Drinking Histories in Alcohol-Use-Disordered Youth: Preliminary Findings on Relationships to Platelet Serotonin Transporter Expression With Genotypes of the Serotonin Transporter*

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ABSTRACT. Objective: The serotonin (5-HT) transporter (5-HTT) is thought to play a key role in the onset of alcohol use, with potential behavioral and biological mechanisms mediated by the level of 5-HT in the synapse and in cerebral spinal fluid. Although 5-HT dysregulation has been related to poor impulse control, the biological mechanism is unknown, although functional control of the serotonergic system has been shown to be regulated in part by differential expression of the 5-HTT. The gene responsible for encoding 5-HTT has a functional polymorphism at the 5'-regulatory promoter region, which results in two forms: long (L) and short (S). The LL genotype is hypothesized to play a key role in the early onset of alcohol use and may be related to poor impulse control. The objective of this pilot study is to determine whether adolescents with a current alcohol-use disorder (AUD) ($N = 21$) have platelet measures of the 5-HTT functioning that are related to

5-HTT genotype and poor impulse control. Specifically, we wanted to examine the relationships between the following: platelet 5-HTT and 5-HTT genotype; platelet 5-HTT parameters and age at onset, as well as duration of drinking; and 5-HTT genotype and impulse control. **Method:** Adolescents with current AUD were recruited from the community to participate in a cross-section pilot study. **Results:** Our main findings showed significantly higher paroxetine binding (density of 5-HTT) in LL genotype versus S carriers (SS or SL genotypes); also, the LL group had a significantly earlier age at onset of drinking and longer duration of drinking, and poorer impulse control. **Conclusions:** These findings provide partial support for the hypothesis that, among currently drinking adolescents with an AUD, differential expression of 5-HTT may play an important role in the onset of adolescent AUD. (*J. Stud. Alcohol Drugs* 70: 899-907, 2009)

IMPAIRED CENTRAL NERVOUS SYSTEM (CNS) serotonin (5-HT) function has been implicated in the regulation of mood, impulsivity, and alcohol use (Johnson and Ait-Daoud, 2000; Kruesi, 1989, 1990; LeMarquand et al., 1994, 1999; Stoltenberg, 2003) that may help explain the variation in the age at onset of drinking and alcohol-use disorders (AUDs; Cloninger, 1987; Fils-Aime et al., 1996; LeMarquand et al., 1999; Linnoila et al., 1983; Swann et al., 1999; Virkkunen et al., 1987, 1996). The brain 5-HT system—originating in the raphe nuclei and projecting to the cortex, hippocampus, and subcortical brain regions—is thought to influence drinking behavior both directly by modulating the reinforcing effects of alcohol (Grunbaum et

al., 2002) and indirectly by processes regulating impulsivity and affect (Stoltenberg, 2003). Findings from animal studies have shown that pharmacological enhancement of 5-HT activity inhibits alcohol intake (McBride and Li, 1998). Human studies have shown that low CNS 5-HT turnover is associated with poor impulse control (Virkkunen et al., 1995) and alcohol-seeking behavior (Virkkunen and Linnoila, 1997). Lower CNS 5-HT turnover (i.e., lower 5-hydroxyindoleacetic acid in cerebrospinal fluid) has been reported in early-onset alcohol-dependent (EOA) adults, compared with late-onset alcohol-dependent (LOA) adults (Fils-Aime et al., 1996; Virkkunen et al., 1996), and the lowest CNS 5-HT turnover occurs in EOA adults when both parents have alcohol dependence (Fils-Aime et al., 1996). Together, these findings support the hypotheses that low CNS 5-HT availability and function regulate adolescent-onset drinking-related behaviors that are characterized by poor impulse control. What remains unknown is how low CNS 5-HT relates to onset and duration of drinking in adolescence, and what are the specific biological mechanisms that occur at the synaptic level in the 5-HT system that mediate onset and duration of drinking. The purpose of this pilot study is to determine whether adolescents with a current AUD have platelet measures of 5-HT transporter (5-HTT) functioning that are related to 5-HTT

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genotype, which may help explain mechanisms that occur at the synaptic level in the 5-HT system in these individuals.

