



Published in final edited form as:

Psychiatry Res. 2017 July ; 253: 211–219. doi:10.1016/j.psychres.2017.03.033.

Associations between serotonin transporter and behavioral traits and diagnoses related to anxiety

Ardesheer Talati, Ph.D.^{1,2,5,*}, Zaga Odgerel, Ph.D.², Priya J. Wickramaratne, Ph.D.^{1,2,3}, Andrea Norcini-Pala, Ph.D.¹, Jamie L. Skipper, M.A.², Jay A. Gingrich, M.D., Ph.D.^{1,2,4}, and Myrna M. Weissman, Ph.D.^{1,2,4,5}

¹Department of Psychiatry, Columbia University Medical Center, New York, NY

²Division of Epidemiology, New York State Psychiatric Institute, New York, NY

³Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY

⁴Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY

⁵Sackler Institute for Developmental Psychobiology, Columbia University, New York, NY

Abstract

The role of the serotonin transporter promoter-linked polymorphism (*5-HTTLPR*) in psychiatric disease remains unclear. Behavioral traits could serve as alternative outcomes that are stable, precede psychopathology, and capture more sub-clinical variation. We test associations between *5-HTTLPR* and (1) behavioral traits and (2) clinical diagnoses of anxiety and depression. Second and third generation participants (N=203, 34.2±13.8 years, 54% female) at high- or low-familial risk for depression (where risk was defined by the presence of major depression in the 1st generation) were assessed longitudinally using the Schedule for Affective Disorders and Schizophrenia-lifetime interview, Barratt Impulsiveness Scale-11, Buss-Perry Aggression Questionnaire, and the NEO-Five Factor Inventory. High (but not low)-risk offspring with two risk (short, s) alleles had higher impulsivity (+13%), hostility (+31%) and neuroticism (+23%). SS was associated higher rates of panic (OR=7.05 [2.44, 20.38], p=0.0003) and phobic (OR=2.68[1.04, 6.93], p=0.04), but not other disorders. Impulsivity accounted for 16% of associations between *5-HTTLPR* and panic, and 52% of association between *5-HTTLPR* and phobias. We show that *5-HTTLPR* predicts higher impulsivity, hostility, and neuroticism, and that impulsivity could serve as a useful independent outcome or intermediary phenotype in genetic studies of anxiety.

*Corresponding Author: Ardesheer Talati, PhD, Columbia University & New York State Psychiatric Institute, 1051 Riverside Drive, Room 2428, Unit 24, New York, NY 10032. Phone: 646.774.6421, talatia@nyspi.columbia.edu; at2071@cumc.columbia.edu.

Financial Disclosures:

In the past three years, Dr. Weissman has received royalties from the Oxford University Press, Perseus Press, the American Psychiatric Association Press, and Multihealth Systems. None pose a conflict. None of the other authors have disclosures or conflicts.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The serotonin transporter gene (*SLC6A4*), whose product is the target of the selective serotonin reuptake inhibitor (SSRI) class of antidepressants, is among the most extensively studied genes in the psychiatric literature. A polymorphism in its promoter region (5-HTTLPR) has elicited particular interest, as it occurs primarily as either a shorter (S, 14-repeat) or longer (L, 16-repeat) repeat sequence, which are associated with lower and higher transcriptional efficiency respectively (Bradley et al., 2005; Lesch et al., 1996). Despite many years of investigation, the precise molecular and cellular basis of 5-HTTLPR in relation to anxiety and affective disorders remains unclear. Some studies report a link between 5-HTTLPR and depression, anxiety, or personality traits related to them such as neuroticism, equally as many others report either no associations, or associations only in the presence of specific early life stressors (Brandt et al., 2015; Caspi et al., 2003; Clarke et al., 2010; Mazzanti et al., 1998; Munafo et al., 2008; Odgerel et al., 2013; Risch et al., 2009).

There are several possible explanations for these contrary findings. One is that 5-HTTLPR (or the serotonin transporter more generally) does not play a causal role in mood disorders. But it may also be that there *is* a role, but one that is difficult to identify by targeting outcomes solely defined by clinical phenomenology. Depression and anxiety disorders are particularly heterogeneous disorders, with wide ranges of qualifying symptoms and severity. Expression of symptoms further varies across age and culture, adding heterogeneity. While not a direct stand-in for psychopathology, behavioral traits may provide a *complementary* approach to help identify the clinical sequelae posed by genetic risk factors. Unlike diagnoses, which manifest in episodes, behavioral traits are more stable and less vulnerable to assessment timeframe. Many have established indices of validity, reliability, and population norms, making them useful research tools (Rush AJ, 2008). By being scalar variables, they also offer greater statistical power and the ability to capture additional sub-clinical variation lost in a binary classification. Examining behavioral traits, whether as independent outcomes, or as intermediary points on the pathway to diagnoses, may be useful, particularly in genetic studies, where statistical power is at a premium.

We implement the above strategy within a longitudinal study of major depressive disorder (MDD), in which we had previously reported that 5-HTTLPR genotypes varied by familial risk for depression (Gori et al., 2014; Rocha et al., 2015). Specifically, 2nd or 3rd generation offspring who were at high familial risk for depression (by virtue of coming from a family where the 1st generation proband had MDD) were more likely to have two copies of the short allele than offspring at low risk (i.e., no depression in the 1st generation). Since that time, additional family members have aged into the study, and we have acquired new trait measures related to anxiety and depression. We focus on three of them—impulsivity, aggression, and neuroticism—that are well suited to this analysis by virtue of their being (1) stable constructs that run in families, (2) predictive of psychopathology, and (3) tunable by serotonergic neurotransmission (Balestri et al., 2014; Fineberg et al., 2014; Waltes et al., 2015). Impulsivity, broadly defined, is a predisposition to rapid or unplanned action without consideration of consequences; aggression, a predisposition to overt hostility; and neuroticism, a predisposition to negative responses to threat and difficulty. Genetic variation within the serotonin transporter has been previously associated with neuroticism (Lesch et

al., 1996), impulsivity (Sakado et al., 2003; Stoltenberg et al., 2012; Varga et al., 2012) and aggression (Baler et al., 2008; Conway et al., 2012), although the directionality of effect has not always been consistent. Correspondingly, serotonin transporter density and binding levels also have been shown to correlate with neuroticism (Chang et al., 2017), impulsivity (Marazziti et al., 2010; Patkar et al., 2003) and aggression (Coccaro et al., 2010; Marseille et al., 2012; Patkar et al., 2003) scores.

The goal of the present study is to examine associations between 5-HTTLPR and (1) behavioral traits related to anxiety and depression, and (2) clinical diagnoses of anxiety and depression; and (3) to explore whether the behavioral traits mediate the relationship between 5-HTTLPR and anxiety or depression. We hypothesize that low-transcription (i.e., short) alleles at 5HTTLPR will be associated with increased behavioral trait scores and diagnoses related to depression and anxiety, and that the traits will mediate the relationship between genotype and diagnosis. Finally, our primary analyses define 5-HTTLPR genotype by the aforementioned allele length (that is, short versus long, or bi-allelic). We will also explore a second tri-allelic classification increasingly adopted in the literature [e.g. (Hildebrandt et al., 2016; Schiele et al., 2016)], that is defined as a change-of-function haplotype between 5-HTTLPR and a neighboring polymorphism, rs25531. 5-HTTLPR and rs25531 interact functionally such that a G allele at rs25531 diminishes transcriptional ability of the long allele at 5HTTLPR. Decreased transcriptional efficiency of 5HTTLPR can be obtained either by the presence of the short allele at 5HTTLPR itself, or the presence of the longer allele in conjunction with the G allele at rs25531 (L-G). Convergence across these approaches will give us increased confidence in findings.

2. Methods

2.1 Cohort description

The sample has been previously described in several publications (Weissman et al., 2006; Weissman et al., 2005). Briefly, the study began in 1982 with simultaneous recruitment of two groups of probands (Generation (G)1). Depressed probands were selected from outpatient psychiatric clinics for the treatment of mood disorders in the New Haven, CT, area and were required to have moderate-to-severe MDD with impairment. Non-depressed probands were selected from the same community, and were required to have no lifetime history of psychiatric illness, based on several interviews. All probands were of European, primarily Southern Italian, ancestry. Their biological children (G2), and subsequently, grandchildren (G3) were followed prospectively over time. The offspring of the depressed probands formed the “high-risk” group, and those of the non-depressed probands, the “low risk” group (Weissman et al., 2006; Weissman et al., 2005).

