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Quantitative Trait Locus Analysis Identifies Rat Genomic Regions Related to Amphetamine-Induced Locomotion and $G\alpha_{i3}$ Levels in Nucleus Accumbens

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Abstract

Background: Identification of the genetic factors that underlie stimulant responsiveness in animal models has significant implications for better understanding and treating stimulant addiction in humans.

Methods: F_2 progeny derived from parental rat strains F344/NHsd and LEW/NHsd, which differ in responses to drugs of abuse, were used in quantitative trait locus (QTL) analyses to identify

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Conflicts of Interest/Disclosure

All of the authors report that they have no conflicts of interest over the past three years to report as related to the subject of the report. Dr. Potenza has received financial support or compensation for the following: Dr. Potenza consults for and is an advisor to Boehringer Ingelheim; has consulted for and has financial interests in Somaxon; has received research support from the National Institutes of Health, Veteran's Administration, Mohegan Sun, and Forest Laboratories, Ortho-McNeil and Oy-Control/Biotie pharmaceuticals; has participated in surveys, mailings or telephone consultations related to drug addiction, impulse control disorders or other health topics; has consulted for law offices and the federal public defender's office in issues related to impulse control disorders; has performed grant reviews for the National Institutes of Health and other agencies; has given academic lectures in grand rounds, CME events and other clinical or scientific venues; has generated books or book chapters for publishers of mental health texts; and provides clinical care in the Connecticut Department of Mental Health and Addiction Services Problem Gambling Services Program. Dr. Brodkin has received financial support or compensation for the following: Dr. Brodkin has performed grant reviews for the U.S. Civilian Research and Development Foundation, the New Jersey Governor,s Council on Autism, and the U.S. Department of Defense Autism Spectrum Disorder Research Program (grant review organized by Constella Group, Inc); is a member of the Gerson Lehrman Group Healthcare Council (although have not performed any services or received any compensation from Gerson Lehrman Group to date); has received research support from the National Institutes of Health, the Burroughs Wellcome Fund, the Cure Autism Now Foundation, NARSAD, the Philadelphia Foundation, and the Department of Veteran Affairs; and has given academic lectures in grand rounds and other clinical or scientific venues. Dr. Gelernter has received financial support or compensation from the following: related to consultation for Columbia University, the Thailand Center for Excellence for Life Sciences (TCELS), the University of CT Health Center, NIH, and Faegre & Benson; related to grant reviews for the National Institutes of Health; and related to academic lectures and editorial functions in various scientific venues (including for the ACNP). Drs. Yang, Birnbaum and Nestler do not have any additional financial support, compensation or personal financial holdings to disclose according to journal policy.

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genomic regions associated with amphetamine-induced locomotion (AIL) and G-protein levels in the nucleus accumbens (NAc).

Results: The most robust QTLs were observed on chromosome 3 (maximal log ratio statistic score (LRS_{max})=21.3) for AIL and on chromosome 2 (LRS_{max}=22.0) for $G\alpha_{i3}$. A "suggestive" QTL (LRS_{max}=12.5) was observed for AIL in a region of chromosome 2 that overlaps the $G\alpha_{i3}$ QTL. Novelty-induced locomotion showed different QTL patterns from AIL, with the most robust QTL on chromosome 13 (LRS_{max}=12.2).

Conclusions: Specific unique and overlapping genomic regions influence AIL, novelty-induced locomotion, and inhibitory G-protein levels in the NAc. These findings suggest that common genetic mechanisms influence certain biochemical and behavioral aspects of stimulant responsiveness.

Keywords

Addiction; F344 rats; Lewis rats; genetics; QTL; amphetamine; novelty-induced locomotion; nucleus accumbens; G proteins

Introduction

Differences in individual responses to stimulants exist in humans and other species. Some exposed individuals become dependent on amphetamine, methamphetamine, or cocaine, while others do not (Barr *et al*, 2006; Sofuoglu and Kosten, 2006). Genetic influences on psychiatric disorders associated with stimulant usage are also significant, with the genetic contributions to drug dependence (including to stimulants) estimated at 30%-60% (Tsuang *et al*, 1998). Divergent responses to stimulants are also important in the clinical arena, in which some individuals with attention deficit hyperactivity disorder (ADHD) are treated effectively with amphetamine or methylphenidate (Biederman *et al*, 2004). Cocaine dependence risk loci have been mapped in humans via genetic linkage (Gelernter *et al*, 2005); animal studies, however, provide the opportunity to identify risk loci for specific traits associated with pharmacological responses and other phenotypes that cannot be readily ascertained in human subjects.

Amphetamine-induced locomotion (AIL) is a well-studied phenomenon in animal models of psychiatric disorders, including stimulant addiction and psychoses (Blackburn and Szumlinski 1997; Ikemoto and Witkin 2003; Rajakumar *et al*, 2005). AIL, regarded as a measure of mesolimbic dopamine function (Dellu-Hagedorn, 2005), is mediated via dopaminergic transmission in the nucleus accumbens (NAc) (Kim and Vezina, 1998; Chausmer and Ettenberg, 1999; Millan *et al*, 1999; Ikemoto and Witkin, 2003). AIL differs from other types of locomotion, such as novelty-induced locomotion (NIL), which has been associated with stress responses. A complex relationship exists between drug-induced behaviors and novelty responses in rodents (Hiroi and Agatsuma, 2005; Agatsuma *et al*, 2006). AIL is differentially regulated in rats with high or low levels of spontaneous exploratory behavior (Corda *et al*, 2005; Alttoa *et al*, 2007). While AIL and NIL correlate with one another in some studies, genetically distinct animal strains show differences in AIL and NIL, and these animals provide an opportunity for identifying genetic factors influencing stimulant responsiveness (Brodkin *et al*, 1998; Marley *et al*, 1998; Stohr *et al*, 1998; Conversi *et al*, 2006). For example, Fisher (F344/NHsd) as compared with Lewis (LEW/NHsd), rats show more robust AIL, and roughly equivalent NIL (Brodkin *et al*, 1998; Stohr *et al*, 1998).

F344/NHsd and LEW/NHsd (referred to hereafter as F344 and LEW, respectively) rat lines have been used as models for multiple psychiatric disorders including addiction (Nestler *et*

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al, 1996; Kosten and Ambrosio, 2002), schizophrenia (Lipska and Weinberger, 1996), and depression (Lahmame *et al*, 1997). Previously, we reported differences in AIL and related phenotypes in these strains and their F2 progeny that were generated in preparation for quantitative trait locus (QTL) analysis (Brodkin *et al*, 1998). Multiple biochemical phenotypes related to striatal dopamine function, and implicated in stimulant addiction, were measured by western blotting specifically in the NAc, including inhibitory G protein subunits ($G\alpha_{i1,2}$ and $G\alpha_{i3}$), the dopamine transporter, the transcription factor ΔFosB, and the protein phosphatase inhibitor DARPP-32. Among the phenotypes investigated, levels of $G\alpha_i$ subunits appeared particularly promising for further study in QTL analyses (Brodkin *et al*, 1998). QTL analysis has been used previously to identify genomic regions contributing to a variety of quantitative traits, for example, stress-responsiveness, alcohol or morphine consumption, aggressive behaviors, and behavioral reactivity and emotionality (Moisan *et al*, 1996; Remmers *et al*, 1996; Bice *et al*, 1998; Ramos *et al*, 1999; Brodkin *et al*, 2002; Potenza *et al*, 2004; Ferraro *et al*, 2005). QTL analysis appears particularly applicable to the study of phenotypes related to psychiatric disorders, given its ability to identify genomic contributions to phenotypes determined by multiple genes. However, the genomic regions identified are usually large and contain multiple candidate genes, and additional investigation is typically required to identify specific genes.

