ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

Differential Impact of Serotonin Transporter Activity on Temperament and Behavior in Persons with a Family History of Alcoholism in the Oklahoma Family Health Patterns Project

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Background: Central serotonergic (5-HT) function is implicated in pathways to alcohol dependence, including dysphoria manifested by symptoms of anxiety and depression. However, little is known about genetic variation in central 5-HT function and its potential impact on temperament and behavior in persons with a family history of alcoholism (FH+).

Methods: We tested 314 healthy young adults (23.5 years of age, 57% female; 193 FH− and 121 FH+) enrolled in the Oklahoma Family Health Patterns project, a study of alcoholism risk in relation to temperament and behavioral dyscontrol. Dysphoria was assessed using the Eysenck neuroticism and Beck depression scales, and Cloninger's Tridimensional Personality Questionnaire. Risk taking was assessed with the Iowa Gambling Task (IGT) and Balloon Analogue Response Task (BART). All subjects were genotyped for a functional polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene (*SLC6A4*).

Results: FH+ subjects with the gain-of-function 5-HTTLPR genotype scored higher in neuroticism, harm avoidance, and symptoms of depression (p-values ≤ 0.03). No effect of 5-HTTLPR genotype was seen in FH−. FH+ carriers of the gain-of-function 5-HTTLPR genotype played to minimize their frequency of losses in the IGT, whereas FH− carriers played a balanced strategy ($p < 0.003$). No 5-HTTLPR effects were seen in the BART. Results were unaffected by sex, education, drug use, and antisocial characteristics.

Conclusions: The functional 5-HTTLPR polymorphism predicted significant variation in negative moods and poorer affect regulation in FH+ persons, with possible consequences for behavior, as seen in a simulated gambling task. This pattern may contribute to a drinking pattern that is compensatory for such affective tendencies.

Key Words: Serotonin Transporter, Family History of Alcoholism, Anxiety, Depression.

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THE OKLAHOMA FAMILY Health Patterns (OFHP) project is devoted to the intensive study of healthy young adults with a family history of alcoholism (FH+) in order to identify personal characteristics that may increase risk for the disorder. Alcohol dependence is more prevalent in FH+ persons, and inherited variation accounts for much of this increased risk, based on a range of twin studies (Cloninger et al., 1981; Goldman et al., 2005). In the present study, we compared unaffected FH+ and FH− persons carrying variations in a functional polymorphism (5-HTTLPR) located in the promoter region of the serotonin (5-HT) transporter (5-HTT) gene (*SLC6A4*). 5-HTTLPR is a common polymorphism that includes a variable number of tandem repeats (44-base pair [bp] insertion/deletion) in the promoter region of the gene that alter transcription such that the less common, short "S" allele is associated with an approximately 50% reduction in 5-HTT availability. This leads to reduced presynaptic reuptake of 5-HT and an increase in synaptic 5-HT availability (Heils et al., 1996). 5-HTTLPR triallelic genotyping (incorporating the functional single

nucleotide polymorphism rs25531) identifies the low transcriptional activity S allele but additionally separates the high activity L_A allele from a third allele, L_G which also displays low transcriptional activity similar to the S allele (Hu et al., 2006). Therefore, these 5-HTTLPR genotypes can be grouped as Low activity (SS, SL_G, L_GL_G), Medium activity (SL_A, L_AL_G), and High activity (L_AL_A) 5-HTT.