There were six waves of assessments including at year 0 (baseline/recruitment) and approximately 2, 10, 20, 25, and 30 years thereafter. Diagnostic assessments were completed at each wave; DNA samples were collected at year 25 or 30, and trait measures were collected at the most recent wave, when all participants were 18yrs or older. Each family member was interviewed independently and blind to the clinical status of other family members, by trained doctoral- and masters-level mental health professionals (reliability has been documented elsewhere (Weissman et al., 2006; Weissman et al., 2005)). All procedures

were approved by the institutional review board at New York State Psychiatric Institute, and informed consent was obtained.

2.2 Participants and assessments

The target sample was the 248 2nd and 3rd generation biological offspring of the probands who had completed the behavioral assessments and clinical interviews at the most recent (6th) wave. The 203 (82%) of these who provided DNA form the analytic sample. The 203 individuals were from 60 original proband families; 113 (56%) were 2nd and 90 (44%) 3rd generation. Participants with/without DNA did not vary by any clinical or behavioral outcomes (all $ps > 0.5$).

Diagnostic Interviews were conducted using the adult (Mannuzza et al., 1986) or child (6–17 years) (Kaufman et al., 1997) version of the semi-structured Schedule for Affective Disorders and Schizophrenia–Lifetime interview. The first interview assessed the lifespan to that point; follow-up interviews assessed catch-up periods; diagnoses are therefore cumulative. Assessments were administered by trained doctoral- and masters-level mental health professionals, blind to genotype or family history. Final diagnoses were made using the best-estimate procedure (Fried et al., 1987) by two experienced clinicians, also blind to genotype.

Behavioral Measures were administered by a trained Masters' level research assistant in person or over the telephone (scores did not vary by method). The Barratt Impulsiveness Scale-11 (BIS-11) (Patton et al., 1995) is one of the most frequently used measures of trait impulsiveness. It includes 30 questions that yield a total score, as well as 6 first order (attention, cognitive complexity, cognitive instability, motor, perseverance, and self-control), and three 2nd-order factors (attention, which includes the 1st order attention and cognitive instability factors; motor, which includes the motor and perseverance factors; and non-planning, which includes the cognitive complexity and self-control factors). A total score of 70 or higher typically classifies an individual as highly impulsive. Internal consistency is high, with coefficients ranging from .79 to .83 for undergraduate, substance abusing, and general psychiatric patients, and the scale has good reliability and validity with other measures of impulsiveness (Petry, 2001; Rush AJ, 2008). Given questions in the literature about the best application of BIS-11 factors (Reise et al., 2013; Steinberg et al., 2013), and that we found greater internal consistency for the full scale (Cronbach alpha = 0.83) and 2nd-order factors (alpha, 0.63–0.78) than 1st-order factors (alpha, 0.34–.74), we use the total and second-order factors as our primary outcomes (main findings from Table 1 are also shown in E-Table 2 using the first-order factors).

The Buss Perry Aggression Questionnaire (Buss and Perry, 1992; Gerevich et al., 2007), includes 29 questions grouped into four factors: physical aggression, verbal aggression, anger and hostility. The scale has been validated in both college students and in population-based samples, and has high internal consistency (Buss and Perry, 1992). Neuroticism was evaluated using the neuroticism domain of the 60-item NEO five-factor inventory (NEO-FFI) (McCrae and Costa, 1997; McCrae et al., 1999) self-report, which operationalizes a hierarchical structure based on the five factor model of personality (neuroticism-extraversion-openness-agreeableness-conscientiousness), and has high test-retest reliability,

construct and predictive validity, heritability, and evidence for cross-cultural universality (McCrae and Costa, 1997; McCrae et al., 1999). There were no missing data for impulsivity and aggression. Neuroticism data were collected as part of a separate study, and were only available on 132 (64%) of individuals. Individuals with and without neuroticism data did not vary by genotype or by mean impulsivity or aggression scores. Despite the missing data, we include neuroticism, given the theoretical framework and hypothesized associations with internalizing disorders and with serotonergic transmission.

2.3 Genotyping

DNA collection and error control have been described previously (Odgerel et al., 2013; Talati et al., 2015; Talati et al., 2013), and thus only briefly summarized here. DNA was extracted from saliva collected using Oragene DNA Self Collection Kit following the protocol provided by the manufacture (Oragene Genotek, Ontario, Canada). The region encompassing 5-HTTLPR was amplified with primers (forward: 5'-TCCTCCGCTTTGGCGCCTCTTCC-3'; reverse 5'-TGGGGGTTGCAGGGGAGATCCTG-3') via a polymerase chain reaction in multiplex master mix (Qiagen, CA). Amplicon was resolved on a 2.3% UltraPure™ Agarose (Invitrogen, CA), and visualized under the UV transilluminator. The 512bp and 469bp bands were called as L and S alleles, respectively. For rs25531, amplicon was digested with restriction endonuclease MspI (New England Biolabs@Inc., Boston, MA, USA), and the product resolved in a 2.9% UltraPure Agarose (Invitrogen) and visualized under the UV transilluminator. Digested fragments of 402 bp were called as G at rs25531. Parallel analysis of amplicon and restriction fragment products allowed us to determine a phase of the 5-HTTLPR/rs25531 haplotype in each individual. Genotype calling was blind to subject familial risk group or MDD status. Ninety eight percent of participants were genotyped twice, and there were no discrepancies between original and replication calls. Mendelian errors were controlled for using famtypes software (Baldacara et al., 2008), and quality control analysis using PLINK (Purcell et al., 2007).

2.4 Classification of genotypes

Two types of association tests were performed. Primary tests were based on 5HTTLPR genotype only (i.e., LL, SL, SS). We then tested a change-of-function haplotype at SLC6A4 promoter composed by 5-HTTLPR and rs25531. The phase of this two-SNP haplotype was derived directly from the genotyping assay (as described above). Because the G allele at rs25531 leads to diminished SLC6A4 transcription efficiency, we recoded possible haplotypes as follows, to reflect the corresponding functionality: S-A, S-G and L-G haplotypes were recoded as “low-function”, and L-A as “high-function”. Each participant could thus have two low-function, two high-function, or one high-, one low-function allele.

Concordance between the primary (P) and haplotype (H)-defined variants was high. Of 193 (95.1%) individuals successfully genotyped at rs25531, 179 (93%) were fully concordant; that is, 27 individuals with short/short genotypes under the primary definition were also low/low in the haplotype definition; 108 short/long were also low/high; and 44 long/long were also high/high. Only 14 (7%) individuals changed grouping: 10 long/long became low/high, and 4 short/long became low/low.

2.5 Statistical Analysis

Analyses were conducted using Statistical Analysis Software (SAS®) 9.4 (Cary, NC). Group differences among continuous outcomes by genotypes were tested using a 3-way ANOVA, followed by post-hoc tukey's tests when the omnibus test was significant. For diagnoses, a chi-square test was used. To formally estimate the effect parameters of genotype on outcome while adjusting for potential non-independence of outcomes among offspring from the same family, we used a generalized estimating equations (GEE) approach, by means of the GENMOD procedure in the SAS software package. Behavioral traits (continuous) or diagnoses (categorical) served as the outcome variable, genotype as the independent variable, with age and sex as co-variates. Nominal p values are presented; findings that survive family-wise error rate (FWE) corrections are indicated alongside in table 1. While p values are important, effect size and directions should be also be considered when interpreting results, as 5-HTTLPR is a known polymorphism with existing directional hypothesis we are trying to replicate and extend. [The null hypothesis thus is not 0 (Caspi et al., 2010)].

