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Genetic analysis of the psychostimulant effects of nicotine in chromosome substitution strains and F2 crosses derived from A/J and C57BL/6J progenitors

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Abstract Previous research utilizing the AcB/BcA recombinant congenic strains (RCS) of mice mapped provisional quantitative trait loci (QTLs) for the psychostimulant effects of nicotine to multiple regions on chromosomes 7, 11, 12, 14, 16, and 17. The current study was designed to confirm these QTLs in an A/J (A) × C57Bl/6J (B6) F2 cross and a panel of B6.A chromosome substitution strains (CSS). The panel of B6.A CSS consists of 21 strains, each carrying a different A/J chromosome on a B6 background. The A × B6 F2, CSS, A, and B6 mice were tested for sensitivity to the effects of nicotine on locomotor activity using a computerized open-field apparatus. In A × B6 F2 mice two QTLs were identified which confirm those previously observed in the AcB/BcA RCS. Significant differences in the expression of nicotine-induced activity were associated with loci on chromosome 11 (*D11Mit62*) and chromosome 16 (*D16Mit131*) in the A × B6 F2. At the chromosome 11 QTL, an A allele was associated with lower nicotine-induced activity scores relative to the B6. In contrast, the A allele was associated with greater relative nicotine activity values for the chromosome 16 QTL. A survey of the CSS panel confirmed the presence of QTLs for nicotine activation on chromosomes 2, 14, 16, and 17 previously identified in the AcB/BcA RCS. In the informative CSS strains, A alleles were consistently associated with greater nicotine-induced activity scores compared to the B6. The results of the present study are the first to validate QTLs for sensitivity to the effects of nicotine across multiple strains of mice. QTLs on chromosomes 2, 11, 14, 16, and 17 were confirmed in CSS and/or F2 mice. Significantly, the identification of a QTL on chromosome 16 has now been replicated in three crosses derived from the A and B6 progenitors.

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Introduction

Estimates of the genetic and environmental effects on smoking initiation and maintenance have consistently shown that there are strong genetic factors influencing the onset of regular smoking (Stallings et al. 1999) as well as the persistence of smoking behavior (True et al. 1999) and smoking dependence (Li et al. 2008; Vink et al. 2005). In recent years, genome-wide linkage analyses have identified several loci across the human genome that may contain genes that influence nicotine dependence or the number of cigarettes smoked (Ehlers and Wilhelmsen 2007; Gelernter et al. 2004, 2007; Goode et al. 2003; Li et al. 2003, 2007a). Studies suggest that nicotine addiction is a complex disorder that is modulated by numerous interacting genes distributed throughout the human genome. However, few of the loci implicated by linkage studies have been replicated in subsequent research (Li et al. 2008; Portugal and Gould 2008). Several factors likely contribute to this inconsistency across studies, including the difficulty in identifying differences in a heterogeneous pool of subjects (Portugal and Gould 2008).

Animal experiments complement human genome-wide linkage analyses and offer the opportunity to systematically examine the biological influence of specific genes on nicotine sensitivity. In a recent study, a quantitative trait loci (QTLs) mapping approach was used to identify

chromosomal regions that influence free-choice nicotine consumption in male and female F2 mice derived from a cross between B6 and C3H/HeJ mice (Li et al. 2007b). The QTL with the largest LOD score was located on distal chromosome 1, and additional QTLs were identified on chromosomes 4, 7, and 15. It was estimated that the four putative QTLs were responsible for up to 62% of the phenotypic variance in oral nicotine consumption (Li et al. 2007b).

In our laboratory natural variation in sensitivity to the psychostimulant effects of nicotine and the potential genetic factors mediating this variation have been examined. Activity-related phenotypes were examined due to the robust relationship demonstrated between the propensity of drugs to elicit psychomotor activation and their inherent capacity to act as a reinforcer in man (Wise 2004; Wise and Rompre 1989). Gill and Boyle (2005) mapped genes involved in regulating the psychostimulant effects of nicotine in the A/J and B6 progenitors as well as in AcB/BcA RCS. Locomotor activity was measured following subcutaneous (SC) administration of nicotine, and a wide range of locomotor excitation and depression was observed among the recombinant congenic strains (RCS), suggesting the presence of a quantitative trait involving the additive effects of several genes. Single-locus association analysis identified QTLs for the psychostimulant effects of nicotine on chromosomes 11, 12, 13, 14, and 17, and one QTL for nicotine-induced depression on chromosome 11. In the BcA RCS, nicotine-induced locomotor activity was associated with seven putative regions on chromosomes 2, 7, 8, 13, 14, 16, and 17.

The objective of the present study was to confirm QTLs for the expression of nicotine-induced activity on chromosomes 7, 11, 12, 14, 16, and 17 previously identified in the RCS. This was accomplished by completing a targeted genetic analysis of nicotine-induced locomotor activity in an A × B6 F2 cross and by conducting a phenotypic survey of the effects of nicotine across a panel of B6.A chromosome substitution strain (CSS) mice. The present study represents one component of a multistage strategy for mapping QTLs in which provisional associations are confirmed in independently derived crosses. It has been suggested that a multistage approach for linkage reduces the probability of chance associations (Buck 1995).

