

Chromosomal loci that influence oral nicotine consumption in C57BL/6J × C3H/HeJ F₂ intercross mice

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Several studies have demonstrated that there are genetic influences on free-choice oral nicotine consumption in mice. In order to establish the genetic architecture that underlies individual differences in free-choice nicotine consumption, quantitative trait loci (QTL) mapping was used to identify chromosomal regions that influence free-choice nicotine consumption in male and female F₂ mice derived from a cross between C57BL/6J and C3H/HeJ mice. These two mouse strains were chosen not only because they differ significantly for oral nicotine consumption, but also because they are at or near phenotypic extremes for all measures of nicotine sensitivity that have been reported. A four-bottle choice paradigm was used to assess nicotine consumption over an 8-day period. The four bottles contained water or water supplemented with 25, 50 or 100 µg/ml of nicotine base. Using micrograms of nicotine consumed per milliliter of total fluid consumed per day as the nicotine consumption phenotype, four significant QTL were identified. The QTL with the largest LOD score was located on distal chromosome 1 (peak LOD score = 15.7). Other chromosomes with significant QTL include central chromosome 4 (peak LOD score = 4.1), proximal chromosome 7 (peak LOD score = 6.1) and distal chromosome 15 (peak LOD score = 4.8). These four QTL appear to be responsible for up to 62% of the phenotypic variance in oral nicotine consumption.

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Genetic factors appear to be important determinants that influence whether or not an individual will become a regular smoker. Twin studies, beginning with those of Fisher (Fisher 1958a; 1958b), have demonstrated that the heritability of smoking ranges from 0.2 to 0.8 with a mean estimate of heritability of 0.53 (Sullivan and Kendler, 1999; Li 2003). In other words, approximately 50% of the variance of whether an individual is a smoker can be attributed to genetic factors. Genetic factors influence multiple aspects of tobacco use including initiation (Heath *et al.* 1993), persistence (Heath 1990; Heath and Martin 1993), number of cigarettes smoked (Carmelli *et al.* 1990) and the ability to stop smoking (Carmelli *et al.* 1992). Due to the considerable genetic influence on smoking-related behaviors, several groups have attempted to identify susceptibility loci for nicotine addiction/dependence. These studies have ranged from candidate gene association studies to genome-wide linkage analyses (see Li, 2006, for recent review). Although considerable progress is being made in this arena, the identification of genes that influence liability to nicotine addiction has proven difficult, at best. This may be due to the many complications including lack of environmental control, the use of different instruments to assess nicotine dependence and disparities in defining nicotine addiction.

Like smoking behavior in humans, the behavioral and physiological effects of nicotine in mice are influenced by genetic factors. For example, Marks *et al.* demonstrated that there is a two- to six-fold difference in ED₅₀ values for a battery of tests for nicotine sensitivity across 19 inbred strains (Marks *et al.* 1989). Miner and Collins (1989) reported similar results for nicotine-induced seizure sensitivity for the same 19 inbred strains. Genetic influences on the development of tolerance to nicotine (Marks *et al.* 1991), nicotine oral self-selection (Robinson *et al.* 1996; Butt *et al.* 2005; Li *et al.* 2005) and conditioned place preference (Schechter *et al.* 1995) also have been reported. Heritability estimates for some of these behaviors were obtained and found to range from 0.3 (for nicotine-induced hypothermia; Marks *et al.* 1984) to 0.63 (for nicotine-induced seizure sensitivity; Miner *et al.* 1984). One of the aforementioned behaviors, nicotine oral self-selection is a measure that assesses free-choice nicotine consumption. Although this measure clearly is not a direct model of smoking, it does possess several features similar to the smoking phenotype. For example, nicotine consumption occurs intermittently through the normal awake period and

sparingly during the normal sleep phase. In addition, similar to what is observed in humans, chronic oral nicotine consumption leads to tolerance development to the effects of nicotine and an increase in the number of high affinity nicotinic receptors in brain (Sparks and Pauly 1989). Moreover, Brunzell *et al.* (2003) demonstrated that chronic oral nicotine consumption leads to alterations in the expression of genes in signaling pathways thought to contribute to the addiction process. Also similar to what is observed in humans, there are significant differences in the willingness of mice to consume nicotine. Some mouse strains avoid nicotine nearly completely while others consume significant levels of the drug (Robinson *et al.* 1996; Butt *et al.* 2005; Li *et al.* 2005; Siu *et al.* 2006).

Based upon these observations, it seems reasonable that identification of genes that influence individual differences in oral nicotine consumption in the mouse may provide some insight into the genetic architecture of nicotine dependence in humans. In the study reported here, quantitative trait loci (QTL) mapping was used to identify chromosomal loci that influence free-choice nicotine consumption in an F_2 intercross between C57BL/6J and C3H/HeJ. These two mouse strains are near phenotypic extremes for nearly every measure of nicotine sensitivity assessed, including oral nicotine consumption (Marks *et al.* 1989, 1991; Miner and Collins 1989; Robinson *et al.* 1996; Butt *et al.* 2005; Li *et al.* 2005). Results indicate that there are at least four significant QTL that influence nicotine consumption in this population. Moreover, these four QTL appear to be responsible for up to 62% of the phenotypic variance in oral nicotine consumption.