Functional control of the central serotonin system is hypothesized to be regulated in part by differences in 5-HTT expression (Heinz et al., 2001, 2004). The gene responsible for encoding 5-HTT expression has a functional polymorphism at the 5'-regulatory promoter region (Heils et al., 1996, 1997). The diallelic polymorphism contains an insertion/deletion mutation with the long (L) variant having 44 base pairs that are absent in the short (S) variant. In normal controls, the LL genotype, compared with S carriers (i.e., SS and SL genotypes), has greater 5-HT uptake in human platelets (Greenberg et al., 1999) and in lymphoblasts (Lesch et al., 1996), as well as greater 5-HTT binding (reflecting either greater expression or less turnover) in human raphe nuclei (Heinz et al., 2000). Assuming that individuals with the LL genotype have greater 5-HTT expression rates, these individuals would be hypothesized to have increased 5-HTT numbers and function, as well as greater presynaptic 5-HT uptake in the CNS. Hence, LL individuals would have *lower* intrasynaptic 5-HT levels and, therefore, reduced intrasynaptic 5-HT neurotransmission, both in vivo and in vitro.

Differential expression of 5-HTT is hypothesized to interact with chronic alcohol use, and this interaction is thought to play an important role in the etiology and pathogenesis of alcoholism (Heinz et al., 2004; Meltzer and Arora, 1988), especially for early-onset alcoholism (Johnson, 2000). More specifically, adolescents with the LL genotype who begin drinking may have specific pre-existing vulnerabilities related to the LL genotype, including increased 5-HTT number and function, greater presynaptic 5-HT uptake, and reduced intrasynaptic 5-HT levels and reduced 5-HT neurotransmission. Together, these factors are hypothesized to increase the urge to drink (Ait-Daoud et al., 2009; Bleich et al., 2007) and in adolescents may increase the risk of developing alcoholism. Family and high-risk studies provide support for this hypothesis. In a sample of men at risk for alcohol dependence, the LL genotype was more prevalent among those who developed alcohol dependence (Schuckit et al., 1999). Ernouf and colleagues (1993) have shown that platelet 5-HT uptake was higher in alcohol-dependent parents and their children, compared with age-matched controls. Rausch and colleagues (1991) showed that adult males with alcohol-dependent fathers had higher mean maximum 5-HT uptake rate (V_{max}) for platelet 5-HT uptake, compared with controls. Together, findings from the aforementioned studies of at-risk samples lead to the conclusion that both 5-HTT genotype and 5-HTT function may be related to the risk for alcoholism in high-risk samples.

Population studies provide additional evidence that 5-HTT genotype and 5-HTT function are related to the age at onset of alcohol dependence. In a Japanese sample, alcoholics with the L allele (i.e., LL and LS genotypes) had significantly earlier onset of alcohol dependence, compared

with those with the SS genotype (Ishiguro et al., 1999). In a Korean male sample, the frequency of the L allele was significantly higher in alcohol-dependent individuals, compared with controls (Kweon et al., 2005). However, in European and Mexican-American samples, the SS genotype, not the LL genotype, has been associated with an antisocial type of alcoholism (Feinn et al., 2005; Konishi et al., 2004). Therefore, the genetic risk for alcoholism is clearly not as simple as a single allelic variant increasing risk for alcohol dependence. It may be the case that differential expression of 5-HTT, in interaction with chronic alcohol use, contributes to early-onset alcoholism. Our group has reported that 5-HT uptake into platelets was greater among EOA adult males, compared with LOA adult males and healthy controls (Javors et al., 2000). Although in normal samples of adults, platelet 5-HT uptake is greater among L carriers compared with individuals with the SS genotype (Greenberg et al., 1999), we recently found that, among adults with chronic alcohol dependence, 5-HT uptake was greater and [3H]paroxetine binding to 5-HTT was reduced among L carriers (i.e., LL and LS) compared with SS homozygotes (Johnson et al., 2008). These reductions in platelet 5-HT function were related to years of drinking and are consistent with Heinz's hypothesis (Heinz et al., 2000, 2004) that chronic alcohol use causes a downregulation or reduction in the expression of 5-HTT among L carriers. However, what remains unanswered is whether adolescents with AUDs (who are by definition "early onset") have 5-HTT platelet 5-HT function that is similar to that found in adult normal samples, because these adolescents have been using alcohol for a shorter period, compared with EOA adults.