Previously, we used QTL analysis to identify regions on chromosomes 4 and 10, which contribute to differences in peak corticosterone levels in F344 and LEW rats (Potenza *et al*, 2004). Here, we use a similar approach to investigate genetic contributions to AIL and levels of $G\alpha_{i1,2}$ and $G\alpha_{i3}$ in the NAc, using NIL as a behavioral control condition for AIL. We hypothesized that: 1) we would identify genomic regions contributing to AIL, NIL, $G\alpha_{i1,2}$ levels, and $G\alpha_{i3}$ levels; 2) genomic regions contributing to AIL would be distinct from those contributing to NIL; and 3) genomic regions contributing to AIL would partially overlap with those contributing to levels of $G\alpha_{i1,2}$ and $G\alpha_{i3}$ in the NAc.

Methods and Materials

Animal Procedures

The animal care and use committee at Yale University approved the study. The research was performed in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals. F344 and LEW rats were maintained and characterized as described previously (Brodkin *et al*, 1998; Potenza *et al*, 2004). F344 and LEW rats were obtained from Harlan-Sprague Dawley (Indianapolis, IN) at 35-45 days of age. F_1 progeny were generated by both F344 (female) X LEW (male) and LEW (female) X F344 (male) crosses and F_2 intercross progeny were derived from mating of both (F344 X LEW)F_1 X (F344 X LEW)F_1 and (LEW X F344)F_1 X (LEW X F344)F_1 pairs. F_2 progeny were weaned at 21 days of age. Animals were housed in groups of two to four with food (Purina chow) and tap water ad libitum in a temperature-controlled colony with a 12-hour light/dark cycle (lights on at 0700 hour). Only males were included in this study, to limit variation in measures associated with the estrus cycle in females, and given known sex differences in F344/NHsd and LEW/NHsd rats in AIL and NIL (Stohr *et al*, 1998).

Locomotor Activity Measures

Rats were assessed for locomotor activity between 8 and 10 am and between the ages of 50 and 60 days using a concentric circular device as described previously (Brodkin *et al*, 1998). Activity was recorded for 60 min. Data for the first ten min of activity in the novel environment were used to assess NIL, as prior studies showed that differences among rats in NIL were most pronounced during this time (Brodkin *et al*, 1998). Immediately following the 60 min test period, animals received injections of DL-amphetamine hemi-sulfate (2.0

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mg/kg s.c.), and locomotor activity was measured for an additional 60 min. Amphetamine was supplied by the National Institute on Drug Abuse (Baltimore, MD), made in isotonic saline and administered in a volume of 1 ml/kg. AIL data were calculated as cumulative activity over the 60-min test period.

Serum Levels of Amphetamine

F344 and LEW rats were given amphetamine (2 mg/kg, intraperitoneally) and trunk blood was obtained 40 min later, at the peak of behavioral effects of amphetamine. Samples were centrifuged for 10 min at 3000 g, the serum removed and stored at −20°C until analysis. Before analysis, amphetamine-d3 (50ng/ml) was added to each sample as an internal standard. Serum amphetamine levels were detected using a Micromass Ultima liquid chromatography/tandem mass spectrometry system (Waters Corp; Milford, MA) in positive ion mode and a BDS-C18 column (ThermoFischer Scientific, Inc.; West Palm Beach, FL) with a 0.8 ml/min flow rate as previously described (Hendrickson *et al*, 2004). Mobile phase was 20% acetonitrile, 0.05% acetic acid and 5 mM ammonium acetate. Amphetamine signals were quantified by comparison of multiple reaction monitoring signals to the internal standard.

G Protein Measures

Five to eight days after behavioral testing, animals were killed by decapitation between 3 and 5 pm as described previously (Brodkin *et al*, 1998). This was performed in a separate area from animal housing, with each animal retrieved individually from the housing area, and with glove changing between animals to minimize possible stress-related alterations in mesolimbic functioning. Brains were removed rapidly and cooled in ice-cold physiological buffer (Brodkin *et al*, 1998). The nucleus accumbens samples were obtained from 1 mm thick coronal cross-sections by use of a 12-gauge syringe needle and were stored at −70°C until Western blotting was performed as reported previously (Brodkin *et al*, 1998).

DNA Extraction, Purification, Amplification and Analysis

As described previously (Potenza *et al*, 2004), genomic DNA was obtained from frozen liver tissue of F344 and LEW parental animals and F_2 progeny via alkaline lysis and column purification strategy (Qiagen; www.qiagen.com). DNA quality was assessed by agarose gel electrophoresis. DNA amplification was performed using the polymerase chain reaction and primers obtained from Research Genetics (www.resgen.com), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (Bethesda, MD) (Remmers *et al*, 1996), or Applied Biosystems Incorporated (ABI; www.appliedbiosystems.com). DNA analysis was performed via size fractionation, either via agarose gel electrophoresis and ethidium bromide visualization, or acrylamide gel electrophoresis and fluorescence detection using an ABI 377 semiautomated sequencer. Data from these gels were independently read by two individuals and double-entered prior to analysis.

QTL Analysis

QTL analysis was performed as described previously (Remmers *et al*, 1996; Potenza *et al*, 2004). Sample sizes were based on power analyses and prior studies that successfully identified QTLs in rats (Remmers *et al*, 1996). One hundred eighty-eight of 298 F_2 progeny, including those with phenotypic extremes for AIL, NIL, $G\alpha_{i1,2}$ levels, and $G\alpha_{i3}$ levels (top and bottom 5-15%), were analyzed at 178 genetic loci distributed across the rat autosomes (average (SD) spacing of 8.65 (4.62) cM). We investigated power for the QTL analysis using the method of revolving power (Darvasi and Soller, 1997), which was calculated under the assumption of an infinite number of markers and was found to be similar to the 95% confidence interval (CI) of QTL map location using moderate marker spacing, e.g., 10

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to 20 cM. Our analysis showed that with a sample size of 188, the 95% CI is 20 cM for a standardized dominant effect of d=0.63. The average spacing (8.65 cM) of the 178 genetic loci used in this study is substantially smaller than 20 cM, and with denser marker spacing, smaller effect sizes can be detected. Based on these analyses, we have a good coverage of marker variation and adequate power to detect QTL of magnitudes of d=0.63 or less.