The B6.A CSS is a panel of 21 strains, each carrying a different A/J chromosome transferred intact onto the B6 background. The unique aspect of CSS (compared with RC strains) is that the genetic background is homogeneous. Screening CCS strains has been proposed as a simple, rapid method of determining the chromosomal location of QTLs (Nadeau et al. 2000). This method consists of phenotyping the entire panel of B6.A CCS strains; individual strains showing significant differences compared to the B6 progenitor are known to carry a QTL. The B6.A strains have been used to identify QTLs for several complex traits, including anxiety (Singer et al. 2004). The CSS model has been demonstrated to be more efficient than traditional crosses in that it requires the testing of fewer progeny in order to detect a specific effect, or allows smaller effects to be detected with a given number of subjects (Belknap 2003). Furthermore, the ability to detect QTLs is enhanced since all but the target chromosome are fixed, eliminating variance due to segregating QTLs on other chromosomes.

Materials and methods

Mice

Breeder pairs of the A/J, B6, and 20 strains of the B6.A CSS (19 autosomes and X chromosome) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Breeder pairs of A/J × B6 F2 and all CSS strains were set up in the Research Institute of the McGill University Health Centre, with strict adherence to protocols ensuring uniformity with regard to housing, lighting, handling, cage changing, noise, and exposure to stressors.

F1 and F2 mice were constructed using reciprocal crosses of the progenitor mice A/J and B6. For example, F1 crosses (A/J × B6 F1, B6 × A/J F1) were mated in all possible combinations to produce F2 (AB6F1 × AB6F1, AB6F1 × B6AF1, etc.), and sex and cross type were considered in all subsequent analyses. B6 and A/J progenitor mice were bred concurrently to assure a consistent exposure to environmental conditions. All mice were housed with same-sex littermates until 8 weeks of age when testing commenced. All breeder pairs and pups were housed in an animal colony controlled for temperature and humidity on a 12-h day/night cycle (lights on between 0600 and 1800 h), in standard polycarbonate, shoebox, solid-bottom cages with beta chip bedding and nestlet pads with Harlan Teklad (Global 18% protein rodent diet) mouse chow and water *ad libitum*. Animals were tested under identical conditions in mixed squads (sex and strain) of approximately 24-32 mice over the course of a 2-year period. The Facility Animal Care Committee, in compliance with the Canadian Council on Animal Care, approved all procedures.

Drug injections

Nicotine solutions were prepared from nicotine hydrogen tartrate salt (Sigma Chemical Co., St. Louis, MO, USA) dissolved in a sterile saline solution. The nicotine solution was injected subcutaneously (SC) in a fixed volume of 0.01 ml/g body weight, at a dosage of 0.8 mg/kg (0.28 mg/

kg base). The dose of nicotine used in the present study was based upon prior research (Gill and Boyle 2005).

Locomotor activity testing

Locomotor activity was measured in open field boxes constructed of Plexiglas, measuring 30 cm × 30 cm × 40 cm high. Six intersecting light-photocell assemblies placed inside the walls of the chambers, 3 cm above the floor, monitored the locomotor activity by automatic registration on a computer connected to the photocells. Counts were automatically registered at 1-min intervals throughout the sessions. A custom-designed computer software program monitored activity on the photocells, providing measures of horizontal activity and stereotypy. Horizontal activity was operationally defined as the number of beam breaks made over the course of the test period. Horizontal locomotor counts were automatically filtered to suppress activity resulting from the repetitive breaks of a single beam due to grooming. Testing was performed under dim red light (40 W) in a sound-insulated room between 1600 and 1800 h immediately prior to lights out.

Naive male and female mice were habituated to the handling and injection procedures and to the test chambers prior to nicotine testing. Habituation was carried out on two successive days (days 1 and 2) where naive animals were transported to the testing room, weighed, and injected with sterile 0.9% NaCl saline solutions (0.01 ml/g body weight). Activity was monitored in the test chambers for 30 min following each SC saline injection. Nicotine-induced activity and procedures specific to each cross type are described below for the F2 and CSS mice.

Nicotine-induced activity in A × B6 F2 mice

Following habituation, F2 mice were tested for responses to nicotine on two additional test sessions spaced at 2-day intervals. On each of these test sessions, mice received a single drug injection (nicotine: 0.8 mg/kg SC) and activity was monitored for 30 min. Note that all F2 mice were tested twice for sensitivity to the effects of nicotine in order to calculate a coefficient of variation and determine the reliability of the individual F2 scores.

