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Tryptophan Hydroxylase 2 Gene and Alcohol Use among College Students

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Abstract

Objective—Genes that regulate serotonin activity are regarded as promising predictors of heavy alcohol use. *Tryptophan Hydroxylase (TPH2*) plays an important role in serotonergic neurotransmission by serving as the rate-limiting enzyme for serotonin biosynthesis in the midbrain and serotonergic neurons. Despite the link between *TPH2* and serotonergic function, *TPH2*'s role in the pathogenesis of alcohol use disorders remains unclear. The goal of this study was to examine whether variation in the *TPH2* gene is associated with risky alcohol consumption. Specifically, this study examined whether the *TPH2* G-703T polymorphism predicted alcohol consumption among college students.

Methods—In two successive years, 351 undergraduates were asked to record their alcohol use each day for 30 days using an internet-based electronic diary. Participants' DNA was collected and polymerase chain reaction genotyping was performed.

Results—Alcohol consumption was not associated with the *TPH2* G-703T polymorphism alone, or the interaction of *TPH2* with two other candidate polymorphisms (*TPH1* C218A, and the *SLC6A4* tri-allelic 5-HTTLPR) or negative life events.

Conclusions—This study supports recent null findings relating *TPH2* to drinking outcomes. It also extends these findings by showing null interactions with the *TPH1* C218A polymorphism, the *SLC6A4* tri-allelic 5-HTTLPR polymorphism, and environmental stressors in predicting sub-clinical alcohol use among Caucasian American young adults.

Keywords

alcohol; college drinking; gene-environment interaction; heavy drinking; tryptophan hydroxylase; TPH2

Introduction

The serotonergic system is involved in the regulation of alcohol preference and intake (Naranjo et al., 1986), and abnormalities in serotonergic neurotransmission are associated with an increased risk for alcohol use disorders (LeMarquand et al., 1994; Virkkunen and Linnoila, 1997). To date, several serotonergic genes have been shown to play a role in alcohol-related

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phenotypes, including *SLC6A4*, which encodes the serotonin transporter (Covault et al., 2007; Kaufman et al., 2007), various serotonin receptor genes (Fehr et al., 2000; Lappalainen et al., 1998; McBride et al., 2004) and *TPH1*, which encodes the enzyme tryptophan hydroxylase 1 (Sun et al., 2005). Another isoform of tryptophan hydroxylase, TPH2 (encoded by *TPH2*), has also been found to play a pivotal role in serotonergic neurotransmission by serving as the rate-limiting enzyme for serotonin biosynthesis in midbrain and serotonergic neurons (Walther et al., 2003). Despite the link between *TPH2* and serotonergic function, its role in alcohol use remains unclear. The goal of this study is to determine whether variation in the *TPH2* gene is associated with alcohol consumption.

Polymorphisms in the *TPH2* gene that alter serotonin levels are promising candidates for alcohol related outcomes. There are several known functional polymorphisms in the *TPH2* gene. One rare polymorphism in *TPH2* (G1463A) results in an arginine to histidine substitution, which reduces brain serotonin by 80% (Zhang et al., 2005). Another polymorphism, T-473A, (rs11178997), which reduces binding of the POU3F2 transcription factor *in vitro* and has been shown to reduce *TPH2* transcriptional activity in primary serotonergic neurons (Scheuch et al., 2007). However, both of these polymorphisms are relatively rare.

The *TPH2* T-473A polymorphism is in strong linkage disequilibrium with another moderately common promoter region polymorphism G-703T (rs4570625). Studies of this more common *TPH2* polymorphism indicate that human subjects with the –703T allele may have reduced CNS serotonin compared to those with the G-variant (Sacco et al., 2007; Stoltenberg et al., 2006) and increased amygdala reactivity to emotional stimuli (Brown et al., 2005; Canli et al., 2005). The increased amygdala reactivity for carriers of the 703T allele is analogous to that found among carriers of the *SLC6A4* 5-HTTLPR S-allele, a known risk factor for alcohol dependence (Feinn et al., 2005), heavy drinking (Herman et al., 2003), and stress-related drinking (Covault et al., 2007; Kaufman et al., 2007). Taken together, these findings suggest that the *TPH2* -703T polymorphism may be a genetic risk factor for serotonin-related behaviors such as heavy drinking.

