Research report

Genetic correlation between the free-choice oral consumption of nicotine and alcohol in C57BL/6J × C3H/HeJ F2 intercross mice

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Abstract

Previous studies in humans have demonstrated a high co-morbidity between alcoholism and smoking. This co-morbidity between alcohol and nicotine dependence can be attributed, in part, to common genetic factors. In rodents, behavioral and physiological responses to alcohol and nicotine also appear to share common genetic influences. In this report, the genetic correlation between free-choice oral nicotine and oral alcohol consumption was evaluated using an ascending two-bottle choice paradigm in C57BL/6J × C3H/HeJ F2 intercross mice. For all concentrations of nicotine (25, 50, and 100 μg/ml) and alcohol (3, 6, and 10%) tested, nicotine consumption was significantly correlated with alcohol consumption. Nicotine consumption at the highest nicotine concentration tested (100 μg/ml) showed low, but significant, correlations with the number of [3H]-cytisine binding sites in the hippocampus (r = 0.307) and the number of [125I]-bungarotoxin binding sites in the cortex (r = −0.328). No significant correlations between alcohol consumption and the number of either [3H]-cytisine or [125I]-bungarotoxin binding sites was observed. A polymorphism in the nicotinic receptor α4 subunit gene, Chrna4, showed a trend with nicotine consumption and a significant association with alcohol consumption in female but not male mice. These results indicate that common genetic factors influence nicotine and alcohol consumption in mice. However, neither individual differences in the expression of [3H]-cytisine or [125I]-bungarotoxin binding nicotinic receptors nor the polymorphism in Chrna4 likely contribute to the genetic overlap that influences the consumption of both of these drugs of abuse in C57BL/6 × C3H/HeJ F2 mice.

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1. Introduction

Studies in humans have demonstrated a high co-morbidity between alcoholism and smoking [3,21]. Approximately 80% of alcoholics are smokers while about 24% of the total adult population smoke cigarettes. Moreover, there is a direct correlation between the amount of alcohol consumed and the number of cigarettes consumed among alcoholics [2,9,22]. It also has been established that there is a considerable genetic influence on both alcoholism and smoking. Studies have estimated that approximately 50–60% of individual variation in either alcohol or tobacco consumption can be attributed to genetic factors [7,18–20,38] and a considerable amount of the genetic variance for alcoholism and smoking may be attributed to common genetic factors [43].

In rodents, it has been established that responses to alcohol and nicotine, the primary addictive agent in tobacco, also are significantly influenced by genetic factors. As is the case in humans, responses to alcohol and nicotine appear to share common genetic components. For example, several responses to nicotine and alcohol were correlated in a classic genetic cross between the selected mouse lines long-sleep (LS) and short-sleep (SS) [12]. In addition, the LS and SS mice, which...
were selected for individual differences in sensitivity to the sedative effects of alcohol, exhibit significant differences in sensitivity to nicotine [33]. Other rodent lines selected for differences in various alcohol-related behaviors also have been found to differ in sensitivity to nicotine [10,11,13,16,17,23].

One potential site of convergence of the actions of nicotine and alcohol is the family of neuronal nicotinic acetylcholine receptors (nAChRs) expressed in the brain. In mammals, the neuronal subfamily of nAChRs comprise an indeterminate number of subtypes that are composed of pentameric combinations of the subunits α2–α7 [37]. nAChRs expressed in the brain. In mammals, the neuronal subfamily of nAChRs comprise an indeterminate number of subtypes that are composed of pentameric combinations of the subunits α2–α7 [37]. nAChRs expressed in the brain. In mammals, the neuronal subfamily of nAChRs comprise an indeterminate number of subtypes that are composed of pentameric combinations of the subunits α2–α7 [37]. nAChRs expressed in the brain. In mammals, the neuronal subfamily of nAChRs comprise an indeterminate number of subtypes that are composed of pentameric combinations of the subunits α2–α7 [37]. nAChRs expressed in the brain. In mammals, the neuronal subfamily of nAChRs comprise an indeterminate number of subtypes that are composed of pentameric combinations of the subunits α2–α7 [37]. nAChRs expressed in the brain. In mammals, the neuronal subfamily of nAChRs comprise an indeterminate number of subtypes that are composed of pentameric combinations of the subunits α2–α7 [37].