To improve our understanding of the relationships between the 5-HTT genotype, 5-HTT expression or binding, and alcohol drinking in the early-onset stages of development, we conducted a pilot study of youth with a current AUD. Our primary aims were to determine whether 5-HTT genotype (LL vs S carriers) differentiated adolescents with an AUD, who by definition are all early onset, with respect to their current and lifetime drinking patterns. We also wanted to examine adolescent's serotonergic activity (measured by 5-HTT density and function in platelets), and determine whether there would be differences in impulsivity between LL and S carriers. We hypothesized that platelet measures of 5-HTT binding and function are related to the 5-HTT genotype and that there would be greater 5-HTT density and poorer impulsivity in the LL genotype because of the relatively short histories of alcohol use, compared with their adult EOAs. Specifically, we wanted to determine whether the shorter histories of alcohol use produced any evidence for reductions in 5-HTT expression in LL genotype adolescents. We predicted that adolescents with a current AUD would have higher 5-HTT function and 5-HTT density and poorer impulse control in the LL genotype compared with the S genotypes (LS, SS), possibly because of less downregula-

tion or less reduction in expression of 5-HTT in the former genotype group. We also tested whether history of drinking, current amount of drinking, or both, determine how 5-HTT genotype is related to 5-HTT function in adolescents with an AUD.

Method

Participants

Participants were youths ($N = 21$; ages 18-20 years; mean age = 18.7 years) who were recruited from the community using radio advertisements and were not seeking treatment for psychiatric or substance-use disorders. Volunteers had current alcohol abuse or alcohol dependence, using criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994). All volunteers were in good physical health (determined by a complete physical examination, electrocardiogram within normal limits, and laboratory screening tests within acceptable clinical parameters), were consuming at least three standard drinks per drinking day (mean [SD] = 9.4 [7.2]), had breath alcohol concentration of zero at screen, and were literate in English. Exclusionary criteria included current or lifetime Axis I DSM-IV substance-use disorder other than an AUD or a cannabis-use disorder. Substance use in the past 30 days other than alcohol or marijuana use was exclusionary. Exclusionary criteria included current major depressive disorder, bipolar disorder, posttraumatic stress disorder, psychosis, or attention-deficit/hyperactivity disorder that was medicated within the previous 30 days. Other exclusionary criteria included elevated liver enzymes greater than four times the normal range and/or elevated bilirubin ($>110\%$ per limits of normal); serious medical co-morbidity requiring medical intervention or close supervision; clinically significant alcohol withdrawal; treatment for alcohol abuse or dependence within the last 30 days; or, if female, pregnancy. All participants gave written informed consent. The study was approved by the University of Texas Health Science Center Institutional Review Board.

Diagnostic measures and measure of impulsivity

Therapists were trained to use the Children's Interview for Psychiatric Syndromes (ChIPS). The ChIPS adheres strictly to DSM-IV criteria for psychiatric disorders and has been shown to be accurate and provide valid diagnoses of psychiatric disorders in adolescents and young adults to 20 years of age (Rooney et al., 1999). Current and lifetime substance-use disorders were diagnosed in a structured clinical interview using the Adolescent Diagnostic Interview (Winters and Henley, 1993; Winters et al., 1993). The principal investigator established reliability of interviews by monitoring the consistency of participant's self-report and using the

Best Estimate Diagnostic Procedure (Kosten and Rounsaville, 1992; Leckman et al., 1982). Recent reported drinking and lifetime drinking were determined by patient interview using Timeline Followback procedures (Sobell and Sobell, 1992).