Difference scores (total horizontal activity on nicotine day minus total horizontal activity for saline day 2) were calculated for each nicotine test session (days 3 and 4) and the scores were averaged. Total horizontal activity was calculated as a function of the number of horizontal beam breaks observed over the 30-min period. A total of 351 F2 mice were phenotyped, and 211 mice representing the top and bottom 30% from the extremes of the phenotypic distribution were genotyped (93 females and 118 males).

Genotyping

Genomic DNA samples were prepared from tail clips using standard extraction methods (tissue lysis, proteinase K, ethanol precipitation) according to protocols described by Hofstetter et al. (1997). MIT mouse MapPair primers were purchased from Invitrogen (Carlsbad, CA, USA), and polymerase chain reaction (PCR) was conducted in 96-well microtiter plates using standard recommended conditions. PCR products were electrophoresed on agarose gels, stained, and scanned to a computer using a GeneGenius gel documentation system. Genotypes were scored independently by two individuals, entered into Microsoft Excel, and exported to MapManager QTX (Manly et al. 2001). Previously identified QTL regions were targeted for genotyping in the A × B6 F2 mice. For each provisional QTL identified in the RC strains, at least three microsatellite markers were used for confirmation of the QTL. One marker was selected nearest the peak of the QTL, with two flanking markers within the 95% confidence interval (1-LOD support interval) of the QTL. A total of 21 markers on chromosomes 7, 11, 12, 14, 16, and 17 were genotyped.

Data analysis

Each mouse was assigned a unique identifier that was used to track and coordinate all aspects of the data collection (study ID#, date, batch, cross type, phenotypic data, tail clip, DNA tube, genotypes). Data were coded and entered into a central tracking database using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Prism (GraphPad Software Inc., La Jolla, CA, USA). Subsequent statistical analysis was conducted using the microcomputer version of SPSS (ver. 13.5 for Windows; SPSS Inc., Chicago, IL, USA).

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

Linkage analysis was performed to confirm previously identified QTLs using Windows QTL Cartographer 2.5 (Wang et al. 2006). Confirmatory QTL mapping in the present study proceeded in two steps. Initially, an analysis at defined loci (simple marker associations) was performed.

Confirmation in the F2 mice of previously identified QTLs on chromosomes 7, 11, 12, 14, 16, and 17 was based on a nominal p value of 0.01, as proposed by Lander and Kruglyak (1995). The alpha levels were adjusted for multiple tests using the false discovery rate (FDR). Secondly, an analysis of positions inferred between loci with statistical control for other loci known to affect the trait (composite interval mapping, CIM) was conducted. (Significant and suggestive QTL regions were selected as controls.) Permutation tests were performed on the data to empirically estimate the threshold for suggestive and significant loci (1000 permutations at 1-cM intervals).

Nicotine activity testing in the CSS panel

Males and females for each of the 20 CSS and progenitors were assessed for sensitivity to the activating effects of nicotine. Response to nicotine (0.8 mg/kg) in naive male and female CSS mice was assessed on day 3, following habituation to the handling and injection procedures as described above on days 1 and 2. Difference scores (total horizontal activity on nicotine day 3 minus total horizontal activity for saline day 2) were calculated. A total of 422 male and female CSS mice were evaluated (average of 23 mice/strain), along with 34 B6 and 33 A/J mice. The CSS-13 and -19 strains were not included in the analysis due to poor breeding performance.

Data analysis

Each mouse is assigned a unique identifier that is used to track and coordinate the data collection as described above for the F2 mice. Subsequent statistical analysis was conducted using the microcomputer version of SPSS (ver. 13.5 for Windows). Data were tested for normality and outliers using the SPSS Explore function, and scores outside the 95% confidence interval for each inbred CSS strain were removed from subsequent analyses. A total of 11 mice randomly distributed across strains were removed.

The statistical analysis of the phenotypic differences between the B6 and CSS strains was accomplished using a two-factor (strain × sex) ANOVA. The identification of informative CSS strains (i.e., those strains carrying a donor A/J chromosome that produced a significantly different phenotype compared with the B6) was conducted using simple main-effects contrasts (planned comparisons). A nominal $p \leq 0.01$ was used as a threshold for establishing confirmation of any locus that was hypothesized *a priori* to mediate the expression of nicotine-induced locomotor activity in the CSS strains. Novel QTL identified in the CSS were considered to be provisional, requiring further study for confirmation.

Results

A × B6 F2 Mice

Allelic influences on the expression of nicotine-induced activity

Descriptive analyses indicated that nicotine activity values (difference scores) in the F2 population were normally distributed with a mean of −82.47 (±10.03). Mean nicotine activity values for the F2 mice were assessed as a function of sex and F2 cross type (AB6, B6A). A two-way ANOVA (sex × cross type) indicated no significant main effects for sex [$F(1, 211) = 2.045$, $p = 0.154$] or cross type [$F(1, 211) = 0.018$, $p = 0.892$] or sex × cross interactions [$F(1, 211) = 1.497$, $p = 0.223$] in the expression of nicotine-induced locomotor activity.