Various nAChRs were measured in all mice by radio-ligand binding assays in four brain regions (cortex, hippocampus, midbrain and striatum). In addition, the relationship between Chrna4 genotype and the consumption of nicotine and alcohol was evaluated in these animals.

2. Methods

2.1. Animals

C57BL/6J, C3H/HeJ, and (C57BL/6J female × C3H/HeJ male) F1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6J × C3H/HeJ F2 intercross mice were produced in-house by mating the F1 animals purchased from The Jackson Laboratory. The F2 animals were weaned at 21 days and group housed by sex. The mice were maintained on a 12 h light/dark schedule and had free access to food (Harlan Teklad, Indianapolis, IN) and water. For preference testing, mice were singly housed in a standard mouse cage and provided with food and two bottles of fluid. One bottle contained the test drug dissolved in water and the other bottle contained water only. Each drug concentration was tested for a period of 4 days and the bottle positions were rotated every day. The mass of each animal was measured at the beginning and end of each drug concentration. For all animals, alcohol preference was measured first starting with 3% alcohol, followed immediately by 6% alcohol and then 10% alcohol. Six days following the completion of the 10% alcohol trial, nicotine preference was initiated. Between the alcohol and nicotine trials, the mice only had access to water. The first nicotine concentration tested was 25 μg/ml nicotine solution followed immediately by 50 and 100 μg/ml nicotine solutions.

2.2. Receptor binding

The binding of [3H]-cytisine to particulate fractions from cortex, hippocampus, midbrain and striatum was measured using methods similar to those described for [3H]-nicotine binding in Marks et al. [28]. Particulate fractions obtained from P2 preparations of the four brain regions were incubated with 10 nM [3H]-cytisine in 100 μl of Krebs–Ringers–HEPES (118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 20 mM HEPES, pH 7.4) (KRH) for 1.5 h at 4 °C. Incubations were conducted in 96-well polystyrene plates. Non-specific binding was determined by including 10 μM unlabeled (−)nicotine in the incubation. The binding reaction was terminated by filtration of the particulate fractions onto glass fiber filters that were soaked in 0.5% polyethylenimine in KRH. After filtration, the filters were washed six times with ice-cold KRH. All filtration was done using a Tomtec (Hamden, CT) Mach II harvester. The filters were collected and placed in scintillation vials. Following the addition of scintillation fluid, the radioactivity was measured using a liquid scintillation counter. The binding of [3H]-α-bungarotoxin ([3H]-αBTX) was performed as described for [3H]-cytisine binding with the following exceptions. Particulate fractions were incubated with 2 nM [3H]-αBTX for 3 h at 37 °C and non-specific binding was determined by the inclusion of 1 mM (−)nicotine in the incubation. Filtration of the samples was done using glass fiber filters that were soaked in 0.5% polyethylenimine and 5%...
non-fat dry milk in KRH. Filters were counted on a Packard Cobra II gamma counter (Perkin-Elmer, Boston, MA). Homogenate protein levels were determined by the method of Lowry et al. [26].

2.3. Chrna4 genotyping

Genomic DNA from the 50 F2 mice was isolated from splenic tissue by standard proteinase K digestion/phenol extraction methodology as described previously [36]. A region of Chrna4 that spanned the SNP at nucleotide position 1587 was amplified by a reaction that included 50 ng of genomic DNA, 1× PCR buffer II (PE Biosystems, Foster City, CA), 2.5 mM MgCl$_2$, 200 μM each, dGTP, dATP, dCTP, dTTP, 20 μmol of each amplification primer (5′-GGTCCCTGAGCGTCCAGCA TG-3′ and 5′-GGTCCTA TCTGGGTCGGGGTG-3′), 2.5 units AmpliTaq Gold DNA polymerase (PE Biosystems) in a reaction volume of 50 μl. Amplification of the DNA was accomplished using a touchdown protocol with an initial annealing temperature of 65 °C and final amplification conditions of 94 °C, 30 s; 55 °C, 30 s; 72 °C, 1 min, for 30 cycles. This amplification reaction generates a product of 405 bp that spans from 185 bp upstream of the Chrna4 SNP at nucleotide position 1587 to 220 downstream of this SNP. Following amplification, 5 μl of the PCR reaction was digested with StuI in a final volume of 20 μl and subsequently electrophoresed on a 1.8% agarose gel. The restriction enzyme StuI (recognition sequence AGGCCCT) will cut the PCR product if the alanine codon, GCC, is present at codon position 529 but will not cut the PCR product if the threonine codon, ACC, is present at this position.