Methods

Animals

C57BL/6J \times C3H/HeJ F_2 intercross mice were used for QTL analysis. In order to generate the F_2 population, F_1 mice were first produced by reciprocal crosses between the parental strains. F_1 mice were then crossed in all pairwise combinations to produce the F_2 intercross animals. Mice were weaned at 21 days of age and housed with same sex siblings with free access to food and water. The mice were maintained on a 12h:12h light/dark cycle with lights on at 0600 h and lights off at 1800 h. All animal care and experimental procedures were approved by and performed in accordance with the guidelines of the Animal Care and Utilization Committees of the University of the University of Michigan, Ann Arbor, MI, and the University of Colorado, Boulder, CO.

Nicotine consumption

Nicotine consumption was measured using the four-bottle choice paradigm. Briefly, mice were individually housed with free access to food and provided with four water bottles, one that contained water only and three that contained water supplemented with different concentrations of nicotine. The nicotine concentrations used were 25, 50 and 100 $\mu\text{g}/\text{ml}$.

New or freshly distilled (–) nicotine free base (Sigma-Aldrich, St Louis, MO) was used for solution preparations. Each day the position of the bottles were rotated to eliminate preference based on the location of the bottle. The test was conducted for 8 days so that each bottle was located in each of the four possible positions twice. A set of dummy bottles (bottles with liquid placed in cages with no mice) were included in each round of testing in order to assess liquid loss due to evaporation and general handling. Fluid consumption values were adjusted for this non-specific fluid loss. The bottles were weighed at the beginning and end of each 4-day trial and the volume consumed from each drinking solution was determined. The mice were weighed on the first, fifth and last day of the experiment. Nicotine consumption (in μg) per day was determined by multiplying the concentration of nicotine solution times the volume of nicotine consumed from each concentration per day. The daily amount of nicotine consumed from the 25, 50 and 100 $\mu\text{g}/\text{ml}$ nicotine-containing bottles was summed to give a total consumption value. Overall nicotine consumption was calculated as micrograms of nicotine consumed per milliliter of total fluid consumed per day (total nicotine consumed per day divided by total fluid consumption per day) and dose of nicotine consumed per day (total nicotine consumed/day divided by the average weight of the mice). A total of 584 mice were tested in groups of approximately 50 mice at approximately 2-week intervals per group. Mice in each test group were matched by age and approximately equal numbers of male and female mice were included per group. Testing was initiated between 55 and 65 days of age.

Genotyping

F_2 animals with the highest and lowest consumption values were chosen for genotypic analysis. A total of 203 animals, 107 male and 96 female mice, were genotyped. DNA was extracted from splenic tissue from these mice by overnight digestion in a solution of 10 mM Tris, pH 8, 5 mM ethylenediaminetetraacetic acid (EDTA), pH 8, 100 mM NaCl, 0.5% sodium dodecyl sulphate and 100 $\mu\text{g}/\text{ml}$ proteinase K, extracted with an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) and precipitated by the addition of one volume of isopropyl alcohol. The samples were resuspended in TE (10 mM Tris, pH 8, 1 mM EDTA, pH 8). For genotyping, microsatellite marker-specific polymerase chain reaction (PCR) primers (0.45 μM each) (MapPairs, Research Genetics/Invitrogen, Carlsbad, CA, USA) were included in a 7.5- μl reaction that contains 40 ng DNA, 1 \times PCR buffer (Amplitaq Gold buffer II), 0.25 mM dNTP mix, 2.5 mM MgCl_2 and 0.3 U AmpliTag Gold DNA polymerase (Applied Biosystems, Branchburg, NJ, USA). Samples were amplified in either an MJ Research PTC-100 thermal cycler (Bio-Rad, Hercules, CA, USA) or a Bio-Rad iCycler thermal cycler according to the conditions recommended by Research Genetics (95°C for 12 min followed by 10 cycles of 94°C for 15 seconds, 55°C for 15 seconds, 72°C for 15 seconds followed by an additional 20

cycles of 89°C for 15 seconds, 55°C for 15 seconds, 72°C for 15 seconds and completed by 72°C for 7 min). Samples were electrophoresed on 3–4% Metaphor agarose (Cambrex, Rockland, ME, USA) gels, stained with ethidium bromide and genotypes were determined by visual inspection by at least two independent observers. Eighty markers were used for genotyping including 77 microsatellite markers and 3 single nucleotide polymorphism (SNP) markers. The microsatellite markers were selected from the Mouse Genome Informatics Database (www.informatics.jax.org) and were all markers from the Massachusetts Institute of Technology collection. The SNP markers were chosen to fill in gaps on distal chromosome 2, proximal chromosome 7 and central chromosome 15. They were selected using the Oxford mouse SNP database (the markers used were all polymorphic between C57BL/6J and C3H/HeJ mice and each SNP resulted in a unique restriction site that was used to identify each allele). The markers were chosen to produce an average inter-marker interval of approximately 20 cM.

