outbred populations and it is not possible to manipulate either the genome or the environment).

An alternative approach to finding clinically relevant QTLs is to first detect and map the QTLs using highly informative animal models and then look for the QTL in the homologous region of the human genome. Because the homologous regions of the mouse and human chromosomes are so well defined, it is possible to identify the chromosomal location of a gene in humans by mapping it in mice, often with far greater precision than human mapping studies (Copeland et al., 1993). Validation of this approach is seen in the recent report of Lemberget et al. (1997), who identified a putative QTL for human obesity on chromosome 20 based on homology with mouse chromosome 2, in which a significant obesity QTL had been mapped. In the application of the

animal-to-human approach, alcoholism has a significant advantage over other areas of substance abuse. Studies on the genetics of alcohol-related behaviors have covered a wide variety of phenotypes and experimental strategies. Most investigations have focused on ethanol preference and choice, ethanol withdrawal, and/or acute ethanol sensitivity. Several recent studies have illustrated that QTL analysis can be successfully applied to such phenotypes, and importantly, some QTLs have been independently confirmed (Phillips et al., 1994; Rodriguez et al., 1995; Melo et al., 1996; Belknap et al., 1997). The work of the current study falls in the category of phenotypes related to acute ethanol sensitivity. QTL data are available for such diverse phenotypes as loss of the righting reflex (LORR), hypothermia, and locomotor activity (Crabbe et al., 1994; Cunningham, 1995; Phillips et al., 1995; Markel et al., 1997). These phenotypes may be relevant to alcoholism because one of the factors that appears to be inherited in alcoholic families is a difference in alcohol sensitivity (Schuckit et al., 1994); family history-positive subjects are generally less sensitive than family history-negative subjects to the effects of alcohol.

The data presented here confirm and extend the QTL analysis of the Crabbe et al. (1983) data set (Gora-Maslak et al., 1991). In this analysis, four provisional QTLs were detected on chromo-

Figure 6. Summary of the screening results for ethanol response QTLs on chromosomes 1, 2, 4, and 6. The 0–5 and 5–20 min phenotypic extremes of the F_2 intercross were genotyped for microsatellite markers on each of the four chromosomes identified from analysis of the RI strain means to contain ethanol response QTLs. The relative placement of each marker (based on the consensus map) is indicated. Each marker was analyzed independently (Kanes et al., 1996) and the χ^2 value obtained was transformed to an LOD score (Lynch and Walsh, 1998).

somes 2, 4, 9, and 13; only the QTL on chromosome 4 was significant at $p < 0.01$. The analysis of the BXD RI data in Table 2 confirmed the QTLs on chromosomes 2 and 4 and detected two additional QTLs on chromosomes 1 and 6. However, only the QTL on chromosome 2 was confirmed in the F_2 analysis. Although the false-positive rate for the RI analysis was high (75%), it was not unexpected given the numerous comparisons being made (Belknap et al., 1996) and was similar to previous results from this laboratory (Kanes et al., 1996). None of the QTLs detected at $p < 0.01$ in the studies of Cunningham (1995) and Phillips et al. (1995) (chromosomes 3, 17 and 18) was confirmed in the present study. In this regard, it is important to note that these authors used a higher dose of ethanol (2 gm/kg) and different apparatus for measuring activity. Erwin et al. (1997b) examined the locomotor response to various doses of ethanol in the LS $\times$ SS RI series (24 strains). These authors found there was only one common QTL between those calculated for the ethanol activation slope and 2.0 gm/kg activity; the activation slope was calculated from the dose–response data obtained at 1, 1.25 and 1.5 gm/kg ethanol. Erwin et al. (1997b) concluded that for the LS $\times$ SS RI series, the 1.5 gm/kg dose of ethanol demarcates the end of the mainly stimulatory effects of ethanol and that the 2

Figure 7. LOD plot of chromosome 2 for the 5–20 min ethanol response data based as determined by an interval mapping approach (MAP-MAKER/QTL). The markers used to generate the plot are found on the x-axis. Data were obtained by genotyping the NN and RR phenotypic extremes from the F_2 intercross and ~100 of the intermediate phenotypes. Curves are presented for the free, additive, dominant, and recessive models. The schematic chromosome indicates the location of some potential candidate genes.