Finally, we explored whether behavioral traits may account for an association between genes and psychiatric disorders, using path analyses. The model assumes that causation flows only in one direction, and there is no feedback loop. Diagnoses serve as the (categorical) endogenous variables, and genotype and behavioral traits (mediator, M) as the exogenous variables (see discussion). Age and gender were included as covariates in all models. We also included family ID as a cluster-level variable in the analysis, to account for potential non-independence of outcomes among related individuals. The following equations were modeled: Eq.1: diagnosis (Y)= constant+ β_1 _{genotype}+ β_2 _{trait}+ β_3 _{age}+ β_4 _{gender}; Eq.2: trait (M)=constant+ β_1 _{genotype}+ β_2 _{age}+ β_3 _{gender}. The product of the trait coefficient (β_2) from Eq.1 and the genotype coefficient (β_1) from Eq.2 models the indirect gene effect on outcome; β_1 in Eq.1 models the direct effect.

3. Results

Mean sample age was 34.17±13.8 (yrs/std), 53.8% female, and 64% from families at high risk for depression. Familial risk, gender, generation, and age did not vary by whether the participant provided DNA.

3.1 Associations between genotype and trait measures of depression and anxiety

Impulsivity, aggression, and neuroticism scores did not vary significantly by gender, age, generation, or presence/absence of DNA, with one exception: females had lower scores than males for physical aggression (21.13 vs 28.7, $t=5.39$, $p<0.0001$).

We first examined associations between 5-HTTLPR and each trait measure independently (note, correlation between traits is shown in e-Table 2). As shown in Table 1a, 5-HTTLPR was associated with impulsivity and (trend) neuroticism. *Post-hoc* contrasts (right-column) showed that individuals with two risk alleles (i.e., SS) had higher impulsivity or neuroticism than those with either one or no s alleles. We next examined the above associations within high-risk (Table 1b, middle panel) and low-risk (1C, bottom panel) separately. All

associations were specific to the high-risk group, where SS was associated with higher impulsivity (attentional and non-planning but not motor), hostility, and neuroticism. There were no significant associations in the low-risk offspring.

Given the above patterns, we collapsed the SL and LL genotypes among high-risk offspring to model the overall effect conferred by having two risk alleles. After adjusting for age, sex, and inter-family relatedness (see methods), offspring with the two risk alleles (i.e., SS genotype), had significantly higher impulsivity (~13% higher), hostility (31% higher) and neuroticism (23% higher) than those with one or no risk alleles (Table 2). These associations did not vary by gender (genotype x gender interaction, $p > 0.40$ for all outcomes, not shown). The association between 5-HTTLPR genotype and impulsivity remained significant after co-varying for either hostility (beta = 4.48 (s.error, 1.89), $p = 0.019$) or neuroticism (beta = 7.16 (2.94), $p = 0.017$); however the relationship between 5-HTTLPR and neuroticism, and between 5HTTLPR and hostility, did not survive adjustments for other traits (see Table 2, right columns for details).

3.3 Exploration of alternative change-of-function haplotype

We also explored the alternative classification of transcriptional efficiency based on the change-of-function haplotype at 5HTTLPR-rs25531 (see methods). Distributions of trait scores by haplotype are shown in e-Table 3. Akin to the 5HTTLPR findings, 5-HTTLPR-rs25531 was associated with higher rates of impulsivity and neuroticism, and these increases were exclusively accounted for by individuals who had two copies of the risk (low-functioning) alleles.

3.2 Associations between genotype and clinical diagnoses

We examined associations between genotypes and clinical outcomes (Table 3). In high-risk offspring, 5-HTTLPR was associated with panic disorder and phobic disorders, but not major depression or any other depressive disorders. *Post hoc* tests showed that for panic there was an additive effect of genotype, where individuals with SS were significantly more likely than those with SL, who were in turn more likely than those with LL genotypes, to have the disorder. For phobias, individuals with SS had higher rates than those with either one or no S alleles.

Formally modeling the effects of genotype on these disorders in the high risk group, while further accounting for age, sex, and family inter-relatedness, showed that individuals with SS, as compared to other genotypes, had higher rates of panic (Adjusted Odds Ratio, AOR, 7.05 [2.44, 20.38], $p = 0.0003$) and phobic (AOR = 2.68 [1.04, 6.93], $p = 0.04$) disorders. Also, though not statistically significant, onset age for panic disorder was a full decade earlier in those with the SS (14.5 ± 9.6 yrs) as compared to other (25.6 ± 18.7 yrs) genotypes ($t = 1.66$, $d.f. = 18.8$, $p = 0.11$). Age of onset for phobias however were similar across those with SS (10.2 ± 10.4 yrs) vs other (8.9 ± 10.8 yrs) genotypes ($t = 0.43$, $d.f. = 61$, $p = 0.67$).

3.4 Linking 5-HTTLPR, behavioral traits and clinical diagnoses

Given that 5-HTTLPR predicted both behavioral traits (higher impulsivity, hostility, and neuroticism) and diagnoses (higher rates of panic and phobic disorders), we wanted to

further test whether the traits played a role in the latter association between genotype and diagnoses. Specifically, we wanted to distinguish between two alternative models: one in which 5-HTTLPR increased risk for behavioral traits and for diagnoses, but these two risks were independent of each other; and the other, where 5-HTTLPR increased risk for behavioral traits, which in turn increased risk for diagnoses, such that conditional on traits, there was no direct association between 5-HTTLPR and diagnosis.

We first examined direct associations between behavioral traits and diagnosis (Table 4). Panic disorder (left) was associated with increased impulsivity (attention and planning), aggression (anger and hostility but not physical or verbal aggression). Phobic disorders were associated with all factors of impulsivity and aggression (except verbal). Associations remained significant when adjusting models for 5-HTTLPR genotype, indicating that behavioral traits and anxiety disorders are not related to each other solely through genotype. Finally, we used path models to more formally test whether impulsivity might explain the relationship between 5-HTTLPR genotype and panic disorder (Fig 1A) or between 5-HTTLPR and phobic disorders (1B). As shown in Figure 1, 16% of the association between 5-HTTLPR and panic disorder was explained by total impulsivity, and 48% for phobic disorders (see discussion for interpretive limitations).

4. Discussion

We showed that individuals at high familial risk for depression who had two copies of the shorter (lower transcribed) allele at the serotonin transporter promoter linked polymorphism (5-HTTLR) had (1) higher impulsivity, hostility, and neuroticism, and (2) higher rates of fear-based anxiety disorders but not other diagnoses. The association with impulsivity was strongest, surviving corrections for multiple comparisons, and explaining 16% and 52% of the associations of 5-HTTLPR with panic and phobic disorders respectively. Similar findings are obtained when using an alternate change-of-function haplotype that is associated with 5-HTTLPR.

The BIS-11 is commonly used to assess stable cognitive domains of impulsivity in both clinical and healthy populations. Total scores ≥ 70 suggest pathological impulsivity, and ≥ 75 , the presence of an impulse control disorder (Barratt ES, 2005; Patton et al., 1995). In the present study, which was not based on a clinical population, mean scores in the highest risk (SS) group were 64.6 ± 11 , with 32% of individuals scoring ≥ 70 . SS was associated with total impulsivity as well as two of the three BIS-11 2nd-order factors: attentional impulsivity, which indexes attention, concentration, and evenness of thought; and non-planning impulsivity, which indexes complexity of cognitive thoughts and self-control. Motor impulsivity, which assesses facets of imminent behavioral action was not associated with serotonergic transmission, suggesting that serotonin transmission is more directly tied to cognitive impulsivity (that is, impulsivity of thought) rather than behavioral impulsivity (impulsivity of action), a distinction also discussed in more detail elsewhere (Reise et al., 2013).

Though a number of studies have identified relationships between serotonergic genes and impulsivity, the majority of those examining the serotonin transporter gene have identified

significant associations only when considering the gene's interaction with other genetic or environmental factors. For example, studies have found that individuals who have risk variants at serotonergic genes (e.g., serotonin receptor 2A or monoamine oxidase (MAO-A)) had higher trait impulsivity (Paaver et al., 2007; Stoltenberg et al., 2012). Another study similarly showed no independent effect of 5-HTTLPR on impulsivity, but found that the genotype moderated the associations between early childhood adversity and adulthood impulsivity (Wagner et al., 2009). A study of Japanese adult males reported a direct association between the 5-HTTLPR SS genotype and BIS-11 total impulsivity (Sakado et al., 2003), consistent with our work. However, Asian populations have higher rates of the s allele than Caucasians (Odgerel et al., 2013), so direct comparisons may be problematic. Regardless, the present study is the first to our knowledge to extend this work to test the role that impulsivity traits play on the pathway from serotonin transporter gene variation to psychiatric diagnoses.