Variation in 5-HTT activity is associated with differences in response to emotional stimuli (Munafo et al., 2008) and with symptoms of depression and anxiety (Goldman et al., 2010). Regulation of 5-HT function may also moderate activity of dopaminergic neurons in mesolimbic reward circuitry (Budde et al., 2010) thus affecting motivated behavior. It is presently unclear if 5-HTTLPR is associated with risk for alcohol use disorders and if FH+ persons respond differently to variations in 5-HTT activity than FH−. In our prior work, we have reported that FH+ persons differ from FH− in showing greater symptoms of depression and higher neuroticism scores (Acheson et al., 2009; Saunders et al., 2008; Sorocco et al., 2006). FH+ also use different strategies in playing the Iowa Gambling Task (IGT), and they show increased activation in striatal brain regions during play on this task (Acheson et al., 2009). Based on this background, we examined the potential for a differential impact of polymorphisms of the promoter region of the 5-HTT gene in FH+ and FH− persons on mood stability, as indexed by scores on the Eysenck neuroticism scale (Eysenck and Eysenck, 1964), negative affect as indexed by the Beck depression inventory (Beck et al., 1996), and we used the Tridimensional Personality Questionnaire (TPQ) to examine novelty seeking, harm avoidance, and reward dependence (Cloninger et al., 1991). Reward dependence and harm avoidance are reflective of conceptually opposing tendencies to require positive feedback from the environment but also to avoid risks. We also examined 2 behavioral tasks thought to be sensitive to a person's risk-taking tendencies, the IGT (Bechara et al., 1997), and the Balloon Analogue Response Task (BART; Lejuez et al., 2002). Performance on the IGT differs between substance abusers and nonabusers (Bechara et al., 2002), and riskier behavior on the BART is seen in smokers versus nonsmokers (Lejuez et al., 2003a). The question we addressed here is whether differing 5-HTT activity levels would account for differences in these measures in either FH+ or FH− persons or in members of both groups.

MATERIALS AND METHODS

Volunteers were recruited through advertisement in the Oklahoma City area. The sample included 314 persons (23.5 ± 0.3 years of age, 57% females) screened for the OFHP who provided reliable FH reports, gave consent for collection of specimens for DNA analysis, and listed their race as white or Native American. Participants were in self-reported good health, free of prescription medications, and did not meet criteria for a current Axis I mental health disorder as defined by the Diagnostic and Statistical Manual of Mental disorders, 4th ed. (American Psychiatric Association, 1994) based on the CDIS-4 interview (Blouin et al., 1988). All participants signed an informed consent form approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center and the Veterans Affairs Medical Center in Oklahoma City, OK, and were paid for participating. The genotyping and genetic analyses were deemed by the NIH Office of Human Subjects Research to be exempt from NIH IRB review.

Exclusion Criteria

Prospective participants were excluded if they had any of the following: a history of alcohol or drug dependence; diagnosis of substance abuse within the past 2 months; current use of any abused drug; history of any Axis I disorder other than depression assessed by psychiatric interview; depression within the past 2 months; Axis II disorders in clusters A or C assessed by Structured Clinical Interview for Diagnosis-II questionnaire and interview, or a history of serious medical disorder, including neurological disorders, cardiovascular diseases, or diabetes.

Screening and Testing

An initial telephone screening to ensure general conformity with inclusion and exclusion criteria was followed by a screening at the laboratory conducted by a trained interviewer supervised by a licensed clinical psychologist.

Family History of Alcoholism. Persons were considered FH+ if either biological parent met criteria for alcohol or substance use by subject report. FH− were those reporting an absence of alcohol or substance use disorders in their biological parents and grandparents. Family history classification was established using the Family History Research Diagnostic Criteria (Andreasen et al., 1977; Zimmerman et al., 1988) as described previously (Lovallo et al., 2012). Individuals were excluded if either they or a parent indicated possible fetal exposure to alcohol or other drugs. Parent interviews to verify FH status were successfully conducted for 80% of the subjects, and subject reports were confirmed in 90% of these cases. Subjects with conflicting reports were excluded or their FH status was reassigned based on information from the parent, resulting in an estimated 97% correct classification in the total sample (Lovallo et al., 2012) in agreement with other studies (Schuckit et al., 1995).

Current alcohol and drug use were assessed through the Cahalan Drinking Habits Questionnaire (Cahalan et al., 1969), the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001), and a Drug Use Questionnaire (Cognitive Studies Laboratory, 1994). Socioeconomic status (SES) was measured using the Hollingshead scale (Hollingshead, 1975) with updated occupational categories and was based on the primary occupation of the main breadwinner in the household in which the subject grew up.

Subjects completed the Beck Depression Inventory II (Beck et al., 1996), the Eysenck Personality Inventory (Eysenck and Eysenck, 1964), and the TPQ (Cloninger et al., 1991). Antisocial and disinhibitory characteristics were assessed using Gough's Sociability scale from the California Personality Inventory (CPI-So; Gough, 1994).