Fig. 1. Measurement of alcohol preference and alcohol consumption in male and female C57BL/6 and C3H/HeJ mice. Male and female mice of the inbred mouse strains C57BL/6J (n = 5 males and 5 females) and C3H/HeJ (n = 6 males and 6 females) were tested in an ascending alcohol two-bottle choice paradigm and preference ratios (percent of total fluid consumed from the alcohol-containing bottle) and alcohol consumption (grams alcohol consumed/kg body mass) were determined for each alcohol concentration. (A) Alcohol preference. For alcohol preference ratio, a significant between-subject effects were observed for strain (P < 0.001), but not for sex. A significant concentration-strain interaction was detected for within-subject effects (P < 0.001). (B) Alcohol consumption. Between-subject measures for alcohol consumption detected significant effects of strain (P < 0.001), sex (P < 0.001) and a strain–sex interaction (P < 0.05). Significant effects of alcohol concentration (P < 0.001), and concentration × strain (P < 0.001), concentration × sex (P < 0.001) and concentration × strain × sex (P < 0.05) interactions were observed for within-subject tests. All data are presented as mean ± S.E.M.

2.4. Statistics

All statistical analyses were performed using the SPSS 12.0 software package (SPSS, Chicago, IL). Consumption measures (drug consumption and preference ratio) for both nicotine and alcohol in the inbred strains were assessed using a general linear model for repeated measures. Consumption measures for both nicotine and alcohol in the F2 intercross mice were evaluated using two-way ANOVA. The relationship between nicotine and alcohol consumption as well as the relationship between drug consumption and [3H]-cytisine and [125I]-αβTX binding levels were evaluated using bivariate correlational analysis (Pearson’s two-tailed correlation coefficient). The relationship between Chrna4 genotype and drug intake was performed using the general linear model for repeated measures.

3. Results

3.1. Alcohol consumption in C57BL/6J and C3H/HeJ inbred mice

Alcohol consumption was measured in C57BL/6J and C3H/HeJ mice using the two-bottle choice paradigm as described in Section 2. Testing was conducted over a 12-day period. During each of three separate 4-day periods, the mice were provided with bottles containing water and water supplemented with 3% (v/v) alcohol (first 4 days), 6% (v/v) alcohol (second 4 days) and 10% (v/v) alcohol (last 4 days). For the alcohol preference measure (Fig. 1A), significant between-subject effects were observed for strain
(F(1,22) = 48.47, P < 0.001), but not for sex. A significant concentration-strain interaction was detected for within-subject effects (F(2,22) = 16.78, P < 0.001). Between-subject measures for alcohol consumption (Fig. 1B) detected significant effects of strain (F(1,22) = 71.26, P < 0.001), sex (F(1,22) = 16.64, P < 0.001) and a strain-sex interaction (F(1,22) = 7.19, P < 0.05). Significant effects of alcohol concentration (F(2,22) = 47.2, P < 0.001), and concentration × strain (F(2,22) = 41.4, P < 0.001), concentration × sex (F(2,22) = 8.38, P < 0.005) and concentration × strain × sex (F(2,22) = 3.66, P < 0.05) interactions were observed for within-subject tests.