To date, only one study has expressly examined the relationship between *TPH2* gene polymorphisms and alcohol-related phenotypes. In that study, Zill and colleagues (Zill et al., 2007) found no association between *TPH2* variants (including the G-703T polymorphism) and alcohol dependence. However, Zill and colleagues' (2007) null findings could reflect the nature of their sample, which was limited to participants of Finnish and African-American descent diagnosed with alcohol dependence. There are several other possible explanations for their null findings. Zill and colleagues (2007) suggested that the *TPH2* G-703T polymorphism could be related to alcohol use only among individuals with a particular *TPH1* genotype. It is also possible that the association between *TPH2* variants and alcohol use is masked by potential modulating effects of the 5-HTTLPR polymorphism, as prior research has revealed that the of the G-703T T-allele may form a higher order association with the 5-HTTLPR S-allele (Herrmann et al., 2007). Finally, emerging research on gene-environment interactions, and particularly evidence of gene × stress interactions (Caspi and Moffitt, 2006), suggests the possibility that the *TPH2* G-703T polymorphism may predict drinking only among individuals who have experienced greater life stress.

Our study addresses each of these possibilities to further elucidate the role of the *TPH2* G-703T polymorphism in predicting drinking among a sample of North American Caucasian college students. According to Wechsler and colleagues (Wechsler et al., 1995), approximately six million of the eight million college students in the U.S. binge drink, and a reported 45% of students nationwide binge drink at least once every two weeks (Wechsler et al., 2002). In light of this demographic, we first examined whether college students with the *TPH2* G-703T T-allele consumed more alcohol than those with the G-allele. Second, we examined the

interaction between the *TPH2* G-703T polymorphism and the *TPH1* C218A polymorphism in predicting alcohol consumption. Third, we examined the interaction between the *TPH2* G-703T polymorphism and the *SLC6A4* tri-allelic 5-HTTLPR polymorphism in predicting alcohol consumption. Finally, we examined the interaction between the *TPH2* G-703T polymorphism and negative life events in predicting alcohol consumption.

Materials and Methods

Participants

This study's sample is identical to that of Covault and colleagues (2007) who reported an interaction between 5-HTTLPR and negative life events in predicting college student drinking and drug use. Our sample consisted of 351 undergraduates at a large northeastern public university (54.4% women). At study entry, 60% of participants were freshmen and 33% were sophomores, their average age was 18.65 +/− 0.86 years, all were unmarried and almost all participants were fulltime students. All participants provided written informed consent to participate in this study, which was conducted under guidelines approved by the institutional review boards at the University of Connecticut, Storrs and the University of Connecticut Health Center, Farmington.

Participants were recruited from an introductory psychology subject pool. Enrolled participants were representative of introductory psychology students and the overall student population. To be eligible for the study, students had to report having consumed alcohol on at least two occasions over the previous 30 days, and report no history of alcohol treatment. This study represents the first two years of an ongoing 4-year study of daily alcohol use and college life.

Procedure

Drinking and life stress data were collected using an internet-based data collection platform, the Daily Experiences and Alcohol Use Survey (DEAUS). Participants began each year by completing, over the internet, the Life Events Scale for Students (LESS), among other background questionnaires. Next, they signed onto a secure website to complete the 5-minute DEAUS between 3:30 pm and 7:00 pm each day for 30 days. Questions about last night's alcohol consumption were contained in the daily survey. Prior college student studies have shown that internet-based daily surveys are convenient and yield response rates >80% (Mohr et al., 2005; Park et al., 2004). Furthermore, the DEAUS allowed for close to real-time data entry, which minimized recall error and bias in reports of alcohol use (Tennen et al., 2005).