Impulsivity was measured using the Barratt Impulsivity Scale, Version 11 (BIS-11; Patton et al., 1995), which is typically considered a measure of impulsive personality traits. This is a 30-item questionnaire in which participants rate the frequency of several common impulsive (e.g., "I do things without thinking") or nonimpulsive (e.g., "I am self-controlled") behavioral traits on a scale from 1 (i.e., "rarely/never") to 4 (i.e., "almost always/always"). Scores range from 30 to 120, with higher scores indicating more impulsivity. The BIS-11 has been validated in adolescents 12 years and older in our laboratory and ages 14 years and older in other studies (Mann et al., 1999; Soloff et al., 2000). The BIS-11 was used to characterize impulsive traits of our participants.

General design and procedures

The experimental design was a cross-sectional retrospective study. After initial screening, eligible participants gave blood for assay of platelet 5-HT function and genotyping. Fifty milliliters of blood was drawn from each participant to obtain platelets for the measurement of 5-HT uptake into intact platelets and paroxetine binding to platelet membranes. Additionally, a 10 ml sample of blood was drawn for the determination of the 5-HTT genotype.

Platelet suspension and platelet membrane preparation

Fifty milliliters of blood was drawn into 60 ml polypropylene syringes containing 10 ml of Acid-Citrate-Dextrose buffer. The blood was then centrifuged at 150 g at 23° C for 20 min in a Beckman TJ-6 centrifuge to obtain platelet-rich plasma (PRP). Platelet count in PRP was determined with a Coulter Counter Model S-Plus VI and adjusted to 3×10^8 platelets/ml with the addition of platelet buffer (137 mM KCl, 1 mM $MgCl_2$, 5.5 mM glucose, 5 mM HEPES; pH 7.4) to prepare adjusted PRP for the serotonin uptake experiments only. Three milliliters of adjusted PRP was used for platelet serotonin uptake experiments, which were performed on the day of the blood draw. To prepare platelet membranes for paroxetine binding experiments, the remainder of the PRP was used. One microliter of prostaglandin I_2 solution (300 ng/ml) per milliliter of PRP was added to prevent loss of platelets during centrifugation. The sample was then centrifuged at 550 g. The resulting platelet pellet was resuspended in platelet buffer and centrifuged at 35,000 g. The platelet membrane pellet was resuspended in 1 ml of platelet buffer and then stored at -80° C until the day of the assay to measure paroxetine binding.

5-HT uptake into intact platelets

Platelet 5-HT uptake was measured in intact platelets, with the assay starting at 1 hour after the centrifugation step, to obtain PRP from whole blood. The 1-hour delay was required to obtain a platelet count of the PRP from the hospital and to dilute the PRP to prepare adjusted PRP and to equilibrate the platelets after dilution. The adjusted PRP suspension was used to determine platelet 5-HT uptake. Assay tubes were prepared in duplicate with and without 50 μ M fluoxetine, and contained [3 H]5-HT at six different concentrations (62.5 nM–2,000 nM; final concentrations were achieved by mixing [3 H]5-HT and nonradioactive 5-HT solutions), as well as 100 μ M pargyline. These tubes were incubated at 37° C for 5 min; then, the reaction was started by the addition of 100 μ l of adjusted PRP that contained 3×10^7 platelets. The assay tubes were incubated at 37° C for an additional 5 min; then, the reaction was quenched by rapid filtering through Whatman GF/B filters using a Brandel Cell Harvester. The filters were washed three times with 5 ml of ice-cold wash buffer containing 50 mM Tris-HCl, 150 mM NaCl, and 20 mM ethylenediaminetetraacetic acid (EDTA). Filters were placed in scintillation vials containing 5 ml of Beckman Ready Protein⁺ scintillation counting fluid and immediately counted. Specific uptake was calculated by subtracting total uptake from nonspecific uptake (fluoxetine tubes). $V_{\max}$ in platelets was expressed as femtomoles of 5-HT/min $\times 10^7$ platelets, and the kinetic constant (K_m) was expressed as nanomoles. K_m and $V_{\max}$ were calculated using the one-site hyperbolic function in Prism 4 software (Graph-Pad Software, Inc., La Jolla, CA).