3.2. Nicotine consumption in C57BL/6J and C3H/HeJ inbred mice

Nicotine consumption also was measured in C57BL/6J and C3H/HeJ mice using the two-bottle choice paradigm as described in Section 2. Testing was conducted over a 12-day period. During each of three separate 4-day periods, the mice were provided with bottles containing water and water supplemented with 25 μg/ml nicotine (first 4 days), 50 μg/ml nicotine (second 4 days) and 100 μg/ml nicotine (last 4 days). A significant between-subject effect was observed for nicotine preference for strain (F(1,20) = 10.48, P < 0.005), but not for sex (Fig. 2A). A significant effect of concentration also was detected for within-subject effects (F(2,20) = 53.62, P < 0.001). Between-subject measures for nicotine consumption (Fig. 2B) detected significant effects of strain (F(1,20) = 17.52, P < 0.001) and a strain–sex interaction (F(1,20) = 7.81, P < 0.01). Significant effects of nicotine concentration (F(2,20) = 3.78, P < 0.05), and concentration × strain (F(2,20) = 14.75, P < 0.001), and concentration × strain × sex (F(2,20) = 3.8, P < 0.05) interactions were observed for within-subject tests of nicotine consumption.

3.3. Effect of sequential alcohol and nicotine two-bottle choice tests on measures of nicotine preference

In order to evaluate the relationship between the consumption of alcohol and nicotine in the same animal, sequential testing of the two drugs is necessary. Therefore, C57BL/6J and C3H/HeJ mice were evaluated for whether prior testing of mice with the alcohol two-bottle choice paradigm affects measures of nicotine preference in a subsequent nicotine two-bottle choice test. Female and male mice of both inbred strains were tested in the 12-day, two-bottle preference paradigm for alcohol exactly as described earlier. An equal number of animals were tested simultaneously in the two-bottle paradigm in which both bottles contained water only. Six days following the completion of the water/alcohol, water/water two-bottle choice experiments, the mice were tested for nicotine two-bottle choice exactly as described earlier. Although alcohol exposure preceding nicotine preference showed a tendency to decrease nicotine consumption in C57BL/6 mice and to a lesser extent in C3H/HeJ mice, statistical analysis of nicotine preference (Fig. 3A) and consumption (Fig. 3B) between mice that were or were not exposed to alcohol in the initial two-bottle choice test indicated that exposure to alcohol did not significantly alter either nicotine preference (F(1,42) = 0.174, P > 0.5) or nicotine consumption (F(1,42) = 1.12, P > 0.2) in either strain.

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Fig. 2. Measurement of nicotine preference and nicotine consumption in male and female C57BL/6 and C3H/HeJ mice. Male and female mice of the inbred mouse strains C57BL/6 (n = 5 males and 5 females) and C3H/HeJ (n = 6 males and 4 females) were tested in an ascending nicotine two-bottle choice paradigm and preference ratios (percent of total fluid consumed from the nicotine-containing bottle) and nicotine consumption (mg nicotine consumed/kg body mass) were determined for each nicotine concentration. (A) Nicotine preference. A significant between-subject effect was observed for nicotine preference for strain (P < 0.005), but not for sex. A significant effect of concentration also was detected for within-subject effects (P < 0.001). (B) Nicotine consumption. Between-subject measures for nicotine consumption detected significant effects of strain (P < 0.001) and a strain-sex interaction (P < 0.01). Significant effects of nicotine concentration (P < 0.03), and concentration × strain (P < 0.001), and concentration × strain × sex (P < 0.05) interactions were observed for within-subject tests of nicotine consumption. All data are presented as mean ± S.E.M.
Fig. 3. Effect of prior exposure to alcohol on nicotine preference and consumption in C57BL/6J and C3H/HeJ mice. Prior to performing the ascending nicotine two-bottle choice test, C57BL/6J and C3H/HeJ mice were exposed to the ascending alcohol two-bottle choice test (n = 5 males and 5 females for C57BL/6J and 6 males and 6 females for C3H/HeJ) or the two-bottle choice test in which both bottles contained only water (n = 5 males and 5 females for C57BL/6J and 6 males and 4 females for C3H/HeJ). A comparison of nicotine preference (panels A and B) and consumption (panels C and D) between mice that were or were not exposed to alcohol in the initial two-bottle choice test indicated that exposure to alcohol did not alter either nicotine preference (P > 0.5) or nicotine consumption (P > 0.2). All data are presented as mean ± S.E.M.