Data were analyzed as described previously with MAPMAKER/EXP and MAPMAKER/QTL (Remmers *et al*, 1996) and MapManager QT (Manly and Olson, 1999). Data presented are from analyses using MapManager QT. The likelihood ratio statistic (LRS) score, a value that is 4.6 times the lod score value, is used to report magnitude of the QTLs as has been done previously when reporting results from analyses using MapManager QT (McBrearty *et al*, 1998; Hahn *et al*, 2004; Potenza *et al*, 2004). “Suggestive significance” and “genomewide significance” thresholds were those recommended by Lander and Kruglyak (Lander and Kruglyak, 1995), using LRS scores of 9.9 and 15.2 for “suggestive” and [genomewide] “significant” QTLs, respectively. Marker map locations from a SHRSPxBN genetic map as described in the rat genome database (www.rgd.mcw.edu/GENOMESCANNER) were used in the QTL analyses, with study data-derived distances used for six markers (D1Arb8, D1Arb11, D1Arb25, D4Arb17, D12Arb8, and D20Mgh1) not available from the map. Exploration of candidate genes in the vicinities of identified QTL and identification of corresponding regions of human and mouse genomes were performed using the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/).

Mixed data have been reported with respect to the position of one marker (D3Rat63) employed in the present study. The Rat Genome Database (http://rgd.mcw.edu/objectSearch/sslpReport.jsp?rgd_id=35218), the MIT rat genomics website (http://www.broad.mit.edu/rat/public/) and the NCBI website (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=10116&query=d3rat63&qchr=&strain=All&advsrch=off) all list the marker as mapping to chromosome 3. The Ensembl website locates the marker on chromosome 8 but lists the map location on chromosome 3 (http://www.ensembl.org/Rattus_norvegicus/markerview?marker=oxsts6992). Analyzing with MAPMAKER/EXP and MAPMAKER/QTL (Remmers *et al*, 1996) and MapManager QT (Manly and Olson, 1999) the F2 genotype data that we generated, we found that by linkage analysis the D3Rat63 marker grouped with the chromosome 3 markers between D3Rat24 and D3Arb12. This mapping procedure confirms that the map location used for the D3Rat63 marker was correct. This procedure was followed for all markers used in the analyses to verify their orders and positions.

Results

General characteristics of the F344 and LEW parental animals and F_2 intercross progeny used in the QTL analysis have been described previously (Brodkin *et al*, 1998). F344 animals had significantly higher AIL, and higher $G\alpha_{i1,2}$ and $G\alpha_{i3}$ levels in the NAc, than did the LEW rats, although the ranges overlapped for the groups (Brodkin *et al*, 1998). Serum levels of amphetamine did not differ for F344 and LEW rats (mean ± SEM for F344 (125±10.8 ng/ml; n=10) and LEW (110±8.8 ng/ml; n=10); p=0.32) indicating that differences in AIL in F344 and LEW rats was not attributable to differences in amphetamine metabolism. No significant between-group differences in F344 and LEW rats were observed for NIL (Brodkin *et al*, 1998). The F_2 progeny displayed AIL, $G\alpha_{i1,2}$, and $G\alpha_{i3}$ levels intermediate between the F344 and LEW parental strains. Mean AIL scores in F_2 progeny were more similar to LEW than to F344 rats, whereas mean $G\alpha_{i1,2}$ and $G\alpha_{i3}$ levels were more similar to F344 rats than to LEW rats (Brodkin *et al*, 1998). The distributions of the F_2 progeny selected for genotypic analysis are displayed (Figure 1).

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Results of an autosomal genomewide QTL linkage scan employing 178 markers are displayed in Table 1. The broadest significance peak and largest likelihood (log ratio statistics or LRS) score for AIL was observed on chromosome 3 (LRS_{max}=21.3) between the markers D3Rat24 and D3Rat63 (Table 1, Figure 2). This peak reached genomewide significance and was estimated to account for 12% of the phenotypic variance for AIL. QTL peaks in this region did not reach even "suggestive" significance levels for NIL (LRS_{max}=6.1; 4% of variance), $G\alpha_{i1,2}$ levels (LRS_{max}=9.8 [slightly more distal than AIL peak]; 7% of variance), or $G\alpha_{i3}$ levels (LRS_{max}=3.1; 2% of variance). Peaks reaching "suggestive" significance level thresholds for AIL were observed on the proximal region of chromosome 2 between D2Rat182 and D2Rat11 (LRS_{max}=12.5; 8% of variance) and on chromosome 17 between D17Rat117 and D17Rat15 (LRS_{max}=11.3; 7% of variance). X^2 statistics for adjacent loci reaching at least $p<0.05$ significance for each individual locus are shown in Table 2. No other peaks reached "suggestive" significance.

Among the other phenotypes, the most robust QTL peak was observed for $G\alpha_{i3}$ levels in the NAc. The QTL peak was significant at a genomewide level (LRS_{max}=22.0; 15% of variance) and was located on the proximal end of chromosome 2 between D2Rat182 and D2Rat11. This region coincides with a "suggestive" QTL peak for AIL. Within this region, no QTL peaks for NIL (LRS_{max}=3.2; 2% of variance) nor for $G\alpha_{i1,2}$ levels (LRS_{max}=8.1; 5% of variance) reached "suggestive" significance. No other QTL peaks for $G\alpha_{i3}$ levels and none for NIL or $G\alpha_{i1,2}$ reached genomewide significance. A "suggestive" QTL peak for $G\alpha_{i3}$ levels was observed in the vicinity of D9Rat126 (LRS_{max}=11.5; 8% of variance), and "suggestive" peaks for NIL (LRS_{max} =10.3; 6% of variance) and $G\alpha_{i1,2}$ level (LRS_{max}=10.6; 7% of variance) were observed in the same region. An additional "suggestive" peak for NIL was observed on chromosome 13 at 13Mit4 (LRS_{max}=12.2; 8% of variance).

Conclusions

Summary of Findings

The current study investigated genomic contributions to differences in AIL, NIL, and levels of inhibitory G-protein subunits ($G\alpha_{i1,2}$ and $G\alpha_{i3}$) in the NAc in two inbred strains of rats. Our first hypothesis, that we would identify genomic regions contributing to AIL, NIL, $G\alpha_{i1,2}$ levels, and $G\alpha_{i3}$ levels, was partially supported. Specifically, QTLs reaching genomewide significance were identified for AIL and $G\alpha_{i3}$ levels, and additional QTLs reaching "suggestive" significance levels were identified for all four phenotypes. Our second hypothesis, that genomic regions contributing to AIL would be distinct from those contributing to NIL, was largely supported. No QTL for NIL reached "suggestive" significance levels in the vicinities of QTLs reaching "suggestive" or genomewide significance levels for AIL and vice versa. Our third hypothesis, that genomic regions contributing to AIL would partially overlap with those contributing to levels of $G\alpha_{i1,2}$ and $G\alpha_{i3}$ in the NAc, was supported. Specifically, the location of the QTL peak on chromosome 2, which reached genomewide significance for $G\alpha_{i3}$ levels in the NAc, coincided regionally with the QTL peak reaching "suggestive" significance for AIL. Analogously, QTL peaks reaching "suggestive" significance levels were observed in a similar region of chromosome 9 for NIL, $G\alpha_{i1,2}$ levels, and $G\alpha_{i3}$ levels. Together, these findings suggest that distinct genetic mechanisms underlie at least some of the differences in AIL and NIL observed in F344 and LEW rats. The findings also suggest overlapping genetic influences for biochemical and behavioral measures for AIL and $G\alpha_{i3}$ levels and for NIL and $G\alpha_{i1,2}$ and $G\alpha_{i3}$ levels, respectively. The implications of these findings for psychiatric disorders are described below.

