

RAPID COMMUNICATION

SEROTONIN TRANSPORTER PROMOTER POLYMORPHISM AND DIFFERENCES IN ALCOHOL CONSUMPTION BEHAVIOUR IN A COLLEGE STUDENT POPULATION

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Abstract — **Aims and methods:** In the present study, differences in alcohol consumption behaviour associated with the presence of the short variant (S) of the serotonin transporter promoter polymorphism (5-HTTLPR) was investigated in a Caucasian subset ($n = 204$) of 268 college students. **Results:** Students who were homozygous for the S allele were more likely to engage in binge-drinking behaviour, drank more alcohol per occasion, and reported drinking to get drunk more often. **Conclusions:** In this Caucasian sample, the 5-HTTLPR strongly influences alcohol consumption in late pubescence.

INTRODUCTION

Binge drinking is a specific type of alcohol misuse that is a major public health concern affecting more than 6 million full-time college students in the USA (Wechsler *et al.*, 1995), spurring a major initiative led by the National Institute on Alcohol Abuse and Alcoholism aimed at understanding the scope of the problem and developing prevention strategies (Goldman *et al.*, 2002). Genetic factors influence the risk for engaging in this behaviour, but the majority of studies have addressed the modifying influences of the ALDH2*2 allele on alcohol consumption in Asians, Caucasians and Ashkenazic Jewish Americans (Luczak *et al.*, 2001; Shea *et al.*, 2001). We surmised that functional differences in serotonergic function conferred by the serotonin transporter (5-HTT) protein promoter polymorphism (5-HTTLPR) might be associated with differences in alcohol consumption behaviours in college students, an area that has not yet been investigated.

The most common insertion deletion polymorphism in the promoter region of the human 5-HTT gene (*SLC6A4*) gives rise to a biallelic polymorphism designated long allele (L) and short allele (S). The S variant is associated with lower expression of 5-HTT sites and reduced efficiency of 5-HT re-uptake (Lesch *et al.*, 1996; Whale *et al.*, 2000). A higher frequency of S was associated with higher ethanol tolerance in young adults (<26 years), suggesting that self-regulation of alcohol intake may be influenced by the presence of this allele (Turker *et al.*, 1998). A higher frequency of S homozygotes was present in a sub-group of adult alcoholics who exhibited an increased frequency of binge drinking (Matsushita *et al.*, 2001).

In the present study, we genotyped college students for the 5-HTTLPR and examined the possible association of the S variant with differences in alcohol use behaviours, determined from a set of responses to the College Alcohol Study. We hypothesized that individuals carrying at least one S allele of

the 5-HTTLPR would engage in more frequent binge drinking and that it was likely that the S allele would act in a recessive fashion.

SUBJECTS AND METHODS

The study was approved by the local Institutional Review Board. The population sample was drawn from college students enrolled in a medium-size undergraduate institution. A total of 262 participants completed the study, of which 256 could be stratified as Caucasian, African-American, or Asian, and of which 6 were members of other ethnic groups. Answers to the following survey questions were used in the analyses. Answers to the questions were scored using ordinal scales. The magnitudes associated with the possible responses, which were used to generate graphic and statistical results, are shown within parentheses preceding each answer. This information did not appear on the questionnaires completed by the respondents.

- (I) 'Think back over the last 2 weeks. How many times have you had five (for male students) four (for female students) or more drinks in a row? (0) None; (1) Once; (2) Twice; (3) 3–5 times; (4) 6–9 times; (5) 10 or more times?'
- (II) 'On how many occasions have you had a drink of alcohol in the past 30 days? (0) Did not drink in the past 30 days; (1) 1–2 occasions; (2) 3–5 occasions; (3) 6–9 occasions; (4) 10–19 occasions; (5) 20–39 occasions; (6) 40 or more occasions?'
- (III) 'In the past 30 days, on those occasions when you drank alcohol, how many drinks did you usually have? (0) Did not drink in the past 30 days; (1) 1 drink; (2) 2 drinks; (3) 3 drinks; (4) 4 drinks; (5) 5 drinks; (6) 6 drinks; (7) 7 drinks; (8) 8 drinks; (9) 9 or more drinks?'
- (IV) 'On how many occasions did you drink to get drunk in the past 30 days? (0) Not at all; (1) 1–2 occasions; (2) 3–5 occasions; (3) 6–9 occasions; (4) 10–19 occasions; (5) 20–39 occasions?'

