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Effect of genetic cross on the detection of quantitative trait loci and a novel approach to mapping QTLs

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Abstract

A genome-wide scan was conducted in two F_2 intercrosses, C57BL/6J (B6) × DBA/2J (D2) and BALB/cJ (C) × LP/J (LP), for three different phenotypes: basal locomotor activity, ethanol-induced locomotor activity, and haloperidol-induced catalepsy. For basal activity, significant quantitative trait loci (QTLs, LOD ≥ 4.3) were detected on chromosomes 9 and 19 for the C × LP intercross and chromosome 1 for the B6 × D2 intercross. Significant QTLs for ethanol-induced activation were detected on chromosome 6 for the C × LP intercross, and on chromosomes 1 and 2 for the B6 × D2 intercross. For haloperidol-induced catalepsy, significant QTLs were detected on chromosome 14 (two different QTLs) in the C × LP intercross, and chromosomes 1 and 9 in the B6 × D2 intercross. These data illustrate the importance of the genetic cross for QTL detection. Finally, the data reported here, and elsewhere, are also used to demonstrate a novel approach to QTL detection and localization.

Keywords: Quantitative trait loci; Mouse; Ethanol; Locomotor activity; Haloperidol; Catalepsy

1. Introduction

Quantitative trait loci (QTL) analysis of murine behavioral phenotypes has reached an interesting stage of development. It is now clear that behavioral QTLs can be reproducibly mapped, with high LOD scores [10,12,16], and that these QTL intervals can be reduced to 1–2 cM [5,22]. The recently reported draft of the human genome and the pending completion of the mouse genome will advance QTL analyses by allowing identification of candidate genes within small QTL intervals and determination of functionally relevant polymorphisms.

To date, most behavioral QTL studies (especially those related to alcohol and substance abuse) have relied either on the use of BXD recombinant inbred (RI) series and/or intercrosses derived from the C57BL/6J (B6) and DBA/2J (D2) inbred strains. The B6 and D2 strains are good choices as progenitor strains for QTL studies since they differ markedly on a wide variety of behavioral phenotypes [3], and are highly polymorphic [7]. However, in any single cross, QTLs are identifiable only in those chromosomal regions where the progenitor lines have a relevant functional polymorphism. Most QTL practitioners agree that the ideal situation would be to have data from multiple crosses. Historically, the high cost associated with genotyping large numbers of animals has precluded this strategy. As these costs drop, with the advent of new technologies, the use of multiple crosses becomes more viable. In this article, we summarize published findings [5,16,20,21], and present new work from our laboratory on the QTL analysis of three phenotypes (basal activity, ethanol-induced locomotion, and haloperidol-induced catalepsy) obtained in two different F_2 intercrosses [B6 × D2 and BALB/cJ × LP/J (C × LP)]. The data obtained illustrate that there is largely no overlap in the QTLs obtained from the two crosses. Finally, the basal activity QTL data were combined with the open-field activity data obtained from B6 × C [10] and B6 × A [12] F_2 intercrosses; the results obtained suggest a new strategy for reducing the QTL interval.

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2. Methods and materials

2.1. Animals and sample sizes

Male and female B6, D2, C, LP, and B6 × D2 F_1 mice were obtained from Jackson Laboratories (Bar Harbor, ME). For production of the F_2 mice, D2 × B6, C × LP, and LP × C F_1 mice were bred locally from the Jackson progenitor lines. Reciprocal F_1 crosses were used to generate a total of 1800 B6 × D2 and 900 C × LP F_2 mice from 6/1/1996 to 8/1/1998. The larger B6 × D2 intercross was formed as part of a project to detect gender specific QTLs. Mice were housed two to four per cage in a constant temperature colony room with a 12-h light/dark cycle. Food and water were provided ad libitum throughout the study. 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 the NIH Guidelines for Using Animals in Intramural Research. It should be noted that the same animals were used for the saline–ethanol challenge and haloperidol testing. Preliminary studies were conducted and demonstrated that there was no carry-over effect of the saline–ethanol challenge on haloperidol response, providing a 2-week washout between procedures. The reverse strategy showed marked effects; basal activity was decreased and the ethanol activation response was attenuated.