In addition to impulsivity, we also found associations between 5-HTTLPR and aggression (hostility) and neuroticism. Although 5-HTTLPR, and serotonin transmission more generally, has been associated with aggression (see introduction), no other study has tested the relationship between 5-HTTLPR allele length and aggression traits measured by the Buss Perry Aggression Questionnaire. A study of the monoamine oxidase gene (which degrades serotonin) found that fewer repeats of the variable number tandem repeat (VNTR) region of MAOA were associated with higher BIS-11 as well as physical aggression (Lim et al., 2015). Finally, 5-HTTLPR has been previously linked to neuroticism in a number of studies, including in seminal work by Lesch et al (Lesch et al., 1996) who found individuals with 1 s allele to have 12% higher neuroticism scores. We found a larger increase (+23%), but only when *both* copies of the short allele were present.

Clinical associations with 5-HTTLPR were only observed for panic disorder and phobias. There were no associations for generalized anxiety disorder, and rates of other anxiety disorders (e.g., OCD, PTSD) were too low to permit analyses. Similar to trait findings, the associations were only observed if two copies of the s allele were present. This diagnostic specificity of findings to the fear-based phenotypes of the anxiety spectrum is particularly interesting as 5-HT transmission is intricately associated with fear circuitry: Individuals with s alleles express greater fear-potentiated skin conductance response (Garpenstrand et al., 2001), amygdala reactivity (Lonsdorf et al., 2009) and eye-blink startle reflex (Klumpers et al., 2012). The associations of 5-HTTLPR with phobic and panic outcomes but lack of associations with generalized anxiety is also consistent with work postulating distinct genetic underpinnings for 'anxious-misery' versus 'fear circuitry' driven internalizing disorders (Kendler et al., 2003).

Finally, our analyses suggest that impulsivity may account for some of the relationship between serotonin transmission and fear based anxiety disorders. Based on prior literature, we expected strongest accounting for by neuroticism (see limitations). However, studies have found that patients with panic disorder to have higher overall impulsivity and cognitive defects related to impulsivity (Jakuszkowiak-Wojten et al., 2013), and specific features such as worry about health and disability in panic patients correlate positively with BIS-11 impulsivity scores (Jakuszkowiak-Wojten et al., 2015). Individuals with phobic disorders

also have high impulsivity and impulsivity-related problems such as ADHD, addictive behaviors and gambling (Koyuncu et al., 2016; Porteret et al., 2016). In our study, the indirect (mediated) effect was more prominent for phobic disorders but the direct path more prominent for panic disorder. Furthermore, the total overall association (that is, the sum of the direct and indirect paths in Figure 1) was large for panic disorder, suggesting that serotonin transmission (as proxied by the 5-HTTLPR) plays both a larger, and a more direct role in panic disorder pathology.

There is an important interpretive caveat to this analysis. Even though we show that trait measures explain some of the relationship between genes and clinical anxiety (the converse sequence, i.e., genes to diagnosis to traits is biologically less plausible as a true predisposing trait cannot emerge in response to a clinical outcome), the statistical model does not inform on biological sequence. Furthermore, strongest associations were observed for the fear-based diagnoses emerging in pre- and early teens, a time when cortical regulatory pathways are also still developing and long-term traits also stabilizing. The inability to temporally separate onset of traits from those of diagnoses prevents elucidating a formal sequence of events, and the path analysis should be viewed as an overall illustration rather than a formal temporal mediation model. But even if behavioral traits did mediate effects of early serotonergic variation on clinical diagnoses, there need not be a single etiological path. Impulsivity, for example, is a multidimensional construct determined by several layers of cortical attentional and self-regulatory processing (Fineberg et al., 2014; Meda et al., 2009; Moeller et al., 2001). The serotonin transporter, similarly, is expressed differentially across brain regions regulating emotions (Chen et al., 1992; Whitaker-Azmitia et al., 1990). Bottom-up pathways, including from the 5-HT-rich raphe nuclei, are initially dominant but are followed by top-down cortical control systems that work to create the optimal output. The role of 5-HT is not uniformly distributed across these systems. It could be that the more primitive systems are under 5-HT control (thus explaining the stronger associations between 5-HTTLPR and trait scores), and that broader disturbances in cortical regulatory circuits through a combination of biology and developmental experiences are required for psychiatric phenotypes to emerge.

Other methodological limitations should be acknowledged. First, the sample is drawn from a study of families at high- or low-risk for major depression, neither of which reflect the general population. Because so few individuals in the low-risk group had the SS genotype, we could not directly compare effects in the high- and low-risk groups. We also performed a number of tests in a sample that is relatively small by genetic standards (though similar to some other 5-HTTLPR studies (Lee et al., 2003; Liao et al., 2004; Sakado et al., 2003)); thus findings should be considered provisional until independently replicated. On the other hand, lack of findings (particularly for neuroticism, where data were only available for 65% of the full sample), could be due to lack of power rather than lack of an effect. Finally, the sample was European (mostly Southern-Italian), so findings may not generalize to other populations, particularly given well-documented ethnic differences for 5-HTTLPR (Murdoch et al., 2013).

In the present study, we only examine a single polymorphism. This spotlight is warranted given the ongoing discussion and controversies about the role of 5-HTTLPR. *In situ*

however, more complex interactions between multiple genes and environments will likely contribute to the effects of 5-HT variation in psychiatric illness (Dunn et al., 2015; Thomas, 2010; Thomas et al., 2012).

Despite the limitations, our work suggests that trait measures can serve as useful independent outcomes as well as potential mediators, in genetic studies of psychiatric disorders. Existing studies may wish to test, when data allow, whether inclusion of behavioral variables illuminates prior associations found between genes and clinical disorders. Our findings are also broadly consistent with the NIMH's Research Domain Criteria (RDoC) conceptualization (Cuthbert and Insel, 2013; Insel et al., 2010; Insel, 2014) that behavioral traits lie more 'upstream' to underlying genes than clinical diagnoses, and associations between genes and traits should be *less* noisy and more easily detectable. Indeed, this conceptualization is reflected in our mediation models where the variance of the indirect pathways is smaller than that of the direct paths.

Finally, regardless of any association with specific psychiatric outcomes or genotypes, extreme impulsivity, aggression and neuroticism are each maladaptive traits. Identifying individuals who lie outside population norms can be helpful, as many behavioral therapies are geared to modulate the cognitive malfunctions underlying these traits, and can lead to improved functional outcomes even in the absence of psychopathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources:

The study is funded in part by grants from the National Institute of Mental Health (**R01MH36197**, Weissman PI, and **P50MH090966**, Gingrich/Weissman, PIs), National Institute of Drug Abuse (**K01DA029598**, Talati, PI), NARSAD Young Investigator Awards from the Brain and Behavior Research Foundation (Talati, PI) and the Sackler Institute for Developmental Psychobiology.