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Table 1. Sex, age and genotype of students

Gender	Age (years) (mean ± SE)	LL	LS	SS	Total	P(L)	P(S)
Male	18.98 ± 0.17	18	28	11	57	0.56	0.44
Female	19.18 ± 0.17	44	66	37	147	0.53	0.47
All	19.14 ± 0.09	62	94	48	<i>n</i> = 204		

L = long allele; S = short allele.

A drink of alcohol was defined as a 12-ounce can or bottle of beer, a 4-ounce glass of wine, a 12-ounce bottle or can of wine cooler or a shot of liquor straight or in a mixed drink.

Genetic analysis

DNA was isolated from saliva following the protocol from the Puregene Genomic DNA Purification kit (Minneapolis, MN, USA). Primers used: 5' Texas Red labelled (Sigma Genosys, The Woodlands, TX, USA), sense, 5' to 3' ATGCCAGCACCTAACCCCTAATGTCC; SLC6A4.for; antisense 5' to 3' GAGGGACTGAGCTGGACAACCACG; SLC6A4.rev. PCR was performed using the Roche GC-Rich kit (catalogue no. 2 140 305; Roche, Indianapolis, IN, USA). Amplification buffer contained genomic DNA (2.5–10 ng in 0.25 µL), 0.375 µM forward and reverse primers, carried out in a 50-µL reaction tube containing 0.2 mM of DNTP, 0.4 mM Tris-HCl, pH 8.0, 2 mM KCl and 1 M GC-rich resolution solution with 2 U enzyme mix. The PCR was hot started and run for 40 cycles (30 s at 95°C; 20 s at 64°C; 25 s at 72°C) in a Perkin-Elmer GeneAmp 9600 PCR System (Perkin-Elmer, Wellesley, MA, USA). Expected amplicon length was 470 bp for the short allele and 514 bp for the long allele. The PCR products were sequenced and matched the expected amplicon sequences.

Statistical analyses

To avoid issues of population stratification, only data from the Caucasian (*n* = 204) sub-population was analyzed. Statistical procedures were performed in JMP 5.0 (SAS Institute, Cary, NC, USA). Only outcome variable III was approximately normally distributed, Kolmogorov–Smirnov goodness-of-fit test (*P* > 0.999). An *F*-test for homoscedasticity confirmed equality of variances: *P*-values for LL vs. LS, LL vs. SS, and LS vs. SS being 0.90, 0.73, and 0.63 respectively. We therefore employed a parametric ordinary least-squared linear regression model (ANOVA) to compare and contrast the mean differences between genotypes for responses to question III. A mixed stepwise regression procedure was employed to construct the linear regression model for III, and logistic regression models for I, II and IV, with genotype-, sex- and age-associated regressor terms, with *P* ≤ 0.25 to enter and *P* ≥ 0.10 to remove. Genotype (LL, LS, SS), age and sex (M, F) along with higher order interaction regressor terms were considered.

The responses obtained from the answers to survey questions I, II and IV were analyzed using a multinomial logistic regression to determine whether genotype group influenced the magnitude of the response to the questions. A stepwise approach was used to avoid biasing the results by fitting the logistic curves to a predetermined model assuming a dominant, recessive, or additive effect for each of the genotypes. The

best fit models are reported for answers to survey question I (binge drinking) and (drinking to get drunk). The results of logistic regression for question II (number of occasions) are not reported as no significant genotype effects were found. The model parameters of the best fit logistic regressions are of the form: $P(\text{Yes, Response} \leq X) = 1 / \{1 + \exp(-\text{Intercept}(X) - \text{Genotype}(\text{LL} + \text{LS} \text{ or } \text{SS}))\}$; where $\text{Intercept}(X)$ is associated with Response level *X*, and the $\text{Genotype}(\text{LL} + \text{LS} \text{ or } \text{SS})$ is the parameter associated with the genotype group. The individual probabilities for a particular response are computed by taking the difference of the successive cumulative probabilities. As these represent cumulative probabilities, the probability for the highest response level is simply $P(\text{Yes}, 5) = 1 - P(\text{Yes}, 4)$. Data were fitted to the logistic regressions using the method of maximum likelihood.

