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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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Abstract

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 yr 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

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Analogue Response Task (BART). All subjects were genotyped for a functional polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene (SCL6A4).

Results—FH+ subjects with the gain-of-function 5-HTTLPR genotype scored higher in neuroticism, harm avoidance, and symptoms of Depression (p values $\leq .03$). 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 ($p < .003$). 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.

Keywords

serotonin transporter; family history of alcoholism; anxiety; depression

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 transporter (5-HTT) gene (*SLC6A4*). 5-HTTLPR is a common polymorphism that includes a variable number of tandem repeats (44-basepair 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 SNP 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) (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, Sorocco et al., 2006, Saunders et al., 2008). FH+ also use different strategies in playing the Iowa Gambling Task, 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 two behavioral tasks thought to be sensitive to a person's risk-taking tendencies, the Iowa Gambling Task (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 vs. 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 mo; current use of any abused drug; history of any Axis I disorder other than depression assessed by psychiatric interview; depression within the past 2 mo; 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 (FH-RDC) (Zimmerman et al., 1988, Andreasen et al., 1977) 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 (EPI) (Eysenck and Eysenck, 1964), and the Tridimensional Personality Questionnaire (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 Iowa Gambling Task (IGT) (Bechara et al., 1997, Bechara et al., 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 Balloon Analogue Response Task (BART) assesses behavioral impulsivity (Lejuez et al., 2003a, Lejuez et al., 2010) and is sensitive to nicotine addiction and impulsive behavioral characteristics in adolescents (Lejuez et al., 2002, Lejuez et al., 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 one cent US 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, Ontario, Canada). DNA was extracted and genotyping was performed in two stages using size discrimination for the S (103bp) and L (146bp) alleles and for the rs25531 (L_A (146bp) and L_G (61bp)) alleles in the Laboratory of Neurogenetics at NIAAA. The *SCL6A4* 5-HTTLPR region was amplified in a 20 μ l reaction: 1 $\times$ Optimized Buffer A, 1 $\times$ PCR enhancer, 0.25 μ M of each primer [FAM-ATCGTCTCCTGCATCCCCATTAT (forward primer), GAGGTGCAGGGGGATGCTGGAA (reverse primer)], 0.125 μ M of dNTP, 10ng of DNA, 1.25u of Platinum Taq polymerase (all from Invitrogen Corp). The PCR conditions were: 95°C (5 min), 40 cycles of 94°C (30sec), 52 °C (30sec), 68°C (1 min), and a final elongation, 68°C (10 min). S and L genotypes were discriminated directly from the PCR reaction products, The rs25531 L_A and L_G genotypes was determined by digesting 10 μ l PCR mix with 50units of MspI (37°C, for 1 hour, 1 $\times$ restriction buffer). Samples were mixed with deionized formamide and GeneScan™-500 ROX Size Standard (Applied Biosystems), 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 was initially recruited but since 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: 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 $\times$ 5-HTTLPR interaction terms are shown with effect size estimates (partial eta squared, η^2) and were followed by planned comparisons using Students *t* tests with Tukey-Kramer adjustment for multiple comparisons. The model we tested was first run with no covariates included. However, since a range of variables could modify the relationship between FH groups, we tested four 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, USA.

Results

Demographic characteristics of the four FH $\times$ 5-HTTLPR genotype groups are shown in Table 1.

Psychological and behavioral data are shown in Figure 1 for the four FH $\times$ 5-HTTLPR activity groups. Multivariable models indicated statistically significant FH $\times$ 5-HTTLPR interaction terms for Neuroticism ($F = 5.05$, $p = .0253$, $\eta^2 = .016$), symptoms of Depression ($F = 6.80$, $p = .0096$, $\eta^2 = .022$), and for Harm Avoidance ($F = 7.58$, $p = .0063$, $\eta^2 = .024$). The results across these psychological phenotypes are consistent; Low/Med vs. High activity 5-HTTLPR groups were not different within the FH– group ($ts < 1.0$, $ps > .49$), whereas among the FH+, the High activity 5-HTTLPR subgroup reported more symptoms of Depression ($t = 3.31$, $p = .0024$), and had higher Neuroticism ($t = 2.84$, $p = .0184$) and Harm Avoidance scores ($t = 3.00$, $p = .0236$) 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 $\times$ 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 $\times$ 5-HTTLPR interaction ($F = 8.86$, $p = .0032$, $\eta^2 = 0$), in which case High activity FH– subjects avoided the B and D decks while FH+ chose from them most frequently ($t = 3.59$, $p < .004$), suggesting loss-aversion in the latter subgroup (Erev and Barron, 2005). FH had no impact on IGT choice behavior within the Low/Mod activity group ($t < 1.0$). Performance on the BART yielded no FH or 5-HTTLPR group main effects or interactions ($ps \geq .20$).