References

- Balestri M, Calati R, Serretti A, De Ronchi D. Genetic modulation of personality traits: a systematic review of the literature. *International clinical psychopharmacology*. 2014; 29:1–15. [PubMed: 24100617]
- Baler RD, Volkow ND, Fowler JS, Benveniste H. Is fetal brain monoamine oxidase inhibition the missing link between maternal smoking and conduct disorders? *Journal of psychiatry & neuroscience*. 2008; 33:187–195. [PubMed: 18592036]
- Barratt ESLM, Moeller FG. When does impulsivity become pathologic? *Psychiatric Times*. 2005; 22:23–26.
- Bradley SL, Dodelzon K, Sandhu HK, Philibert RA. Relationship of serotonin transporter gene polymorphisms and haplotypes to mRNA transcription. *Am J Med Genet B Neuropsychiatr Genet*. 2005; 136B:58–61. [PubMed: 15858822]
- Brandt H, Umbach N, Kelava A. The Standardization of Linear and Nonlinear Effects in Direct and Indirect Applications of Structural Equation Mixture Models for Normal and Nonnormal Data. *Frontiers in psychology*. 2015; 6:1813. [PubMed: 26648886]

- Buss AH, Perry M. The aggression questionnaire. *J Pers Soc Psychol.* 1992; 63:452–459. [PubMed: 1403624]
- Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psych.* 2010; 167:509–527.
- Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* 2003; 301:386–389. [PubMed: 12869766]
- Chang CC, Chang HA, Fang WH, Chang TC, Huang SY. Gender-specific association between serotonin transporter polymorphisms, 5-HTTLPR and rs25531 and neuroticism, anxiety and depression in well-defined healthy Han Chinese. *J Affect Disord.* 2017; 207:422–428. [PubMed: 27788383]
- Chen HT, Clark M, Goldman D. Quantitative autoradiography of 3H-paroxetine binding sites in rat brain. *Journal of pharmacological and toxicological methods.* 1992; 27:209–216. [PubMed: 1421530]
- Clarke H, Flint J, Attwood AS, Munafo MR. Association of the 5-HTTLPR genotype and unipolar depression: a meta-analysis. *Psychol Med.* 2010; 40:1767–1778. [PubMed: 20380781]
- Conway CC, Keenan-Miller D, Hammen C, Lind PA, Najman JM, Brennan PA. Coaction of stress and serotonin transporter genotype in predicting aggression at the transition to adulthood. *Journal of clinical child and adolescent psychology. American Psychological Association, Division.* 2012; 53(41):53–63.
- Coccaro EF, Lee R, Kavoussi RJ. Inverse relationship between numbers of 5-HT transporter binding sites and life history of aggression and intermittent explosive disorder. *J Psychiatr Res.* 2010; 44:137–142. [PubMed: 19767013]
- Cuthbert BN, Insel TR. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC medicine.* 2013; 11:126. [PubMed: 23672542]
- Dunn EC, Brown RC, Dai Y, Rosand J, Nugent NR, Amstadter AB, Smoller JW. Genetic determinants of depression: recent findings and future directions. *Harv Rev Psych.* 2015; 23:1–18.
- Fineberg NA, Chamberlain SR, Goudriaan AE, Stein DJ, Vanderschuren LJ, Gillan CM, et al. New developments in human neurocognition: clinical, genetic, brain imaging correlates of impulsivity and compulsivity. *CNS spectrums.* 2014; 19:69–89. [PubMed: 24512640]
- Fried PA, Watkinson B, Dillon RF, Dulberg CS. Neonatal neurological status in a low-risk population after prenatal exposure to cigarettes, marijuana, alcohol. *J developmental and behavioral pediatrics: JDBP.* 1987; 8:318–326.
- Garpenstrand H, Annas P, Ekblom J, Orelund L, Fredrikson M. Human fear conditioning is related to dopaminergic and serotonergic biological markers. *Behavioral neuroscience.* 2001; 115:358–364. [PubMed: 11345960]
- Gerevich J, Bacskai E, Czobor P. The generalizability of the Buss-Perry Aggression Questionnaire. *Int J Methods Psychiatr Res.* 2007; 16:124–136. [PubMed: 17849418]
- Gori A, Craparo G, Sareri GI, Caretti V, Giannini M, Meringolo P. Antisocial and psychopathic personalities in a sample of addicted subjects: differences in psychological resources, symptoms, alexithymia and impulsivity. *Comprehensive psychiatry.* 2014; 55:1580–1586. [PubMed: 25081732]
- Hildebrandt A, Kiy A, Reuter M, Sommer W, Wilhelm O. Face and emotion expression processing and the serotonin transporter polymorphism 5-HTTLPR/rs22531. *Genes, brain, behavior.* 2016; 15:453–464.
- Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, et al. Research domain criteria, RDoC: toward a new classification framework for research on mental disorders. *Am J Psychiatry.* 2010; 167:748–751. [PubMed: 20595427]
- Insel TR. The NIMH Research Domain Criteria, RDoC Project: precision medicine for psychiatry. *Am J Psychiatry.* 2014; 171:395–397. [PubMed: 24687194]
- Jakuszkowiak-Wojten K, Galuszko-Wegielnik M, Raczak A, Cubala WJ, Wiglusz MS, Herstowska M, et al. Impulsivity in panic disorder: neuropsychological correlates. *Psychiatria Danubina.* 2013; 25(Suppl 2):S149–152. [PubMed: 23995165]

- Jakuszkowiak-Wojten K, Landowski J, Wiglusz MS, Cubala WJ. Impulsivity and Panic Disorder: an exploratory study of psychometric correlates. *Psychiatria Danubina*. 2015; 27(Suppl 1):S456–458. [PubMed: 26417815]
- Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, K-SADS-PL: initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry*. 1997; 36:980–988. [PubMed: 9204677]
- 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]
- Clummers F, Heitland I, Oosting RS, Kenemans JL, Baas JM. Genetic variation in serotonin transporter function affects human fear expression indexed by fear-potentiated startle. *Biological psychology*. 2012; 89:277–282. [PubMed: 22061270]
- Koyuncu A, Celebi F, Ertekin E, Kok BE, Tukul R. Attention deficit and hyperactivity in social anxiety disorder: relationship with trauma history and impulsivity. *Attention deficit and hyperactivity disorders*. 2016; 8:95–100. [PubMed: 26797941]
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996; 274:1527–1531. [PubMed: 8929413]
- Lim JA, Gwak AR, Park SM, Kwon JG, Lee JY, Jung HY, et al. Are adolescents with internet addiction prone to aggressive behavior? The mediating effect of clinical comorbidities on the predictability of aggression in adolescents with internet addiction. *Cyberpsychology, behavior and social networking*. 2015; 18:260–267.
- Lonsdorf TB, Ruck C, Bergstrom Jersson G, Ohman A, Schalling M, et al. The symptomatic profile of panic disorder is shaped by the 5-HTTLPR polymorphism. *Prog Neuropsychopharmacol Biol Psychiatry*. 2009; 33:1479–1483. [PubMed: 19683026]
- Mannuzza S, Fyer AJ, Klein DF, Endicott J. Schedule for Affective Disorders and Schizophrenia--Lifetime Version modified for the study of anxiety disorders, SADS-LA: rationale and conceptual development. *J Psychiatr Res*. 1986; 20:317–325. [PubMed: 3806426]
- Marazziti D, Baroni S, Masala I, Golia F, Consoli G, Massimetti G, Picchetti M, Catena Dell'osso M, Giannaccini G, Betti L, et al. Impulsivity, gender, the platelet serotonin transporter in healthy subjects. *Neuropsychiatric disease and treatment*. 2010; 6:9–15. [PubMed: 20169041]
- Marseille R, Lee R, Coccaro EF. Inter-relationship between different platelet measures of 5-HT and their relationship to aggression in human subjects. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012; 36:277–281. [PubMed: 22019855]
- Mazzanti CM, Lappalainen J, Long JC, Bengel D, Naukkarinen H, Eggert M, et al. Role of the serotonin transporter promoter polymorphism in anxiety-related traits. *Arch Gen Psychiatry*. 1998; 55:936–940. [PubMed: 9783565]
- McCrae RR, Costa PT Jr. Personality trait structure as a human universal. *The American psychologist*. 1997; 52:509–516. [PubMed: 9145021]
- McCrae RR, Costa PT Jr, Pedrosa de Lima M, Simoes A, Ostendorf F, Angleitner A, et al. Age differences in personality across the adult life span: parallels in five cultures. *Dev Psychol*. 1999; 35:466–477. [PubMed: 10082017]
- Meda SA, Stevens MC, Potenza MN, Pittman B, Gueorguieva Rrews MM, et al. Investigating the behavioral and self-report constructs of impulsivity domains using principal component analysis. *Behavioural pharmacology*. 2009; 20:390–399. [PubMed: 19724194]
- Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC. Psychiatric aspects of impulsivity. *Am J Psychiatry*. 2001; 158:1783–1793. [PubMed: 11691682]
- Munafò MR, Brown SM, Hariri AR. Serotonin transporter 5-HTTLPR genotype and amygdala activation: a meta-analysis. *Biol Psychiatry*. 2008; 63:852–857. [PubMed: 17949693]
- Murdoch JD, Speed WC, Pakstis AJ, Heffelfinger CE, Kidd KK. Worldwide population variation and haplotype analysis at the serotonin transporter gene SLC6A4 and implications for association studies. *Biol Psychiatry*. 2013; 74:879–889. [PubMed: 23510579]