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Importance of Current Findings

This study represents the first to our knowledge to identify via QTL analysis in any species genomic regions linked to inhibitory G protein levels in the NAc, and to identify genomic regions associated with AIL and NIL in rats. Although QTL and other linkage analyses have been used in studies of numerous psychiatric disorders in humans and related phenotypes in animal models, few have addressed stimulant-related behaviors, and even fewer biochemical markers within the NAc, and we believe that ours is the first to do so simultaneously in any species. One prior study using recombinant inbred mice identified a region of chromosome 19 that influences dopamine transporter (DAT) binding, which correlated with cocaine- and methamphetamine-induced locomotion (Janowsky *et al*, 2001). Another study of recombinant inbred mice found a QTL associated with copper levels in NAc on a region of chromosomes 5 in males (Jones *et al*, 2006). Brain copper concentrations were associated with cocaine-related open-field behavior in these mice (Jones *et al*, 1999). A third study found that mice lacking the M5 muscarinic receptor gene (located on chromosome 2) showed increased dopamine D2 receptor expression in the NAc and diminished AIL (Wang *et al*, 2004). A fourth study used gene expression microarray analyses to identify differences in gene expression in the NAc in two mouse lines selected for differences in methamphetamine-induced locomotion (Palmer *et al*, 2005). Using an internet database, the authors identified a region on chromosome 15 that co-mapped with the behavioral QTL for methamphetamine-induced locomotion. Similar regions of murine chromosome 15 have been associated with stimulant-, phencyclidine- and ethanol-induced locomotion (Alexander *et al*, 1996; Grisel, Belknap *et al*, 1997; Phillips *et al*, 1998; Jones *et al*, 1999; Boyle and Gill, 2001; Downing *et al*, 2003). Together, these studies indicate that stimulant-induced locomotion is a poly-genetically determined behavior. With respect to the present findings, only the chromosome 2 falls within a homologous region implicated in the current analysis, corresponding to the region of rat chromosome 3 with the most robust QTL for AIL.

Genomic Regions, Candidate Genes and Implications

Our study, unlike any prior report, directly searched for QTL associated with AIL using F2 progeny generated from an intercross of two rat strains showing parental differences in AIL. Consistent with prior studies, multiple genomic regions were identified in association with AIL. QTL analyses define relatively large intervals that are highly likely to contain multiple candidate genes that might contribute to the phenotype under investigation. Consequently, candidate genes within the region, while important to consider, should be viewed cautiously.

The most robust QTL for AIL was found on chromosome 3 in a location corresponding to regions of chromosome 11p, 15q and 20q in humans and chromosome 2 in mice. Regions of human chromosome 11p have been identified in autosome/genomewide scans as contributing to tobacco smoking, alcoholism and opioid dependence (Long *et al*, 1998; Gelernter, Liu *et al*, 2004; Gelernter *et al*, 2006). The region of human chromosome 15 homologous to the identified region of rat chromosome 3 has been implicated in multiple studies of alcoholism and tobacco smoking and contains a GABA receptor gene cluster and the gene encoding the nicotinic acetylcholine α7 subunit (Leonard *et al*, 2000; Song *et al*, 2003). The region of human chromosome 20q homologous to the identified region of rat chromosome 3 has been implicated in studies of cigarette traits (Saccone *et al*, 2003). Other regions of the human genome map to the region of rat chromosome 3 containing the QTL. For example, the gene encoding brain-derived neurotrophic factor (BDNF), located on human chromosome 11p, is within the identified region of rat chromosome 3. BDNF is induced by chronic cocaine (Kumar *et al*, 2005), regulated by CREB (Choi *et al*, 2006), and implicated in cocaine withdrawal (Grimm *et al*, 2003; Pu *et al*, 2006) and cocaine-induced locomotion and reward (Horger *et al*, 1999; Hall *et al*, 2003), and possibly associated with substance dependence (e.g., (Zhang *et al*, 2006)). Other candidate genes including ones

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included within the identified region of rat chromosome 3 include those coding for the M5 muscarinic cholinergic receptor and ΔFosB/FosB. The muscarinic M5 receptor has been previously implicated in NAc function and AIL in mice (Wang *et al,* 2004). ΔFosB has been widely implicated in addictive processes, including the rewarding and locomotor effects of stimulants (Nestler *et al,* 2001; Zhu *et al,* 2007).

Among the genomic regions reaching suggestive significance for AIL, the one on chromosome 2 overlapped in proximity with the most robust QTL identified in the study, that for $G\alpha_{i3}$ in the NAc. This region is homologous to regions of chromosome 5q in humans and chromosome 13 in mice. Although this region appears distinct from a GABA(A) gene cluster implicated in alcoholism (Radel *et al,* 2005), regions of human chromosome 5q closer to the homologous region of rat chromosome have been implicated in alcohol craving (Ehlers and Wilhelmsen, 2005) and event-related brain potentials in families with a history of alcoholism (Almasy *et al,* 2001). One of the genes in this region of rat chromosome 2 is that encoding the serotonin 1A receptor is expressed in the NAc (Luna-Munguia *et al,* 2005), couples through $G\alpha_i$ with strongest affinity for $G\alpha_{i3}$ (Pucadyil *et al,* 2005), influences cocaine-induced dopamine levels in the NAc (Andrews *et al,* 2005) and cocaine-induced locomotion (Carey *et al,* 2005), and has been implicated in aggressive behaviors, including those induced by cocaine (Knyshevski *et al,* 2005). A nearby gene is that for tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, which has been implicated in cocaine self-administration (Self *et al,* 2004). Also in this genomic region is the gene for the peptide CART (cocaine and amphetamine related transcript), which has been implicated in addictive processes including cocaine dependence (Jaworski and Jones, 2006). CART is expressed in mesolimbic regions including the NAc (Philpot and Smith, 2006), is regulated by stimulant exposure, dopaminergic transmission, and the cAMP pathway in the NAc (Hunter *et al,* 2006; Jones and Kuhar, 2006), influences cocaine-induced locomotion (Jaworski *et al,* 2003), and is elevated the NAc of people who have abused cocaine (Albertson *et al,* 2004).