As a final validity check on the FH $\times$ 5-HTTLPR interactions, we ran the analysis entering the four covariates listed above and including only persons with genotypes of Caucasian ancestry, excluding genotypes of non-African, non-Caucasian ancestry. The p and η^2 values for the FH $\times$ 5-HTTLPR interaction terms for Eysenck Neuroticism, Beck Depression, and Harm Avoidance scores, and attention to losses were, respectively: .0395 and .015, .0038 and .025, .0130 and .022, and .0009 and .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+ vs. 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 (Haenisch et al., 2013, Goldman et al., 2010, Enoch 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 $\times$ 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 stable mood than persons with Low/Mod 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 is 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 two 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 over-represent 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 $FH \times 5-HTTLPR$ 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 (Twitchell et al., 1998, Twitchell et al., 2001, Sadeh et al., 2010), 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 et al., 1998, Merikangas, 1990). 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 (Kendler et al., 1995, Kendler et al., 2003, Anthenelli et al., 1998, 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 serotonin transporter genotype may strongly modify negative affective tendencies in persons with a family history of alcoholism and have little impact on these characteristics in persons with no such history. The exploration of 5-HTTLPR influences among persons with a family history of alcoholism may yield useful findings in understanding genetic contributions behaviors associated with risk for this disorder.

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References

- Acheson A, Robinson JL, Glahn DC, Lovallo WR, Fox PT. Differential activation of the anterior cingulate cortex and caudate nucleus during a gambling simulation in persons with a family history of alcoholism: Studies from the Oklahoma Family Health Patterns Project. *Drug Alcohol Depend.* 2009; 100:17–23. [PubMed: 19026496]
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders.* Washington, D.C.: American Psychiatric Association; 1994.
- Andreasen NC, Endicott J, Spitzer RL, Winokur G. The family history method using diagnostic criteria. Reliability and validity. *Archives of General Psychiatry.* 1977; 34:1229–1235. [PubMed: 911222]
- Anthenelli RM, Tipp J, Li TK, Magnes L, Schuckit MA, Rice J, Daw W, Nurnberger JI Jr. Platelet monoamine oxidase activity in subgroups of alcoholics and controls: results from the Collaborative Study on the Genetics of Alcoholism. *Alcohol Clin Exp Res.* 1998; 22:598–604. [PubMed: 9622437]

- Babor, TF.; Higgins-Biddle, JC.; Saunders, JB.; Montiero, MG. The Alcohol Use Disorders Identification Test: Guidelines for use in primary care. Geneva, Switzerland: World Health Organization: Department of mental health and substance dependence; 2001.
- Bechara A, Damasio H, Tranel D, Damasio AR. Deciding advantageously before knowing the advantageous strategy. *Science*. 1997; 275:1293–1295. [PubMed: 9036851]
- Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE. Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia*. 2001; 39:376–389. [PubMed: 11164876]
- Bechara A, Dolan S, Hindes A. Decision-making and addiction (part II): myopia for the future or hypersensitivity to reward? *Neuropsychologia*. 2002; 40:1690–1705. [PubMed: 11992657]
- Beck AT, Steer RA, Ball R, Ranieri W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess*. 1996; 67:588–597. [PubMed: 8991972]
- Blouin AG, Perez EL, Blouin JH. Computerized administration of the Diagnostic Interview Schedule. *Psychiatry Res*. 1988; 23:335–344. [PubMed: 3387505]
- Budde H, Sander T, Wernicke C, Muller A, Gallinat J, Schmidt LG, Smolka MN. Serotonin transporter promoter polymorphism and dopaminergic sensitivity in alcoholics. *J Neural Transm*. 2010; 117:133–138. [PubMed: 19885717]
- Cadoret RJ, O'Gorman TW, Troughton E, Heywood E. Alcoholism and antisocial personality. Interrelationships, genetic and environmental factors. *Arch Gen Psychiatry*. 1985; 42:161–167. [PubMed: 3977542]
- Cahalan, U.; Cisin, I.; Crossely, HM. American Drinking Practices. Newark, NJ: Rutgers Center for Alcohol Studies; 1969.
- Cloninger CR, Bohman M, Sigvardsson S. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. *Arch Gen Psychiatry*. 1981; 38:861–868. [PubMed: 7259422]
- Cloninger CR, Przybeck TR, Svrakic DM. The Tridimensional Personality Questionnaire: U.S. normative data. *Psychol Rep*. 1991; 69:1047–1057. [PubMed: 1784653]
- Cognitive_Studies_Laboratory. The Drug Use Inventory. Oklahoma City, OK: Center for Alcohol and Drug Related Studies, University of Oklahoma Health Sciences Center; 1994.
- Crean J, Richards JB, de Wit H. Effect of tryptophan depletion on impulsive behavior in men with or without a family history of alcoholism. *Behav Brain Res*. 2002; 136:349–357. [PubMed: 12429396]
- Enoch MA, Hodgkinson CA, Gorodetsky E, Goldman D, Roy A. Independent effects of 5' and 3' functional variants in the serotonin transporter gene on suicidal behavior in the context of childhood trauma. *J Psychiatr Res*. 2013; 47:900–907. [PubMed: 23558235]
- Erev I, Barron G. On adaptation, maximization, and reinforcement learning among cognitive strategies. *Psychol Rev*. 2005; 112:912–931. [PubMed: 16262473]
- Ert E, Yechiam E, Arshavsky O. Smokers' decision making: more than mere risk taking. *PLoS One*. 2013; 8:e68064. [PubMed: 23844156]
- Eysenck SB, Eysenck HJ. An improved short questionnaire for the measurement of extraversion and neuroticism. *Life Sci*. 1964; 305:1103–1109. [PubMed: 14225366]
- Finn PR, Kleinman I, Pihl RO. The lifetime prevalence of psychopathology in men with multigenerational family histories of alcoholism. *J Nerv Ment Dis*. 1990; 178:500–504. [PubMed: 2380695]
- Frick PJ, Bodin SD, Barry CT. Psychopathic traits and conduct problems in community and clinic-referred samples of children: further development of the psychopathy screening device. *Psychol Assess*. 2000; 12:382–393. [PubMed: 11147105]
- Goldman D, Oroszi G, Ducci F. The genetics of addictions: uncovering the genes. *Nat Rev Genet*. 2005; 6:521–532. [PubMed: 15995696]
- Goldman N, Glei DA, Lin YH, Weinstein M. The serotonin transporter polymorphism (5-HTTLPR): allelic variation and links with depressive symptoms. *Depress Anxiety*. 2010; 27:260–269. [PubMed: 20196101]
- Gough H. Theory, development, and interpretation of the CPI socialization scale. *Psychological Reports*. 1994; 75:651–700. [PubMed: 7809335]