Data analysis

Data were analyzed using Map Manager QTX (Manly *et al.* 2001). Initially, permutation analysis (1000 permutations at 1 cM intervals) was performed on the data to empirically estimate thresholds for suggestive, significant and highly significant QTL (Churchill and Doerge 1994). Essentially, in this test the trait values are randomly permuted among the progeny, destroying any relationship between the trait values and the genotypes of the marker loci. The regression model is fitted for the permuted data at multiple analysis points across the genome (matching the points used for detecting QTLs) and the maximum LRS is recorded. This procedure is repeated a thousand times, giving a distribution of statistic values, which we would expect if there were no QTL linked to any of the marker loci. The threshold values of the permutation test, which are labeled suggestive, significant and highly significant are taken from the guidelines of Lander and Kruglyak (1995) and correspond to the 37th, 95th and 99.9th percentiles, respectively. These thresholds correspond to genome-wide α values of 0.63, 0.05 and 0.001, respectively. For the $\mu\text{g/ml}$ consumption data, thresholds were estimated as LOD 2.0 for suggestive, LOD 3.38 for significant and LOD 4.97 for highly significant. Once genome-wide thresholds were estimated, regression analysis using the free model was utilized to identify markers that were associated with the nicotine consumption phenotypes.

We also performed regression analysis separately for each sex and utilized the method previously described by Bergeson *et al.* (2003) to identify sex-specific or sex-influenced QTL. Briefly, the marker LOD scores for each sex were subtracted from one another, and this difference in LOD score between female and male mice was converted back to a P value. The calculated P value is an estimate of P for the sex difference. Interconverting LOD and P were done by using the formulas $\text{LOD} = -\log_{10}(P)$ or $P = 10^{-\text{LOD}}$. Because 15 provisional QTL

were searched in the F_2 for gender differences, we used a Bonferroni correction of 15-fold. Therefore, the significance threshold was defined as $P = 0.05/15$ or $P = 0.003$. This converts into a LOD score difference of 2.5 as the threshold for a sex difference. Any QTL that met or exceeded the 2.5 LOD difference between sexes were further defined as sex-specific if the marker exceeded the significance threshold in one sex only or sex-influenced if the marker surpassed the significance threshold in both sexes.

Interval mapping was performed on chromosomes containing at least one marker that met the significant threshold. All mapping methods used by Map Manager QTX are based upon the Maximum Likelihood approach of Lander and Botstein (1989). Data also were evaluated using QTL Cartographer (Wang *et al.* 2005b) with essentially identical results.

Results

Nicotine consumption in C57BL/6J \times C3H/HeJ F_2 intercross mice

A total of 584 BxH F_2 mice (306 female and 278 male) were tested for nicotine consumption in a four-bottle choice paradigm as described in the Methods. The main phenotypic measure used to assess average daily nicotine consumption was micrograms of nicotine consumed per milliliter of total fluid consumed per day ($\mu\text{g/ml/day}$). When using this measure, no sex differences in nicotine consumption were observed (16.1 ± 0.42 and 15.7 ± 0.44 $\mu\text{g/ml/day}$ for female and male mice, respectively ($t = -0.625$, $P > 0.5$)) (Fig. 1a). In addition, there were no sex differences in daily total fluid consumption (6.30 ± 0.06 and 6.28 ± 0.06 ml/day for female and male mice, respectively ($t = -0.378$, $P = 0.711$)) (Fig. 1b). However, for a second measure of nicotine consumption, the mean dose of nicotine consumed, sex differences were noted. The daily dose of nicotine consumed by female mice (4.7 ± 0.12 mg/kg/day) was significantly greater ($t = -8.28$, $P < 0.001$) than the dose consumed by male mice (3.37 ± 0.10 mg/kg/day) (Fig. 1a). For both measures of nicotine ingestion, the data were skewed toward low nicotine consumption (Fig. 2) while total fluid consumption exhibited a normal distribution (Fig. 3).

Identification of QTL related to nicotine consumption

To locate chromosomal loci that are associated with nicotine consumption, 203 animals (100 female, 103 male) at the phenotypic extremes (96 from the low consumption extreme and 107 from the high consumption extremes) were genotyped for 80 markers across all 19 autosomal chromosomes. Primary analyses to identify QTL were performed for the micrograms of nicotine consumed per milliliter of total fluid measure because there was no sex difference for this phenotype. The mean nicotine consumption from the low and high end extremes was 8.5 ± 0.15 and 25.1 ± 0.63 $\mu\text{g/ml/day}$, respectively.