Behavioral Tasks. The IGT (Bechara et al., 1997, 2001) is a simulated card game that assesses decision behavior in a risky and uncertain setting. The subject is free to choose cards from 4 decks stacked to provide differing levels of risky or safe plays and resulting in greater final wins and losses. Full details are provided in Supplementary Material. Preliminary examination of the final simulated winnings on the IGT showed no difference in performance as a function of FH, 5-HTTLPR, or their interaction. We next examined a measure shown to index a cautious approach to this task, a measure we term "sensitivity to frequent losses" (Ert et al., 2013). In the present analysis, we measured a subject's tendency to play from Decks B + D because these produce not only consistent winnings at first, but also few penalties, and so this tendency to play from Decks

B and D is viewed as an attempt to avoid frequent losses (Erev and Barron, 2005). This analysis is described in greater detail elsewhere (Ert et al., 2013).

The BART assesses behavioral impulsivity (Lejuez et al., 2003a, 2010) and is sensitive to nicotine addiction and impulsive behavioral characteristics in adolescents (Lejuez et al., 2002, 2003a). The subject viewed a computer screen with an image of a partially inflated balloon. The subject was told to tap a computer key to pump up the balloon and that the monetary value of the balloon would increase on each inflation. Each balloon was set to burst after an unpredictable number of pumps. The subject was free to stop pumping at any point, and the current value of the balloon on that trial would be added to their bank account. If the balloon burst, the value on that trial went to zero. The performance measure was the average of the number of pumps on all unexploded balloon trials during the game (Lejuez et al., 2003b). Subjects were paid 1 cent U.S. for each dollar shown on the screen at the end.

Genotyping

Subjects provided a saliva sample by passive drool into an Oragene collection and preservation kit (DNA Genotek, Inc., Kanata, ON, Canada). DNA was extracted and genotyping was performed in 2 stages using size discrimination for the S (103 bp) and L (146 bp) alleles and for the rs25531 (L_A (146 bp) and L_G (61 bp)) alleles in the Laboratory of Neurogenetics at the National Institute on Alcohol Abuse and Alcoholism. The *SLC6A4* 5-HTTLPR region was amplified in a 20-μl reaction: 1× Optimized Buffer A, 1× polymerase chain reaction (PCR) enhancer, 0.25 μM of each primer (FAM-ATCGCTCCTGCATCCCCCATTAT [forward primer], GAGGTGCAGGGGGATGCTGGAA [reverse primer]), 0.125 μM of dNTP, 10 ng of DNA, 1.25 unit of Platinum Taq polymerase (all from Invitrogen, Pittsburgh, PA). The PCR conditions were as follows: 95°C (5 minute), 40 cycles of 94°C (30 seconds), 52°C (30 seconds), 68°C (1 minute), and a final elongation, 68°C (10 minutes). S and L genotypes were discriminated directly from the PCR products. The rs25531 L_A and L_G genotypes were determined by digesting 10-μl PCR mix with 50 units of MspI (37°C, for 1 hour, 1× restriction buffer). Samples were mixed with deionized formamide and GeneScan^{TM}-500 ROX Size Standard (Applied Biosystems, Pittsburgh, PA), and the genotypes were resolved on a 3730 DNA Analyzer (Applied Biosystems).

Genotyping accuracy was determined empirically by duplicate genotyping of 25% of the samples selected randomly. The error rate was <0.005, and the completion rate was >0.95.

Ethnicity was self-described: 291 (93%) individuals were of European ancestry, 12 were Native American, 5 were Hispanic, and 6 were "other" (not African ancestry). A group of 24 African Americans were initially recruited, but as their 5-HTTLPR allele frequencies differed markedly from the rest of the sample, they were excluded from this analysis. Frequencies of the 5-HTTLPR alleles in the 291 Caucasians were as follows: S = 0.44, L_A = 0.48, L_G = 0.08. The allele frequencies were similar among the 23 non-Caucasian, non-African individuals: S = 0.41, L_A = 0.48, L_G = 0.11. Therefore, the sample was analyzed as a whole (N = 314), and genotypes were grouped as Low activity (SS, SL_G, L_GL_G; 0.25), Medium activity (SL_A, L_AL_G; 0.53), and High activity (L_AL_A; 0.22) based on published results (Hu et al., 2006).