ANOVAs were used to determine whether there were significant differences in mean phenotype values of progeny with different genotypes (A, B6, heterozygote) at specific markers. Significant differences in nicotine difference scores were observed as a function of allelic status at *D11Mit62* (1.5 cM) on chromosome 11 [$F(2, 205) = 5.315$, $p = 0.006$]. Similarly, differences were observed at *D16Mit131* (4.3 cM) [$F(2, 209) = 4.749$, $p = 0.009$] on chromosome 16. The results indicated that on chromosome 11 the A/J allele was a "decreaser" allele relative to the B6. In contrast, the A/J allele on chromosome 16 was an "increaser" allele associated greater relative nicotine activity scores compared with the B6. The relationship between allelic status for representative loci on chromosomes 11 and 16 and nicotine-induced activity is presented in Fig. 1.

Quantitative trait loci analysis

A test of simple associations was conducted to identify associations between the genotyped markers and nicotine difference scores. QTLs ($p < 0.05$) for nicotine difference scores detected by Windows QTL Cartographer are described in Table 1. The results of the analysis provided support for an association between the markers on chromosomes 11 and 16. Specifically, a loci at 1.5 cM on chromosome 11 (*D11Mit62*) was associated with decreased nicotine-induced activity. The peak LRS value on chromosome 16 was observed for *D16Mit131* at 4.3 cM, for the interval between 4.3 and 21.5 cM.

CIM analysis was used to control for the influence of unlinked QTLs. Target loci associated with nicotine difference scores were mapped while controlling for the influence of other QTLs. A significant loci on chromosome 16 between 4.5 and 22.5 cM was identified, confirming a QTL previously identified in the RCS mice. A peak LOD

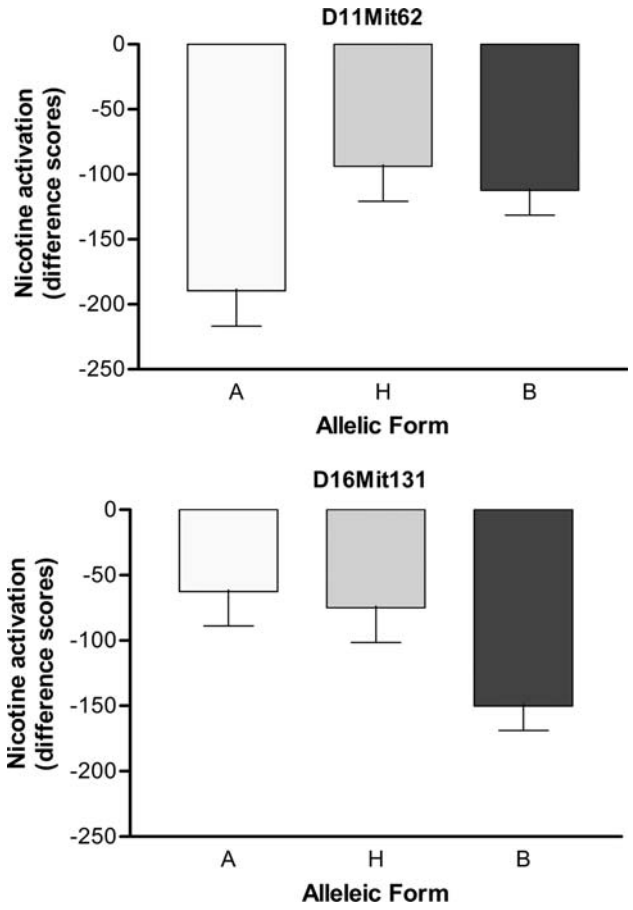

Fig. 1 Illustration of nicotine activity (combined females and males) in the A × B F2 mice presented as a function of allelic status for the QTLs at *D11Mit62* (top) and *D16Mit131* (bottom). B designates homozygous B6 alleles, A designates homozygous A alleles, H indicates heterozygotes

score of 2.7 was obtained for this region. The mapping results for the chromosome 16 QTL are presented in Fig. 2.

CSS strain survey

Males and females for each of the 20 strains of the CSS panel and parental strains were assessed for sensitivity to the activating effects of nicotine. A two-way ANOVA with sex and strain was used to examine the nicotine activity phenotypes (nicotine difference scores). Planned comparisons (simple main effect contrasts) were used to evaluate the significance of main-effect differences between each CSS strain and the B6 parental strain.