A region of chromosome 17 corresponding to areas of chromosome 6p in humans and 13 in mice reached suggestive significance for AIL. In humans, chromosome 6p has been implicated in tobacco smoking (Fust *et al,* 2004) and intelligence in individuals with alcoholism and their families (Dick *et al,* 2006). Of several genes in this region of rat chromosome 17, prolactin has been implicated in multiple studies of people with cocaine dependence. Prolactin levels have been associated with severity of cocaine use (Patkar *et al,* 2006), cocaine administration increases prolactin levels (Elman and Lukas, 2005), serotonergically induced prolactin release is blunted in cocaine dependent subjects (Patkar *et al,* 2006), and this effect is associated with high behavioral disinhibition and aggression (Patkar *et al,* 2006). Another gene in this vicinity is that encoding protein phosphatase 1, which has been implicated in the function of NAc neurons and in their response to cocaine (Hu *et al,* 2005; Svenningsson *et al,* 2005; Zachariou *et al,* 2006). Also present is the gene for Cdk5, a protein kinase, which has been implicated in cocaine-mediated dopamine signaling (Chergui *et al,* 2004; Takahashi *et al,* 2005), is increased following chronic cocaine or methamphetamine exposure (Bibb *et al,* 2001; Benavides and Bibb, 2004; Chen and Chen, 2005), interacts with tyrosine hydroxylase (Kansy *et al,* 2004), is regulated by ΔFosB (Kumar *et al,* 2005), and has been implicated in NAc-mediated methamphetamine-induced locomotion (Chen and Chen, 2005).

Dopamine systems contribute to exploratory behaviors in rodents (Kliethermes and Crabbe, 2006) and differences in the genetic influences that contribute to exploratory and drug-induced locomotion have been reported (Hiroi and Agatsuma, 2005; Agatsuma *et al,* 2006). A region of chromosome 9 contained suggestive QTL for NIL and levels of $G\alpha_{i1,2}$ and $G\alpha_{i3}$ in the NAc. This region corresponds to areas of chromosome 2q in humans and 1 in mice. In

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humans, a similar genomic region of chromosome 2q has been implicated in co-occurring alcoholism and depression (Nurnberger *et al,* 2001). Among genes in this region of rat chromosome 9 is that for CREB, which is widely implicated in G-protein-related signal transduction in the NAc and animal models of stress and addiction (Barrot *et al,* 2005; Carlezon *et al,* 2005; Green *et al,* 2006; Nestler and Carlezon, 2006).

Study Limitations and Future Directions

There exist multiple limitations in the present study. First, we only examined males, and future research in female populations is needed. Second, other inbred rat strains may also show differences in AIL, NIL, and levels of G_{α} subunits in NAc, and in their relationship to other specific behavioral or biochemical measures of psychiatric relevance; we cannot apply any direct conclusions to these others strains at this point. However, although certain QTLs may be strain-specific, we speculate that common QTLs will influence phenotypes across strains and outbred rats. Also, it is likely that other strains may be used to identify additional QTLs relevant to stimulant responsiveness and biochemical measures in the NAc. A third limitation is that the findings were obtained in rats and the extent to which they are applicable to humans warrants further investigation. A fourth limitation is inherent to QTL analyses like the one employed here. Specifically, large genomic regions are identified that contain many candidate genes. Additional research is necessary to determine the extent to which specific genes are implicated and to identify the nature of the genetic differences generating the biochemical and behavioral differences observed in the parental rat strains. Such work could involve gene expression investigations or the identification of strain-related differences in coding regions of candidate genes within the regions defined by the QTLs. A fifth limitation involves the use of an F2 design with respect to the investigation of dominant versus recessive loci or on loci with effects dependent on interactions with strain specific alleles at the X-chromosome or maternal environment. A sixth limitation involves the complex nature of behavioral responses like AIL and NIL, and future investigations could examine the extent to which specific genetic factors contribute to specific aspects (e.g., the temporal magnitudes) of the responses.

Despite the limitations of the present study, the findings identify rat QTLs underlying differences in stimulant responsiveness and NAc functioning. As such, the investigation is important in several ways. Few prior studies have attempted to map neurochemical QTLs in inbred strains of rats. Since much work has been devoted to defining the neurochemistry in rat models of psychiatric disorders, this line of research is important in that it circumvents the need to translate phenotypes well-described in rats to genetic models in mice. As rat genomics become increasingly well-defined and more frequently used, this line of research will become increasingly important. Likewise, few prior studies have simultaneously mapped behavioral and neurochemical QTLs concurrently. The simultaneous assessment of QTLs provides insight into the molecular mechanisms underlying behaviors with psychiatric relevance. Finally, and most importantly, the findings of specific genomic regions influencing stimulant responsiveness provide a basis for future investigations into the genetic basis for multiple psychiatric conditions in humans.

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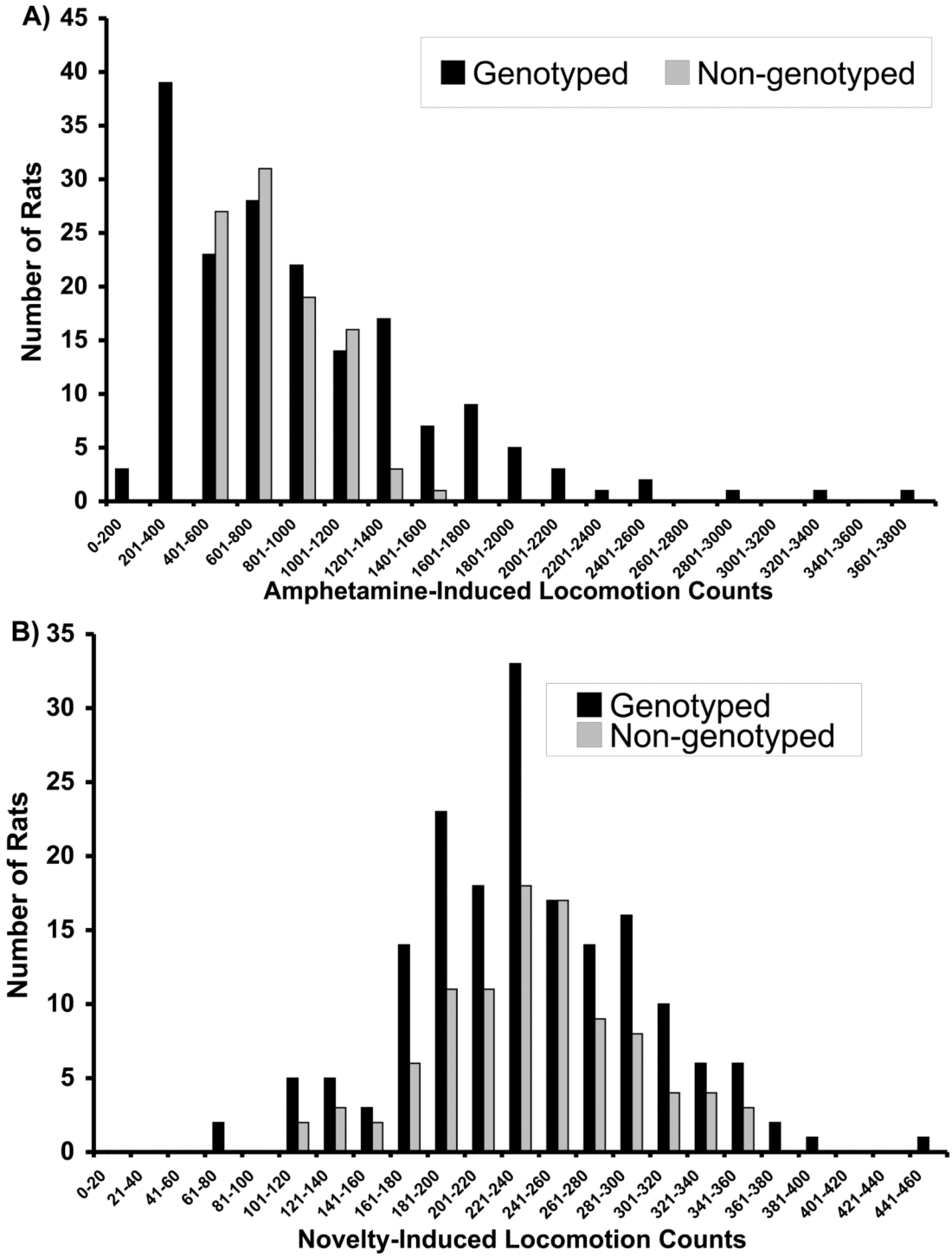