Statistics

Due to small sample size, the Low and Medium activity 5-HTTLPR genotypes were combined into a single Low/Med group based on previous studies indicating similar behavioral and neurofunctional consequences of SS homozygotes and SL heterozygotes (Lesch et al., 1996). Psychological reports and behavioral data were analyzed using a series of multivariable models with 2 between-subjects predictor variables FH (FH+ and FH−) and 5-HTTLPR genotype (Low/Med and High). Each model included the separate grouping variables and the interaction term with sex and SES tested as covariates. Type III sums of squares were used to avoid potential problems of collinearity. Significant FH × 5-HTTLPR interaction terms are shown with effect size estimates (partial eta squared, η^2) and were followed by planned comparisons using Student's t-tests with Tukey–Kramer adjustment for multiple comparisons. The model we tested was first run with no covariates included. However, as a range of variables could modify the relationship between FH groups, we tested 4 covariates including sex, drug use, CPI-So scores, and education; chosen because they differed between FH groups in the current analysis (sex and drug use) or were shown to differ in characteristics of the FH groups in prior work from this project (Lovallo et al., 2013; Saunders et al., 2008). Data were analyzed using SAS software, Ver. 9.2 for Windows (Copyright© 2008 SAS Institute Inc., Cary, NC).

RESULTS

Demographic characteristics of the 4 FH × 5-HTTLPR genotype groups are shown in Table 1.

Psychological and behavioral data are shown in Fig. 1 for the 4 FH × 5-HTTLPR activity groups. Multivariable models indicated statistically significant FH × 5-HTTLPR interaction terms for neuroticism (F = 5.05, p = 0.0253, η^2 = 0.016), symptoms of depression (F = 6.80, p = 0.0096, η^2 = 0.022), and for harm avoidance (F = 7.58, p = 0.0063, η^2 = 0.024). The results across these psychological phenotypes are consistent; Low/Med versus High activity

Table 1. Group Demographics

Family history	FH−		FH+		G × FH
5-HTTLPR activity	Low/Med	High	Low/Med	High	*p*-Value
N	152	41	94	27	
Age (year)	24 (0.2)	24 (0.5)	23 (0.3)	23 (0.5)	—
Sex (% female)	50	61	69	78	0.004
Race (% white)	94	100	90	82	—
SES	50 (1.0)	52 (1.5)	43 (3.9)	46 (2.5)	—
Education (year)	16.0 (0.2)	16.1 (0.2)	15.1 (0.2)	15.7 (0.4)	—
Mental age (year)	18.0 (0.1)	18.4 (0.1)	17.7 (0.1)	17.8 (0.1)	—
Cahalan (oz/month)	19.7 (2.3)	23.1 (4.5)	21.3 (3.2)	19.2 (4.4)	—
AUDIT	3.8 (0.2)	4.3 (0.5)	4.4 (0.4)	3.5 (0.6)	—
Drugs used (no.)[a]	1.00 (0.1)	0.83 (0.2)	1.59 (0.2)	1.48 (0.3)	—
Smokers (%)[a]	7	5	12	11	—
CPI-So	33.4 (0.4)	32.8 (0.7)	30.3 (0.5)	30.0 (0.8)	—
IGT Bank ($)	172 (38)	206 (93)	245 (52)	263 (90)	—

Entries show M ± SEM. FH+, FH−, persons with and without a family history of alcoholism. Race: sample includes no persons identifying as African American. Nonwhite includes American Indian and Other. SES: Hollingshead Socioeconomic Status score. Mental Age measured using the Shipley Institute of Living Scale. Cahalan: Drinking Habits Questionnaire. AUDIT: Alcohol Use Disorders Information Test. CPI-So: Socialization Scale of the California Personality Inventory, low scores are more antisocial. IGT: Iowa Gambling Task final value of subject's bank.

[a]FH+ versus FH−, $p < 0.05$.