Paroxetine binding to platelet membranes

Platelet membranes were used to determine platelet paroxetine binding. Assay tubes were prepared in duplicate with and without 150 μ M fluoxetine, and containing incubation buffer containing 50 mM Tris-HCl, 5 mM KCl, and 120 mM NaCl, and [3 H]paroxetine at six different concentrations (0–2 nM). The actual concentration of paroxetine in each tube was determined using a 40 μ l aliquot taken from each tube before the addition of platelet membranes. The experiment was started by the addition of 80 μ g of platelet membrane protein, then the assay tubes were incubated for 1 hr at 23° C. The reaction was quenched by addition of ice-cold wash buffer (50 mM Tris-HCl, 150 mM NaCl, and 20 mM EDTA) and rapid filtering through Whatman GF/B filters treated with 0.3% polyethyleneimine using a Brandel Cell Harvester. Filters were washed three times with ice-cold wash buffer, dried overnight, placed in scintillation vials containing 5 ml of Beckman Ready Protein⁺ scintillation counting fluid, and counted in a Beckman LS-6500 liquid scintillation counter. Disintegrations per minute from the 40 μ l aliquots were converted into nanomoles of paroxetine to obtain the actual

concentrations in each tube. Total and nonspecific binding of paroxetine was plotted against each actual concentration. Specific binding was calculated by subtracting nonspecific binding from total binding. K_d and $B_{\max}$ of paroxetine binding were calculated using Prism 4 software. Paroxetine binding ($B_{\max}$) was expressed as femtomoles per milligram of platelet membrane protein and the dissociation constant (K_d) as nanomoles. Protein concentrations were measured using the BioRad method and a SPECTRAMax PLUS³⁸⁴ Microplate Spectrophotometer.

Genotyping

The blood sample for the determination of 5-HTT genotype was drawn at enrollment. White blood cells were separated from plasma and resuspended. DNA was isolated using PUREGENE Gentra Systems (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's protocol. The 5'-HTTLPR 44 base pair promoter region repeat polymorphism was amplified by polymerase chain reaction (PCR) from ~ 50 ng of DNA using two primers—(1) 5'-CGT TGC CGC TCT GAA TGC CAG-3' and (2) 5'-GGA TTC TGG TGC CAC CTA GAC GCC-3'—and in a 25- μ l final volume consisting of 0.5 units of Tfl DNA polymerase (EPICENTRE Biotechnologies, Madison, WI), 1X PCR buffer, 1.5 ml MgCl, 200 μ M deoxyribonucleotide triphosphate, 1X enhancer, and 0.6 μ M of each primer. The PCR conditions were as follows: 94° C for 30 seconds, 70° C for 30 seconds, and 72° C for 30 seconds; a final extension of 72° C for 7 minutes; and terminal hold at 4° C. Separation by gel electrophoresis using 4% MetaPhor agarose (Cambrex, Rockland, ME) allowed visualization by ethidium bromide/ultraviolet detection of the two variants of the 5-HTT genotype: (1) L and (2) S. Fragment sizes were 464 base pairs and 420 base pairs, respectively, of the promoter region of the *SCL4A* gene (-1,415 to -951) (Heils et al., 1996).

Statistical analyses

Statistical analyses were conducted using PASW statistics, Version 17.0 (SPSS Inc., Chicago, IL).