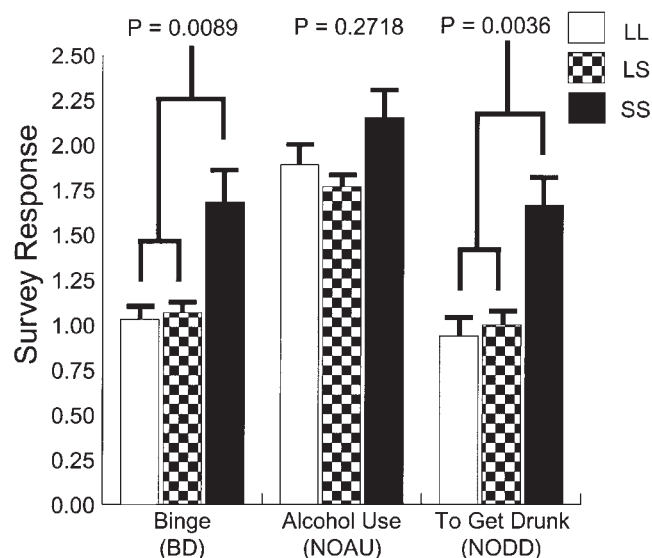


Fig. 1. Association of 5-HTTLPR genotype and means of survey responses in a study on serotonin transporter promoter polymorphism and differences in alcohol consumption behaviour in a college student population.

Number of occasions of binge drinking in past 2 weeks (BD, question I); number of occasions of alcohol use in the past 30 days (NOAU, question II); number of occasions of drinking to get drunk in the past 30 days (NODD, question IV) are given as means ± SEM (bars). There was a significant difference in the magnitude of the response to questions I and IV between (LL + LS) and SS. The significance levels for the differences were obtained from the logistic regressions, and are equal to the genotype group effects shown in Table 2. There were no significant differences in the magnitude of the response to question II between genotype groups.

Table 2. Results of best-fit logistic regression models^a

Regression parameters	Binge drinking (I) Coefficient \pm SE; <i>P</i> -value	Drinking to get drunk (IV) Coefficient \pm SE; <i>P</i> -value
Intercept (Response = 0)	-0.573 \pm 0.153; 0.0002	-0.744 \pm 0.174; <0.0001
Intercept (Response = 1)	0.332 \pm 0.150; 0.0273	0.595 \pm 0.171; 0.0005
Intercept (Response = 2)	1.181 \pm 0.172; <0.0001	1.605 \pm 0.213; <0.0001
Intercept (Response = 3)	2.439 \pm 0.262; <0.0001	2.474 \pm 0.292; <0.0001
Intercept (Response = 4)	3.477 \pm 0.415; <0.0001	5.114 \pm 0.154; <0.0001
Genotype (LL + LS)	0.385 \pm 0.147; 0.0089	0.448 \pm 0.154; 0.0036
Genotype (SS)	-0.385 \pm 0.147; 0.0089	-0.448 \pm 0.154; 0.0036
Model χ^2 ; d.f.; <i>P</i> -value	6.828; 1; 0.0089	8.324; 1; 0.0036

^aCumulative probability fit to logistic regression with $P(\text{Yes, Response} \leq X) = 1 / \{1 + \exp(-\text{Intercept}(X) - \text{Genotype}(\text{LL} + \text{LS} \text{ or } \text{SS}))\}$, where Intercept (*X*) is associated with Response level *X*, and the Genotype(LL + LS or SS) is the parameter associated with the genotype group.