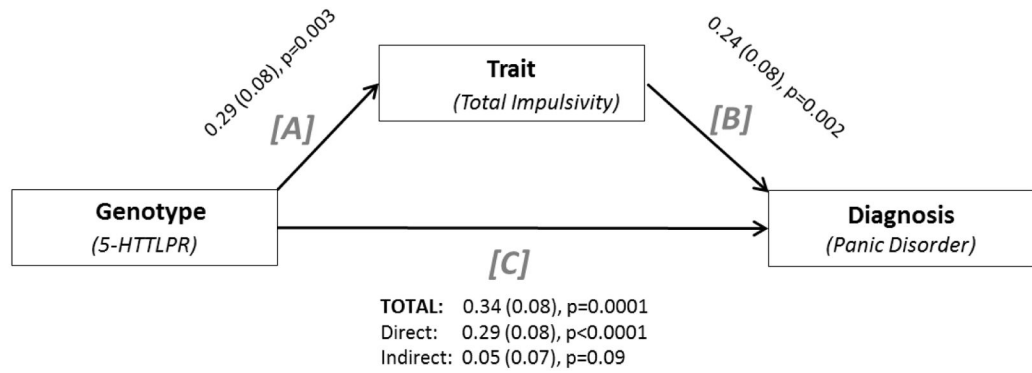
- Odgerel Z, Talati A, Hamilton SP, Levinson DF, Weissman MM. Genotyping serotonin transporter polymorphisms 5-HTTLPR and rs25531 in European- and African-American subjects from the National Institute of Mental Health's Collaborative Center for Genomic Studies. *Transl Psychiatry*. 2013; 3:e307. [PubMed: 24064711]
- Paaver M, Nordquist N, Parik J, Harro M, Oreland L, Harro J. Platelet MAO activity and the 5-HTT gene promoter polymorphism are associated with impulsivity and cognitive style in visual information processing. *Psychopharmacology*. 2007; 194:545–554. [PubMed: 17628790]
- Patkar AA, Gottheil E, Berrettini WH, Hill KP, Thornton CC, Weinstein SP. Relationship between platelet serotonin uptake sites and measures of impulsivity, aggression, craving among African-American cocaine abusers. *The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions*. 2003; 12:432–447.
- Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. *Journal of clinical psychology*. 1995; 51:768–774. [PubMed: 8778124]
- Petry NM. Substance abuse, pathological gambling, impulsiveness. *Drug and alcohol dependence*. 2001; 63:29–38. [PubMed: 11297829]
- Porteret R, Bouchez J, Bayle FJ, Varescon I. ADHD/D and impulsiveness: Prevalence of impulse control disorders and other comorbidities, in 81 adults with attention deficit/hyperactivity disorder, ADHD/D. *L'Encephale*. 2016; 42:130–137.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007; 81:559–575. [PubMed: 17701901]
- Reise SP, Moore TM, Sabb FW, Brown AK, London ED. The Barratt Impulsiveness Scale-11: reassessment of its structure in a community sample. *Psychological assessment*. 2013; 25:631–642. [PubMed: 23544402]
- Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, et al. Interaction between the serotonin transporter gene, 5-HTTLPR, stressful life events, risk of depression: a meta-analysis. *JAMA*. 2009; 301:2462–2471. [PubMed: 19531786]
- Rocha MV, Nery-Fernandes F, Guimaraes JL, de Quarantini LC, de Oliveira IR, Ladeia-Rocha GG, et al. Normal Metabolic Levels in Prefrontal Cortex in Euthymic Bipolar I Patients with and without Suicide Attempts. *Neural plasticity*. 2015; doi: 10.1155/2015/165180
- Rush, AJ., FM, Blacker, D. *Hand Book of Psychiatric Measures*. 2. Arlington, VA: American Psychiatric Association Press; 2008.
- Sakado K, Sakado M, Muratake T, Mundt C, Someya T. A psychometrically derived impulsive trait related to a polymorphism in the serotonin transporter gene-linked polymorphic region, 5-HTTLPR in a Japanese nonclinical population: assessment by the Barratt impulsiveness scale, BIS. *Am J Med Genet B Neuropsychiatr Genet*. 2003; 121B:71–75. [PubMed: 12898578]
- Schiele MA, Ziegler C, Holitschke K, Schartner C, Schmidt B, Weber H, et al. Influence of 5-HTT variation, childhood trauma and self-efficacy on anxiety traits: a gene-environment-coping interaction study. *Journal of neural transmission*. 2016; 123:895–904. [PubMed: 27145764]
- Steinberg L, Sharp C, Stanford MS, Tharp AT. New tricks for an old measure: the development of the Barratt Impulsiveness Scale-Brief, BIS-Brief. *Psychological assessment*. 2013; 25:216–226. [PubMed: 23148649]
- Stoltenberg SF, Christ CC, Highland KB. Serotonin system gene polymorphisms are associated with impulsivity in a context dependent manner. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012; 39:182–191. [PubMed: 22735397]
- Talati A, Guffanti G, Odgerel Z, Ionita-Laza I, Malm H, Sourander A, et al. Genetic variants within the serotonin transporter associated with familial risk for major depression. *Psychiatry Res*. 2015; 228:170–173. [PubMed: 25920807]
- Talati A, Weissman MM, Hamilton SP. Using the high-risk family design to identify biomarkers for major depression. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2013; 368:201–20129.
- Thomas D. Gene-environment-wide association studies: emerging approaches. *Nat Rev Genet*. 2010; 11:259–272. [PubMed: 20212493]

- Thomas DC, Lewinger JP, Murcray CE, Gauderman WJ. Invited commentary: GE-Whiz! Ratcheting gene-environment studies up to the whole genome and the whole exposome. *Am J Epidemiol*. 2012; 175:203–207. discussion 208–209. [PubMed: 22199029]
- Varga G, Szekely A, Antal P, Sarkozy P, Nemoda Z, Demetrovics Z, Sasvari-Szekely M. Additive effects of serotonergic and dopaminergic polymorphisms on trait impulsivity. *Am J Med Genet B Neuropsychiatr Genet*. 2012; 159B:281–288. [PubMed: 22259185]
- Wagner S, Baskaya O, Lieb K, Dahmen N, Tadic A. The 5-HTTLPR polymorphism modulates the association of serious life events, SLE and impulsivity in patients with Borderline Personality Disorder. *J Psychiatr Res*. 2009; 43:1067–1072. [PubMed: 19358998]
- Waltes R, Chiochetti AG, Freitag CM. The neurobiological basis of human aggression: A review on genetic and epigenetic mechanisms. *Am J Med Genet B Neuropsychiatr Genet*. 2015; 171(5):650–75. [PubMed: 26494515]
- Weissman MM, Wickramaratne P, Nomura Y, Warner V, Pilowsky D, Verdeli H. Offspring of depressed parents: 20 years later. *Am J Psychiatry*. 2006; 163:1001–1008. [PubMed: 16741200]
- Weissman MM, Wickramaratne P, Nomura Y, Warner V, Verdeli H, Pilowsky DJ, et al. Families at high and low risk for depression: a 3-generation study. *Arch Gen Psychiatry*. 2005; 62:29–36. [PubMed: 15630070]
- Whitaker-Azmitia PM, Shemer AV, Caruso J, Molino L, Azmitia EC. Role of high affinity serotonin receptors in neuronal growth. *Annals of the New York Academy of Sciences*. 1990; 600:315–330. [PubMed: 2252318]