DNA was collected during the second year of the study. Among the 575 participants initially enrolled in the study, 535 (active and still living in the area) were invited to take part in the DNA sub-study. Of these, 416 (77.8%) consented to provide DNA samples, 69 (12.9%) declined, 34 (6.4%) did not respond, and 16 (2.9%) had moved out of the area. Study participants who provided DNA did not differ from those who did not provide DNA in their alcohol consumption or recent life events. However, individuals who provided DNA were younger ($\chi^2 = 20.76$, $p < 0.01$) and more likely to be female ($\chi^2 = 4.82$, $p<0.05$) than non-providers. To avoid potential confounds due to ethnic differences in allele frequencies and drinking behavior, analyses were limited to self-reported non-Hispanic Caucasian subjects ($n = 361$) who provided drinking data (a minimum of 15 of the possible 30 diary records) in at least one of the two study years ($n = 351$). Of these 351 participants, 308 (88%) provided two years of usable data, and an additional 43 subjects (12%) provided one year of usable data. The number of daily reports ranged from 15 to 30 with a mean (+/− SD) of 25.5 +/− 3.6 reporting days in year 1 and 25.3 +/− 3.9 reporting days in year 2. Response rates were generally better for women, who completed more daily surveys than men in year 1 (25.2 vs. 24.3; $t = 96.73$, $p<0.001$) and year 2 (25.3 vs. 23.3; $t = 83.41$, $p<0.001$). There was no relationship between

the number of daily surveys participants completed and any of the drinking outcome variables after controlling for gender.

Life Events

The 36-item Life Events Scale for Students (LESS; Clements and Turpin, 1996; Linden, 1984) is an empirically derived checklist from which participants endorse the stressful life events that they experienced during the past year (e.g., breaking up with boy/girlfriend; failing a course; family health problems; financial problems). Of the 36 events included in the checklist, we followed Covault et al. (2007) and retained the 25 unambiguously negative events for analysis. These selected items were summed to yield a score ranging from 0 to 25, indicating the number of negative life events that occurred in the past year. Life events were reported in each year of data collection.

Alcohol Consumption

In each daily survey, a standard drink was defined as one 12-oz can or bottle of beer, one 5-oz glass of wine, one 12-oz wine cooler, or one 1.5-oz measure of liquor straight or in a mixed drink. Participants recorded the number of alcoholic drinks they consumed the previous night. We transformed the daily alcohol data into four aggregated alcohol quantity and frequency variables for each person. Drinking episode quantity was defined as the number of alcoholic beverages consumed on each drinking day divided by the total number of drinking days. Heavy drinking episode quantity was defined as the total number of alcoholic beverages consumed on days in which a participant consumed four or more drinks for women and five or more drinks for men (Wechsler et al., 1995) divided by the total number of heavy drinking days. Drinking frequency was defined as the proportion of days in which a participant consumed one or more alcoholic beverages (the number of days with drinking divided by total days surveyed). Heavy drinking frequency was defined as the proportion of days during which a participant consumed four or more drinks for women and five or more drinks for men.

Genotyping

Genomic DNA was extracted and purified from mouthwash-stabilized samples using a commercial DNA isolation protocol (PureGene, Gentra Systems, Minneapolis, MN). The DNA collected from the participants was genotyped for three polymorphisms: *TPH2* G-703T (rs4570625) *TPH1* C218A (rs1800532), and the *SLC6A4* tri-allelic 5-HTTLPR polymorphism.

The *TPH1* and *TPH*2 polymorphisms were genotyped using PCR-based TaqMan allelic discrimination assays designed using Primer Express v2.02 software (ABI, Applied Biosystems Inc., Foster City, CA). A 10 ul PCR reaction was performed in 1x ABI TaqMan Universal master mix (ABI, Applied Biosystems Inc.) containing 100nM of each TaqMan MGB probe (*TPH1*: FAM labeled T-allele AATAGCAGCTATCACCTAAT and VIC labeled G-allele AATAGCAGCTAGCACCTAA; *TPH*2: FAM labeled T-allele CACAAAATTATAATATGTCAAGTC, and VIC labeled G-allele CACAAAATTAGAATATGTCAAGT), and 600nM of each primer (*TPH1*: ATTTTTTTCAGTGTTACATTCCCTATGC and TCCATGCTCTATATGTGTTAGCCATT; *TPH*2: ATTACACATATATACACTCACACATTTGCA and CTCATTGACCAACTCCATTTTATGTT). The polymerase chain reaction was performed with the following cycling parameters, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, followed by 60°C for 60 seconds. End point FAM and VIC fluorescence levels were captured using an ABI 7500 Sequence Detection System (ABI, Applied Biosystems Inc., Foster City, CA) and genotype calls were made based on the level of fluorescence signal. The genotyping assay was repeated for 14% of samples with no discrepancy in genotype calls.