On average, 120 each of the phenotypic extreme mice (>1 and <1 S.D. from the mean) and 240 of the intermediate phenotypic mice were genotyped for each of the markers used in the genome-wide scan. The intermediate phenotypic data were obtained as part of other ongoing experiments with this data set. Both the phenotypic extremes and intermediates were pseudorandomly selected. Effectively, the animals genotyped provided 90% of the power to detect QTLs from a sample of $N = 720$ [18]. The minimum QTL effect size that could be detected was estimated from $N\ (720) = (Z_\alpha + Z_\beta)^2/(s_{QTL}{}^2/s_{RES}{}^2)$, where Z_α and Z_β are the normal variates for 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. For $\alpha = .0001$ [$Z_\alpha = 3.89$ (two-tailed)] and $\beta = .1$ ($Z_\beta = 1.28$), $s_{QTL}{}^2 = 0.036$. Increasing the α threshold to .00001, increases $s_{QTL}{}^2$ to 0.041 while decreasing the threshold to 0.001, decreases $s_{QTL}{}^2$ to 0.029. These estimates are based on the assumption that all QTLs have equal and additive effects [1,9]. Since this assumption is most certainly violated, the power of the analysis will be somewhat less.

2.2. Measurement of locomotor activity

Mice were removed from the home cage, administered saline, and placed individually in the testing arena; the arena floor was covered with standard laboratory bedding. Locomotor activity was monitored for 20 min. On the next day, the procedure was repeated except that the animals were administered 1.5 g/kg ethanol dissolved in saline (20% v/v). The difference score between the ethanol and saline days was taken as the ethanol response. One week later, the saline/ethanol days were repeated; the data from the two tests were averaged [19].

Locomotor activity was assessed using a San Diego Instruments Flex Field locomotor system. The apparatus comprised a 4 × 8 array of photocells mounted in a 25 × 47-cm metal frame, situated 1 cm off the floor, and surrounding a 22 × 42 × 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.

2.3. Measurement of catalepsy

One week after the locomotor testing, animals were removed from the home cage and placed in individual cages for 30 min. Animals were then administered the typical antipsychotic drug, haloperidol, by intraperitoneal injection. Fifteen minutes after injection, the animals were tested for catalepsy as described in Hitzemann et al. [13]. For a positive response, the animal must maintain a fixed rearing posture against the side of the cage for 30 s. To minimize the possible effect of differences in metabolism or sensitivity, no subsequent time points were evaluated. In some preliminary studies, the ED_{50} was determined in both the F_1 and the F_2 crosses using the *up-and-down* method [8]. For both crosses, the ED_{50} was approximately 4 mg/kg. This dose of haloperidol was then used to screen the F_2 animals as haloperidol responders and nonresponders. One week later, the responders and nonresponders were challenged with 0.06 and 7.5 mg/kg, respectively. This challenge provided four phenotypic categories: very responsive (RR), responsive (R), nonresponsive (N), and very nonresponsive (NN). The doses of haloperidol used in the second challenge were empirically determined to provide RR and NN samples of equal size, with each containing approximately 18% of the total F_2 population.

2.4. DNA isolation and genotyping the microsatellite polymorphisms

High molecular weight genomic DNA was isolated as described in Demarest et al. [5]. All of the genotyping involved the $-(CA)_n-$ repeating microsatellites first described by Dietrich et al. [6]; assays were performed as described in Demarest et al. [5].