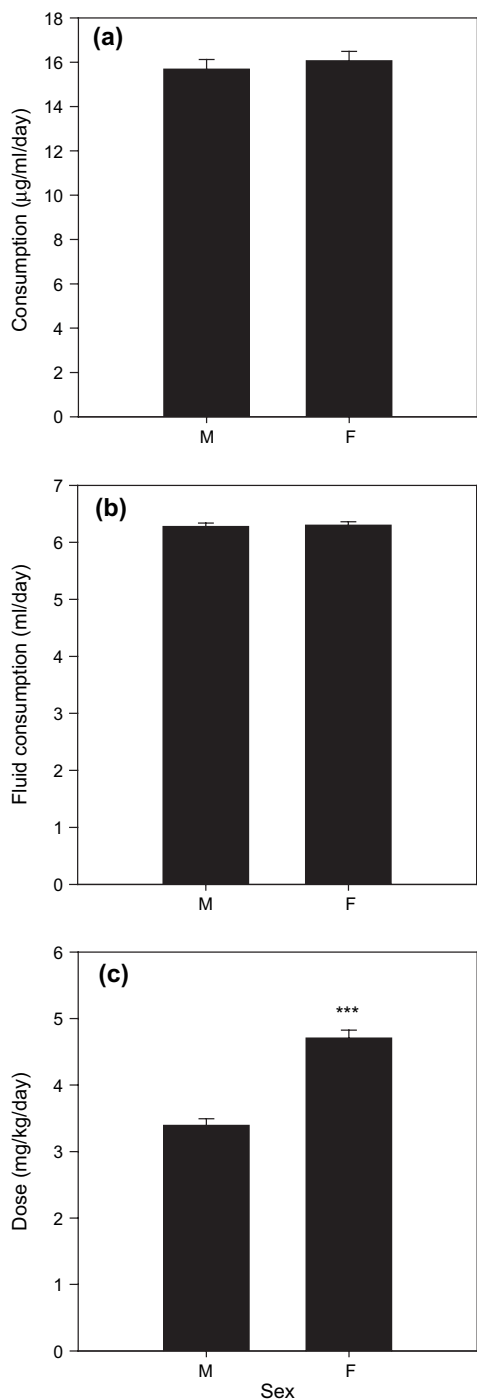


Figure 1: Influence of sex of mice on phenotypic measures. (a) No effect of sex was observed when micrograms of nicotine consumed per milliliter of total fluid consumed per day was used as the phenotypic measure. (b) Daily fluid consumption (nicotine plus water) also was not different between male and female mice. (c) A significant effect of sex ($P < 0.001$) was detected when dose of nicotine consumed (mg/kg/day) was used as the phenotypic variable for nicotine consumption. *** $P < 0.001$. Data presented represent mean \pm SEM.

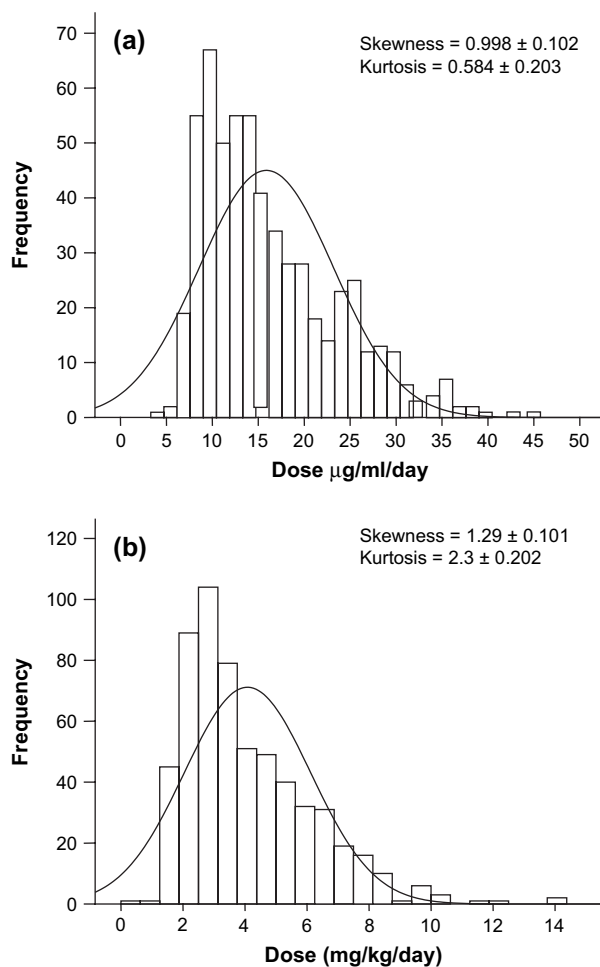


Figure 2: Distribution of nicotine consumption in 584 C57BL/6J \times C3H/HeJ F_2 intercross mice. Nicotine consumption when measured either by $\mu\text{g/ml/day}$ (a) or mg/kg/day (b) of nicotine drank per day exhibits a distribution that is positively skewed. The mean amount of nicotine consumed for the two measures, respectively, was $15.88 \pm 0.30 \mu\text{g/ml/day}$ and $4.08 \pm 0.084 \text{ mg/kg/day}$. The solid line represents a normal distribution.

Regression analysis

Permutation analysis of the data from the phenotypic extremes established empirical LOD score values of 2.0, 3.4 and 5.0 as thresholds for suggestive, significant and highly significant QTL. Fifteen markers were identified that are associated with nicotine consumption at the suggestive LOD score value of 2.0 or greater (Table 1). The list of markers includes three each on chromosomes 1, 4 and 15, four on chromosome 7 and one each on chromosomes 14 and 18. Regression analysis also was performed on each sex separately (Table 1). Although no additional QTL were detected, it was ascertained that of the 15 QTL identified in combined sex data, 2 meet the criteria defined in the methods for sex-specific QTL (D1MIT308 and D4MIT175) and 1 meets the criteria defined in the methods for a sex-influenced QTL (D1MIT206). For all markers on

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were performed for dose, nearly identical results were obtained (data not shown).