The results of the 2-way ANOVA indicated significant main effects for strain [$F(19, 489) = 2.747$, $p < 0.001$], sex [$F(1, 489) = 3.934$, $p = 0.048$], and a significant sex × strain interaction [$F(19, 489) = 1.875$, $p = 0.014$]. An analysis of simple main effect contrasts (males and females combined) indicated that when compared with the parental B6 strain, the CSS show evidence for increased nicotine activation ($p < 0.05$) on chromosomes 2, 4, 10, and 15 ($p < 0.05$), and on 14, 17, and X ($p < 0.01$) relative to the B6 progenitor strain. A similar pattern was observed in the females when assessed independently. However, in the females the CSS-16 strain was also identified as informative ($p \leq 0.01$). The CSS strain distribution pattern (for males and females combined) is presented in Fig. 3. The informative CSS-2, 14, 16 (females) and CSS-17 strains provide confirmation of QTLs previously identified in the AcB/BcA RCS. Confirmation was established as a function of both the identification of informative strains (a nominal $p \leq 0.01$) and the demonstration of concordance with the direction of allelic influences on increasing or decreasing the phenotypic scores. The QTLs for nicotine-induced activity independently mapped in the AcB/BcA RCS, A × B6 F2 cross, and the B6.A CSS panel are presented in Table 2. In the informative CSS strains, greater nicotine-induced activity scores were consistently observed in those strains carrying the A/J chromosome on a B6 background.

Table 1 QTL analysis (simple associations) derived from the output from WinQTL Cartographer using the A × B6 female F2 mice

Chromosome	Marker	cM	Genome coordinates	*p* value
7	*D7Mit222*	52.6	120077980–120078126	0.779
7	*D7Mit66*	57.5	127029510–127029671	0.718
7	*D7Mit22105*	63.5	135707912–135708169	0.225
11	*D11Mit62*	1.5	8926228–8926377	0.011*
11	*D11Mit82*	14.0	25961780–25961942	0.200
11	*D11Mit206*	20.0	44459827–44459969	0.308
11	*D11Mit245*	44.8	76777286–76777433	0.794
11	*D11Mit39*	49.0	87613874–87614042	0.384
12	*D12Mit233*	52.0	109494466–109494615	0.224
12	*D12Mit280*	55.0	110455669–110455860	0.542
14	*D14Mit141*	15.0	47379212–47379351	0.180
14	*D14Mit233*	19.5	52795622–52795821	0.699
14	*D14Mit37*	27.5	63002280–63002418	0.341
14	*D14Mit34*	40.0	72048013–72048168	0.231
16	*D16Mit131*	4.3	7319135–7319274	0.009**
16	*D16Mit57*	21.5	29406628–29406739	0.036*
16	*D16Mit84*	34.2	45397811–45397949	0.361
16	*D16Mit152*	57.0	85804079–85804183	0.290
17	*D17Mit142*	47.4	79365240–79365386	0.835
17	*D17Mit76*	54.6	86033231–86033354	0.637
17	*D17Mit221*	56.7	90487044–90487182	0.963

* Significant at the 0.05 level

** Significant at the 0.01 level

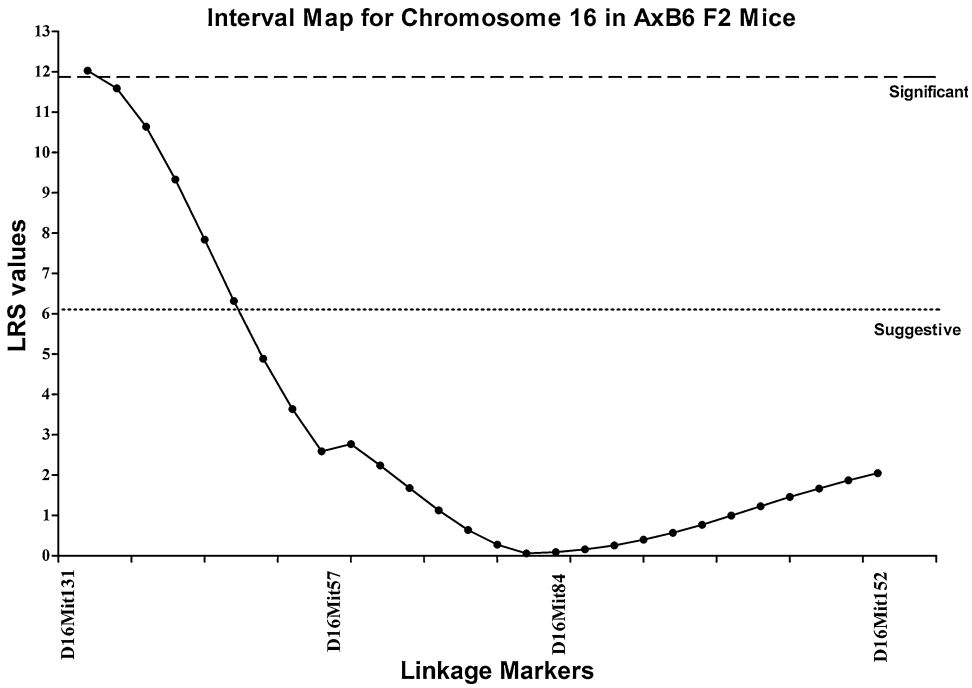

Fig 2 An interval map for the QTL on Chr 16 was derived from the output of WinQTL Cartographer using the A/J × B6 F2 mice. The interval map data indicate that a peak LRS was obtained for the region bounded by the markers *D16Mit131* and *D16Mit57*