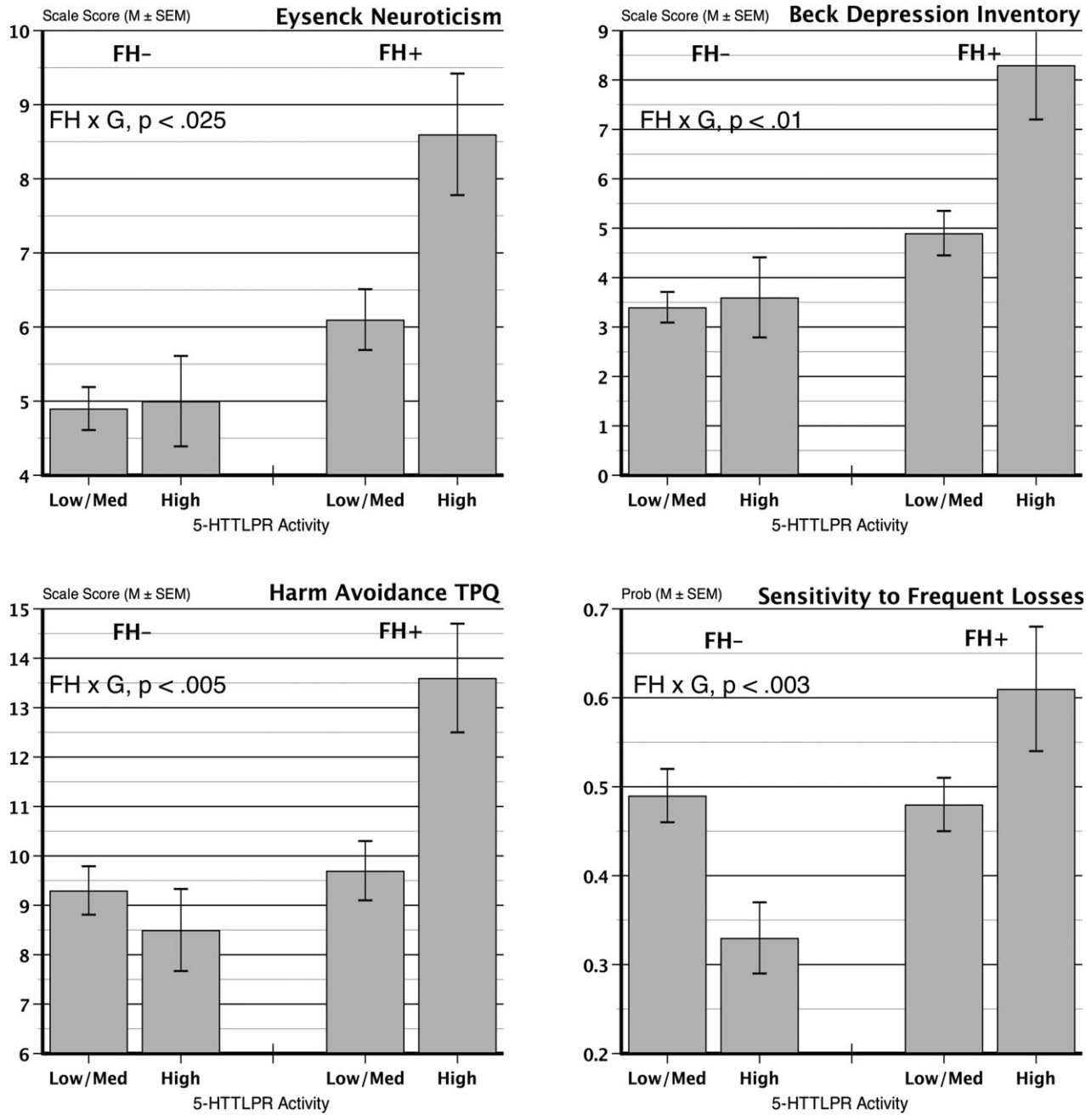

Fig. 1. The effect of serotonin transporter activity level (Low/Med vs. High) on psychological and behavioral characteristics of persons with and without a family history of alcoholism (FH+, FH−). Entries show M ± SEM and *p*-values refer to the significance level of the interaction between FH (+, −) × Genotype (Low/Med, High activity 5-HTTLPR) interaction term.

5-HTTLPR groups were not different within the FH− group ($ts < 1.0$, $ps > 0.49$), whereas among the FH+, the High activity 5-HTTLPR subgroup reported more symptoms of depression ($t = 3.31$, $p = 0.0024$) and had higher neuroticism ($t = 2.84$, $p = 0.0184$) and harm avoidance scores ($t = 3.00$, $p = 0.0236$) compared to their Low/Med counterparts, suggesting negative and unstable affective tendencies in the High activity subgroup, which are consistent with their reported desire to avoid harmful outcomes. The FH × 5-HTTLPR groups did not differ on novelty seeking or reward dependence on the TPQ.