Interval mapping for both measures of nicotine consumption

Chromosomes for which at least one marker was identified as being significantly associated with nicotine consumption were further evaluated by interval mapping. Due to the positive skewing of the raw phenotypic data, log transforms of the data were used for interval mapping. Using the free regression model for interval mapping, highly significant QTLs for micrograms of nicotine consumed per milliliter of total fluid were identified on distal chromosome 1 (peak LOD score = 15.7) and proximal chromosome 7 (peak LOD score = 6.1), while significant QTLs were found on central chromosome 4 (peak LOD score = 4.1) and distal chromosome 15 (peak LOD score = 4.8) (Fig. 5). The estimated percentage of the phenotypic variance that can be explained by the QTL on chromosomes 1, 4, 7 and 15 was 30%, 9%, 13% and 10%, respectively. Interval mapping also was performed on the data from each sex for chromosomes 1 and 4 because QTL on these chromosomes appear to be either sex-influenced or sex-dependent (Fig. 5a,b). Interval mapping using dose as the phenotype yielded essentially identical results although with slightly lower peak LOD scores (Chr 1, LOD = 12.6; Chr 4, LOD = 4.4; Chr 7, LOD = 4.6; Chr 15, LOD = 4.4).

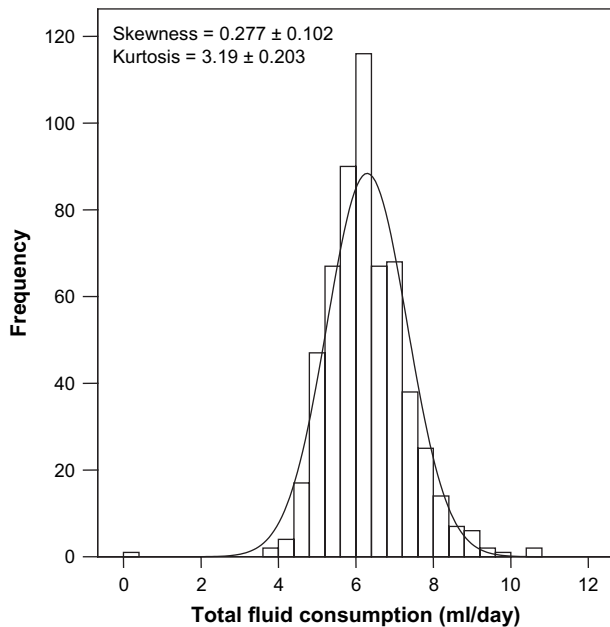


Figure 3: Distribution of total fluid consumption. Total fluid consumption (ml nicotine solutions consumed plus ml water consumed) exhibits a normal distribution in the F_2 animals. Mean fluid consumption was 6.34 ± 0.09 ml/day. The solid line represents a normal distribution.

chromosomes 1, 4, 14, 15 and 18, the C57BL/6 allele was associated with higher nicotine consumption. In contrast, for each marker on chromosome 7, the C3H/HeJ allele was associated with greater nicotine consumption. The relationship between the markers on each chromosome with the highest LOD score and nicotine consumption ($\mu\text{g/ml/day}$ data are shown) is shown in Fig. 4. When the same regression analyses

Discussion

Four significant QTL for nicotine consumption have been identified in F_2 intercross mice derived from the parental strains C57BL/6J and C3H/HeJ. The significant QTL are located on chromosomes 1, 4, 7 and 15. Each of the four

Table 1: Marker regression analysis for nicotine consumption ($\mu\text{g/ml/day}$ nicotine)

Chromosome	Marker	Location (cM)	LOD score combined	LOD score female	LOD score male	LOD score difference
1	D1MIT308	62.1	6.25	1.11	6.14	5.03
1	D1MIT206	95.8	15.38	10.2	4.27	5.93
1	D1MIT511	109.6	7.92	3.45	3.14	0.31
4	D4MIT175	49.6	3.93	4.32	0.82	3.5
4	D4MIT203	60	2.47	2.54	0.46	2.08
4	D4MIT42	81	2.54	2.32	0.63	1.69
7	rs13479172	~13	5.18	2.95	2.17	2.78
7	D7MIT228	18	5.90	2.65	3.56	0.91
7	D7MIT91	28.1	2.97	1.06	2.23	1.17
7	D7MIT323	50	2.54	1.48	1.65	1.7
14	D14MIT39	30	2.23	0.59	1.34	0.75
15	D15M70	47.7	3.99	2.62	2.60	0.02
15	rs3667785	~56	4.47	3.73	1.65	2.08
15	D15M161	69.2	3.77	3.90	1.63	2.27
18	D18MIT7	50	2.62	2.0	0.15	1.85