RESULTS

Genotypes and age are shown in Table 1. There were no differences in age between the men and the women ($P = 0.34$, unpaired *t*-test) for the Caucasian subset which was analyzed. Population frequencies for the L and S variants were 53 and 47%,

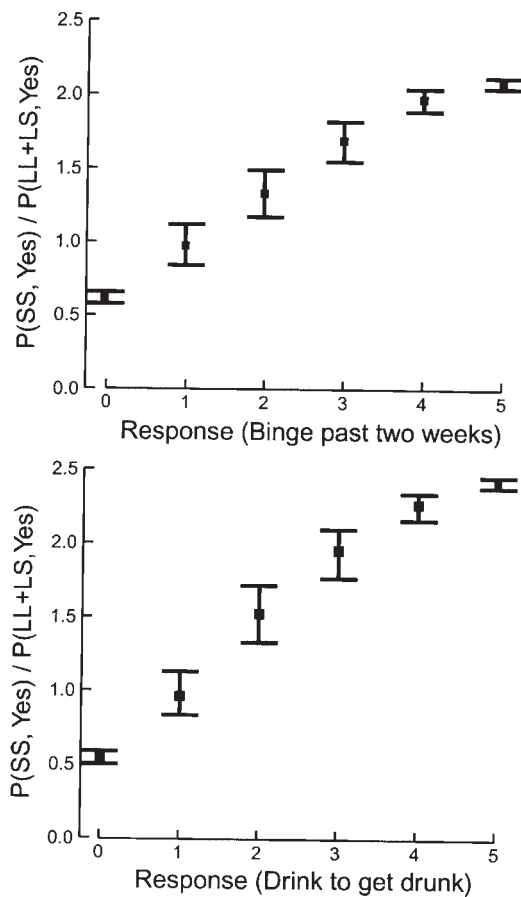


Fig. 2. Probability ratios of 'Yes' responses to the different levels of the survey questions.

Ratios are related to binge drinking (top) and drinking to get drunk (bottom). The ratio is computed as the probability of a 'Yes' response at each level, shown on the *x*-axis, for individuals with an SS genotype, $P(\text{SS, 'Yes'})$ divided by the probability of a 'Yes' response for individuals with LL or LS genotypes $P(\text{LL} + \text{LS, 'Yes'})$. The probability values and associated errors were computed from the logistic regressions. A ratio of one implies an equal chance for a 'Yes' response. The means \pm SEM (bars) are shown.

respectively. Genotypes were distributed in accordance with Hardy–Weinberg equilibrium, ($\chi^2 = 0.996$; d.f. = 2; $P = 0.61$), similar to the previously reported L and S allele frequencies of 57 and 43%, respectively, in 505 healthy Caucasians (Lesch *et al.*, 1996). Hardy–Weinberg equilibrium was maintained across sex: for female students, L and S allele frequencies were 53 and 47% ($\chi^2 = 0.379$; d.f. = 2; $P = 0.83$) and for male students 56 and 44% ($\chi^2 = 0.997$; d.f. = 2; $P = 0.61$) respectively. Delbruck described a novel extra long allelic variant (Delbruck *et al.*, 1997) which tends to be exclusively present in individuals of African origin. We did not note any novel alleles.

We report summary data for questions I, II and IV in Fig. 1. The results of the best-fit models for the logistic regressions are shown in Table 2. For response variables I and IV, the results of the logistic regressions are depicted in Fig. 2. Neither age nor sex were retained in the final logistic fit for both I and IV, and the recessive model (LL + LS vs. SS) provided the best fit. The logistic regressions for II resulted in a best-model fit with $P = 0.2718$, therefore no other results are reported. Results of the linear regression model for III are shown in Table 3. In this case, regressor terms for all genotype groups were found to explain a significant portion of the variance, suggesting a mixed additive/recessive effect of the S allele on the number of drinks consumed per drinking occasion.

