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Quantitative trait loci (QTL) for physical activity traits in mice

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The genomic locations and identities of the genes that regulate voluntary physical activity is presently unknown. The purpose of this study was to search for quantitative trait loci (QTL) that were linked with daily mouse running wheel distance, duration, and speed of exercise. F_2 animals (n=310) derived from high active C57L/J and low active C3H/HeJ inbred strains were phenotyped for 21 days. After phenotyping, genotyping using a fully informative SNP panel with an average intermarker interval of 13.7cM was used. On all three activity indices, sex and strain were significant factors, with the F_2 animals similar to the high active C57L/J mice in both daily exercise distance and duration of exercise. In the F_2 cohort, female mice ran significantly farther, longer, and faster than male mice. QTL analysis revealed no sex-specific QTL, but at the 5% experimentwise significance level, did identify one QTL for duration, one QTL for distance, and two QTL for speed. The QTL for duration (*DUR13.1)* and distance (*DIST13.1)* colocalized with the QTL for speed (*SPD13.1).* Each of these QTL accounted for approximately 6% of the phenotypic variance whereas *SPD9.1*, (Chr. 9, 7 cM*),* accounted for 11.3% of the phenotypic variation. *DUR13.1, DIST13.1, SPD13.1, SPD9.1* were subsequently replicated using haplotype association mapping. The results of this study suggest a genetical basis of voluntary activity in mice and provide a foundation for future candidate gene studies.

Introduction

There is substantial evidence that physical inactivity contributes to a large variety of chronic health conditions (e.g. 3, 28). Several studies with animals and humans have suggested that genetic factors may play a significant role in regulating voluntary daily activity (e.g. 12, 16, 19, 31, 35). However, no investigations have reported the location or identity of the genetic factors involved in regulating voluntary physical activity.

Previous attempts at identifying locomotor-related behavior genetic linkage has provided tantalizing suggestions regarding possible chromosomal locations of regulating genes. Conti, et al. (5) identified two significant QTL related to open-maze locomotion behavior, Gershenfeld and colleagues (8) identified several significant QTL related to open-field activity, and Toth and Williams (33) have identified several suggestive QTL linked to the amplitude of the oscillation in daily activity in mice. Recent data have suggested that short-term behavioral locomotor tests, such as maze locomotion and open-field activity, are more tests of fear and anxiety and do not correlate well with home cage activity (23, 24). Therefore, previously identified QTL associated with short-term locomotor behaviors may not be associated with activity that is truly spontaneous and voluntary.

We therefore initiated a study to search for QTL that are associated with how far (distance), how long (duration), and how fast (speed) mice ran on a running wheel permanently mounted in their home cage. This study involved a full genome scan for QTL for these traits in the F_2 descendants of two inbred strains that were previously shown (19) to exhibit significant differences in daily physical activity (C57L/J - high active; C3H/HeJ - low active). Several QTL were discovered for each of the activity traits in this population of mice, and their individual additive and dominance effects on the traits were estimated. We also replicated the existence and refined the genomic position of the two QTL with the largest effect on activity using haplotype association mapping in 27 inbred mouse strains.

Methods

Animals: The UNC Charlotte Institutional Animal Care and Use Committee approved the procedures in this study. We followed the guidelines for ethical use of animals from the American Physiological Society and the American College of Sports Medicine.

In a previous study (19) we identified C57L/J inbred mice (generation F_{130}) as having high daily physical activity (HI-PAL) and C3H/HeJ inbred mice (generation F_{170}) as having low daily physical activity (LO-PAL). Ten C57L/J (5 males and 5 females) mice and 10 C3H/HeJ mice (5 females and 5 males) underwent a reciprocal breeding protocol which produced 63 F_1 mice; 32 C3C57 F_{1a} mice from C3H/HeJ mothers and C57L/J fathers and 31 C57C3 F_{1b} mice from C57L/J mothers and C3H/HeJ fathers. The F_1 mice were weaned at 21-28 days, phenotyped for voluntary daily activity (see below), and then 10 C57C3 F_{1b} (5 males and 5 females) and 10 C3C57 F_{1a} (5 males and 5 females) mice were randomly chosen for reciprocal crossing.

An attempt was made to produce at least 300 F_2 offspring from these F_1 matings. This sample size was expected to be a reasonable compromise between the labor-intensive difficulties associated with the phenotyping process (see below) and the achievement of an acceptable level of statistical power for the detection of QTL (18). With a sample size of 300, the statistical power to detect a QTL that contributes 5% of the total variation in a trait is 0.98 if its effects are entirely additive or 0.90 if there is complete dominance (22). For a QTL contributing only 2.5%, the statistical power is still relatively high: 78% if there is no dominance and 61% for complete dominance (22). Although it is known that the effects of QTL detected with very low statistical power may be considerably overestimated, those estimated with 60 to 98% statistical power have very little inflation (1).

The F_1 crosses were successful in producing a total of 310 F_2 progeny. There were four resulting types of F_2 offspring (i.e. F_2 substrains): $F_{2v} = F_2$ mice that had C57C3 F_{1b} mothers / C3C57 F_{1a} fathers (C57C3/C3C57; n=100); $F_{2w} = F_2$ mice that had C3C57/C57C3 progenitors (n=84); $F_{2x} = F_2$ mice that had C3C57/C3C57

progenitors (n=71); and F_{2y} = F_2 mice that had C57C3/C57C3 progenitors (n=55). The F_2 mice of all four types were weaned at 21-28 days and phenotyped. All mice were housed in the same room in the University Vivarium that was maintained at 18-21°C and 20-40% humidity with 12 hour light/dark cycles. Food (Harland Teklad 8604 Rodent Diet, Madison, WI) and water and were provided *ad libitum*. Each mouse was weighed to the nearest tenth of a gram weekly.