Distributions of platelet outcome variables (K_m , $V_{\max}$, K_d , and $B_{\max}$) were examined. Nonnormal distributions of platelet outcome variables were transformed. Planned analyses included Pearson correlations to examine the relationships among the aforementioned platelet variables. For the dependent variables for platelet function ($B_{\max}$, K_d , $V_{\max}$, and K_m), *t* tests were used to determine whether there were group differences between LL genotypes versus S carrier genotypes. The *t* tests were also used for group differences in current and lifetime drinking and trait impulsivity, and chi-square tests were used for categorical variables, including gender, ethnicity, and current psychiatric disorders. We also used Pearson correlations between BIS total score and

age to further examine the relationship between impulsivity and age.

Results

The distribution of genotypes was as follows: LL, $n = 8$; LS, $n = 9$; and SS, $n = 4$. Our rationale for pooling LS and SS for analysis of group differences was based on the following factors: The sample size was small ($N = 21$); the genotypes were not in Hardy-Weinberg equilibrium (Sasieni, 1997); S carriers (LS/SS) were the predominant genotypes in the sample; and other studies and reviews have shown that that the LL genotype may be the critical distinction for our testing our hypothesis (Heinz et al., 2001; Johnson, 2000; Shuckit et al., 1999). However, there was no evidence of significant differences in duration of alcohol use and age at onset of drinking among the LS and SS subgroups.

There were no statistically significant differences in age or ethnicity between the LL and S carriers (see Table 1). However, results in Table 1 showed that the LL group had a significantly earlier age at onset (i.e., 13.5 vs 15.2 years of age; $p = .03$) and longer duration of alcohol use ($p < .01$) but did not have significant differences in quantitative measures of recent drinking. The Pearson correlation between age and BIS total score was not significant ($r = -.10$, $p = .65$), and the differences in BIS total scores did not appear related to age. However, age at onset of alcohol use was significantly correlated with BIS total score ($r = -.45$, $p < .05$).

As hypothesized, the LL group also had significantly higher inattention ($p = .03$) and motor ($p = .02$) components of trait impulsivity (Patton et al., 1995) and a trend toward significant differences in total BIS-11 score of trait impulsivity ($p = .05$; see Table 1). Subsequent analyses of variance—with genotype as the grouping variable, age at onset

TABLE 1. Demographics, drinking history, and current psychiatric disorders

Variable	Genotype				<i>p</i>
	LL (<i>n</i> = 8)		LS/SS (<i>n</i> = 13)		
	Mean	(SD)	Mean	(SD)	
Age, years	18.9	(0.6)	18.5	(0.5)	.20
Trait impulsivity, BIS-11					
Nonplanning	26.0	(4.7)	24.4	(6.9)	.57
Inattention	20.5	(3.4)	16.5	(3.8)	.03
Motor	27.8	(3.2)	23.9	(3.2)	.02
Total Score	74.3	(7.4)	64.9	(11.3)	.05
Lifetime drinking, years					
Age at onset of alcohol use	13.5	(1.2)	15.2	(1.9)	.03
Duration of alcohol use	5.4	(0.9)	3.3	(1.8)	<.01 ^a
Recent drinking					
DD	3.0	(1.7)	3.9	(5.3)	.98
DDD	9.9	(5.7)	7.8	(7.8)	.27
PDA	67.6	(16.1)	52.7	(25.7)	.16
	No.	% of LL participants	No.	% of LS/SS participants	
Gender					.97
Male	5	62.5	8	61.5	
Female	3	37.5	5	38.5	
Ethnicity					.38
White	2	25.0	3	23.1	
Hispanic	3	37.5	9	69.2	
Biracial or mixed	3	37.5	0	0.0	
American Indian	0	0.0	1	7.7	
ADHD	3	37.5	3	25.0	.48
ODD	1	12.5	3	25.0	.55
CD	6	75.0	9	75.0	.92
GAD	0	0.0	4	44.0	.81
Alcohol dependence	8	100.0	10	83.3	.14
Alcohol abuse	0	0.0	3	16.7	^b
Cannabis dependence	1	12.5	6	50.0	.11