- Haenisch B, Herms S, Mattheisen M, Steffens M, Breuer R, Strohmaier J, Degenhardt F, Schmal C, Lucae S, Maier W, Rietschel M, Nothen MM, Cichon S. Genome-wide association data provide further support for an association between 5-HTTLPR and major depressive disorder. *J Affect Disord.* 2013; 146:438–440. [PubMed: 22921522]
- Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP. Allelic variation of human serotonin transporter gene expression. *J Neurochem.* 1996; 66:2621–2624. [PubMed: 8632190]
- Hollingshead, AB. Four factor index of social status. New Haven, CT: Yale University; 1975.
- Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, Murphy DL, Goldman D. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet.* 2006; 78:815–826. [PubMed: 16642437]
- Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch Gen Psychiatry.* 2003; 60:929–937. [PubMed: 12963675]
- Kendler KS, Walters EE, Neale MC, Kessler RC, Heath AC, Eaves LJ. The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. *Arch Gen Psychiatry.* 1995; 52:374–383. [PubMed: 7726718]
- Lejuez CW, Aklin WM, Jones HA, Richards JB, Strong DR, Kahler CW, Read JP. The Balloon Analogue Risk Task (BART) differentiates smokers and nonsmokers. *Exp Clin Psychopharmacol.* 2003a; 11:26–33. [PubMed: 12622341]
- Lejuez CW, Aklin WM, Zvolensky MJ, Pedulla CM. Evaluation of the Balloon Analogue Risk Task (BART) as a predictor of adolescent real-world risk-taking behaviours. *J Adolesc.* 2003b; 26:475–479. [PubMed: 12887935]
- Lejuez CW, Magidson JF, Mitchell SH, Sinha R, Stevens MC, de Wit H. Behavioral and biological indicators of impulsivity in the development of alcohol use, problems, and disorders. *Alcoholism, Clinical and Experimental Research.* 2010; 34:1334–1345.
- Lejuez CW, Read JP, Kahler CW, Richards JB, Ramsey SE, Stuart GL, Strong DR, Brown RA. Evaluation of a behavioral measure of risk taking: the Balloon Analogue Risk Task (BART). *J Exp Psychol Appl.* 2002; 8:75–84. [PubMed: 12075692]
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science.* 1996; 274:1527–1531. [PubMed: 8929413]
- Lovallo WR, Farag NH, Sorocco KH, Acheson A, Cohoon AJ, Vincent AS. Early life adversity contributes to impaired cognition and impulsive behavior: studies from the oklahoma family health patterns project. *Alcohol Clin Exp Res.* 2013; 37:616–623. [PubMed: 23126641]
- Lovallo WR, Farag NH, Sorocco KH, Cohoon AJ, Vincent AS. Lifetime adversity leads to blunted stress axis reactivity: studies from the oklahoma family health patterns project. *Biological Psychiatry.* 2012; 71:344–349. [PubMed: 22112928]
- Merikangas KR. The genetic epidemiology of alcoholism. *Psychol Med.* 1990; 20:11–22. [PubMed: 2181516]
- Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, Rounsaville BJ. Familial transmission of substance use disorders. *Arch Gen Psychiatry.* 1998; 55:973–979. [PubMed: 9819065]
- Munafo MR, Brown SM, Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biol Psychiatry.* 2008; 63:852–857. [PubMed: 17949693]
- Nurnberger JI Jr, Wiegand R, Bucholz K, O'Connor S, Meyer ET, Reich T, Rice J, Schuckit M, King L, Petti T, Bierut L, Hinrichs AL, Kuperman S, Hesselbrock V, Porjesz B. A family study of alcohol dependence: coaggregation of multiple disorders in relatives of alcohol-dependent probands. *Arch Gen Psychiatry.* 2004; 61:1246–1256. [PubMed: 15583116]
- Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nat Neurosci.* 2005; 8:828–834. [PubMed: 15880108]