2.5. Detection and mapping of QTLs

For the saline and ethanol activity data, the Phenotype × Genotype interaction was analyzed separately for each marker using standard ANOVA procedures. The F_2 data are presented graphically as the F value obtained for each marker. With the sample sizes used, an F value >11.3, exceeds the LOD threshold of 4.3 [17]. The strategy for

analyzing the categorical catalepsy data was somewhat different. Twenty-five each of the phenotypically extreme mice were randomly selected for genotyping. The screening threshold for a significant segregation of the alleles was set at $P < .1$. For markers meeting the screening threshold, 25 each of the phenotypically extreme mice were again randomly selected and genotyped to confirm a significant effect; in this second step, mice were randomly selected from the entire population, and thus, one or two of the animals from Step 1 were included in Step 2. For markers now significant at $P < .01$, the remainder of the RR and the NN phenotypic extreme mice were genotyped ($n = 120–150$ for both groups). Genotypic data were principally analyzed using the χ^2 statistic; LOD scores were estimated from $\text{LOD} = 0.2173(\chi^2)$, for an additive ($df = 1$) model [18].

3. Results

3.1. Genome scans

The genome-wide scans for basal activity, ethanol-induced activity, and haloperidol-induced catalepsy in the C × LP and B6 × D2 intercrosses are presented in Figs. 1 and 2. Only QTLs with an LOD > 4.3 are discussed here; the approximate two LOD support interval for each QTL is given in parentheses. Basal activity was determined from the 0–20-min interval of the saline test day (see Methods). For basal activity, significant QTL in the C × LP intercross were found on chromosome 9 (25–55 cM) and chromosome 19 (0–35 cM); in the B6 × D2 intercross, a significant QTL was found only on chromosome 1 (55–85 cM) (Fig. 1). Ethanol-induced activity for the 0–20-min interval was taken as the difference in activity between the ethanol (1.5 g/kg) and saline test days (see Methods). For the C × LP

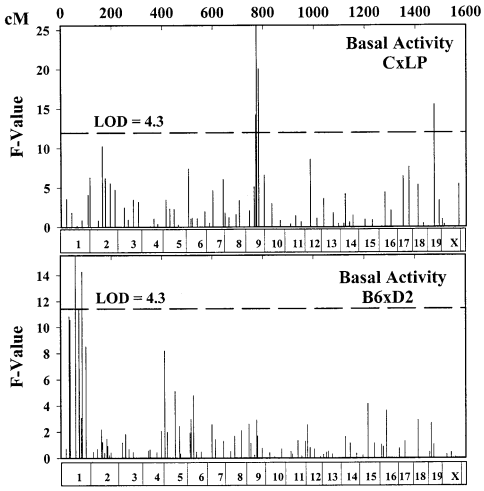

Fig. 1. Genome-wide scan for basal activity QTLs in C × LP and B6 × D2 F_2 intercrosses. Basal locomotor activity was measured using a test/retest design over 0–20 min after placement in the activity apparatus. Details on the phenotypic data are found in Koyner et al. [16]. From each intercross, on the average, 120 each of the extreme phenotypes (>1 and <1 S.D. from the mean) and 240 of the intermediate phenotypes were genotyped for each of the markers used in the genome-wide scan. Each individual was genotyped for 80–90 microsatellite markers [7]. For each marker, standard ANOVA techniques were used to calculate the F value. F value ≥ 11.3 meet the threshold for significance (LOD = 4.3) [17]. The data for each marker are represented by a bar in the graphs.

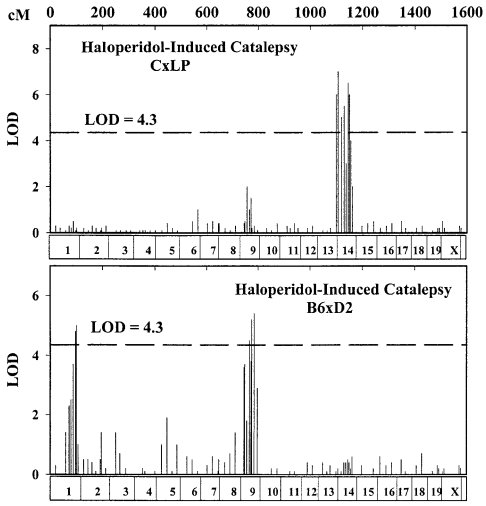

Fig. 2. Genome-wide scan for ethanol-induced activity QTLs in C × LP and B6 × D2 F_2 intercrosses. Ethanol-induced activity was the difference score between the activity in response to 1.5 g/kg of ethanol and the response to saline. Phenotypic details are found in Ref. [5]. See legend to Fig. 1 for additional details.