Phenotyping daily wheel running activity level: Phenotyping of daily activity was conducted as in past studies from our lab (19, 35). Briefly, after weaning, mice were housed individually in a rat cage with a solid surface running wheel mounted in the cage (145 mm diameter, Ware Manufacturing, Phoenix, AZ). A magnetic sensor was glued to the running wheel that was interfaced to a small bicycle computer (either BC600 or BC500, Sigma Sport, Olney, IL) that counted the total wheel revolutions and time each mouse spent exercising (19). Total daily distance (kilometers) and total daily exercise time (minutes) were recorded every 24 hours for each mouse, with average daily running speed (meters/minute) being subsequently calculated by dividing distance by duration. For the F_1 and F_2 cohorts, these three physical activity level (PAL) phenotypes were measured continuously for 21 days beginning when the mice were 63 days old (9 weeks) to account for any fluctuations in daily wheel running due to menstrual cycling in the female mice and to prevent confounding due to the mice learning to run on the wheels. The male parental mice were also phenotyped between 9 and 11 weeks of age. Due to time constraints, the female parental mice were not phenotyped, but representative female data from earlier strain screens (19) were used for phenotypic comparisons. During phenotyping, each mouse used the same wheel throughout the 21-day monitoring period and the wheels were checked on a daily basis to insure that they turned freely.

Genotyping: Within seven days after the phenotyping was completed the mice were anesthetized using 2-4% inhaled isofluorine. Kidneys were then harvested, flash frozen in liquid nitrogen, and stored at -80°C for later genotyping. DNA extraction from kidneys was performed as previously described (21) using a DNeasy kit (Qiagen, Inc., Valencia, CA). Genotyping of 129 single-nucleotide polymorphisms (SNPs) was conducted by

GeneSeek Inc. (Lincoln, NE) using a matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) system. All SNPs chosen were polymorphic between the progenitor strains (Wellcome-CTC Mouse Strain SNP Genotype Set - ref. 36) and covered the entire mouse genome with an average intermarker interval of 13.7 cM. Results are reported using NCBI Build 36.1 SNP locations.

Statistics: General linear modeling (GLM – JMP 5.1 SAS Institute, Cary, NC) was used to test for the contribution of strain (i.e. C57L/J, C3H/HeJ, F_1, F_2), substrain group within the F_2 cohort, gender, weight, and all appropriate interactions on each activity phenotype (i.e. daily distance, duration, and exercise speed). Alpha values were set *a priori* at 0.05 and variables that did not contribute significantly to the models were dropped and the analysis repeated using standard ANOVA methods. To determine pairwise differences (e.g. between genders and strains) in each trait, subsequent *post hoc* testing was completed using Tukey-Kramer HSD. Broad-sense heritability was calculated from the F_2 cohort using the degree of genetic determination (d^2) which was estimated by the following formula: $d^2 = (V_{F2} - V_{F1})/V_{F2}$ where V_{F1} and V_{F2} equal the variances of the F_1 and F_2 populations. The standard error of the degree of genetic determination was estimated using the following formula: $s_d^2 = (1- d^2)[2(1/N_1 + 1/N_2]^{1/2}$ where N_1 and N_2 are the degrees of freedom in the F_1 and F_2 populations respectively (7).

<u>*Preliminary Statistical Adjustment:*</u> Prior to the QTL analyses, we first tested the three activity traits (i.e. distance, duration, speed) for potential differences due to sex, litter size, rearing block, and body weight effects. These tests were accomplished in analyses of covariance in which sex, litter size, and block effects were treated as classification factors and body weight was treated as a covariate. If any of these factors were significantly associated with any of the activity traits, we then adjusted the data for these nongenetic effects. Normality tests were then conducted on the data to determine the need for subsequent transformation to normalize the data.

<u>*Experimental QTL Determination*</u>: Separate QTL analyses were run for the three traits using the interval mapping model (10) with a canonical correlation approach that we have previously described (15). To

implement this approach, additive genotypic index values of -1, 0, and +1 and dominance genotypic index values of -0.5, +0.5, and -0.5 were assigned for C3H/HeJ homozygotes, heterozygotes, and C57L/J homozygotes, respectively, at the site of each SNP marker. We also imputed genotypic index values for all locations 2 cM apart between flanking SNP markers on each chromosome using the recombination percentages given in the Mouse Genome Database (6) and the equations in Haley and Knott (10). The canonical analyses generated *F* values with their associated probabilities that were converted to a linear scale by logarithmic transformation [LPR = $\log_{10}$(1/Prob.)] to make the results comparable to LOD scores obtained via maximum likelihood analysis (13). If the highest LPR calculated for a given chromosome exceeded a specific threshold value, a QTL was considered to be present at the position associated with that LPR score.