The 5-HTTLPR tri-allelic insertion$_A$/insertion$_G$/deletion polymorphism was genotyped using a two-stage TaqMan™ 5′nuclease allelic discrimination assay modified from that originally described by (Hu et al., 2005; Hu et al., 2006). This method identifies the presence of the 14- vs. 16-repeat variable number of tandem repeats [short (S) vs. long (L)], as well as two subtypes of the 16-repeat variant, L_A, and L_G, which are the products of an A→G single nucleotide polymorphism present at the sixth nucleotide of the first of two 23-bp repeat elements present in the 16-repeat L-allele (Nakamura et al., 2000). Twenty-five μL PCR reactions were performed, each containing 200 nM each of forward and reverse primers (5′ GCAACCTCCCAGCAACTCCCTGTA-3′ and 5′ GAGGTGCAGGGGGATGCTGGAA-3′), 1M Betaine, 1X ABI TaqMan Universal master mix (Applied Biosystems Inc., Foster City, CA), 25 ng genomic DNA, 120nM of an L-allele specific Fam-labeled probe (6FAM-TGCAGCCCCCCCAGCATCTCCC-MGB) and 60 nM of a Vic-labeled internal control probe (VIC-TCCCCCCCTTCACCCCTCGCGGCATCC-MGB) whose target is present in the 5-HTTLPR region adjacent to the L-specific insertion, which served to distinguish the L vs. S insertion/deletion status. Samples were heated to 95°C for 10 minutes, followed by 40 thermal cycles of 98°C for 15 sec, followed by 62.5°C for 90 sec. The number of L-alleles (0, 1, or 2) for each patient was identified by examination of scatter plots of endpoint Fam vs. Vic fluorescence levels captured using an ABI 7500 Sequence Detection System. A second TaqMan™ 5′nuclease allelic discrimination assay served to distinguish L_A - vs. L_G-alleles by using the same primers and amplification conditions as for the L- vs. S-allele assay but using L_A- vs. L_G-allele specific probes, (6FAM-CCCCCCTGCACCCCCAGCATCCC-MGB and VIC-CCCCTGCACCCCCGGCATCCCC-MGB, respectively). We validated the closed-tube fluorescent assay of 5-HTTLPR L- vs. S-allele by comparing results obtained for 492 samples in another sample (Covault, unpublished) using this 5′nuclease TaqMan assay with those from a traditional 5-HTTLPR agarose gel-based PCR fragment length assay, with 100% agreement between methods. Additionally, we sequenced 8 samples for each of the genotypes (L_AL_A, L_AL_G and L_GL_G) with 100% agreement between direct sequencing and the TaqMan L_A vs. L_G assay. We did not observe the G-allele in samples from S-allele homozygotes, which is consistent with (Hu et al., 2006). The S- and Lg-alleles have similar transcriptional effects (reduced compared with the La-allele) and are combined for statistical analysis purposes (Hu et al., 2006).

Statistical Analysis

Least squares multiple regression was used to test the main effect of the *TPH2* G-703T polymorphism, which we coded as 0, 1, and 2 for the number of T-alleles, in predicting the four drinking outcomes. Additional regression models were run to test the *TPH2* G-703T polymorphism and its interaction with the *TPH1* C218A polymorphism, which we coded as 0, 1, 2 for the number of A-alleles; its interaction with the tri-allelic 5-HTTLPR polymorphism, which we coded as 0, 1, 2 for the number of S- and L_G-alleles; and its interaction with negative life events (0 to 25, mean centered), in predicting the four drinking outcomes. Analyses were run separately for year 1 and year 2 data. All regression models controlled for sex because men drank more than women. Additionally, alpha was set to 0.01 to control for the number of statistical tests performed. A power analysis revealed that our sample provided power >80% to detect a gene-gene interaction or a gene-negative life events interaction, assuming an interaction effect size accounting for 3% of the variance (Aiken and West, 1991; Cohen and Uhlenbeck, 1971). The 3% effect size was based on the interaction of 5-HTTLPR and negative life events reported by Covault et al. (2007) using this dataset.