Turning to the behavioral tasks, on the IGT, we observed a potential behavioral counterpart of the effects of 5-HTTLPR on negative affect and harm avoidance. We examined the probability of draws from Decks B + D that are stacked to provide frequent rewards and few losses (Ert et al., 2013). Here, we observed a significant FH × 5-HTTLPR interaction ($F = 8.86$, $p = 0.0032$, $\eta^2 = 0.030$), in which case High activity FH− subjects avoided the B and D decks while FH+ chose from them most frequently ($t = 3.59$, $p < 0.004$), suggesting loss aversion in the latter subgroup (Erev and Barron, 2005). FH had no impact on IGT choice

behavior within the Low/Med activity group ($t < 1.0$). Performance on the BART yielded no FH or 5-HTTLPR group main effects or interactions ($ps \geq 0.20$).

As a final validity check on the FH × 5-HTTLPR interactions, we ran the analysis entering the 4 covariates listed above and including only persons with genotypes of Caucasian ancestry, excluding genotypes of non-African, non-Caucasian ancestry. The p and η values for the FH × 5-HTTLPR interaction terms for Eysenck neuroticism, Beck depression, and harm avoidance scores, and attention to losses were, respectively: 0.0395 and 0.015, 0.0038 and 0.025, 0.0130 and 0.022, and 0.0009 and 0.041. Exclusion of persons of non-African, non-Caucasian ancestry, and entry of these covariates therefore did not materially affect the results.

DISCUSSION

To our knowledge, the present study is the first to explore the impact of 5-HTTLPR activity genotype in healthy young adult FH+ versus FH− persons. The results indicate that 5-HTTLPR genotype has a larger impact within the FH+ group and little impact in the FH− group. This finding raises the question of whether 5-HTT activity is a direct contributor to risk for excessive drinking, perhaps for mood regulation purposes, or whether 5-HTT activity has an indirect impact in FH+ individuals, who may have other unmeasured diatheses associated with maladaptive behavioral and affective regulation.

The 5-HTTLPR polymorphism has attracted extensive interest because it is associated with variation in anxious temperament and may predict proneness to psychiatric disorders such as obsessive–compulsive disorder and depression and vulnerability to suicidality (Enoch et al., 2013; Goldman et al., 2010; Haenisch et al., 2013; Hu et al., 2006). In this study, we examined the triallelic 5-HTTLPR polymorphism in a sample of young adults classified as FH+ or FH− for family alcoholism. Among FH+, but not FH−, High 5-HTT activity was associated with higher Eysenck neuroticism scores, greater numbers of symptoms on the Beck Depression Inventory, and higher TPQ harm avoidance scores. We also observed a related behavioral effect in the IGT, such that FH+ carriers of the High activity genotype selected preferentially from card decks that avoided losses even at the expense of total gains (Erev and Barron, 2005). There was no performance variation across FH × 5-HTT groups on a widely used measure of impulsive behavior, the BART (Lejuez et al., 2002). An examination of Table 1 does not suggest a disproportionate impact of genotype on drinking, drug use, smoking, or on disinhibitory or antisocial characteristics. This may reflect our use of restrictive entry criteria to achieve a goal of studying FH+ with minimal comorbidities, such as substance dependence, and this procedure produced a sample with normative alcohol and drug experimentation habits. Inclusion of a wider range of alcohol and other drug intake behaviors could therefore potentially lead to different findings. Another limitation is that we tested multiple dependent variables in a relatively small sample for a study of this sort. The relative independence of the independent variables did not, however, appear to require correction for multiple statistical tests (Proschan and Waclawiw, 2000). However, the sample size and novelty of the findings point toward the desirability of replication with independent, and perhaps larger, samples.

A common thread to these results is that FH+ persons with the High activity 5-HTTLPR genotype were more likely to report negative and less stabile mood than persons with Low/Med genotypes, and they appear more cautious in their behavioral strategies. The present findings of greater neuroticism scores, symptoms of depression, and harm avoidance scores among High activity FH+ persons are consistent with this literature. Overall, people with the High activity 5-HTTLPR genotype were responsible for the greater variation in psychological state and behavior within and between FH groups. The finding of greater mood instability among FH+ is consistent with much literature indicating depressive tendencies in such persons. However, the impact of the High activity genotype appears inconsistent with other literature, indicating that anxiety/dysphoria and negative emotionality are more often associated with low activity or S genotypes of the 5-HTTLPR (Pezawas et al., 2005). However, because there are no published studies on the impact of 5-HTTLPR variation in FH+ persons, there is little basis for a direct comparison with other findings. This again points toward the need for converging studies distinguishing FH+ and FH− individuals with differing 5-HTT genotypes.