The effective marker number approach of Cheverud (4) was used to generate specific threshold values for each chromosome (chromosomewise 5% values) as well as an overall 5% experimentwise threshold value. This approach does not require permutation runs but instead calculates 5% chromosomewise threshold values by division of 0.05 by the number of effective markers on each chromosome (obtained from the variance of the eigenvalues of intermarker correlations) and the 5% experimentwise threshold value by dividing 0.05 by the sum of the effective number of markers over all chromosomes (4). The 5% experimentwise specific threshold value for our F_2 mouse population was determined to be equal to 3.39 (i.e. significant LPR value = 3.39) while the 5% chromosomewise specific threshold values ranged from 1.77 to 2.13 with a mean of 1.97. LPR scores exceeding their appropriate chromosomewise threshold value were considered *suggestive* of linkage whereas those exceeding the experimentwise threshold value were taken as *significant* evidence of linkage. Confidence intervals for each QTL were determined by the one-LOD rule (22).

Each chromosome was also tested for two-QTL and sex-specific QTL effects. Sex-specific effects were tested by first assigning a code for the sex of each individual. We than ran a whole genome scan for each trait and tested for the interaction of sex with the additive and dominance genotypic index values (partialling the main effects due to genotypic values and sex). Any interactions exceeding their appropriate chromosomewise

threshold values were taken to represent QTL whose effects differed in the two sexes. In those instances where the interactions exceeded the threshold values, the QTL analyses were repeated for the separate sexes. Tests for two QTL on a given chromosome were done by comparing the chi-square values generated for the one- and two-QTL models. Where two QTL on a given chromosome were indicated, confidence intervals for each QTL were obtained as before, but partialling out the effects of the other QTL on that chromosome.

Multiple regressions of each trait on the additive and dominance genotypic index values were run for all QTL affecting that trait to estimate additive (*a*) and dominance (*d*) genotypic values and to test them for significance (using individual *t*-tests). The *a* values estimate one-half of the difference between the average phenotypic values of the two homozygotes and the *d* values estimate the difference between the average phenotypic value of the heterozygotes and the midpoint between the two homozygote genotypic values. These values are therefore useful in expressing the magnitude of effect of the QTL and were standardized (divided by the standard deviation) to permit comparisons of these effects among the three traits.

Epistasis Analysis: Once QTL were discovered for each of the three activity traits, significant interactions (epistasis) among each pair of QTL on different chromosomes were investigated. We expected to be able to detect any epistasis present because significant epistatic interactions among QTL for various traits have been discovered in other mouse populations of similar size (e.g. 37). Epistasis determination was accomplished by multiple regression of each trait on the additive and dominance genotypic index values for each of two QTL and the four interactions of the additive and dominance values. Tests for the overall significance of epistasis for each combination of QTL were made with an *F* statistic (numerator df = 4) generated from testing the four interaction terms. We used the conventional 5% significance level without adjustment for multiple comparisons problems since the locations for epistasis testing were chosen without any prior knowledge of potential epistatic effects (9). The regression coefficients associated with these four interactions estimated additive by additive (*aa*), additive by dominance (*ad*), dominance by additive (*da*), and dominance by dominance (*dd*) genotypic

epistatic terms. For those pairs of QTL reaching significance, testing for the individual significance of each of these four genotypic epistasis terms was done via individual *t*-tests.

Replication of experimentally derived QTL: Replication of significant experimentally-identified QTL found was accomplished with haplotype association mapping or HAM (also known as *in silico* analysis) using the bayesian imputation-based association mapping approach (30). In this analysis, distance, duration, and speed data from 27 inbred strains of mice (A/J, AKR/J, Balb/cJ, C3H/HeJ, C3Heb/FeJ, C57B/6J, C57L/J, CAST/EiJ, CBA/J, DBA/2J, MRL/MpJ, NZB/BinJ, SWR/J, and SPRET/EiJ from ref. 19; 129S1/SvImJ, C57Bl/10J, CE/J, LP/J, PL/J, SM/J, and WSB/EiJ – Lightfoot, personal communication; and BTBR_T+_tf/J, DBA/1J, FVB/NJ, NOD/LtJ, RIIIS/J, and SJL/J - extrapolated from ref. 29) were associated with 1,272 SNPs derived from the Wellcome-Trust SNP database (36). The wild-type strains (CAST/EiJ, SPRET/EiJ, and WSB/EiJ) were included in this dataset to increase the genomic diversity of the SNP-haplotype map (38). This dataset averaged 636 SNPs on the two chromosomes analyzed with an average distance between each SNP of 181.8 kbp (0.13cM). Output from this analysis was a set of Bayesian factors calculated at each SNP site; p-values were calculated for each Bayesian factor on each chromosome using 1000 permutations. Significance of each SNP Bayesian factor was set *a priori* as 0.01.