Results

Descriptive Statistics

The distributions of all genotypes are shown in Table 1. The *TPH2* G-703T polymorphism, *TPH1* C218A polymorphism, and the *SLC6A4 5-HTTLPR* genotype distributions were all in Hardy-Weinberg equilibrium ($\chi^2 = 1.02$, $p = .31$;$\chi^2 = 0.04$, $p = .84$; $\chi^2 = 0.05$, $p = .84$, respectively). The observed T-allele frequency for the *TPH2* G-703T polymorphism (0.22) was typical of non-Hispanic European American samples (Mossner et al., 2006), as was the A-allele frequency for the *TPH1* C218A polymorphism (0.39) (Li and He, 2006), and the L_G-allele frequency for the tri-allelic 5-HTTLPR polymorphism (0.12) (Hu et al., 2005). No sex differences were found in the genotype distributions. The correlations among genotypes are shown in Table 2. There was a small negative correlation between the *TPH2* G-703T polymorphism and the *TPH1* C218A polymorphism.

The negative life events count for the overall sample ranged from 0 to 15, with a mean of 4.23 +/− 2.85 in year 1, and ranged from 0 to 16, with a mean of 3.63 +/− 2.77 in year 2. Significantly fewer negative life events were reported in year 2 than in year 1 ($t = 3.90$, $p<0.001$). Women reported more negative life events than men during both years (year 1, $t = 27.78$, $p<0.001$; year 2, $t = 24.37$, $p<0.001$). Controlling for gender, a greater number of negative life events was associated with greater drinking frequency in year 2 (b=0.01, SE<0.01, r^2=0.09; $p<0.01$) and a trend for greater drinking frequency in year 1 (b=0.01, SE<0.01, r^2=.01; $p<0.05$). Genotypes were not generally associated with negative life events, with one exception: In year 2 a trend emerged in which G-homozygotes of the *TPH2* G-703T polymorphism reported more life stressors than T-carriers ($t = 2.11$, $p <0.05$). No other associations between negative life events and genotype were observed.

Table 3 presents the descriptive statistics for the daily drinking outcomes for the sample overall. As shown in row 1, participants reported drinking on 21% of study days and heavy drinking on 13% of study days in year 1, which increased to 25% and 15% in year 2, respectively ($t = 5.62$, $p<0.01$; $t = 3.50$, $p<0.01$). Drinking quantity remained consistent across years, averaging 5.3 and 7.6 drinks per drinking and heavy drinking occasions, respectively. Additionally, in both years, 8.5% of the students reported not drinking during the study period. There were no differences in the proportion of non-drinkers by TPH2 genotypes. Table 4 presents the descriptive drinking statistics for each of the *TPH2* G-703T genotypes. As can be seen from this table, few genotype differences were observed. The one exception was that, contrary to prediction, individuals with the TT genotype (n = 20) appeared to drink *less* frequently than the other genotype groups.

Regression Results

Table 5 presents the results for all regression models testing the main effects of the *TPH2* G-703T genotype (column 1) and, for completeness, the main effects of *TPH1* C218A (column 2) and 5-HTTLPR (column 3) on the four alcohol variables, as well as the interactions between the *TPH2* G-703T genotype and the other polymorphisms (*TPH1* C218A, 5-HTTLPR) and negative life events (columns 4 – 6, respectively). All results were null. Only one test approached but did not reach significance: the effect of genotype on drinking frequency in year 1 (b=−0.027, SE=0.014, r^2=0.10; $p<0.05$), reflecting TT subjects' less frequent drinking.

Discussion

The findings of this longitudinal study of college drinking are consistent with the null findings reported by Zill and colleagues (2007), and extend their work by suggesting that the *TPH2* G-703T polymorphism is not associated with alcohol consumption in a non-clinical population.

Our study also showed no interactive effects of the *TPH2* G-703T polymorphism with the *TPH1* C218A polymorphism on the quantity or frequency of drinking. Additionally, no interactive effects of the *TPH2* G-703T polymorphism and the *SLC6A4* tri-allelic 5-HTTLPR polymorphism on drinking behavior were detected. Nor did we observe an interaction between negative life events and the *TPH2* G-703T polymorphism in predicting alcohol consumption. This interaction was null despite previous evidence of a negative life stress × gene interaction in this same sample for the 5-HTTLPR S- vs L-allele (Covault et al., 2007).