Notes: BIS-11 = Barrett Impulsivity Scale-11; DD = average drinks per day in past 90 days; DDD = average drinks per drinking day in past 90 days; PDA = percentage days abstinent in past 90 days; ADHD = attention-deficit/hyperactivity disorder (was unmedicated at least 30 days); ODD = oppositional defiant disorder; CD = conduct disorder; GAD = generalized anxiety disorder. ^aDuration of alcohol use remains significant after including BIS total as covariate; ^bthree participants met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria for alcohol abuse.

TABLE 2. Group differences in measures of serotonin uptake and paroxetine binding

Variable	Genotype				<i>p</i>
	LL (<i>n</i> = 8)		LS/SS (<i>n</i> = 13)		
	Mean	(SD)	Mean	(SD)	
Paroxetine binding					
B _{max} , fmol/mg protein	802.0	(254.2)	504.3	(199.8)	.02
K _d (nM)	0.7	(0.5)	0.4	(0.3)	.03
5-HT uptake					
V _{max} , fmol/min x10 ⁷ platelets	181.6	(128.4)	200.1	(113.5)	.46
K _m , nM	445.9	(409.3)	323.2	(136.4)	.40

Note: Data for K_d , $V_{\max}$, and K_m were transformed to the natural log scale for *t* test analyses.

of alcohol use as the covariate, and dependent variables of the BIS inattention subscale ($p = .2$), motor subscale ($p = .06$), and total BIS-11 score ($p = .3$)—were not significant.

Table 2 and Figure 1 present the results from the platelet studies. Participants having the LL genotype had significant differences in $B_{\max}$ and K_d , compared with the S carriers,

indicating greater maximal binding of 5-HTT with lower affinity for paroxetine binding (Pratt and Taylor, 1990). There was no significant correlation between $B_{\max}$ and BIS total score (Spearman correlation = .06; $p = .81$). Also, there were no significant genotype group differences in the functional measures of 5-HT uptake.

FIGURE 1. Platelet paroxetine binding ($B_{\max}$) for LL and LS/SS genotypes; HTTLPR = functional polymorphism of the 5' flanking region of the serotonin transporter gene; $\blacklozenge$ = data points for LL genotypes; $\blacksquare$ = data points for LS/SS genotypes

Discussion

Our main findings were that the 5-HTT genotype predicted differences in age at onset and duration of drinking, and differences in the platelet binding profile of 5-HTT among adolescents with a current AUD. Specifically, adolescents with the LL genotype began drinking at a younger age, showed greater [³H]paroxetine binding than did S carriers, and had poorer impulse control.

Although both genotype groups had an onset of AUD during adolescence and were of the same current age, participants with an LL genotype had significantly earlier age at onset of drinking, compared with the S carriers. This finding is consistent with a hypothesis described by Johnson (2000) that predicts the LL genotype is associated with an earlier age at onset of problem drinking. Also consistent with the hypothesis, the LL group had higher levels of behavioral vulnerability (i.e., trait impulsivity) than did the S carriers (Heinz et al., 2001, 2004; Johnson, 2000; Johnson and Ait-Daoud, 2000). This latter finding is interesting in light of the literature suggesting that, among early-onset alcoholics, LL genotypes may have lower levels of 5-HT in the *synaptic cleft* that are associated with higher levels of impulsivity (Heinz et al., 2001, 2004; Johnson, 2000; Johnson and Ait-Daoud, 2000). The finding that LL homozygotes, in comparison with S carrier genotypes, had greater paroxetine binding to 5-HTT in adolescents with current AUDs also fits this hypothesis and appears to contrast with drinking-related reductions in 5-HTT binding and activity that is seen with alcohol-dependent adults who are carriers of the L allele. One explanation for these differences between adolescent and adults may be that chronic drinking of alcohol is required to produce reductions in 5-HTT binding and possibly 5-HTT activity (Johnson et al., 2008). Interestingly, there were no significant group differences in the current levels of drinking, which contrasts with reports in college students that show S carriers having heavy drinking patterns (Covault et al., 2007). Although somewhat speculative, it may be the case that the adolescents in our study who had the LL genotype and high levels of impulsivity represent a distinct subgroup of early-onset drinkers on a continuum of severity, because they all had a current AUD.