3.4. Alcohol consumption in F2 intercross mice

Alcohol consumption was measured in 50 F2 intercross animals (26 female and 24 male mice) using the two-bottle choice paradigm as described in Section 2 (Fig. 5). Testing was conducted over a 12-day period. During each of three separate 4-day periods, the mice were provided with bottles containing water and water supplemented with 3, 6, and 10% (v/v). Female mice consumed 49.8 ± 3.8, 57.3 ± 5.3, and 61.3 ± 6.1% of their total fluid from the 3, 6, and 10% ethanol solutions, respectively (Fig. 4). In contrast, male mice consumed 28.2 ± 4.1, 26.3 ± 5.5, and 22.9 ± 5.7% of their total fluid from the alcohol-containing solutions. The average consumption of alcohol during the 3, 6, and 10% trials was 6.16 ± 0.62, 7.23 ± 0.8, and 12.0 ± 1.2 g/kg for female mice and 2.53 ± 0.37, 2.39 ± 0.49, and 3.31 ± 0.81 g/kg for male mice. A significant effect of sex was detected for both the alcohol preference ratio measure (P < 0.001) and consumption of alcohol (F(1,50) = 60.31, P < 0.001). There was also a significant effect of alcohol concentration on the consumption of alcohol (P < 0.001) and a significant sex–alcohol concentration interaction (P < 0.001).

3.5. Nicotine consumption in F2 intercross mice

After completion of the two-bottle choice test with alcohol, nicotine consumption was measured in the same 50 C57BL/6 × C3H/2 F2 intercross mice using the two-bottle choice paradigm as described in Section 2. During the test period, the mice were provided with bottles containing water and water supplemented with 25 µg/ml nicotine for the first 4 days, water and water supplemented with 50 µg/ml
nicotine for the next 4 days and water and water supplemented with 100 μg/ml for the last 4 days. During the 25, 50, and 100 μg/ml trials, female mice consumed 43.7 ± 2.4, 29.9 ± 3.8, and 14.3 ± 2.5% of their total fluid from the nicotine-laced solution, respectively (Fig. 5). Male mice consumed 38.4 ± 2.9, 28.0 ± 3.8, and 13.6 ± 2.4%, respectively, of their total fluid from the nicotine-containing solutions. The average consumption of nicotine during the 25, 50, and 100 μg/ml trials was 2.74 ± 0.17, 3.79 ± 0.45, and 3.62 ± 0.58 mg/kg for female mice and 2.02 ± 0.18, 2.96 ± 0.39, and 2.7 ± 0.5 mg/kg for male mice. There was a significant effect of nicotine concentration on the preference ratio (P < 0.001) but not for nicotine consumption. Although female mice tended to consume more nicotine at each nicotine concentration relative to their male counterparts, there was no significant effect of sex on nicotine consumption.

3.6. Relationship between alcohol and nicotine consumption

In order to evaluate whether individual differences in alcohol consumption were related to individual differences in
nicotine consumption, the two measures were compared at all three concentrations of each drug (Fig. 6). Since sex differences were detected for the alcohol consumption measures, bivariate correlational analysis was performed separately for the female and male mice. For all concentrations of nicotine and alcohol tested, nicotine consumption was significantly correlated with alcohol consumption in both female and male mice. The highest correlation between alcohol and nicotine consumption in female mice was observed for the comparison between 25 μg/ml nicotine and 6% alcohol (0.641, $P < 0.001$) and the lowest correlation for these measures in female mice was between 100 μg/ml nicotine and 10% alcohol (0.378, $P < 0.05$). In male mice, the highest correlation between alcohol and nicotine consumption was observed for the comparison between 100 μg/ml nicotine and 3% alcohol (0.829, $P < 0.001$) and the lowest correlation for these measures in male mice was between 50 μg/ml nicotine and 10% alcohol (0.434, $P < 0.05$). These results suggest that in females, between 41% (25 μg/ml nicotine versus 6% alcohol) and 14% (100 μg/ml nicotine versus 10% alcohol) of the variance in nicotine and alcohol consumption may be attributed to common genetic factors. In comparison, between 69% (100 μg/ml nicotine versus 3% alcohol) and 19% (50 μg/ml nicotine versus 10% alcohol) of the variance in nicotine and alcohol consumption in male mice may be attributed to common genetic factors. Similar results were obtained for comparisons between alcohol and nicotine preference ratios (data not shown).