Results

Cohort Demographics and Activity Traits: As expected, male mice were significantly ($p<0.05$) heavier than female mice in all strains and substrains (Table 1). There was no difference found between the four F_2 substrains in any of the activity traits (i.e. distance, duration, or speed) and thus, these F_2 substrains were pooled for subsequent analyses. Weight and age did not exert significant effects on any of the activity traits and thus were dropped from further analyses. Strain ($p<0.005$, power>0.85 all traits) and sex ($p<0.0001$, power=0.99 all traits) were significant main effects in all traits with *post hoc* analysis indicating that in general, the F_2 cohort had activity levels similar to the C57L/J progenitors which were significantly higher than the C3H/HeJ progenitor strain (Figs. 1-3). *Post hoc* analysis indicated that the C3H/HeJ strain ran shorter distances than the

other three groups (Fig 1; approximately 117% less on average than the F_2 cohort) and ran less on a daily basis than the C57L/J and F_2 mice (Fig. 2; F_2 mice ran ≈ 81% longer than the C3H/HeJ mice). *Post hoc* testing revealed that mice in all of the strains ran significantly different speeds from each other (Fig. 3 - C57L/J > F_1 > F_2 > C3H/HeJ). The female F_2 mice exhibited significantly higher activity than the male F_2 mice, running on average 47% farther, 39% longer, and 9% faster. Broad-sense heritability (d^2) was high for all activity phenotypes: distance d^2= 0.495 ± 0.100 (mean ± SE); duration d^2= 0.586±0.82; and speed d^2 = 0.469±0.105.

QTL Analyses:

Non-genetic adjustments and sex-specific QTL analysis: For the genetic analysis, the three activity traits were adjusted for sex, litter size, and rearing blocks to decrease trait variation and enhance the likelihood of identifying QTL. The standard deviation of distance, duration, and speed was reduced on average by approximately 14% by the nongenetic variable adjustment. Normality tests showed that adjusted values for all three traits were normally distributed (data not shown). Not unexpectedly, all three traits were positively correlated ($p<0.01$; duration and distance, $r = 0.92$; duration and speed, $r = 0.45$; and distance and speed, $r = 0.71$). Given the sex differences we observed in the activity traits (Fig. 1-3), sex-specific QTL effects were tested. These tests indicated possible sex-QTL differences for distance and duration on chromosome 3 and for duration on chromosome 17. However, separate QTL analyses of the male and female cohorts for these three trait/chromosome combinations were not significant, showing no evidence of sex-specific QTL for any of the three activity traits. Therefore, all subsequent QTL analyses were based on the entire cohort without regard to sex.

One significant QTL (Table 2 – *DUR13.1*) and three suggestive QTL were discovered for duration. These QTL contributed on average nearly 5% of the variation in duration and a multiple regression model using *a* and *d* values from all four QTL showed that they jointly accounted for 14% (adjusted R^2 = 11%) of the total variance of duration. *DUR13.1* exhibited a large positive additive genotypic value and a non-significant positive dominance value, suggesting that the C57L/J allele increased duration.

At the 5% experimentwise level, one significant distance QTL (*DIST13.1*) was discovered, colocalizing in a similar location as *DUR13.1* – as did several of the suggestive distance and duration QTL – which probably represented pleiotropic QTL affecting both traits. The LPR score for *DIST13.1* was 4.14,and this QTL contributed 6.36% of the total variance in distance. Collectively, the significant and suggestive QTL for distance accounted for 21% (adjusted R^2 value = 18%) of the total variance of distance. *DIST13.1* was similar to *DUR13.1* in exhibiting significant positive genotypic values indicating that the C57L/J allele acts to increase distance.

Two QTL, *SPD9.*1 and *SPD13.1,* were significantly associated with speed of activity. *SPD13.1* probably represents the same QTL previously found for duration and distance (*DIST13.1, DUR13.1*) and *SPD9.1* appears to colocalize with the suggestive QTL *DIST9.1*. The LPR score for *SPD9.1* was 7.51, the highest achieved among the QTL for all three traits. The contribution of *SPD9.1* and *SPD13.1* were 11.27% and 6.10% , respectively and altogether, the QTL for speed accounted for 34% (adjusted R^2 value = 30%) of the total variance. Similar to the significant QTL for distance and duration, the positive additive values for *SPD9.1* and *SPD13.1* indicate that the C57L/J allele increased speed.

Epistasis Analyses: Almost no evidence for epistasis amongst the detected QTL was found. *F* tests for the pairwise interactions among QTL for duration and speed were all non-significant ($p > 0.05$). However, one of the 13 pairwise interactions among the QTL for distance, *DIST5.1* with *DIST9.1*, was significant ($p = 0.012$). Of the four epistatic genotypic components associated with this interaction, *dd* (standardized value = -0.599) was significant ($p = 0.009$). This dominance by dominance epistasis suggests that the single-locus dominance genotypic value (*d*) for *DIST5.1* changed depending on the genotype at *DIST9.1* and *vice versa*. Inclusion of this epistatic term in the single-locus multiple regression model for distance increased the genetic contribution to the total variance by 3% to 24% (adjusted $R^2 = 0.21$).

Haplotype Association Mapping: We conducted HAM analyses for only chromosomes 9 and 13 since the QTL on these chromosomes had the greatest effects (Table 3). Several significant ($p<0.01$) HAM QTL were found within the confidence intervals of the significant experimental QTL we identified on chromosomes 9 (*DIST9.1, SPD9.1)* and 13 (*DIST13.1, DUR13.1, SPD13.1).* The significant HAM QTL colocalizing within the identified experimental QTL were not present when the C57L/J phenotype/genotype data were omitted from the HAM analysis (data not shown). Suggestive HAM QTL were also found for both *DUR13.2* (rs3683883, 27.1cM, p=0.15), and *DIST13.2* (rs13481817, 27.107cM, p= 0.021), further refining the possible location of genes that play a role in regulating voluntary daily activity. The HAM analysis also identified six additional potential QTL linked to distance (five on chromosome 9 and one on chromosome 13), and 12 additional potential QTL linked to speed (eleven on chromosome 9 and one on chromosome 13). In particular, the area surrounding the site at 35 cM on chromosome 9 and 40 cM on chromosome 13 were strongly linked with both distance and speed (Table 3) indicating the potential for further QTL not revealed in the present F_2 cohort.