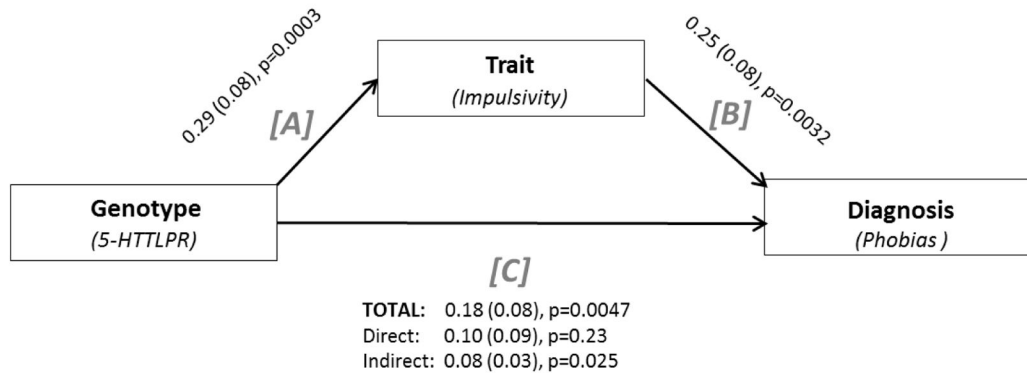
HIGHLIGHTS

- The role of serotonin transporter promoter-linked polymorphism (*5-HTTLPR*) in psychiatric disease remains unclear.
- We tested associations between 5-HTTLPR and behavioral traits and clinical diagnoses related to anxiety
- 5HTTLPR risk alleles were related to higher impulsivity, neuroticism, and hostility, and to fear-based (panic, phobia) but not other anxiety disorders
- Impulsivity traits partially explained associations between gene and diagnosis, suggesting that behavioral traits may provide informative phenotypes in genetic studies of anxiety

A. 5-HTTLPR → Impulsivity → Panic Disorder



B. 5-HTTLPR → Impulsivity → Phobic Disorders

**Figure 1.**

Direct and Indirect Associations between 5-HTTLPR genotype and anxiety disorders
 The figure illustrates a potential path between the serotonin transporter S/S genotype (the predictor) and two groups of DSM anxiety disorders: panic disorder (upper panel) and phobic disorders (bottom). The path model tests whether the relationship between genotype and diagnoses is explained by impulsivity.

The 'A' path represents the independent effect of genotype on impulsivity; the B path represents the independent effect of impulsivity on diagnosis; the 'C' path reflects the relationship between gene and diagnosis. To test the mediation hypothesis, the C path is 'broken' into two portions: a direct path which reflects the association between gene and diagnosis that is *independent* of impulsivity, and the indirect path, which reflects the relationship between gene and outcome that is *dependent* upon (i.e., explained by) impulsivity. The sum of the direct and indirect effect makes up the total effect (100%). And the percent of the total effect that is contributed to by the indirect path reflects the degree to which the association between gene and diagnosis is mediated by impulsivity. As shown in the C paths, approximately half (52%) of the association between 5-HTTLPR and phobias, and 16% of that between 5-HTTLPR and panic disorder, were explained by impulsivity.

A note of interest is that even though the magnitude of the direct and indirect effects are similar for phobias, the confidence intervals for the direct effect are much larger, suggesting that the association between genotype and clinical outcome is more heterogeneous than that between genotype and trait.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Associations between 5-HTTLPR and behavioral traits related to depression and anxiety

	Mean Score (95% CI)		Mean Score (95% CI)	F(df=2), p	Contrast ^d
	LL (N=59, 29%)	SL (N = 116, 57%)			
[A] All Participants					
Impulsivity ^f					
Total Score	58.48 (55.63, 61.33)	57.78 (55.88, 59.69)	62.50 (58.12, 66.88)	2.11, p=0.12	
- Attention	14.93 (13.88, 15.98)	14.05 (13.31, 14.80)	16.46 (14.83, 18.10)	4.01, p=0.020	SS>SL/LL
- Motor	21.05 (20.01, 22.09)	20.92 (20.20, 21.65)	21.43 (19.62, 23.23)	0.17, p=0.84	
- Non Motor Planning	22.44 (21.22, 23.66)	22.74 (21.97, 23.52)	25.14 (23.47, 26.82)	3.90, p=0.022	SS>SL/LL
Aggression ^f					
Total Score	81.00 (73.02, 88.98)	77.06 (72.12, 82.00)	81.35 (69.29, 93.40)	0.48, p=0.63	
- Anger	18.43 (16.26, 20.59)	17.78 (16.28, 19.28)	20.12 (17.02, 23.21)	2.12, p=0.12	
- Hostility	19.86 (16.99, 22.73)	19.09 (17.26, 20.92)	23.85 (19.03, 28.66)	2.12, p=0.12	
- Physical Aggression	26.14 (23.48, 28.80)	24.23 (22.30, 26.15)	23.08 (18.65, 27.50)	0.95, p=0.39	
- Verbal Aggression	16.57 (14.73, 18.41)	15.96 (14.85, 17.08)	14.31 (12.04, 16.57)	1.15, p=0.32	
Personality Factors ^{f,2}					
- Neuroticism ^{f,2}	1.60 (1.48, 1.72)	1.46 (1.36, 1.57)	1.77 (1.42, 2.12)	3.00, p=0.053	
[B] High Risk only					
Impulsivity ^f					
Total Score	59.92 (56.38, 63.46)	56.37 (54.26, 58.47)	64.64 (60.04, 69.23)	7.34 p=0.001*	SS>SL
- Attention	15.37 (14.28, 16.46)	14.01 (13.04, 14.99)	17.13 (15.38, 18.87)	5.66, p=0.0044	SS> SL
- Motor	21.16 (19.83, 22.48)	20.56 31.32 (19.58, 21.07)	22.21 (20.28, 24.14)	2.26, p=0.11	
- Planning	23.11 (21.43, 24.78)	22.15 (21.28, 23.02)	25.75 (23.93, 27.57)	6.04, p=0.003*	SS> SL
Aggression ^f					
Total Score	83.83 (73.69, 93.98)	76.95 (70.62, 83.28)	87.00 (74.27, 99.73)	1.31, p=0.27	
- Anger	19.81 (16.96, 22.65)	18.42 (16.47, 20.36)	21.50 (18.18, 24.82)	1.22, p=0.29	
- Hostility	20.11 (16.55, 23.67)	19.69 (17.31, 22.07)	25.80 (20.62, 31.02)	3.33, p=0.039	SS>SL/LL
- Physical Aggression	26.72 (23.31, 30.13)	22.68 (20.15, 25.20)	24.77 (20.01, 29.54)	1.71, p=0.19	
- Verbal Aggression	17.19 (14.82, 19.57)	16.17 (14.76, 17.58)	14.91 (12.44, 17.38)	0.90, p=0.41	

	Mean Score (95% CI)	Mean Score (95% CI)	Mean Score (95% CI)	F (df=2), p	Contrast ⁴
Personality Factors ^{1,2}					
- Neuroticism ^{1,2}	1.58 (1.43, 1.74)	1.49 (1.32, 1.65)	1.88 (1.60, 2.15)	3.57, p=0.033	SS>SL
[C] Low-Risk only Impulsivity¹	LL (N=21, 30%)	SL (N = 47, 65%)	SS (N = 4, 5%)	t⁴ (df=66),	
Total Score	55.90 (51.17, 60.63)	59.86 (56.39, 63.34)	50.75 (46.29, 55.21)	1.16, p=0.25	
- Attention	14.14 (11.95, 16.33)	14.11 (12.94, 15.27)	12.50 (10.18, 14.82)	0.26, p=0.77	
- Motor	20.86 (19.14, 22.58)	21.82 (20.42, 23.22)	16.75 (15.82, 17.68)	2.22, p=0.12	
- Non Motor Planning	21.24 (19.73, 22.75)	23.61 (22.20, 25.02)	21.50 (19.47, 23.53)	2.46, p=0.093	
Aggression¹					
Total Score	78.76 (64.33, 93.19)	75.87 (67.77, 83.97)	50.25 (36.64, 63.86)	0.76, p=0.70	
- Anger	15.95 (12.90, 19.00)	16.87 (14.51, 19.22)	12.50 (9.47, 15.53)	0.01, p=0.99	
- Hostility	19.40 (14.43, 24.37)	18.22 (15.35, 21.09)	13.00 (6.94, 19.06)	0.95, p=0.34	
- Physical Aggression	25.10 (20.81, 29.39)	26.47 (23.59, 29.34)	13.75 (6.31, 21.19)	0.01, p=0.98	
- Verbal Aggression	15.45 (12.56, 18.34)	15.67 (13.85, 17.48)	11.00 (5.78, 16.22)	0.18, p=0.86	
Personality Factors^{1,2}					
- Neuroticism ^{1,2}	1.63 (1.42, 1.84)	1.45 (1.30, 1.60)	n/e ³	1.50, p=0.14	

* Survived further FWE corrections p<0.05

¹For each score, higher score indicates a worse outcome.