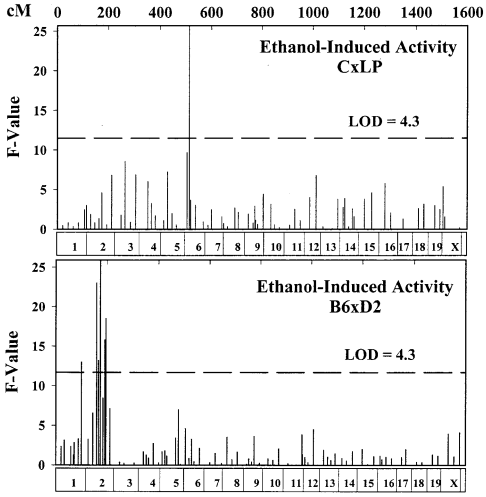

Fig. 3. Genome-wide scan for haloperidol-induced catalepsy QTLs in C × LP and B6 × D2 F_2 intercrosses. Haloperidol-induced catalepsy was measured using a two-step procedure. For the first step, the animals were challenged with 4 mg/kg, ip; this dose divided the animals into roughly equal numbers of responders and nonresponders. One week later, the animals were challenged either with 0.06 or 7.5 mg/kg haloperidol (responders/nonresponders, respectively). The second step isolated the RR and NN phenotypic extremes, which comprised 18–20% each of the total population. These phenotypic extremes were subjected to a genome-wide scan as described in Methods. The Genotype × Phenotype association was determined using the χ^2 statistic; LOD scores were estimated from $\text{LOD} = 0.2173(\chi^2)$ for an additive ($df = 1$) model [18].

intercross, only a single QTL on chromosome 6 (20–55 cM) was detected for ethanol-induced activity; in the B6 × D2 intercross, QTLs were detected on chromosomes 1 (70–100 cM) and 2 (50–90 cM) (Fig. 2).

The procedure for detecting QTLs for haloperidol-induced catalepsy differed somewhat from that for the activity phenotypes, which were continuous, normally distributed variables. In contrast, catalepsy was defined as a categorical variable, following a two-dose challenge procedure (see Methods); the intercross progeny were categorized as RR, R, N, or NN. The QTL analysis focused on the phenotypic extreme mice; effects were detected by χ^2 analysis, and the χ^2 values were converted to LOD scores. For the C × LP intercross, two significant QTLs were detected on chromosome 14 (0–20; 35–60 cM); for the B6 × D2 intercross, QTLs were detected on chromosome 1 (70–100 cM) and chromosome 9 (20–50 cM) (Fig. 3).