Discussion:

The results of this investigation, to our knowledge, represents the first identification of genomic locations associated with the regulation of voluntary wheel-running activity in mice. In this study, we have also supported previous observations of the heritability of wheel-running behavior, differential activity patterns between sexes, and have noted a lack of differences between the F_2 substrains suggesting that the genetic influences on physical activity were not transferred predominantly through either the maternal or paternal lines. Further, in the F_2 cohort, we have identified two significant and several suggestive experimentally-derived QTL that are linked with one or more of our three indices of activity. The significant QTL on chromosome 9 and 13 were confirmed using a haplotype association mapping analysis, which also indicated several sites in these regions for further candidate gene investigation. Given the conserved synteny between human and mouse genomes, these results provide a significant foundation for further research investigating the genetic regulation of voluntary daily activity in rodents and humans (2).

It has only become recently accepted that voluntary daily activity has a significant heritable component (12, 16, 19, 31, 35). Supporting this contention, we found that the broad-sense heritability of wheel-running behavior was substantial, ranging between 49-58% depending upon the activity measurement considered, which is similar to previously reported values (16, 19). Not surprisingly, our broad-sense estimates of heritability are somewhat higher than previous non-adjusted narrow-sense heritability estimates for wheel-running (0.12-0.24, ref. 32); however, the additive genetic component estimates in the current study and previous studies (adjusted realized heritability = 0.28, ref. 32) are similar.

Whereas the portion of the heritability/phenotypic variance that was accounted for by the QTL ranged from 11-34% depending upon the activity index used, it is probable that there are other QTL or genetic factors that explain more of the variability that were not uncovered in our limited cohort of F_2 animals. For example, Tsao, et al. (34) demonstrated that an overexpression of GLUT4 glucose transporters leads to a four-fold increase in daily activity in male mice. However, the GLUT4 gene is located on chromosome 11 (40cM), an area in which none of the QTL we identified in the current study were found. Our haplotype mapping results certainly indicated other sites on Chromosome 9 and 13 that may contain QTL that influence activity. Additionally, while the near absence of significant epistasis among the identified QTL was somewhat surprising (37, 39), this does not rule out the possibility of epistasis among loci that were not detected as main effect QTL. While these possibilities will require further investigation to identify all of the chromosomal locations that are linked to the regulation of physical activity, the current study has identified two major and several other suggestive loci that control voluntary daily activity in mice.

As additional QTL for measures of voluntary daily activity are eventually discovered in other mouse models, it will be interesting to see whether their mode of action is comparable to that seen for the QTL we have located. For example, it is unclear whether the lack of sex-specific QTL for the activity traits measured here indicates that the sex-mediated regulation of activity is a result of other biological factors not related to genetic regulation (i.e. ‘downstream’ of genetic regulation) or whether we simply did not have sufficient power to detect them in

our mouse population (see below). Certainly QTL that act differentially in the sexes have been detected for other traits in mice (14, 21). The mean levels of the standardized additive/dominance genotypic values and percentage contributions for our QTL, however, are generally quite comparable to those reported by Kenney-Hunt et al. (11) for a large number of QTL influencing a battery of body size components in a LG/J X SM/J intercross population of mice.

While no other study has identified QTL directly linked to the activity traits we used (i.e. wheel-running activity), other studies have identified QTL linked to other locomotion-related behaviors. In 25 recombinant inbred (RI) mouse strains developed from C57Bl/6J and DBA/2J inbred strains, Phillips, et al. (25) identified five QTL in 87-day-old female mice associated with the magnitude of horizontal movement in an activity monitoring chamber. Of these five QTL, three (Chrm. 9, 26-36cM; Chrm. 13, 9-10cM; and Chrm. 5, 20-30cM) colocalize with QTL identified in the present study - the significant QTL we identified on chromosomes 9 (*DIST9.1* and *SPD9.1)* and 13 (*DUR13.1, DIST13.1,* and *SPD13.1*), as well as the suggestive QTL*SPD5.1* . Unfortunately, Phillips and colleagues only reported measures of correlation and did not report any measure of QTL strength; thus, it is unknown if these QTL reached either experimentwise or chromosomewise level of significance (25).

Using twenty-two recombinant inbred rat strains - the HXB/BXH RI strains derived from SHR/O1a and the inbred congenic BN.*LA*Cub strain – that were 11-13 weeks old, Conti, et al. (5) identified two significant QTL on chromosomes 3 (47 cM; D3Rat180 proximal marker) and 18 (40 cM; D18Rat55 proximal marker) related to open-maze locomotion behavior. Additionally, Gershenfeld and colleagues (8) investigated the genetic regulation of open-field behavior (e.g. vertical rearing and response to novelty) and identified several significant QTL on chromosomes 1, 3, 10, and 19 and five additional suggestive QTL in 10-11 week old F_2 mice derived from A/J and C57Bl/6J progenitor strains. Comparisons of the results from Conti, et al. (5) and Gershenfeld, et al. (8) to those in the current study are difficult due to the shorter time interval of the locomotor phenotyping used by Conti, et al. (5 min observation) and Gershenfeld, et al. (15 min observation) as compared to our longer

term measurements (21 days). Additionally, Mill, et al. (23) observed that home cage activity in mice, similar to our measures of activity, was not correlated with open-field locomotor testing measures similar to what was employed by Gershenfeld, et al. (8). Further, open-field testing and maze testing, similar to that used by Conti, et al. (5) is now widely considered a measurement of fear and anxiety rather than voluntary locomotion in rodents (24). Thus, it is not surprising that none of the QTL (or QTL homologs) identified by either Conti and colleagues (5) or Gershenfeld, et al. (8) are similar to the QTL associated with the longer-term indices of activity that we used in this study.