Secondary analyses also revealed two additional findings of potential interest. First, the main effect of the *TPH1* A218C polymorphism on drinking behavior was null. This pattern is surprising in view of previous studies that have linked the A-allele of the *TPH1* A218C polymorphism with alcohol dependence (Sun et al., 2005), alcoholism (Ishiguro et al., 1999), and an earlier age of onset of alcoholism (Chung et al., 2005). However, our study is the first to examine the association of this polymorphism with drinking behavior in a non-clinical population. Second, some studies have suggested that any results for the *TPH2* G-703T promoter polymorphism should also pertain to the *TPH2* T-473A promoter polymorphism, since they are in strong linkage disequilibrium. Therefore, in analyses not reported, we analyzed the less frequent *TPH2* T-473A polymorphism (rs11178997) and found that the *TPH2* T-473A polymorphism did not predict any of the alcohol outcome variables nor did it interact with the *TPH2* G-703T, the *TPH1* C218A, the tri-allelic 5-HTTLPR polymorphism, or life stress to predict any of the alcohol outcomes.

Our findings must be viewed in the context of several limitations. First, our study sample included only North American Caucasian college students. Future research should examine these patterns in other ethnic and socio-economic groups, e.g., community dwelling young adults. Second, life stressors were measured as negative life events that occurred over the past year; early trauma life stressors were not considered. However, this same measure of life stress interacted with the 5-HTTLPR genotype to predict drinking (Covault et al., 2007), an effect that is similar to the gene-environment interaction observed by Kaufman and colleagues who measured stressful childhood events (Kaufman et al., 2007). Third, although this is the first study to examine the interaction of *TPH2* with three theoretically relevant factors, the *TPH1* C218A polymorphism, the tri-allelic 5-HTTLPR polymorphism, and life stress, we examined these patterns in a non-clinical population.

Although the *TPH1* and *TPH2* genes have been well studied, they remain poorly understood biologically and their individual roles are continuing to emerge. While TPH2 is more relevant to adult brain serotonin levels, recent results suggests that *TPH1* variations may alter circulating serotonin levels in maternal circulation and thereby in the developing embryo prior to expression of *TPH2* (Cote et al., 2007; Nakamura et al., 2006). Serotonin is known to be important for embryonic brain development. Thus, *TPH1* polymorphic variations that lead to reductions in serotonin production may ultimately lead to developmental malformation of all neocortical layers, disorders of neuronal growth, development and differentiation, and changes in their shape and dimensions (Khozhai and Otellin, 2005). Therefore, it may be that *TPH1* variants predict alcohol consumption as a result of intrauterine effects on serotonin in the early embryonic brain. In light of these recent findings, it appears that a complex relationship may exist among the *TPH1* and *TPH2* genes and alcohol consumption.

In conclusion, the results of this study may have implications for understanding whether this rate-limiting enzyme for serotonin biosynthesis in the adult brain, *TPH2*, is involved in regulating alcohol consumption. Although the current study examined the two most commonly studied polymorphic variants of *TPH1* and *TPH2*, we encourage investigators to examine possible interactions between other *TPH1* and *TPH2* variants in predicting alcohol use.

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Table 1

Genotype Distributions

***TPH2* G-703T**	***N***	***TPH1* C218A**	***N***	***SLC6A4* 5-HTTLPR**	***N***
GG	216	CC	128	L_AL_A	87
GT	113	CA	164	SL_A or L_AL_G	177
TT	20	AA	55	SS or SL_G or L_GL_G	86

Table 2

Correlations among Serotonin-Related Polymorphisms

Polymorphism	***TPH2* G-703T**	***TPH1* C218A**	***SLC6A4* 5-HTTLPR**
***TPH2* G-703T**	--	−0.13[a]	−0.01
***TPH1* C218A**	−0.13[a]	--	−0.05
Tri-allelic 5-HTTLPR	−0.01	−0.05	--

[a]Note. Correlation is significant at the $p < 0.05$ (2-tailed).