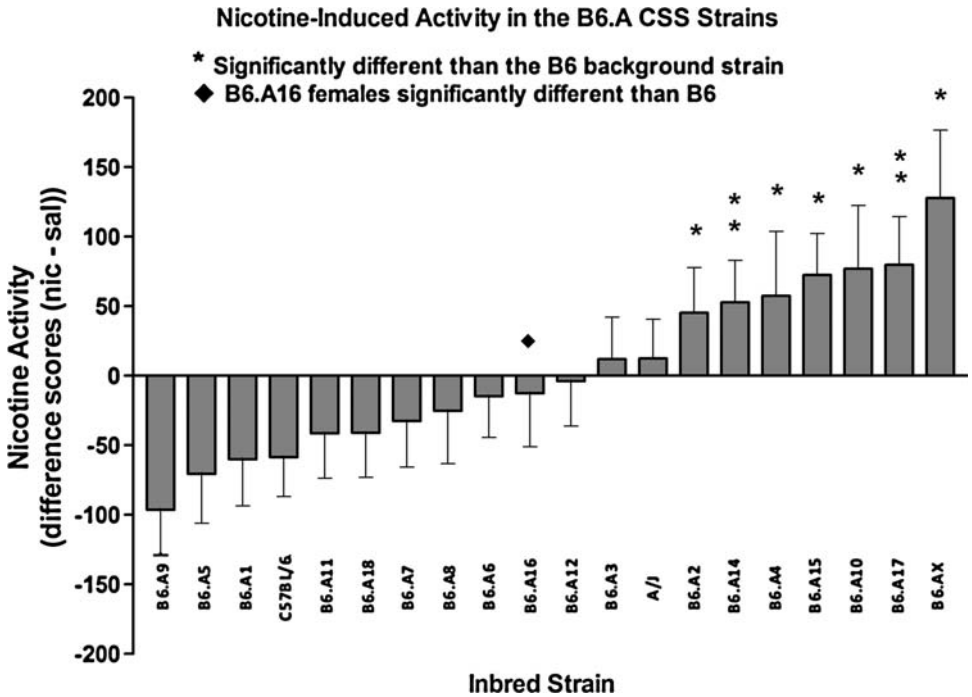

Fig. 3 Mean nicotine activity (difference scores ± SEM) for male and female CSS mice. * denotes CSS strains that are significantly different from the B6 at a nominal p value of 0.01 (corrected for multiple comparisons). ** denotes CSS strains (14 and 17) that confirm QTL previously identified in RC strains. The A/J and B6 progenitor strains are included as reference

Discussion

The results of the present study are the first to validate QTLs for sensitivity to the psychostimulant effects of nicotine across multiple strains of mice. The presence of QTLs on chromosomes 2, 11, 14, 16, and 17 were confirmed in the CSS panel or A × B6 F2 mice. Significantly, the identification of a QTL on chromosome 16 has now been replicated in three crosses derived from the A and B6 progenitors. The QTLs on these chromosomes exhibited consistent directionality of effects across genetic constructs. In particular, the CSS-2, -14, -16, and -17 strains exhibited significantly greater relative nicotine-induced activity compared with the B6 progenitor strain. These results indicate that in the CSS panel, the transfer of chromosomes 2, 14, 16, or 17 derived from the A strain onto a B6 background is associated with greater psychostimulant effects of nicotine. This is consistent with the

Table 2 Significant QTLs for nicotine-induced activity independently mapped in the AcB/BcA RCS, A × B6 F2 cross, and the B6.A CSS panel[a]

	AcB/BcA RCS				A × B6 F2[b]		B6.A CSS[c]		
Chr	cM range	Peak marker[d] (cM)	RC panel	Allele effect[e]	F2	Allele effect[e]	CSS	Allele effect[e]	
2	71.0–96.0	*D2Mit311* (83.1)	BcA	A↑	NS		++	A↑	*
7	54.5–65.6	*D7Mit66* (57.5)	BcA	A↑	NS		NS		
11	1.5–18.0	*D11Mit82* (14.0)	AcB	B↑	1.5	A↓	NS		*
11	47.67–54.0	*D11Mit39* (49.0)	AcB	B↑	NS				
12	52.0–59.0	*D12Mit233* (52.0)	AcB	B↑	NS		NS		
14	52.0–60	*D14Mit165* (52.0)	AcB	B↑	NS		++	A↑	*
	14.5–43.0	*D14Mit155* (25.0)	BcA	A↑					
16	4.3–66.0	*D16Mit131* (4.3)	BcA	A↑	4.3–21.5	A↑	++ (females only)	A↑	*
17	56.7	*D17Mit221* (56.7)	AcB	B↑	NS		++	A↑	*
	44.5–56.0	*D17Mit76* (54.6)	BcA	A↑					