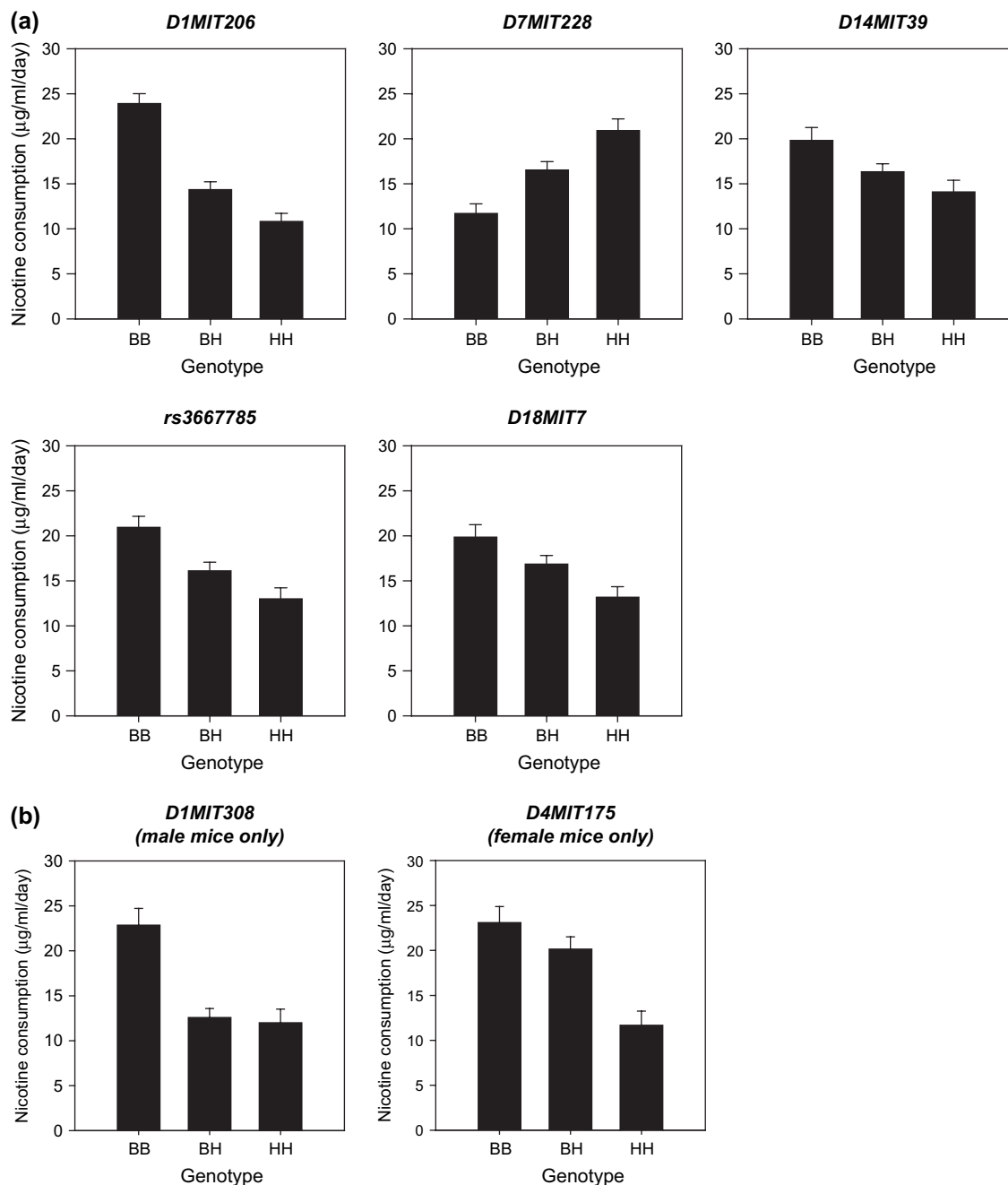


Figure 4: Association between some significant markers and nicotine consumption. Markers on six chromosomes (1, 4, 7, 14, 15 and 18) were detected at a LOD score of 2.0 or greater. The relationship between markers with the highest LOD scores on each of the six chromosomes is shown. (a) Markers with highest LOD scores which are not sex-specific. Data from all F_2 extremes are included. (b) The two significant markers that meet the criteria as sex-specific quantitative trait loci were plotted using data from either male (D1MIT308) or female (D4MIT175) animals only. Data represent mean \pm SEM. BB, homozygous for the C57BL/6 allele of the marker; BH, heterozygous for the marker; HH, homozygous for the C3H allele of the marker. The identity of the evaluated marker is indicated above each graph. LOD scores for each marker can be found in Table 1.

QTL was detected using two different measures of nicotine consumption (μg nicotine/ml fluid or nicotine dose). Although numerous studies have identified QTL for oral consumption of drugs of abuse in animal models (for review see Crabbe *et al.*

1999 and Flint 2003), this is the first report of the identification of QTL for nicotine consumption in an animal model. The results of this study also indicate that as much as 62% of the variance in nicotine consumption may be explained by