3.7. Nicotinic receptor levels and drug consumption

In order to assess whether individual differences in nicotinic receptor levels are correlated with drug consumption,
the binding of [3H]-cytisine and [125I]-BTX were measured in cortex, striatum, midbrain and hippocampus in the 50 F2 mice (Table 1). Due to sex differences in the alcohol measures, correlations between receptor levels and the alcohol phenotypes were made for each sex. No significant correlations were detected between levels of either [3H]-cytisine or [125I]-BTX and alcohol preference or consumption. However, low but significant correlations were observed between the measures of nicotine consumption at the highest nicotine concentration tested and the level of [3H]-cytisine binding sites in the hippocampus and [125I]-BTX binding sites in the cortex.

3.8. Nicotinic receptor genotype and drug consumption

In order to determine the relationship between a previously identified polymorphism in the nAChR subunit gene Chrna4 [37] and drug consumption, the Chrna4 genotype of each animal was determined and compared to drug consumption. Although there was a trend towards lower nicotine preference and consumption in mice homozygous for the C3H allele of Chrna4, no significant association between Chrna4 genotype and either nicotine preference ratio or dose of nicotine consumed was observed (Fig. 7). In contrast, Chrna4 genotype was significantly associated with the alcohol consumption ratio (P < 0.01) and dose of alcohol consumed (P

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Table 1

<table>
<thead>
<tr>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Midbrain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytisine</td>
<td>nBTX</td>
<td>Cytisine</td>
<td>nBTX</td>
</tr>
<tr>
<td>E3% ratio</td>
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<td>0.075</td>
<td>0.1460</td>
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<td>0.055</td>
<td>0.1470</td>
</tr>
<tr>
<td>E6% con</td>
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<td>0.111</td>
<td>0.0580</td>
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<td>N100 ratio</td>
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<td>0.342</td>
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<tr>
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<td>-0.258</td>
</tr>
<tr>
<td>N50 con</td>
<td>0.010</td>
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</tr>
<tr>
<td>N100 con</td>
<td>0.185</td>
<td>0.307</td>
<td>-0.328</td>
</tr>
</tbody>
</table>

Cytisine: [3H]-cytisine binding; nBTX: [125I]-bungarotoxin binding; E3%: 3% ethanol; E6%: 6% ethanol; E10%: 10% ethanol. For ethanol, correlations with both female (given first) and male data are shown. N25: 25 µg/ml nicotine; N50: 50 µg/ml nicotine; N100: 100 µg/ml nicotine; ratio: preference ratio; con: average daily consumption (g/kg alcohol, mg/kg nicotine).
* P < 0.05.

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**Fig. 7.** Association between Chrna4 genotype and nicotine consumption in C57BL/6 × C3HHeJ F2 intercross mice. The 50 F2 mice were genotyped for a previously identified polymorphism in the nAChR subunit gene Chrna4 as described Section 2 and the relationship between Chrna4 genotype and measures of nicotine intake were evaluated. Although mice homozygous for the C3H allele of Chrna4 tended to consume less nicotine than heterozygous mice or C57BL/6 allele homozygotes, the relationship between the Chrna4 polymorphism and either nicotine consumption (A) or the nicotine preference ratio (B) was not significant. B6C3, mice homozygous for the C57BL/6 allele of Chrna4; B6C3, mice heterozygous for the Chrna4 allele; C3C3, mice homozygous for the C3HHeJ allele of Chrna4. All data are presented as mean ± S.E.M.
Fig. 8. Association between the Chrna4 genotype and ethanol consumption in C57BL/6 × C3H/HeJ F2 intercross mice. The 50 F2 mice were genotyped for the polymorphism in Chrna4 and the relationship between Chrna4 genotype and measures of alcohol intake were evaluated. Chrna4 genotype was significantly associated with alcohol consumption ($P < 0.001$) and the alcohol preference ratio ($P < 0.001$) in female, but not male mice. B6B6, mice homozygous for the C57BL/6J allele of Chrna4; B6C3, mice heterozygous for the Chrna4 alleles; C3C3, mice homozygous for the C3H/HeJ allele of Chrna4. All data are presented as mean ± S.E.M.