This leads to several considerations of the existing literature and indications for future research. The present paper is a first attempt to examine the association of 5-HTTLPR polymorphisms in healthy young adult FH groups. Only 2 other studies have studied 5-HTTLPR and FH characteristics in adults, but these both had a different emphasis. One involved 5-HT receptor binding in autopsy samples from FH+ persons (Underwood et al., 2008). An autopsy population is likely to overrepresent suicide and accidental death. The other study involved the acute effects of 5-HT depletion by intake of a tryptophan-free diet, finding increased impulsive behavior in 5-HT-depleted FH+ (Crean et al., 2002). While interesting, acute changes in 5-HT availability might be expected to have different effects than chronic differences in 5-HT availability associated with variations in genotype.

Three other studies in children and adolescents bear on our findings. One study on a small number ($N = 44$) of FH+ and FH− 10-year-olds found that those with more behavior problems also had lower levels of whole blood 5-HT. FH did not interact with 5-HT levels (Twitchell et al., 1998). In a related report from that project, 47 children who were 5-HTTLPR LL carriers had more disinhibitory behavior and negative moods than SS or SL carriers, again with no difference as a function of FH status (Twitchell et al., 2001). The small sample sizes may have precluded finding FH × 5-HTTLPR interactions, and the age difference makes comparisons with our study difficult; however, it is of interest that the LL (gain-of-function) genotype was associated with

negative affect and disruptive behavior. A more recent study of 118 14-year-olds from treatment programs in a mid-sized community and in an urban replication sample of 178 11-year-old community volunteers found that lower SES LL homozygotes scored as more antisocial (using the Antisocial Process Screening Device; Frick et al., 2000) with no relationship among individuals with low and intermediate activity genotypes (Sadeh et al., 2010). These studies agree that children and adolescents who are LL homozygotes may be disinhibited and additionally that this pattern may be modifiable by environmental factors. In summary, studies on children (Sadeh et al., 2010; Twitchell et al., 1998, 2001), adolescents (Sadeh et al., 2010), and young adults in the present sample all show an impact of the LL or High activity 5-HTTLPR genotype on psychological and behavioral characteristics. Younger aged samples appear to show a greater effect of the gain-of-function genotype on externalization than does our older sample, which showed a greater influence on negative affect and harm avoidance. Also, lower SES may increase impulsive characteristics in these younger samples, but no such effect of SES was seen here. Both outcomes may reflect differences in maturational factors.

The contribution of genetic characteristics to risk for alcoholism is well established based on large twin and twin-adoption studies in Scandinavia and the United States (Cloninger et al., 1981; Merikangas, 1990; Merikangas et al., 1998). That said there is a lack of agreement on specific genes or combinations of genes that may be responsible for this association. Alcoholism is often accompanied pre- and postmorbidly by a number of behavioral features, especially depression and antisocial and disinhibitory tendencies, and these characteristics are themselves heritable (Cadoret et al., 1985; Finn et al., 1990). Inheritance patterns suggest direct and interactive pathways by which coinherited traits may reinforce each other and interact with family environment as well (Anthenelli et al., 1998; Kendler et al., 1995, 2003; Nurnberger et al., 2004). Our results do not permit an estimate of the potential contribution of 5-HTTLPR genotype to risk for alcoholism. Our sample is small, and the selection criteria we used eliminated substance use disorders in our sample. Further work on FH+ risk groups will be needed, perhaps with a broadened range of entry criteria.

In addition to the limitations just mentioned, our study has the following strengths. First, the sample is carefully classified as to FH status with most cases being confirmed by parental report. Second, the subjects were free of significant comorbidities. As such, the data are broadly applicable to a healthy young adult sample although the results may not fully reflect the FH $\times$ 5-HTTLPR activity relationships that might prevail within an alcohol dependent or drug abusing population. Third, although our sample is small for a study of genetic influences on disease, it is moderately large for a study of its type, providing some confidence that the results may be replicated in other studies.

In young adults, the presence of a High activity 5-HTT genotype may strongly modify negative affective tendencies in FH + persons and have little impact on these characteristics in persons with no such history. The exploration of 5-HTTLPR influences among FH + persons may yield useful findings in understanding genetic contributions behaviors associated with risk for this disorder.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Supplementary Material.