²For neuroticism, data were only available on 132 (64%) of the participants (see methods).

³Could not be estimated due to low Ns.

⁴Based on tukey's test; only conducted when omnibus test is significant.

⁵Because only four offspring in the low-risk group had the SS genotype, we performed two way t-tests comparing LL with SL groups instead of an F test comparing all three groups.

Table 2

Modeling effects of 5-HTTLPR genotype on depression and anxiety traits

Trait outcome	Beta ¹ (std err), p value	% change ²	Beta ³ (std err), p value, further adjusted for:	Neuroticism	
			Impulsivity	Hostility	
Total Impulsivity	7.02 (2.14), p = 0.0013	+13%	N/A	4.48 (1.89), p = 0.019	7.16 (2.94), p = 0.017
Hostility	5.56 (2.29), p = 0.017	+31%	1.606 (2.07), p = 0.44	N/A	2.39 (2.70), p = 0.38
Neuroticism ⁴	0.35 (0.13), p = 0.013	+23%	0.25 (0.14), p = 0.08	0.18 (0.12), p=0.12	N/A

¹ Betas reflect the difference in trait scores between individuals with the SS genotype, as compared to those with one or more L alleles, after adjusting for age, sex and inter-relatedness among family members

² Percent increase in trait score among those with SS, as compared to SL/LL genotypes.

³ Betas reflect the difference in trait scores between individuals with the SS genotype, as compared to those with one or more L alleles, after adjusting for age, sex and inter-relatedness among family members as well as for the trait reflected in the appropriate column. Thus, in the first row, the 4.48 and 7.16 betas reflect the difference in impulsivity after further adjusting for hostility and neuroticism respectively. The findings show that the associations between 5-HTTLPR and impulsivity remained significant after co-varying the other traits (hostility and neuroticism, top row); however the associations between genotype and hostility (2nd row), or between 5-HTTLPR and neuroticism (bottom row) did not.

⁴ Data only available for 79 individuals

Table 3
Associations between 5-HTTLPR genotypes and DSM diagnoses of depression and anxiety

	LL	SL	SS		Contrasts ⁹
[A] All Participants	N = 59 29%	N = 116 57%	N = 28 14%	Chi-Square, (df=2), p value	
<i>Any Mood Disorder</i> ¹	29 (49)	72 (62)	21 (75)	5.72, p = 0.057	SS>LL
Major Depressive Disorder	22 (37)	60 (52)	16 (57)	4.28, p = 0.11	
<i>Any Anxiety Disorder</i> ²	31 (53)	64 (55)	19 (68)	1.92, p = 0.38	
Phobic Disorder ³	18 (31)	50 (43)	17 (61)	7.30, p = 0.026	SS>LL
Panic Disorder ⁴	1 (2)	12 (10)	11 (39)	26.0, p < 0.0001*	SS>LL; SL>LL; SS>SL
Generalized Anxiety Disorder ⁵	4 (7)	11 (9)	5 (17)	2.61, p = 0.26	
[B] High Risk only	N = 38 29%	N = 69 53%	N = 24 18%	Chi-Square, (df=2), p value	Contrasts ⁹
<i>Any Mood Disorder</i> ¹				3.75, p = 0.15	
Major Depressive Disorder	20 (53)	42 (61)	14 (58)	0.68, p = 0.71	
<i>Any Anxiety Disorder</i> ²	20 (52)	43 (63)	17 (71)	2.11, p = 0.34	
Phobic Disorder ³	12 (32)	36 (52)	16 (67)	7.89, p = 0.019	SS>LL; SL>LL
Panic Disorder ⁴	1 (3)	9 (13)	10 (41)	17.8, p = 0.0001*	SS>LL; SS>SL
Generalized Anxiety Disorder ⁵	4 (10)	8 (12)	5 (21)	1.63, p = 0.44	
[C] Low Risk Only	N = 21 30%	N = 47 65%	N = 4 5%	Chi-Square, (df=1), p value	Contrasts ⁹
<i>Any Mood Disorder</i> ¹	7 (33)	22 (47)	2 (50)	1.07, p=0.29	
Major Depressive Disorder	2 (10)	18 (38)	2 (50)	5.78, p=0.016	
<i>Any Anxiety Disorder</i> ²	11 (53)	21 (45)	2 (50)	0.33, p=0.57	
Phobic Disorder ³	6 (28)	14 (29)	1 (25)	0.01, p=0.91	
Panic Disorder ⁴	0	3 (6)	1 (25)	0.54 ⁷	
Generalized Anxiety Disorder ⁵	0	3 (6)	0	n/e ⁸	

* FWE corrected, p<0.05

¹ Includes major depressive disorder, minor depressive disorder, and dysthymia

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

- 2 Includes generalized anxiety or over-anxious disorder, obsessive compulsive disorder, panic disorder with agoraphobia, panic disorder without agoraphobia, post-traumatic stress disorder, social anxiety disorder (social phobia), specific phobia (note, of these, rates of PTSD and obsessive compulsive disorder were too low to examine individually).
- 3 Includes panic disorder, either with or without agoraphobia
- 4 Includes social anxiety disorder (social phobia), and specific phobia
- 5 Includes generalized anxiety disorder, and (for DSM3) overanxious disorder
- 6 Because there were too few low-risk offspring in the SS group, we conducted chi-square tests comparing the SL and LL offspring only
- 7 Fisher Exact Test.
- 8 Not estimatable.
- 9 Only when omnibus test is significant

Table 4

Associations between behavioral traits and anxiety diagnoses

	-----Panic Disorder-----		-----Phobic ¹ Disorders-----	
	Beta ¹ (s.e.), p value <i>Unadjusted for Genotype</i>	Beta ² (s.e.), p value <i>Adjusted for genotype</i>	Beta ¹ (s.e.), p value <i>Unadjusted for Genotype</i>	Beta ² (s.e.), p value <i>Adjusted for genotype</i>
<i>Impulsivity</i>				
Total Score	0.073 (0.025), p=0.0039	0.056 (0.027), p= 0.037	0.085 (0.02), p=0.0001	0.080 (0.023), p=0.0005
- Attention	0.18 (0.063), p=0.0041	0.15 (0.067), p=0.026	0.17 (0.052), p=0.0010	0.16 (0.054), p=0.0032
- Motor	0.082 (0.060), p=0.18	0.055 (0.065), p=0.40	0.14 (0.054), p=0.0059	0.14 (0.055), p= 0.010
- Non-planning	0.15 (0.058), p=0.0080	0.11 (0.062), p= 0.072	0.16 (0.048), p=0.0007	0.15 (0.049), p= 0.0023
<i>Aggression</i>				
Total Score	0.026 (0.0097), p=0.0069	0.026 (0.010), p=0.011	0.029 (0.008), p=0.0006	0.029 (0.0087), p= 0.0009
- Anger	0.076 (0.030), p=0.011	0.071 (0.032), p=0.026	0.067 (0.025), p=0.0077	0.062 (0.025), p=0.0150
- Hostility	0.087 (0.024), p=0.0004	0.078 (0.026), p=0.0033	0.062 (0.019), p=0.0013	0.057 (0.019), p=0.0035
- Physical Aggression	0.044 (0.026), p=0.082	0.051 (0.027), p=0.056	0.064 (0.022), p=0.0040	0.067 (0.023), p=0.0033
- Verbal Aggression	-0.0097 (0.041), p=0.81	0.012 (0.044), p =0.78	0.045 (0.031), p=0.15	0.055 (0.032), p=0.083
<i>Neuroticism</i>				
	0.83 (0.71), p=0.24	0.377 (0.80), p=0.63	1.077 (0.55), p=0.053	0.73 (0.59), p=0.21

¹ Adjusted for age, sex, interrelatedness among family members.² Adjusted for age, sex, interrelatedness among family members, and 5-HTTLPR genotype (SS vs other).