Table 3

Drinking Summary Data Stratified by Gender

	Mean Drinking Frequency		Mean Heavy Drinking Frequency		Mean Drinking Episode Quantity		Mean Heavy Drinking Episode Quantity	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Total								
(M, SD)	0.21, 0.16	0.25, 0.19	0.13 (0.13)	0.15 (0.14)	5.36 (2.65)	5.33 (2.60)	7.55 (2.47)	7.59 (2.52)
(Mdn, range)	0.18 (0–.93)	0.21 (0–.94)	0.10 (0–.93)	0.13 (0–0.65)	5.00 (1–15.5)	4.85 (1–16)	7.00 (4–15.5)	7.00 (4–16)
Male								
(M, SD)	0.26 (0.17)	0.31 (0.20)	0.17 (0.15)	0.19 (0.15)	6.38 (2.90)	6.49 (2.70)	9.04 (2.31)	9.04 (2.45)
(Mdn, range)	0.25 (0–.93)	0.29 (0–.92)	0.17 (0–.93)	0.15 (0–0.65)	6.63 (1–13.5)	6.25 (1–16)	8.75 (5–15.5)	8.88 (5–16)
Female								
(M, SD)	0.16 (0.14)	0.21 (0.16)	0.10 (0.11)	0.12 (0.12)	4.46 (2.04)	4.34 (2.03)	6.16 (1.67)	6.20 (1.66)
(Mdn, range)	0.13 (0–.88)	0.17 (0–.94)	0.07 (0–.50)	0.10 (0–0.61)	4.44 (1–12.4)	4.00 (1–11.5)	6.00 (4–12.4)	5.94 (4–11.5)

Note. Numbers reflect means (M), standard deviations (SD), medians (Mdn), and ranges

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Table 4

Drinking Summary Data for *TPH2* G-703T Genotype Groups

	Mean Drinking Frequency		Mean Heavy Drinking Frequency		Mean Drinking Episode Quantity		Mean Heavy Drinking Episode Quantity	
Genotype	**Year 1**	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**
GG (n=216)	0.21 (0.17)	0.26 (0.20)	0.14 (0.14)	0.16 (0.15)	5.26 (2.62)	5.31 (2.72)	7.53 (2.51)	7.68 (2.59)
GT (n=113)	0.20 (0.14)	0.25 (0.17)	0.11 (0.12)	0.16 (0.13)	5.45 (2.64)	5.37 (2.43)	7.51 (2.50)	7.50 (2.42)
TT (n=20)	0.14 (0.11)	0.19 (0.16)	0.09 (0.10)	0.12 (0.12)	5.62 (2.87)	5.32 (2.16)	7.81 (1.80)	7.23 (2.48)

Note. Numbers reflect means and (standard deviations); all comparisons are non-significant.

Table 5

Results from Regression Analyses on Drinking Outcomes

Outcome Variables	G-703T *TPH2*	C218A *TPH1*	5-HTTLPR	G-703T *TPH2*× C218A *TPH1*	G-703T *TPH2* × 5-HTTLPR	G-703T *TPH2* × Life Events
Drinking Frequency						
Year 1	−0.027[a] (0.014)	−0.013 (0.012)	0.001 (0.012)	−0.015 (0.021)	−0.021 (0.018)	−0.004 (0.005)
Year 2	−0.024 (0.017)	−0.025 (0.015)	0.024 (0.014)	−0.013 (0.026)	−0.007 (0.022)	−0.004 (0.005)
Heavy Drinking Frequency						
Year 1	−0.013 (–0.011)	−0.005 (0.010)	0.004 (0.010)	−0.016 (0.018)	−0.008 (0.015)	0.001 (0.004)
Year 2	−0.010 (0.013)	−0.019 (0.011)	0.019 (0.011)	−0.017 (0.020)	0.000 (0.016)	0.001 (0.004)
Drinking Episode Quantity						
Year 1	0.184 (0.235)	0.134 (0.203)	−0.142 (0.206)	−0.407 (0.367)	0.265 (0.306)	0.130 (0.089)
Year 2	0.044 (0.235)	0.234 (0.198)	0.240 (0.195)	−0.406 (0.364)	−0.255 (0.297)	0.130 (0.089)
Heavy Drinking Episode Quantity						
Year 1	0.117 (0.209)	−0.177 (0.178)	−0.006 (0.181)	−0.116 (0.325)	0.150 (0.275)	0.069 (0.078)
Year 2	−0.198 (0.218)	−0.327 (0.183)	0.082 (0.186)	−0.274 (0.334)	−0.245 (0.282)	0.069 (0.078)

[a]Note. Unstandardized regression coefficient (with standard error) $p < 0.05$ (2-tailed)