Unfortunately, we did not directly assess alcohol sensitivity or alcohol-induced effects on 5-HT clearance. Therefore, our findings do not directly address results from the literature on 5-HTT genotype and alcohol sensitivity and tolerance (Barr et al., 2003; Corbin et al., 2006; Hinckers et al., 2006; Hu et al., 2005; Shuckit et al., 1999), or reports suggesting that alcohol-induced inhibition of 5-HT clearance may, in some circumstances, be independent of 5-HTT function (Daws et al., 2006).

The major limitations of this study are a result of the small sample size of non-treatment-seeking research volunteers. We were fortunate to have a large enough sample of

LL homozygotes to establish an LL versus S carrier group comparison; but, the sample size was insufficient to fully examine SS versus LS subgroup differences for our main outcome variables or to test more fully the relationships among age, age at onset of alcohol use, impulsivity, and genotype. We also do not have an adolescent age group of healthy comparison participants, which prevents any conclusion about allelic frequency associations with drinking. Also, our genotyping strategy included only the diallelic L versus S polymorphisms and did not include the triallelic A → G polymorphic variant (Hu et al., 2006), which may better characterize true 5-HTT expression rates.

This preliminary study cannot address the frequencies of the L or S alleles in association with AUD among the general adolescent population, and our findings are not in Hardy-Weinberg equilibrium (Sasieni, 1997). Results from previous studies in adult populations have generally suggested that the SS genotype is associated with antisocial types of alcoholism in European and Mexican-American populations (Feinn et al., 2005), whereas the LL genotype has been associated with alcoholism risk in Asian populations (Ishiguro et al., 1999; Konishi et al., 2004). Our population of adolescents included primarily white and "white" Hispanic, with smaller numbers of biracial or mixed participants and one Native-American ancestry participant. However, our results showed that the LL genotype is associated with worse impulse control compared with S carriers, which may help account for the age at onset of drinking in this genetic subgroup of adolescents with AUD.

Our study has a number of strengths. Importantly, the findings provide initial evidence to address questions on whether serotonergic dysregulation exists during the onset of problem drinking or AUD (LeMarquand et al., 1994; Pine et al., 1997; Soloff et al., 2000; Twitchell et al., 2000, 2001). We are aware of no other studies that have used multiple measures of platelet 5-HTT density and function, as well as genotyping of the 5'-promoter of 5-HTT, to assess serotonin dysregulation in adolescents with current AUD. Our findings support the generally accepted hypothesis that peripheral platelet markers of 5-HTT density are related to 5-HTT genotype and that higher 5-HTT density exists in the LL genotype, compared with the S carriers soon after the time of adolescent-onset AUD.

Conclusion

Our preliminary findings provide partial support for the hypothesis that, among currently drinking adolescents with an AUD, individuals having an LL genotype display greater trait impulsivity, an earlier age at onset of drinking, and increased [³H]paroxetine binding to platelet 5-HTT in comparison with S carriers. These findings expand our current understanding of the 5'-promoter of the 5-HTT gene in regulating the 5-HTT in adolescents with an AUD. Future

research will require case-control studies and longitudinal designs to determine the extent to which the LL versus SS genotypes are related to differential risk of heavy drinking and alcoholism and whether serotonergic dysregulation mediates these outcomes. Thus, this study provides preliminary findings demonstrating that platelet and genetic measures of 5-HTT function may be useful measures to predict the complex interplay of biological and environmental factors in the etiology of alcoholism.

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