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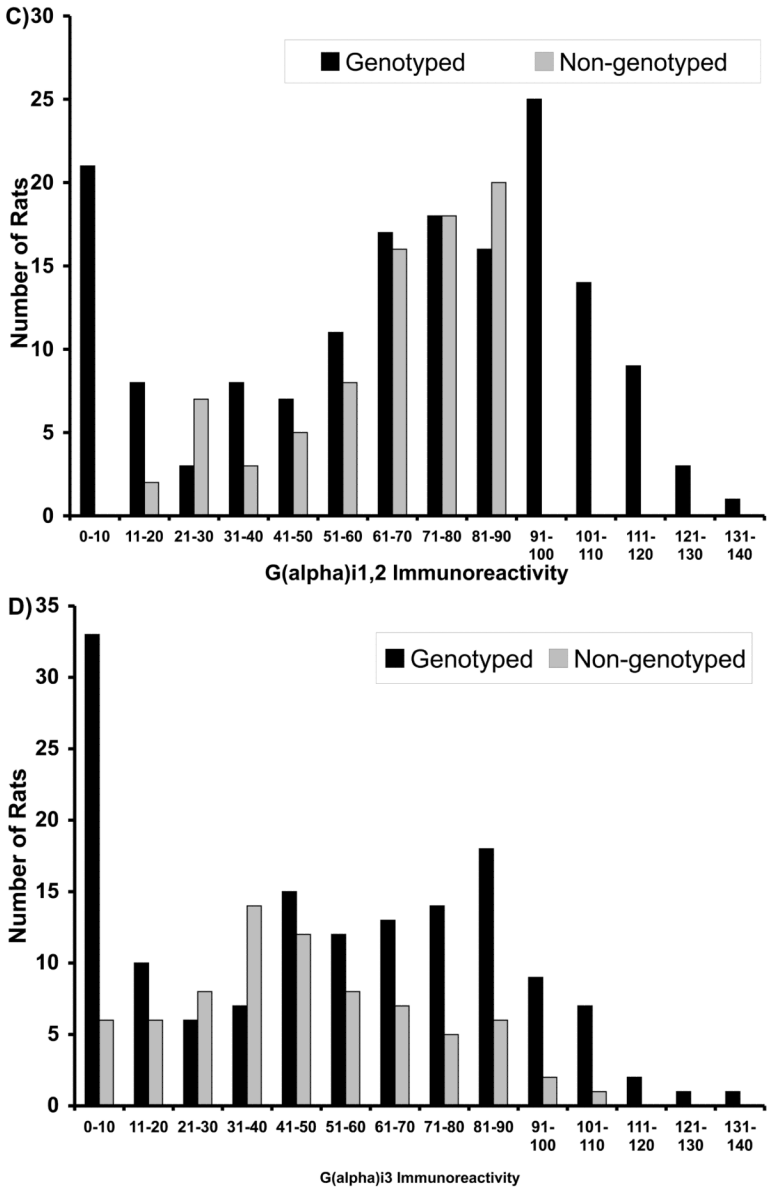

Figure 1.

Distributions of F2 progeny with respect to (A) amphetamine-induced locomotion (AIL), (B) novelty-induced locomotion (NIL), (C) $Ga_{i1,2}$ levels, and (D) Ga_{i3} levels. Progeny selected for genotyping are indicated in dark bars.

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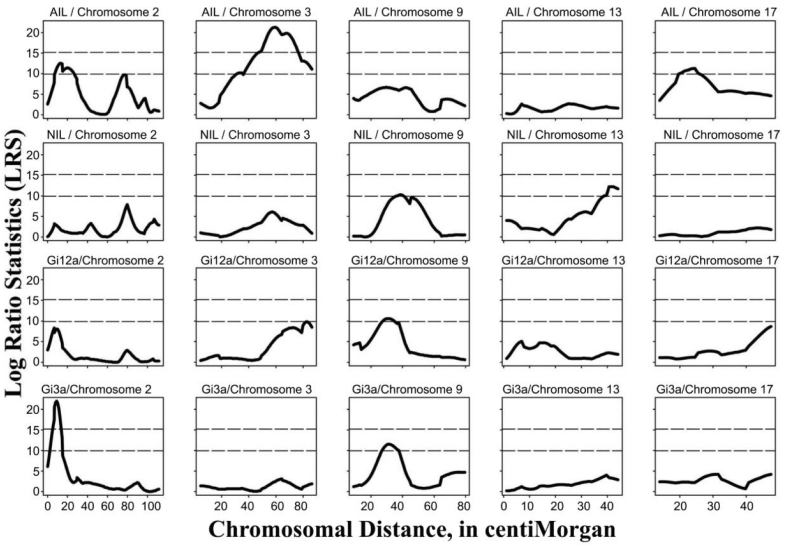

Figure 2.

LRS values for amphetamine-induced locomotion (AIL), novelty-induced locomotion (NIL) and G-protein ($Ga_{i1,2}$ and Ga_{i3}) levels on chromosomes showing suggestive or significant QTLs. Horizontal lines indicate thresholds for suggestive or significant QTL values, as described in the methods.

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Table 1

Log Ratio Statistic Scores for Amphetamine Induced Locomotion, Novelty Induced Locomotion, and Gαi Levels in Nucleus Accumbens at Specific Genomic Locations.

Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, Gαi1,2	Log Ratio Statistic, Gαi3
D1Rat4	1	9.2	1.6	0.3	0.7	0.1
D1Rat7	1	12.5	2.2	0.5	0.7	1.8
D1Rat19	1	23.8	2.6	0.4	0.4	1
D1Arb8	1	35	4.2	0	0.3	0.9
D1Rat256	1	28.3	1.9	0.3	1.7	0.4
D1Rat266	1	46.8	4.5	1	2.4	2.3
D1Mgh7	1	53.8	6.8	0.1	2.2	3.2
D1Arb11	1	57	4.8	0.8	4.5	4.2
D1Rat35	1	59.4	3.7	1.2	5	5.2
D1Rat215	1	74.5	0.1	0	0.7	0.6
D1Rat164	1	83.5	0.5	0.9	0.3	0.4
D1Rat437	1	89.1	0.3	0.7	1.5	0.9
D1Rat67	1	95.9	0.9	2.1	1.7	0.8
D1Rat70	1	106.1	1.2	6.4	2.5	1.4
D1Rat169	1	122	3.2	7.1	0.4	0.7
D1Rat76	1	125.3	1.9	8.8	0.6	1
D1Mgh12	1	133.4	0.5	0.8	1	1.4
D1Arb25	1	139	1.9	3.3	0.2	0.7
D1Rat122	1	143.5	2.6	1.6	0.9	0.1
D2Rat3	2	0.1	2.6	0.1	3	6.1
D2Rat182	2	6.9	9.7	3.2	7.2	20.5
D2Rat11	2	15.1	10.6	1.5	3.5	8.8
D2Mit6	2	29.5	7.7	0.9	0.8	3.4
D2Mgh19	2	35.5	3.7	0.9	0.9	2
D2Rat217	2	43.5	0.9	3.3	0.7	2.1
D2Rat34	2	57.1	0.1	0.1	0.3	1.1
D2Rat170	2	68.2	4.9	1.3	0	0.7

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Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, Gαi1,2	Log Ratio Statistic, Gαi3
D2Rat240	2	79.7	6.8	7.9	2.9	0.7
D2Rat62	2	90.8	1.7	1.4	0.5	2.1
D2Rat185	2	97.7	4	1.6	0.2	0.3
D2Rat69	2	106.8	1.2	4.3	0.4	0.2
D2Rat168	2	111.5	0.9	2.9	0.3	0.6
D3Rat53	3	4.6	2.8	1	0.4	1.4
D3Rat80	3	18.9	4.6	0	0.9	0.6
D3Rat75	3	36	9.8	2	0.7	0.7
D3Rat24	3	49.5	15.6	3.6	1.6	0.6
D3Rat63	3	65.2	19.5	4.6	7.5	2.7
D3Arb12	3	79.9	13.1	2.9	8.9	1.1
D3Mgh10	3	86.5	11.1	0.9	8.5	1.9
D4Arb14	4	0	1.5	0.5	0.6	0.9
D4Rat11	4	18.2	1.1	1.1	1.1	1.1
D4Arb17	4	23	1.1	0.6	1.8	1.3
D4Rat153	4	27.1	1.6	0.5	6	2.2
D4Rat15	4	29.4	5.9	0.6	3.9	2.5
D4Rat24	4	32.8	0.1	0.2	1	0
D4Rat226	4	32.9	0.4	1.1	1.8	1.6
D4Arb8	4	34.1	0.7	2.2	1.3	0.2
D4Rat33	4	40.8	0.8	2	1.6	0.8
D4Rat108	4	41.9	0.9	0.7	2.8	0.7
D4Rat172	4	46.4	0.1	0.9	2	0.7
D4Rat40	4	48.7	0.8	2.6	3.3	0.9
D4Rat48	4	54.3	0.9	2.2	1.1	0.3
D4Rat193	4	62.5	0.3	1.2	2.5	0
D4Rat60	4	71.4	0	1.5	0.6	0.3
D4Rat241	4	77	0.3	3	1.1	0.7
D4Rat66	4	82.6	1.9	1.6	1.8	0.2
D4Mgh30	4	86.7	2.6	1.6	0.3	0.6

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Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, Gαi1,2	Log Ratio Statistic, Gαi3
D4Rat112	4	98.8	0.9	3	0.4	0
D5Rat121	5	3.5	5	1	5.1	3.6
D5Rat126	5	16	8.1	5.3	4.5	2.6
D5Rat82	5	26	7.8	4.7	6.1	8.6
D5Rat10	5	37	8.4	4.5	5.8	7.6
D5Rat85	5	48	5.9	3.1	5.8	9
D5Rat196	5	58.1	5.7	3.4	3.3	4.3
D5Rat30	5	68.3	3	2.2	3.2	0.3
D5Rat171	5	78.5	6.4	2.1	1.6	0.2
D5Rat93	5	85.2	5.4	0.7	0.4	0.4
D5Rat49	5	105.6	2.4	0.9	3	6.4
D6Rat41	6	20.6	1.2	8.4	1.8	1.6
D6Rat29	6	33	0.8	1.1	0.5	0.2
D6Rat133	6	37.5	2.8	0.8	1	0.9
D6Rat23	6	46.6	1	1.3	1	1.2
D6Rat14	6	57.8	1.5	0.8	2	2.6
D6Rat160	6	76	2.3	1.3	0.8	1.8
D6Rat109	6	85.2	0.6	3.4	1.5	2.5
D7Rat113	7	3.1	8.9	3.8	1.2	2
D7Rat37	7	6.8	4.4	0.5	2.5	1.7
D7Rat27	7	22	6	0.1	3.5	1.9
D7Rat51	7	31	6.6	2.8	1.8	0.7
D7Rat22	7	45.5	1.9	6.3	0.6	2
D7Rat139	7	52.3	4.9	4.7	2	1.8
D7Rat11	7	64.7	3.9	0.5	3.3	2
D7Rat81	7	72.4	1	0.5	1.3	0.3
D7Rat4	7	80.4	1.7	1.4	1.1	0.4
D8Rat77	8	0	3	0.1	1.1	0.1
D8Rat55	8	8.4	5.6	0.3	2.7	2.6
D8Rat52	8	14.3	0.8	0.2	7.4	5

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Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, Gαi1,2	Log Ratio Statistic, Gαi3
D8Rat164	8	18.5	0.7	0.1	5.6	5.8
D8Arb6	8	23.1	0.8	0.7	1.8	2.2
D8Arb8	8	34.6	0.7	1	1.6	1.6
D8Rat43	8	36.9	0.4	5.6	0.9	0.7
D8Rat36	8	41.2	0	4.3	2.4	3
D8Rat23	8	46	1.9	6.7	6.9	5.2
D8Rat21	8	47.1	2.1	7	8.9	3.8
D8Rat16	8	57.2	1.2	6	2.7	0
D8Rat123	8	66.3	0.9	3.8	0.1	0.6
D8Rat11	8	71.9	2.1	4.5	0.4	0.1
D8Arb119	8	77.4	0.6	4.1	1	0.1
D9Rat135	9	9.1	4	0.2	4.2	1.2
D9Rat133	9	13.9	4	0.2	3.1	1.5
D9Rat126	9	37.7	5.9	10.2	9.6	10
D9Rat13	9	45.5	6.2	9.7	2.4	1.6
D9Rat110	9	64.7	3.6	0.2	1.2	3.5
D9Rat1	9	79.5	2.2	0.5	0.6	4.7
D10Rat218	10	4.6	3.7	4	0.6	0.9
D10Rat117	10	6.1	2.1	3.8	1.6	0.1
D10Rat45	10	21.6	2.1	0.3	0.8	0.1
D10Rat38	10	34	3.3	1.8	2	1.8
D10Rat164	10	38.5	1.6	0.4	0.2	0.5
D10Rat28	10	47.6	1.6	0.5	1.4	0.9
D10Rat153	10	51	0.8	0.1	1.6	1.2
D10Rat124	10	58.5	1.5	0.9	1.3	1.8
D10Rat142	10	66.5	2.8	0.4	3.1	1.7
D10Rat203	10	71.1	3.2	0.8	6.6	6.2
D10Rat15	10	73.3	3.8	0.8	7.3	6.6
D10Rat11	10	77.1	2.2	2.2	4.5	6
D10Rat8	10	85.9	2.7	0.8	0.5	2.6