gm/kg dose involves both the stimulatory and inhibitory influences of ethanol, making it a mixture of two different quantitative traits, which could be difficult to disentangle. This conclusion is supported in part by the observations of Dudek et al. (1991) that the stimulatory and inhibitory effects of ethanol are genetically independent. The exact dose conclusions of Erwin et al. (1997b) do not seem applicable to the BXD RI strains, because numerous strains show inhibition of activity over a time course very similar to that used to characterize the LS $\times$ SS RI series. However, it is reasonable to assume that for any particular dose of ethanol, one is observing (from a locomotor perspective) a mixture of quantitative traits, and this mixture is dose-dependent. Thus, the behavior seen at 2 gm/kg ethanol probably differs substantially from that found at lower doses. The argument that one is studying a mixture of quantitative traits has important implications for the QTL analysis. If there are separate QTLs for the activating and inhibitory effects of ethanol on locomotor activity, and assuming that one always has some mixture of these influences, then the power of both the RI and F_2 analyses will be substantially reduced. Furthermore, in a genome-wide F_2 analyses we could expect to find QTLs where the genetic segregation is asymmetrical; i.e., the responders but not the nonresponders (or vice versa) show a significant deviation from the expected 1:2:1 ratio of genotypes. However, the QTL on chromosome 2 was reasonably symmetrical; the genotypic ratios for the RR and NN individuals at *D2Mit94* were 2.1:1.4:0.5 and 0.8:1.7:1.5, respectively. These data strongly suggest that this marker is linked to a gene or genes in which the D2 alleles cause activation, whereas the B6 alleles cause inhibition.

The activity QTL on chromosome 2 is in the same general region previously identified to contain QTLs associated with ethanol preference and consumption (Melo et al., 1996; Phillips et

Figure 8. Location of the microsatellite markers used in the genome-wide scan for ethanol response QTLs.

al., 1999) and acute ethanol withdrawal (Buck et al., 1997) and appears to be significantly proximal from the loss of righting reflex QTL (*Lorr2*) identified by Markel et al. (1997). The observation that the ethanol and CDP responses in the RI strains are highly correlated and both map to the same region of chromosome 2, suggests the presence of a gene or genes that could provide a link between the ethanol response and ethanol effects at the GABA-benzodiazepine receptor arrays (see Deitrich et al., 1989). A potential GABA-related candidate gene that is also likely to affect ethanol preference and withdrawal is glutamic acid decarboxylase 1 (*Gad1*) (see Fig. 7). GAD activity among inbred mouse strains, including the B6 and D2 strains, has been reviewed (Hitzemann et al., 1995), and in general there is no substantial evidence for a functional polymorphism; although see Buck (1996). However, it is of interest to note that stress has been reported to increase GABA levels in the amygdala of the D2 but not B6 strain (Simler et al., 1982); recent evidence from this laboratory has suggested that the amygdala and in particular the central nucleus are important in the regulation of the ethanol activity response (Hitzemann and Hitzemann, 1997; Demarest et al., 1998, 1999).

In conclusion, the data presented here are the first to confirm a QTL for the locomotor response to ethanol. This QTL joins a growing list of alcohol-related QTLs that have met or exceeded the generally accepted criteria for acceptance of $\text{LOD} > 4.4$ (Lander and Schork, 1994; Lander and Kruglyak, 1995). This list includes QTLs for alcohol preference (Melo et al., 1996; Phillips et al., 1998), acute ethanol withdrawal (Buck et al., 1997), and loss of the righting reflex (Markel et al., 1997). Although QTL detection for ethanol phenotypes has generally been quite successful,

it is but the first step in the lengthy process of gene identification. The next step will be the fine mapping of the QTLs to intervals of ≤ 1 cM; for this step it will be necessary to use strategies such as advanced intercross lines or interval-specific congenic strains (Darvasi, 1998). The formation of congenic strains will also allow for a direct test of whether the QTLs, such as those identified on chromosome 2, are indeed pleiotropic.

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