Additionally, other locomotor-associated behaviors have been examined genetically, such as the efforts made to determine QTL associated with the amplitude of the daily oscillation in locomotion between day and night (33). This effort monitored activity using a tethered-EMG implant system in C57Bl/6, Balb/cBy, and 13 recombinant inbred strains. Unfortunately, only male mice were monitored for a 48 hr period of time and no significant QTL were discovered associated with the variation in activity (the total 24 hr. activity between all of the strains was not different). However, several suggestive QTL were identified by these authors, one being located on Chr. 12, (23 cM), in a location similar to our *SPD12.1*.

It is tempting to hypothesize that QTL for physiological traits associated with high exercise endurance (e.g. mitochondrial density, cardiac output) are associated with the functional ability to be active for long periods of time. However, these hypotheses are not justified at this time due to two issues. First, several studies, including work from our lab, have shown that physical activity and exercise endurance in mice are not correlated and are therefore, probably distinct phenotypes (e.g. 16, 19, 20). Further strengthening the contention that maximal exercise endurance and high/low physical activity are distinct phenotypes is the fact that the activity QTL noted in the current study do not colocalize with any of the mouse QTL recently published for exercise endurance (21). Secondly, given our inability to determine the length and intensity of the individual exercise bouts that the mice were performing, it is difficult to determine what type of physiological trait might have functionally allowed the increased activity in our F_2 cohort. If the mice were performing multiple short, intense bouts of

exercise, we would naturally wish to investigate colocalization of QTL of physiological traits favoring intense exercise bouts (e.g. percentage of Type II fiber composition, increased LDH, etc.). Conversely, if the mice were completing exercise in longer, less intensive bouts, then we would be interested in QTL for traits leading to longer duration exercise (e.g. Type I fiber composition, increased mitochondrial biogenesis, etc.). These types of QTL comparisons are logical for future studies when the types of activity bouts can be monitored.

Our general finding of higher activity levels in female mice supports results from multiple rodent studies that have found similar results (e.g. 12, 17, 19). However, as noted earlier, the lack of sex-related QTL could indicate that the differential regulation of activity due to sex is downstream of any genetic regulation. In fact, studies have clearly established that estrogen mediates physical activity through the estrogen-receptor α pathway (24). While the subsequent downstream pathways activated by the estrogen-receptor α pathway are still somewhat unclear, it has been postulated that estrogen-receptor α modulates several neurotransmitters, including dopamine in the female, which may lead to increased physical activity (24). Supporting a possible dopamine linkage are two studies (26, 27) that have suggested a role for various dopamine mechanisms in influencing activity. Interestingly, while no genes with known control of any sex hormones are located in the QTL regions identified in the current study, identified QTL that affect the physiological behavior of dopamine colocalize within some of the suggestive QTL sites we identified. *Dbh* (Chr. 2, 15.5cM), a gene that produces dopamine beta-hydroxylase, an enzyme that catalyzes the dopamine to norepinephrine pathway, is located within our *SPD2.1* QTL. Additionally, while no dopamine receptor genes are located within any of our identified significant QTL, there are two QTL, *Drb2* (Chr. 5, 54.0 cM) and *Drb5* (Chr. 12, 25.0 cM), both of which are associated with dopamine receptor binding, that colocalize within our identified *SPD5.1* and *SPD12.1* sites. Therefore, given the location of these dopamine-associated regions within three of our suggestive QTL sites and the apparent lack of estrogen-controlling genes in any of the identified QTL sites, we hypothesize that while the genetic regulation of activity may involve dopamine (i.e. through dopamine receptor and/or dopamine

metabolism), the sex-related estrogenic effects on activity appear to be nongenetic in nature and potentially occur downstream of other genetic regulatory mechanisms.

In summary, this study has experimentally identified four significant and at least 14 suggestive QTL associated with spontaneous activity in mice. The significant QTL on chromosomes 9 and 13 were validated using a haplotype association mapping approach, which also identified several other genomic loci where potential QTL may exist. This study also noted a clear sex-difference in activity patterns, but we hypothesize that this sex-difference results from a nongenetic mechanism functioning downstream of genetic regulation. Future research will focus on reducing the intervals where these identified QTL exist to ultimately identify genes that regulate physical activity.

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Table 1 – Mouse demographic data

			FEMALES		MALES	
Strain	Age at start of testing (days)	Weight (gm)	n	Weight (gm)	n	Weight (gm)
C57L/J	72±4	22.4±3.6	5	21.6±0.9†	5	24.4±1.7*
C3H/HeJ	73±3	23.0±1.9	5	19.2±1.1	5	25.6±1.7*
F_1 mice	63±0	28.2±2.6	25	25.8±1.1	38	29.8±2.1*
F_2 mice	63±0	26.2±3.5	149	23.7±2.2	161	28.5±2.9*

*Significantly ($p<0.05$) heavier than the females within strain.