- Proschan MA, Waclawiw MA. Practical guidelines for multiplicity adjustment in clinical trials. *Control Clin Trials*. 2000; 21:527–539. [PubMed: 11146147]
- Sadeh N, Javdani S, Jackson JJ, Reynolds EK, Potenza MN, Gelernter J, Lejuez CW, Verona E. Serotonin transporter gene associations with psychopathic traits in youth vary as a function of socioeconomic resources. *J Abnorm Psychol*. 2010; 119:604–609. [PubMed: 20677849]
- Saunders B, Farag N, Vincent AS, Collins FL Jr, Sorocco KH, Lovallo WR. Impulsive errors on a Go-NoGo reaction time task: disinhibitory traits in relation to a family history of alcoholism. *Alcohol Clin Exp Res*. 2008; 32:888–894. [PubMed: 18373725]
- Schuckit MA, Klein JL, Twitchell GR. The misclassification of family history status in studies of children of alcoholics. *J Stud Alcohol*. 1995; 56:47–50. [PubMed: 7752632]
- Sorocco KH, Lovallo WR, Vincent AS, Collins FL. Blunted hypothalamic-pituitary-adrenocortical axis responsivity to stress in persons with a family history of alcoholism. *Int J Psychophysiol*. 2006; 59:210–217. [PubMed: 16360227]
- Twitchell GR, Hanna GL, Cook EH, Fitzgerald HE, Little KY, Zucker RA. Overt behavior problems and serotonergic function in middle childhood among male and female offspring of alcoholic fathers. *Alcohol Clin Exp Res*. 1998; 22:1340–1348. [PubMed: 9756051]
- Twitchell GR, Hanna GL, Cook EH, Stoltenberg SF, Fitzgerald HE, Zucker RA. Serotonin transporter promoter polymorphism genotype is associated with behavioral disinhibition and negative affect in children of alcoholics. *Alcohol Clin Exp Res*. 2001; 25:953–959. [PubMed: 11505018]
- Underwood MD, Mann JJ, Huang YY, Arango V. Family history of alcoholism is associated with lower 5-HT_{2A} receptor binding in the prefrontal cortex. *Alcohol Clin Exp Res*. 2008; 32:593–599. [PubMed: 18241316]
- Zimmerman M, Coryell W, Pfohl B, Stangl D. The reliability of the family history method for psychiatric diagnoses. *Arch Gen Psychiatry*. 1988; 45:320–322. [PubMed: 3281625]

Figure 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.

Table 1

Group Demographics

Family History 5-HTTLPR activity	FH- Low/Med	High	FH+ Low/Med	High	G × FH p value
N	152	41	94	27	—
Age (yr)	24 (0.2)	24 (0.5)	23 (0.3)	23 (0.5)	—
Sex (% fem)	50	61	69	78	.004
Race (% white)	94	100	90	82	—
SES	50 (1.0)	52 (1.5)	43 (3.9)	46 (2.5)	—
Education (yr)	16.0 (0.2)	16.1 (0.2)	15.1 (0.2)	15.7 (0.4)	—
Mental Age (yr)	18.0 (0.1)	18.4 (0.1)	17.7 (0.1)	17.8 (0.1)	—
Cahalan (oz/mo)	46 (2.9)	48 (5.1)	50 (3.9)	61 (9.7)	—
AUDIT	3.8 (0.2)	4.3 (0.5)	4.4 (0.4)	3.5 (0.6)	—
Drugs Used (no.) *	1.00 (0.1)	0.83 (0.2)	1.59 (0.2)	1.48 (0.3)	—
Smokers (%) *	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)	—

Note. Entries show $M \pm SEM$. 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.

* FH+ vs. FH-, $p < .05$