[a] There was concordance in the directionality of allelic influences between the BcA RCS and F2 or CSS for QTLs on chromosomes 11, 14, 16, and 17 ($p < 0.01$), suggesting that these chromosomal regions harbor genes that influence sensitivity to nicotine in mice

[b] Genotyping of the A × B6 F2 was conducted with SSLP markers on chromosomes 7, 11, 12, 14, 16, and 17. The QTL region on Chr 2 was not genotyped in the F2

[c] Results from AcB/BcA RCS published by Gill and Boyle (2005). B6.A CSS "++" indicates that the B6.A CSS carrying the designated A/J chromosome displayed a significantly different nicotine activation phenotype compared to the B6 progenitor, indicating the presence of a QTL on the chromosome. NS = nonsignificant loci. * denotes confirmation ($p \leq 0.01$)

[d] Peak marker identified by MapManager QTX, and position provided in recombinant distance in centimorgans from centromere

[e] Allelic effect describes the directionality of allelic influences upon the expression of nicotine activity across strains: A(A/J); B(B6)

Significance of QTLs determined through permutation tests in MapManager QTX following the guidelines of Lander and Kruglyak (1995)

allelic influences observed for the chromosome 16 QTL identified in the A × B6 F2 and those reported previously in the RC strains of mice. In contrast, A/J alleles were associated with lower relative nicotine-induced activity for the QTL on chromosome 11 in both the A × B6 F2 and RCS.

Analysis of the CSS panel resulted in the confirmation of QTLs associated with nicotine-induced activity located on chromosomes 14 and 17 ($p < 0.01$); however, these QTLs were not identified in the A × B6 F2 cross. Similarly, Stylianou et al. (2006) reported the detection of QTLs in the B6.A CSS panel that were not found in an F2 cross. These authors suggested that QTLs were probably masked by epistatic loci segregating in the mixed background of the F2.

The identification of genes underlying complex behavioral phenotypes is a significant challenge. The challenge primarily relates to the difficulty of narrowing QTLs to intervals that contain only a small number of candidate genes. It has been suggested that classical intercross mapping in combination with bioinformatics methods, including gene expression analysis, provides an effective means for narrowing QTL regions (Flint et al. 2005; DiPetrillo et al. 2005; Mackiewicz et al. 2008). Complete genome sequences and dense single nucleotide polymorophism (SNP) maps allow new approaches to identifying QTLs and candidate genes within QTL intervals. While the QTL regions are too broad to effectively identify relevant candidate genes, the present study utilized an in silico approach to narrow the list of potential candidate genes within the 95% confidence interval for the chromosome 16 QTL. This process was initiated with an examination of dense SNP maps available at the Mouse Phenome Database (MPD) (http://www.jax.org/phenome; August 2008). The SNP maps for the significant QTL regions (highlighted by red bars) indicate genomic regions where the A and B6 differ, as shown in Fig. 4. As apparent from Fig. 4, the regions comprising the QTLs on chromosomes 11, 14, and 17 are highly polymorphic, requiring further fine mapping prior to in silico analyses The chromosome 16 QTL interval contained a limited number genotypic regions where the progenitors A/J and B6 differed; thus, it appeared to be the most suitable of the four loci confirmed in the present study for in silico analysis. Interval boundaries derived from the nearest anonymous markers outside the 95% confidence interval of the chromosome 16 QTL were converted to base pair locations using the Ensembl database. An interval from 5193522 to 32312108 bp was identified (27.12 Mb). A search of the database, consisting of the NCBI build 37, identified 322 genes within this region. Subsequently, a survey of polymorphic sites produced a list of nine unique genes producing nonsynonymous substitutions in amino acids of relevant proteins. The list of these genes with SNP differences in coding