Table 2. Experimentally derived QTL from F_2 cohort

QTL	Trait	CH	Marker	M Dist	C Dist	CI	LPR	%	*a/SD*	*d/SD*
DUR5.1	Duration	5	*CEL-5_11773662*	10	95	82—105	3.21	5.10	-0.34**	-0.06
DUR8.1	Duration	8	*rs13479600*	6	8	2—22	2.17	3.20	0.13	-0.36**
DUR13.1	Duration	13	*rs6329684*	10	11	1—15	4.10	6.24	0.92**	0.38
DUR13.2	Duration	13	*rs13481783*	2	21	19—27	3.21	5.01	-0.71**	-0.54*
DIST5.1	Distance	5	*rs13478553*	0	103	93—109	2.82	4.37	-0.31**	-0.06
DIST8.1	Distance	8	*rs13479600*	8	10	4—20	3.36	5.24	0.18*	-0.44**
DIST8.2	Distance	8	*rs3703161*	0	88	78—88	1.32	2.07	-0.04	0.29*
DIST9.1	Distance	9	*rs13480073*	0	1	1—13	3.31	4.93	0.25**	0.23*
DIST13.1	Distance	13	*rs6329684*	10	11	1—15	4.14	6.36	0.84**	0.39
DIST13.2	Distance	13	*rs13481783*	4	23	19—29	2.45	3.88	-0.52*	0.62*
SPD2.1	Speed	2	*rs13476352*	8	19	11—27	2.96	4.74	-0.19*	-0.42**
SPD2.2	Speed	2	*rs3664044*	0	109	63—109	2.44	3.92	-0.24**	0.22
SPD5.1	Speed	5	*mCV22996021*	6	57	27—75	2.72	4.19	0.26**	0.21
SPD8.1	Speed	8	*rs3023193*	0	50	44—52	2.88	4.55	0.19*	-0.35**
SPD8.2	Speed	8	*rs3703161*	0	88	78—88	1.80	2.85	-0.1	0.32**
SPD9.1	Speed	9	*rs13480073*	6	7	1—15	7.51	11.27	0.45**	0.22
SPD12.1	Speed	12	*CEL-12_33256230*	6	27	7—51	2.07	3.25	0.27*	0.10
SPD13.1	Speed	13	*rs6329684*	8	9	1—17	3.98	6.10	0.39**	-0.03

Locations, confidence intervals (CI), LPR scores ($\log_{10}\text{Prob}^{-1}$), percentage of the variation explained (%), and standardized additive (*a*) and dominance *(d)* genotypic values for QTL on all chromosomes (Ch) significantly affecting duration, distance, and speed. Locations are given as estimated map distances from the nearest proximal marker (M Dist) and from the centromere (C Dist), and confidence intervals are expressed from the centromere. All LPR values are significant at the 5% chromosomewise level and those exceeding 3.39 are significant at the 5% experimentwise level. * = $P < 0.05$; ** = $P < 0.01$.

Table 3 – Haplotype Association Mapping QTL

Chromosome	SNP id	Position kbp (cM)*	p-value	Distance between flanking markers –kbp (cM)	Within expQTL CI?
Phenotype = Distance					
9	rs33659069	6854.122 (0.000222)	0.022	808.634 (0.0001)	yes
	rs6217029	29988.19 (14.1874)	0.006	401.515 (0.372)	no
	rs6206353	55716.037 (35.7885)	0.002	245.029 (0.001)	no
	rs3677551	56503.472 (35.9467)	0.011	262.998 (0.001)	no
	rs3700733	74787.967 (49.6453)	0.007	280.061 (1.517)	no
	rs3680245	116352.785 (85.2537)	0.014	143.391 (0.041)	no
13	rs13481721	20572.081 (5.42)	0.016	208.983 (0.094)	yes
	rs3688040	78581.703 (40.83)	0.016	284.317 (0.001)	no
Phenotype = Speed					
9	rs6167265	20875.853 (5.83861)	0.01	558.426 (0.225)	yes
	rs6206353	55716.037 (35.7885)	0.009	245.029 (0.0003)	no
	rs3685822	63891.547 (39.384)	0.007	234.281 (0.141)	no
	rs13480253	67175.969 (42.089)	0.006	505.273 (0.161)	no
	rs13480317	85581.421 (54.6754)	0.012	116.4 (0.281)	no
	rs13480345	92031.249 (56.6244)	0.01	583.418 (0.448)	no
	rs6244819	96717.651 (59.4049)	0.008	672.598 (0.287)	no
	rs6377847	100653.305 (60.9671)	0.009	320.129 (0.0004)	no
	rs3685576	108916.785 (75.6429)	0.004	287.309 (1.52)	no
	rs3694903	113874.665 (81.6974)	0.012	174.257 (0.240)	no
	rs8254361	119186.669 (89.54)	0.007	558.426 (0.225)	no
	rs13480448	119277.02 (89.5405)	0.011	245.029 (0.0003)	no
13	rs6293765	35935.806 (11.4837149)	0.007	265.212 (0.00007)	yes
	rs13481817	54888.709 (27.1007)	0.007	270.778 (0.786)	yes
	rs3690969	96876.114 (51.9225)	0.006	277.15 (0.205)	no
Phenotype = Duration					
13	rs3678616	17074.23 (3.01134)	0.01	125.005 (0.0003)	yes
	rs13481721	20572.081 (5.42048)	0.007	208.983 (0.094)	yes
	CEL-13_27061395†	27161.335 (9.91703)	0.002	483.315 (0.155)	yes
	rs13481751	33727.974 (11.074)	0.01	910.848 (0.183)	yes
	rs13481752	33956.38 (11.1174)	0.002	459.701 (0.088)	yes
	rs13481762	36245.079 (11.48379901)	0.009	206.411 (0.00005)	yes
	rs3088825	37639.427 (14.5418)	0.004	397.518 (3.12)	yes