< 0.05) in females (Fig. 8). Female mice homozygous for the C57BL/6 allele of Chrna4 exhibited less preference for alcohol and consumed less alcohol relative to mice heterozygous for Chrna4 or homozygous for the C3H allele of Chrna4. A significant relationship between Chrna4 genotype and the alcohol consumption measures was not observed in male mice.

4. Discussion

The results of this study provide evidence that there are common genetic determinants for the consumption of nicotine and alcohol in mice. This is the first demonstration of a correlation between alcohol and nicotine consumption in a genetically segregating population of mice. Significant genetic correlations were observed between nicotine and alcohol consumption at all concentrations of nicotine and alcohol examined. In female mice, the correlations ranged from 0.641 ($P < 0.001$) for 25 μg/ml nicotine versus 6% alcohol to 0.378 ($P < 0.05$) for 100 μg/ml nicotine versus 10% alcohol. The correlations between the alcohol and nicotine measures in male mice ranged from 0.829 ($P < 0.001$) for 100 μg/ml nicotine versus 3% alcohol to 0.434 ($P < 0.05$) for 50 μg/ml nicotine and 10% alcohol. The average correlation between alcohol and nicotine consumption was 0.537 for females and 0.599 for males. Therefore, taking all alcohol–nicotine comparisons into account, approximately 29 and 36% of the phenotypic variance in alcohol and nicotine consumption may be attributed to common genetic factors in female and male mice, respectively.

Another potential explanation for the significant correlation between alcohol and nicotine consumption is that prior exposure to alcohol “primes” the mice that drink the alcohol to drink nicotine. However, this does not appear to be the case as previous exposure to alcohol did not significantly affect subsequent nicotine consumption in either of the inbred strains used to generate the F2 mice used in this study. In fact, exposure to alcohol prior to nicotine may actually
The lack of a significant correlation between striatal cytisine as measured by cytisine and BTX binding, respectively, were found to correlate with a few measures of nicotine consumption but not with any measures of alcohol consumption. The lack of a significant correlation between striatal cytisine and BTX binding with alcohol consumption is in contrast to the results of Tizabi et al. This group demonstrated which encodes the $\alpha_4$-nicotinic receptor $\beta_2$ subunit (Chrna4) and the $\beta_3$ nicotinic receptor subunit (Chrnb3) [1]. BTX binding sites in striatum as compared to their non-prefering selected rat line counterparts. The combination of these data indicates that the relationship between levels of nicotinic receptors and alcohol and nicotine consumption are dependent upon the test population. The low correlation values observed between receptor levels and nicotine consumption indicate that individual differences in nicotine receptor levels contribute to only a minor fraction of the phenotypic variance. Furthermore, individual differences in receptor levels cannot explain the genetic correlation between alcohol and nicotine consumption in the population used in this study.

Polymorphisms in the nicotinic receptor subunit gene Chrna4 also cannot account for the genetic correlation between alcohol and nicotine consumption in the C57BL/6J × C3H/HeJ F2 mice. Although a trend was observed for the relationship between Chrna4 genotype and nicotine consumption, the trend was not significant. Recently, Butt et al. (submitted for publication) demonstrated that Chrna4 genotype was significantly associated with nicotine consumption in an F2 intercross between C57BL/6J and A/J mice. The A/J mouse strain possesses the same Chrna4 allele as C3H/HeJ [24]. Like the data reported in this study, F2 mice homozygous for the C57BL/6J allele of Chrna4 consumed the most nicotine. Butt et al. also found that the association between Chrna4 genotype and nicotine consumption was eliminated in F2 mice that carried a null mutation for the gene, Chrnb2, which encodes the $\beta_2$-nicotinic receptor subunit. Most, if not all, $\alpha_4$-containing nicotinic receptors co-assemble with the $\beta_2$ subunit in the brain. Therefore, the finding that the association between Chrna4 genotype and nicotine preference is not observed when nicotinic receptors that contain the $\alpha_4$ subunit are absent from the brain provides evidence that the association between nicotine preference and Chrna4 genotype is due to the polymorphism in Chrna4 and not due to a gene linked to Chrna4.