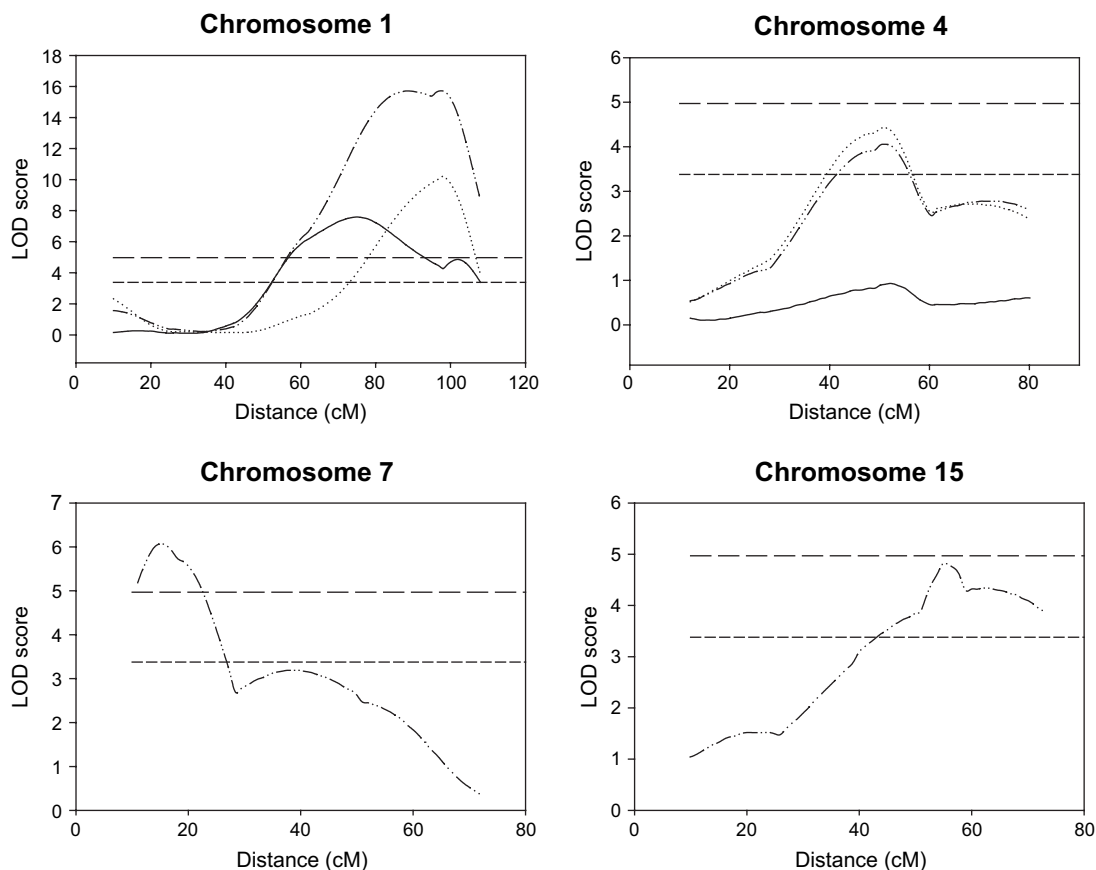


Figure 5: Interval mapping of quantitative trait loci (QTL) on chromosomes 1, 4, 7 and 15. Interval mapping was performed using Map Manager QTX. LOD scores represent the results of a model independent (free) interval analysis. The x-axis represents the approximate genetic map of each chromosome in centiMorgans (cM). The short-dashed line represents the LOD score threshold for a significant QTL and the long-dashed line represents the LOD score threshold for a highly significant QTL. Significance thresholds were determined empirically by permutation analysis. For chromosomes 1 and 4, combined (— · —) as well as specific interval maps for male (—) and female (· · ·) are shown.

the four significant QTL. Thus, oral nicotine consumption appears to be under a strong genetic influence. These results are consistent with studies in humans that indicate that approximately 56% of the variance in smoking initiation (Sullivan and Kendler 1999) and up to 70% of the variance in nicotine dependence (Kendler *et al.* 1999; True *et al.* 1999) can be attributed to genetic factors. Moreover, the largest peak LOD score identified for nicotine consumption in mice is located at around 96 cM on mouse chromosome 1. This region of the mouse genome is syntenic with human chromosome 1 at around 169 cM. To date, the QTL with the greatest effect on nicotine dependence identified in humans is located on human chromosome 1 with a confidence interval of between 168 and 196 cM (Wang *et al.* 2005a). Two additional studies also have identified potential QTL for nicotine dependence in humans located within this region of chromosome 1 (Bergen and Caporaso 1999; Goode *et al.* 2003). Thus, in both mice and humans, the same genomic region appears to influence nicotine consumption/dependence.

The markers for the significant QTL on chromosomes 1, 4 and 15 were associated with nicotine consumption in a manner consistent with parental nicotine consumption (Li *et al.* 2005); mice homozygous for the C57BL/6 allele consumed the most nicotine on average while mice homozygous for the C3H allele consumed the least amount of nicotine on average. In contrast, markers for the significant QTL on chromosome 7 showed the opposite pattern. Mice homozygous for the C3H marker allele consumed more nicotine than did mice homozygous for the C57BL/6 allele. Therefore, despite the fact that C57BL/6 mice consume the most nicotine of any mouse strain evaluated (Robinson *et al.* 1996; Butt *et al.* 2005; Li *et al.* 2005), they do not carry all of the alleles for high nicotine consumption.