*Positions from NCBI Build 36.1. †Location from NCBI Build 34 (i.e. location not available in Build 36.1). An experimental QTL (expQTL) was considered confirmed if the HAM analysis identified a significant QTL within the confidence interval of the experimental QTL (Table 2). Average distance between flanking markers for HAM identified QTL = 363.637kbp (0.37cM).

Figure Legends:

Figure 1 – Daily distance run by sex and strain (mean±SD). Open bars are females, closed bars are males. *Significantly different between sexes within strain. †Significantly less distance than other strains.

Figure 2 – Duration of activity by sex and strain (mean±SD). Open bars are females, closed bars are males. *Significantly different between sexes within strain. †Significantly less duration than C57L/J and F_2 mice.

Figure 3 – Average speed of activity by sex and strain (mean±SD). Open bars are females, closed bars are males. *Significantly different between sexes within strain. All strains ran significantly different speeds from each other.

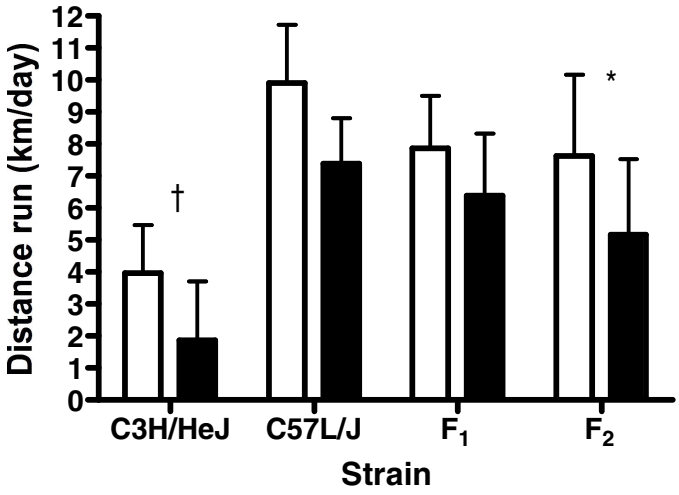
Distance run (km/day)
12
11
10
9
8
7
6
5
4
3
2
1
0
†
*
C3H/HeJ
C57L/J
F1
F2
Strain

Figure 1

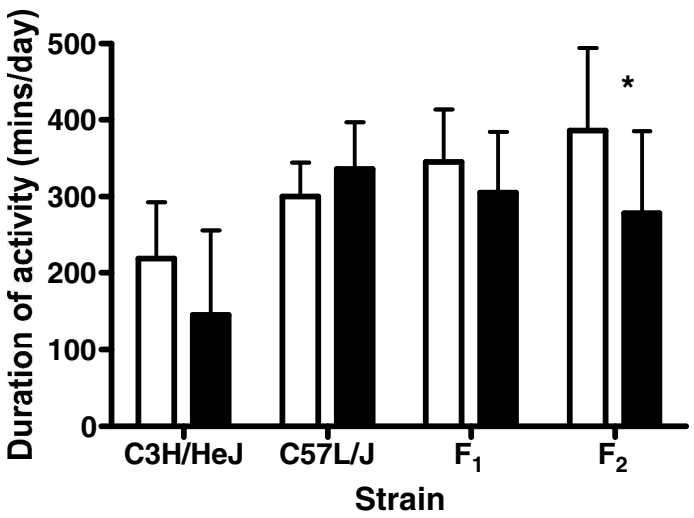
Duration of activity (mins/day)
500
400
300
200
100
0
*
C3H/HeJ
C57L/J
F1
F2
Strain

Figure 2

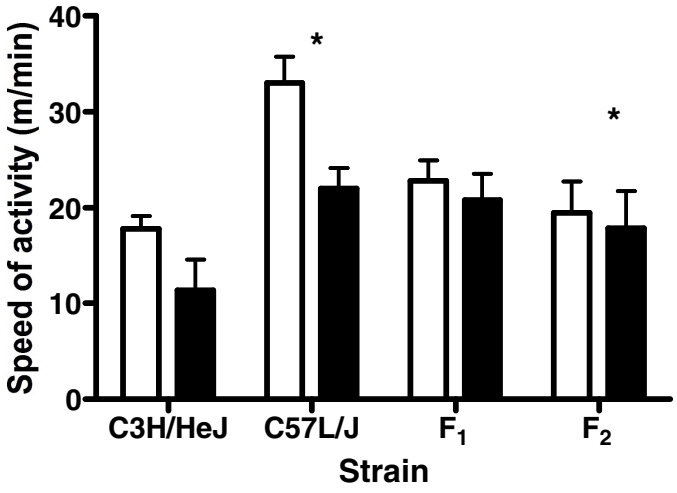

Figure 3