The lack of a significant association between Chrna4 genotype and nicotine consumption in the C57BL/6J × C3H/HeJ F2 mice could indicate that the polymorphism in Chrna4 does not influence nicotine consumption in this particular population. Alternatively, the lack of significance, despite the same trend in the relationship between Chrna4 genotype and nicotine consumption, could be due to a lack of statistical power in the C57BL/6J × C3H/HeJ F2 population. Reduced power to detect an association between Chrna4 genotype and nicotine consumption might be explained by the observation that C57BL/6J and C3H/HeJ F2 mice also are polymorphic for the genes that encode the $\alpha_6$ nicotinic receptor subunit (Chrna6) and the $\beta_3$ nicotinic receptor subunit (Chrnb3) [1]. If this receptor subtype is important for nicotine consumption, then polymorphisms in any of these subunits might affect consumption. Consequently, in the C57BL/6J × A/J F2 mice, function of this receptor subunit would be affected only by the strain variants of the $\alpha_4$ subunit. In contrast, the influence of the variants of the $\alpha_4$ subunit on the function of the putative $\alpha_4\beta_6\beta_3$ nicotinic receptor in C57BL/6J × C3H/HeJ F2 mice might be affected by which strain-specific variants of the $\alpha_6$ and $\beta_3$ subunits are included in the receptor. Chrna4 genotype was associated with alcohol consumption although the association was observed in female mice only. Female mice homozygous for the C3H allele of Chrna4 consumed more alcohol than female mice homozygous for the C57BL/6J allele of Chrna4. A potential role for Chrna4 in alcohol preference previously has been indicated. Gill et al. [15] identified a QTL for preference for 10% alcohol in the A/J × C57BL/6J (AXB) and C57BL/6 × A/J (BXA) recombinant inbred strains that mapped to distal chromosome 2, near the Chrna4 locus. The mouse strain A/J carries the same allele for Chrna4 as C3H/HeJ. In contrast to the results of the current study, the association between distal chromosome 2 Chrna4 and alcohol consumption was observed in both males and females. Moreover, the direction of the effect of distal chromosome 2 Chrna4 was in the opposite direction relative to the C57BL/6J genotype. In the study by Gill et al. [15], the C57BL/6 allele was associated with increased alcohol consumption while the C57BL/6 allele in the C57BL/6 × A/J F2 intercross. Like the results of Gill et al. [15], the study by Butt et al. (submitted for publication) found that the C57BL/6 allele of Chrna4 was associated with higher alcohol preference. However, Butt et al. found that the association between Chrna4 genotype and alcohol preference was maintained in mice that did not express the nicotinic receptor $\beta_2$ subunit. Therefore, the association between alcohol preference and Chrna4 is likely due to a gene that is linked to Chrna4. This may explain why the direction of the effect of distal chromosome 2 Chrna4 and the influence of sex on the relationship between Chrna4 genotype and alcohol consumption appears to be dependent upon the genetic background of the test population. The A/J and C3H/HeJ strains may possess different alleles of a gene linked to Chrna4 that have opposite effects on alcohol consumption.
In summary, the results presented here indicate that there are common genetic influences on nicotine and alcohol consumption in mice. However, the genetic overlap between nicotine and alcohol consumption in mice cannot be attributed to individual differences in expression of α4β2 or α7 nAChRs or to a polymorphism in the nAChR subunit gene <chrna4/H9251. Consequently, the genetic basis for the relationship between nicotine and alcohol consumption remains to be determined.

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