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Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, Gαi1,2	Log Ratio Statistic, Gαi3
D10Rat105	10	92.7	3.7	0.6	0.4	3.9
D10Rat135	10	94.1	4.1	0.6	0.4	4.1
D11Rat73	11	8.2	5.2	0.9	0.1	0.5
D11Mit1	11	11.5	3.4	0.8	0.1	0.8
D11Rat6	11	19.5	2.3	0.8	2.3	1.8
D11Rat91	11	36.5	4.6	2	2.8	1.1
D12Rat59	12	5.9	1.7	3.2	4.3	0.5
D12Arb8	12	11	0.5	3.2	2.3	3.1
D12Rat4	12	17.1	0.8	3	3	3.8
D12Rat51	12	24.1	0.9	4.1	0.5	0.8
D12Rat76	12	33	1	1.3	0.5	2.1
D12Rat52	12	44.3	0.1	1.8	1.7	3.6
D12Rat44	12	54.1	3.4	0.1	5	1.1
D13Rat7	13	1.2	0.3	4	0.9	0.2
D13Arb5	13	7.9	2.2	2.1	3.7	0.9
D13Arb8	13	14.8	0.7	2.1	4.7	1.4
D13Rat126	13	19.3	1.1	0.6	4	1.5
D13Rat85	13	26.1	2.6	4.3	0.9	1.5
D13Rat131	13	33.9	1.5	5.7	0.8	2.4
D13Mit4	13	40.7	1.8	12.2	2.3	3.4
D13Rat153	13	44.1	1.6	11.7	1.9	2.9
D14Rat72	14	2.3	2.1	0.8	0.2	0.2
D14Rat77	14	6.9	0.3	0.6	2.5	1
D14Rat50	14	15.9	1	1.7	1.4	0.5
D14Rat68	14	28.2	2.4	2.2	0.9	0.3
D14Arb10	14	43	4.8	2.6	1.2	0.4
D14Rat49	14	64	0.6	0.2	1.6	1
D15Rat55	15	5.5	2.3	3.5	7.5	2
D15Rat66	15	15.8	0.9	4.3	0.3	0.4
D15Rat116	15	25	0	7	1.5	0.6

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Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, Gαi1,2	Log Ratio Statistic, Gαi3
D15Rat96	15	41.9	1.8	6	0.8	1.2
D15Rat26	15	58.9	3.4	2	0.5	1.9
D16Rat35	16	5.6	6.8	1.5	0.4	0.4
D16Rat67	16	18.1	5	1.6	2.6	1.2
D16Rat53	16	28.3	3.3	0.3	1.5	0.2
D16Rat37	16	38.5	1.9	2.5	0.4	0.3
D16Rat15	16	46.7	3.8	0.4	0.3	0.2
D17Rat115	17	13.9	3.5	0.3	1.1	2.4
D17Rat117	17	19.6	9.9	0.4	0.8	2.2
D17Rat15	17	25.5	10.1	0.2	2.3	2.7
D17Arb7	17	32.6	5.6	1.2	1.8	3
D17Rat130	17	40.8	5.3	2	3.9	2.2
D17Rat154	17	47.5	4.6	1.8	8.7	4.2
D18Rat133	18	2.5	1.1	5	0.3	0.3
D18Rat25	18	11.5	2.9	6.9	0.2	0.2
D18Rat17	18	18.3	0.8	2	1.8	0.5
D18Rat55	18	22.7	0.9	3.7	1.8	1.3
D18Rat8	18	43.2	0.8	0.9	4.9	2.2
D18Rat76	18	48.9	0.6	0.6	4.1	1.9
D19Rat82	19	6.7	0.5	0.5	5.6	1.8
D19Rat12	19	20.2	1.7	1.3	2.6	1.5
D19Rat35	19	27.8	1.5	2	1	1
D19Rat67	19	35.7	1.4	2.3	1	2.6
D19Rat63	19	39.1	3.5	0.7	0	0.5
D19Rat58	19	48.8	3.1	3	0.1	1
D20Rat46	20	0	3.1	0.4	2.1	2
D20Rat31	20	11.5	0.1	1.9	0.1	0
D20Rat34	20	21.5	0.6	2.5	0	0.6
D20Rat39	20	30.9	1.2	0.9	0.4	0.4
D20Mgh1	20	39	4	1.2	0.6	0.7

Marker	Chromosome	Map Location (cM)	Log Ratio Statistic, AIL	Log Ratio Statistic, NIL	Log Ratio Statistic, *Gαi1,2*	Log Ratio Statistic, *Gαi3*
D20Arb10	20	48.2	3.9	1.3	0.7	0.8

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Table 2

Chi-squared statistics for individual markers

Markers	Chromosome	LRS.AIL	LRS.NIL	LRS.Gi12a	LRS.Gi3a	AIL.pvalues	NIL.pvalues	Gi12a.pvalues	Gi3a.pvalues
D2Rat3	2	2.6	0.1	3	6.1	0.27	0.95	0.22	**0.05**
D2Rat182	2	9.7	3.2	7.2	20.5	**0.008**	0.2	**0.03**	**0.00004**
D2Rat11	2	10.6	1.5	3.5	8.8	**0.005**	0.47	0.17	**0.01**
D2Mit6	2	7.7	0.9	0.8	3.4	**0.02**	0.64	0.67	0.19
D3Rat75	3	9.8	2	0.7	0.7	**0.007**	0.37	0.7	0.7
D3Rat24	3	15.6	3.6	1.6	0.6	**0.0004**	0.17	0.45	0.74
D3Rat63	3	19.5	4.6	7.5	2.7	**0.00006**	0.1	**0.02**	0.26
D3Arb12	3	13.1	2.9	8.9	1.1	**0.001**	0.24	**0.01**	0.58
D3Mgh10	3	11.1	0.9	8.5	1.9	**0.004**	0.64	**0.01**	0.39
D9Rat126	9	5.9	10.2	9.6	10	0.05	**0.006**	**0.008**	**0.007**
D9Rat13	9	6.2	9.7	2.4	1.6	**0.05**	**0.008**	0.3	0.45
D13Mit4	13	1.8	12.2	2.3	3.4	0.41	**0.002**	0.32	0.18
D13Rat153	13	1.6	11.7	1.9	2.9	0.45	**0.003**	0.39	0.23
D17Rat117	17	9.9	0.4	0.8	2.2	**0.007**	0.82	0.67	0.33
D17Rat15	17	10.1	0.2	2.3	2.7	**0.006**	0.9	0.32	0.26

Results presented represent findings from multipoint analyses keyed to single marker locations.

Data presented represent two or more contiguous markers at $p<0.05$ in a free regression model for any of the studied phenotypes.

P values significant at $p<0.05$ are bolded. AIL p values for D9Rat126 and D9Rat13 are 0.052 and 0.04505, respectively.