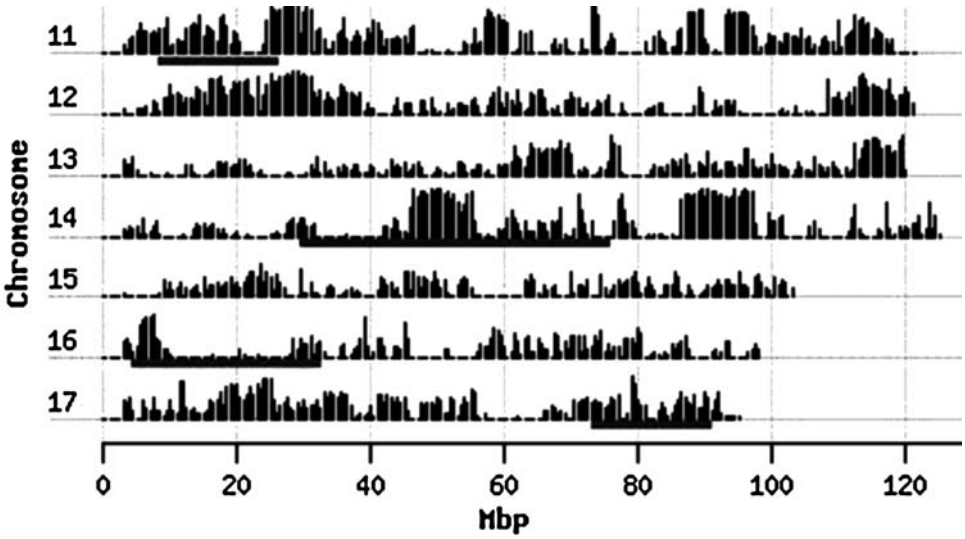

Fig. 4 Graph depicts relative genotypic differences between the A/J and B6 inbred strains as tabulated from the SNP available at the Mouse Phenome Database (MPD) (http://www.jax.org/phenome, August 2008). Each bar represents a bin 0.5 Mb wide. *Bars* are indicative of differences between A/J and B6 strains. *Red bars* indicate the intervals containing significant QTLs

regions is presented in Table 3. *A priori*, the filtering of strong candidate genes is based on the potential for changes in DNA sequence altering the amino acid makeup of the translated protein. In part, this approach is based on the literature which suggests that the value in identifying regulatory polymorphisms is hindered by limited knowledge of functional regulatory elements (DiPetrillo et al. 2005). Additional research will be required to identify and assess the functional impact of polymorphisms in noncoding regulatory DNA sequences. Of the eight genes with nonsynonymous mutations, five were found to be expressed in brain tissue (see Table 3).

The *Abat* (GABA-transaminase) gene was identified as a potential candidate gene. It is of interest to note that irreversible inhibition of GABA-transaminase (the primary enzyme involved in degradation of the neurotransmitter GABA) by the compound gamma vinyl-GABA (GVG or Vigabatrin) dose-dependently decreased both ethanol and cocaine consumption in rats (Stromberg et al. 2001). Similar results were obtained for nicotine-induced place preference conditioning (Ashby et al. 2002) and NAcc DA extracellular concentrations in rats (microdialysis) (Schiffer et al. 2003). A GVG-induced blockade of nicotine-induced increases in synaptic DA was also shown in primates using PET imaging and the D2 receptor tracer 11C-raclopride (Dewey et al. 1999). Thus, inhibition of GABA-transaminase has been shown to block the behavioral effects of both nicotine and cocaine, including self-administration, and to block the neurochemical actions of nicotine in relation to dopamine release in the NAcc. Additional research will be required in order to examine candidate gene expression in relation to differential nicotine sensitivity.

It is of interest that the survey of the CSS resulted in the identification of novel putative QTLs associated with

Table 3 Genes within the chromosome 16 QTL region, with SNP differences between the A/J and B6 inbred strains of mice that lead to nonsynonymous coding differences in amino acids

SNP ID (dbSNP Build 128)	Map position (NCBI Build 37)	Gene : dbSNP function class	Expressed: brain tissue	Variation type	C57BL/6J	A/J	Allele summary (all strains)
rs4159451	Chr16:8614182	Abat : Coding-Nonsynonymous	*	SNP	G	A	A/G
rs4159536	Chr16:8683355	EG436336 : Coding-Nonsynonymous		SNP	C	T	C/T
rs4165166	Chr16:20596264	Vwa5b2 : Coding-Nonsynonymous	*	SNP	G	A	A/G
rs4165236	Chr16:20716011	Clcn2 : Coding-Nonsynonymous Polr2 h : Locus-Region (upstream)	*	SNP	G	A	A/G
rs4167308	Chr16:29248226	Atp13a5 : Coding-Nonsynonymous	*	SNP	C	T	C/T
rs30714458	Chr16:29421820	Atp13a4 : Coding-Nonsynonymous		SNP	G	A	A/G
rs4167976	Chr16:30309136	Gp5 : Coding-Nonsynonymous	*	SNP	A	T	A/T
rs4168038	Chr16:30344625	Atp13a3 : Coding-Nonsynonymous		SNP	C	A	A/C
rs4168058	Chr16:30352467	Atp13a3 : Coding-Nonsynonymous		SNP	A	G	A/G

Data were extracted from the Mouse Genome Database (MGD) (The Jackson Laboratory, Bar Harbor, ME) available at http://www.informatics.jax.org (August 2008)

* Denotes expression observed in brain tissue

nicotine-induced locomotor activity on the 3, 5, 10, 15, and X chromosomes. These loci have not been identified previously with nicotine-induced activity (Gill and Boyle 2005). However, it is noteworthy that a QTL for oral nicotine consumption was similarly identified on chromosome 15 (Li et al. 2007b). Additional research will be required to validate these putative QTLs.

Overall, the results of the present study validate QTLs on chromosomes 2, 11, 14, 16, and 17 for sensitivity to the effects of nicotine across multiple strains of mice. Collectively, the locus on chromosome 16 has now been replicated in at least three independent crosses derived from the A/J and B6 progenitors.

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