4. Discussion

The data presented here confirm and extend previous studies (e.g., Ref. [11]) showing that the detection of specific QTLs is cross-dependent. Most behavioral QTL research has focused on using either the BXD RI series or B6 × D2 intercrosses for QTL detection. This focus has been well placed, given that the strains are highly polymorphic [20], and differ markedly for a wide variety of phenotypes (e.g., Ref. [2]). Thus, the repeated observation that highly significant QTLs can be detected and confirmed [3], in crosses derived from these inbred strains, cannot be unexpected. However, it is important to recognize that these QTLs probably reflect only a small fraction of the available natural variation. Here, we introduce a novel intercross, C × LP, for QTL detection. The choice of these strains stems from studies of haloperidol-induced catalepsy. Of over 45 strains studied to date, the C and LP strains are representative of the extreme phenotypes; the ED_{50} for the C strain = 0.2 mg/kg, and the ED_{50} for the LP strain = 10 mg/kg [14]. Further, among the seven inbred strains in the MIT catalog (D2, C, LP, A/J, AKR/J, C3H/HeJ, CBA/J), the LP strain has the highest striatal D_2 receptor density and the highest number of striatal cholinergic neurons [4,15]. Under conditions of normal laboratory lighting, the C and LP strains differ markedly in terms of basal activity (>100% higher in the C strain) and in terms of ethanol-induced activation (high in the C strain, absent in the LP strain). Overall, for the three phenotypes studied here, the C and LP strains differ either to the same extent or more than the differences found in the B6 and D2 strains (see, e.g., Ref. [5]). The results presented in Figs. 1–3 illustrate that there is no obvious overlap in the QTLs detected in the two intercrosses, illustrating the potential value of using multiple crosses for QTL detection.

The use of multiple crosses may also be of value in narrowing QTL intervals, a point that will be illustrated for basal activity QTLs. Using the procedures outlined in Methods section, it has been observed that the B6 strain shows the highest level of basal activity compared to the other seven strains in the MIT catalog of microsatellite markers [5,16]. From this perspective, it is of interest to note that QTLs for basal/open-field activity are detected on the distal portion of chromosome 1 in the B6 × D2, B6 × C, and B6 × A F_2 intercrosses [10,12,16]. The resolution of the mapping in each of these studies is not sufficient to determine if one is dealing with identical or different QTLs; however, the interval maps from each of these studies suggest that two different QTLs may be present. One QTL is centered at approximately 60–70 cM [16], and is present in the same general region as the QTL for open-field activity detected by Talbot et al. [22] in heterogeneous stock (HS) mice. The second QTL appears to be centered at 90–100 cM [10,12]. However, the precision of the F_2 intercross studies is such that one cannot discount the possibility that the same QTLs were being detected. Given the availability of data from multiple crosses, a strategy evolves for more precisely localizing the QTLs and determining the likelihood that multiple QTLs are present. A review of the MIT catalog of microsatellite markers revealed that for chromosome 1 there were 254 markers distributed across 52–110 cM, and for which data were available for the B6, D2, A, C, and LP strains. A total of 162 of these markers (64%) were polymorphic between the B6 and D2 strains, 117 markers (46%) were polymorphic between the B6 and C strains, and 149 markers (59%) were polymorphic between the B6 and A strains. Panel A illustrates the distribution of the markers polymorphic between the B6 and D2 strains. The markers are not evenly distributed; clusters of markers are found at approximately 68, 75, 82, and 110 cM. Panel B illustrates the distribution of markers where the B6 strain is polymorphic to both the D2 and C strains; 92 of the MIT markers met this criterion. This second filter most significantly reduced the proportion of markers found at approximately 75 cM; other effects observed were a decrease in the markers over the range of 52–65 cM, and an increase in the proportion of markers on the distal (95–110 cM) portion of the chromosome. The third filter (Panel C) imposed the criterion that the B6 strain was polymorphic to the D2, C, and A strains; 72 of the MIT markers met this criterion. This third filter continued the trends seen with the second filter, and, in addition, decreased the proportion of markers clustered at approximately 82 cM. The fourth filter (Panel D) imposed the same criterion as in Panel C plus the criterion of MIT markers, which are not polymorphic between the C and LP strains (note in Fig. 1 that there is no chromosome 1 QTL for this intercross). This final filter reduced the number of markers meeting criterion to 35, and importantly, the residual markers were now clearly defined as two clusters, one peaking at approximately 67 cM, and the other showing a broad distribution over a region from 95 to 105 cM. These are precisely the regions where QTLs were identified in the F_2