The results of this study also suggest that there are sex-influenced as well as sex-specific QTL for nicotine consumption even though sex differences were not observed in the main phenotype used to assess nicotine consumption ($\mu\text{g/ml/day}$). Both a male-specific and sex-influenced QTL

were detected in the distal third of chromosome 1 while the QTL on chromosome 4 appears to be female-specific. However, follow-up studies using independent genetic designs are necessary to confirm the sex-dependence of these QTL as well as to verify each of the QTL identified in this initial report.

There are several possible reasons why there is individual variability in oral nicotine consumption in mice. One possible explanation is that there is no real choice involved but rather, mice that consume more nicotine do so simply because they drink more overall fluid. If this were the case, there would be a strong positive correlation between total fluid consumption and nicotine consumption. However, there is a small and negative correlation ($r = -0.295$; $P < 0.001$) between these two measures. The fact that there is an inverse correlation between daily nicotine consumption and daily fluid consumption argues that individual differences in nicotine consumption are not due to individual differences in overall fluid consumption. A second reason why mice may exhibit differences in nicotine consumption is taste; nicotine is classified as a bitter taste. Therefore, there may be avoidance or preference for nicotine based upon its taste rather than some pharmacological effect. Although an influence of taste on nicotine consumption cannot be entirely ruled out, we do not believe that it is the major determinant for nicotine consumption based upon the following information. First, QTL for bitter taste have been mapped in mice (Harder and Whitney 1998; Le Roy *et al.* 1999) and none of these QTL map to the same chromosomal regions as the QTL for nicotine consumption. Second, there are no known taste receptor genes located within the confidence intervals of the four nicotine consumption QTL. Third, previous studies have demonstrated that masking the taste of nicotine with saccharin does not alter the rank order of nicotine consumption among inbred mouse strains (Robinson *et al.* 1996). Finally, mice exhibit no preference for the less biologically active stereoisomer of nicotine (Butt *et al.* 2005). These combined results indicate that individual differences in nicotine consumption are likely due to a pharmacological effect of the drug (either adverse or reinforcing). This conclusion is further supported by studies which demonstrate that oral nicotine preference is reduced when animals are pretreated with a nicotinic receptor antagonist (Glick *et al.* 1996). The observation that nicotine consumption is reduced by a nicotinic antagonist suggests that oral nicotine consumption is due to the reinforcing properties of the drug and not its aversive effects.

Previously, we have demonstrated that there is a significant genetic correlation between alcohol consumption and nicotine consumption in C57BL/6 \times C3H F₂ intercross mice (Li *et al.* 2005). Moreover, nicotine consumption in inbred mouse strains (data from Butt *et al.*, 2005) is significantly correlated with both preference for a solution containing 10% ethanol (Belknap *et al.* 1993) ($n = 9$, $r^2 = 0.726$, $P < 0.05$) and withdrawal from chronic ethanol exposure (Metten *et al.* 1998) ($n = 9$, $r^2 = 0.707$, $P < 0.05$) (data not shown). These

data suggest that there is genetic overlap between nicotine consumption, alcohol consumption and withdrawal from alcohol. Consistent with this possibility, all four significant QTL for nicotine consumption overlap with previous QTL identified for either alcohol preference or alcohol withdrawal. For example, two QTL for ethanol withdrawal (Buck *et al.* 1997; 2002) exhibit peak LOD scores very near or identical to the regions of chromosomes 1 and 4 where we report peak LOD scores for QTL for nicotine consumption. In addition, QTL for both nicotine consumption and alcohol preference are located in proximal chromosome 7 (Bachmanov *et al.* 2002) and central/distal chromosome 15 (Vadasz *et al.* 2000; Gill and Boyle 2005). Interestingly, for the QTL on chromosome 7, the relationship between genotype and phenotype is opposite that of the parental strains for both nicotine and alcohol consumption. This observation suggests that there is a 'protective' allele or alleles on proximal chromosome 7 that reduces drug consumption in the high drug consuming C57BL/6 mouse strain.

In humans, there is high co-morbidity between alcoholism and smoking (Istvan and Matarazzo 1984; Battjes 1988). In addition, a large number of studies clearly have established that there is a strong genetic influence on both smoking and alcoholism (Istvan and Matarazzo 1984; Carmelli *et al.* 1990; Swan *et al.* 1990; Heath *et al.* 1993; Heath and Martin 1993; Hettaema *et al.* 1999). Moreover, True *et al.* (1999) reported that there are common genetic influences on both alcoholism and smoking and Bergen and Caporaso (1999) identified common loci that show evidence for linkage to both smoking and alcoholism. These findings suggest that alcohol and nicotine dependence may be influenced, in part, by genetic variability in common genes. The fact that QTL for both alcohol- and nicotine-related behaviors map to the same regions of chromosomes 1, 4, 7 and 15 in mice also support this possibility. Consequently, establishing whether the same gene or genes in these chromosomal regions do, in fact, influence both nicotine- and alcohol-related behaviors may provide insight into the molecular genetic basis for the co-morbidity between smoking and alcoholism.

In summary, free-choice oral nicotine consumption in mice appears to be strongly influenced by genetic factors. The data reported here suggest that four QTL account for up to 62% of the variance in nicotine consumption. Although follow-up studies are necessary to confirm these QTL, future studies to identify the genes that underlie the verified QTL will provide insight into the molecular basis of individual differences in nicotine and perhaps alcohol consumption.

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