Aggregate results for mean number of drinks per occasion (III) grouped by genotype are shown in Fig. 3. For LL vs. SS, LL vs. (LS + SS) and LS vs. SS, $P =$ (uncorrected, corrected by Bonferroni–Holm): (0.005, 0.015), (0.016, 0.032) and (0.072, 0.072). Male students consumed more drinks per occasion than did female students, 4.60 ± 0.37 vs. 3.72 ± 0.14 ($P = 0.006$). There was a significant effect of age, with a decrease of approximately one drink per occasion every 3 years for the sample population. None of the higher-order interactive effects were retained in the final model: genotype \times sex ($P = 0.5574$), genotype \times age ($P = 0.4652$), genotype \times sex \times age ($P = 0.3914$). The power to detect meaningful effects at an $\alpha = 0.05$ for group effects was as follows: genotype (0.71), sex (0.79), age (0.92).

DISCUSSION

The findings of the present study reveal a significant association of the 5-HTTLPR polymorphism with increased alcohol consumption behaviour in Caucasian college students. Students homozygous for the short allele (S) of the 5-HTTLPR engaged

Table 3. Analysis of variance results for number of drinks per occasion

Regression parameters	Coefficient	F-ratio	P-value
Intercept	10.869		<0.0001
Genotype (LL)	- 0.334		
Genotype (LS or SS)	0.334	5.9720	0.0225
Genotype(LS)	-0.313		
Genotype(SS)	0.313	3.5127	0.0592
Male	0.451		
Female	-0.451	8.8422	0.0002
Age (years)	-0.353	13.294	0.0022
[Model d.f.]; F, P-value	[4, 174]; 8.009; <0.0001		

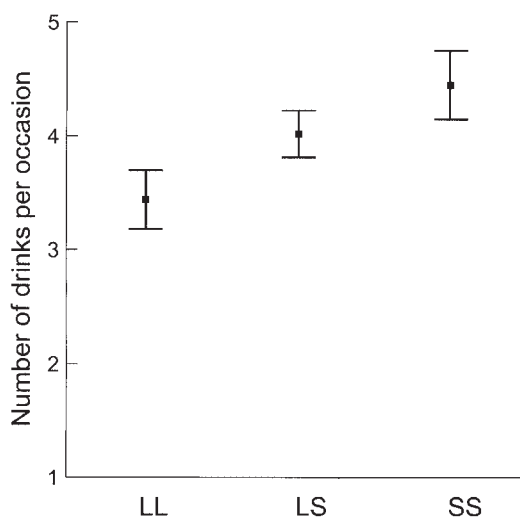


Fig. 3. Allelic dose-response for number of drinks per occasion and genotype. The mean of the number of drinks of alcohol consumed per occasion [\pm SEM (bars)] is represented on the y-axis, and the 5-HTTLPR genotype is indicated on the x-axis.

in a higher frequency of binge drinking, drank more often to get drunk, and consumed an increased number of alcoholic drinks during drinking occasions compared to L homozygotes or heterozygotes. The long variant of the 5-HTTLPR exerted an additive/recessive effect on the number of drinks consumed per occasion (Fig. 3). The frequency of alcohol consumption was not different between genotype groups.

Individuals homozygous for the S variant of the 5-HTTLPR had a higher risk of purposefully engaging in alcohol consumption to induce intoxication. One explanation for this finding might be that individuals homozygous for the S variant exhibit higher levels of baseline anxiety (Lesch *et al.*, 1996; Melke *et al.*, 2001), using alcohol as an anxiolytic. Heavy drinking as a tension reducing strategy was recently described in a college population (Rutledge and Sher, 2001).

There are limitations of the current study which affect the generalizability of the findings. Other unknown positive and negative genetic and environmental modulators of alcohol consumption behaviour may have been present. However, one of the strongest findings is the allelic dose-response, as shown in Fig. 3, fulfilling one of the main criteria outlined by Hill (1965) implying causation rather than simple association. Our results support a biologically derived difference in alcohol consumption.

In conclusion, the results of this study suggest that the 5-HTTLPR genotype strongly influences alcohol drinking

behaviour in this sample of college students, increasing our understanding of biological risk factors which may play a role in determining maladaptive patterns of alcohol consumption. Based on these results, further study of the influence of the 5-HTTLPR on alcohol consumption behaviours in other populations is under way.

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