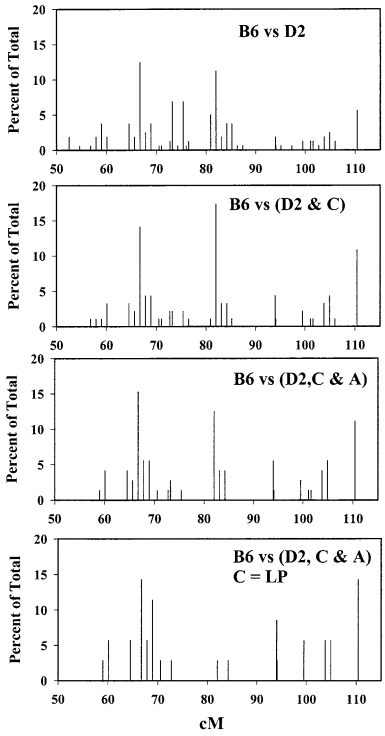

Fig. 4. Distribution of microsatellite markers on the distal portion of mouse chromosome 1. The Whitehead catalog of MIT microsatellite markers were screened for those markers from 52 to 110 cM on chromosome 1, and for which genotypic data were available for the B6, D2, C, A, and LP strains. A total of 254 markers met these criterion. Panel A illustrates the distribution of markers that were polymorphic between the B6 and D2 strains ($N=162$). Panel B illustrates the distribution markers that met the criterion, B6 differs from D2 and C ($N=92$). Panel C illustrates the distribution markers that met the criterion, B6 differs from D2, C, and A ($N=72$). Panel D illustrates the distribution markers that met the criterion, B6 differs from D2, C, and A and C equals LP ($N=35$).

intercross studies [10,12,16], and further, the peak at 67 cM appears to coincide with the QTL identified in HS mice and mapped to a resolution of <2 cM [22]. These may simply be chance occurrences, and additional examples will be required to determine if there is an underlying principle that, heretofore, has not been exploited. However, the data presented in Fig. 4 suggest that there is substantial information content in the strain patterns of the MIT markers. It is likely that even the casual user of microsatellites for QTL analysis has encountered the problem of chromosomal domains where there are no markers polymorphic between strains of interest. The significance of this problem has been largely ignored, and it is generally assumed that one only needs to look to other microsatellites or to single nucleotide polymorphisms to find informative markers. On the other hand, given the relatively high degree of relatedness among the commonly used inbred mouse strains, finding substantial isomorphic domains should not be surprising. This argument then leads one intuitively to propose that the distribution of the MIT markers (or other marker sets) could be used to create plots of polymorphism probability. Such plots could then be used to predict regions where candidate genes will be found. As the number of crosses used increases, the probability plots become more restrictive and, thus, more informative.

A related issue to using multiple line crosses for bringing focus on candidate genes is to use the information to predict functional polymorphisms. Once a QTL interval is reduced to <1 cM (now technically possible [22]), one is currently faced with the daunting task of determining which of approximately 50 genes is(are) key to the QTL. Many of the genes will have unknown function and/or will only be functional during development. In a recent paper describing the interface between genetics and genomics, Wells and Brown [23] noted that associated with the establishment of a mouse mutant resources has been the fear "that moving in a systematic and comprehensive manner from the uncovered phenotypes to the underlying genes would be a slow and resource-intensive task." However, these authors respond by both example and argument "that the rapid pace of developments in genomics would appear to give us confidence that these fears are unfounded." We would argue that a similar conclusion could be reached for moving form QTL to gene. However, the problem of detecting silent from functional polymorphisms will be greater for QTL analysis as opposed to mutagenesis. This particular QTL problem should be largely solved by using multiple line crosses to establish an algorithm for evaluating the polymorphisms. As noted in Fig. 4, the number of markers meeting criterion was reduced from 162 to 35 (-78%). A similar reduction in sorting through the polymorphisms in ORFs and the associated 5′ and 3′ UTRs would be of significant benefit.

Acknowledgments

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