

GENETIC VARIATION IN THE SEROTONIN TRANSPORTER GENE INFLUENCES ADULT ATTACHMENT STYLE

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Abstract

Objective: To ascertain if the 5-HTTLPR short allele was associated with an insecure style of attachment in a mixed sample of healthy subjects and psychiatric patients.

Method: Three hundred and thirty five adults (222 healthy subjects and 113 psychiatric patients) were genotyped for the 5-HTTLPR serotonin transporter polymorphism. Adult attachment style was assessed by using the Relationship Questionnaire (RQ). The personality trait of harm avoidance was assessed by using the Temperament and Character Inventory (TCI). The depression subscale of the Profile of Mood States (POMS-D) was used to assess mood state. Harm avoidance and depression were measured to control their potential confounding effects on the relationship between the 5-HTTLPR serotonin transporter polymorphism and adult attachment style.

Results: We found a significant association between insecure attachment and the 5-HTTLPR short allele. Separate analyses in the subcohorts of healthy subjects and psychiatric patients confirmed the association between attachment style and 5-HTTLPR. Such an association was not a byproduct of the relationship between the serotonin transporter polymorphism and neuroticism or negative affectivity because insecure attachment, but not the harm avoidance scale of the TCI, was associated with the short variant of 5-HTTLPR. Moreover, inclusion of harm avoidance and depressive symptoms into a multiple regression model did not weaken the significant correlation between the 5-HTTLPR and insecure attachment. The effect size seen in our study (Cramer's $\phi = 0.15-0.21$ approximately corresponding to Cohen's $d = 0.30-0.40$) compares favorably to those reported in previous studies of association between the 5-HTTLPR polymorphism and personality traits.

Conclusions: This study suggests that part of the variation in adult attachment styles can be accounted for by genetic differences among individuals.

Key words: 5-HTTLPR, attachment style, adulthood, behavioral phenotype, harm avoidance

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Introduction

Attachment theory is a multifaceted theory of personality structure, functioning, and development, as well as a theory of interpersonal behavior, emotional bonds, and close relationships (Mikulincer and Shaver 2007). One of the key ideas in attachment theory is that early attachment experiences play an important role in shaping the development of mental representations of the self and of the other ("internal working models" in the terminology of attachment theory) that influence later emotional and psychosocial functioning. Adults who recall warm, loving relationships with their parents are more likely to rate themselves as secure in attachment whereas adults who describe their parents as cold or rejecting are more likely to classify themselves as insecure. Attachment insecurities (of both the anxious and avoidant varieties) are associated with depression, clinically significant anxiety, obsessive-compulsive

disorder, post-traumatic stress disorder (PTSD), suicidal tendencies, and eating disorders (see Mikulincer and Shaver 2012 for a comprehensive review). In sum, attachment insecurity can be viewed as a general factor of vulnerability to mental disorders which predisposes a person to break down psychologically in times of crisis.

The complexity of attachment organization suggests that numerous factors contribute to variability in adult attachment styles. Part of the variation in adult attachment styles can be accounted for by genetic differences among individuals, as shown by studies of behavioral and molecular genetics (see Gillath et al. 2016 for a recent review). Picardi et al. (2011) assessed adult attachment style with the Experiences in Close Relationships questionnaire in a sample of 677 Italian twins and found that genetic effects accounted for 45% and 36% of individual differences in anxiety and avoidance, respectively. Crawford et al. (2007) found that 40% of the variance in adult attachment

anxiety was accounted for by genetic influences, and Donnellan et al. (2008) found that additive genetic effects accounted for 45% of the variability in attachment anxiety and 39% of the variability in avoidance. In a sample of 147 undergraduates, Gillath et al. (2008) measured adult attachment styles with the Experience in Close Relationships questionnaire and found that anxious attachment was associated with a polymorphism of the DRD2 dopamine receptor gene and that avoidant attachment was associated with a polymorphism of the 5-HT2A serotonin receptor gene. In a cohort of 185 patients with mood disorders and 192 matched healthy controls, Costa et al. (2009) found associations between the GG genotype of two single nucleotide polymorphisms within the oxytocin receptor gene (OXTR) and attachment scores, such that it was negatively associated with attachment security and positively associated with attachment anxiety and avoidance. In a cohort of 112 psychiatric patients, Troisi et al. (2012) found that variation in the mu-opioid receptor gene (OPRM1) moderates the influence of early maternal care on fearful attachment. Participants expressing the minor 118G allele had similar and relatively high scores on fearful attachment regardless of the quality of maternal care. By contrast, early experience made a major difference for participants carrying the A/A genotype. Those who recalled higher levels of maternal care reported the lowest levels of fearful attachment whereas those who recalled lower levels of maternal care scored highest on fearful attachment.

In the study reported here, we investigated the association between adult attachment style and the serotonin transporter promoter polymorphism (5-HTTLPR). Previous studies of the association between 5-HTTLPR and adult attachment styles have yielded inconsistent findings. In 92 women with bulimic syndromes, Steiger et al. (2007) found that carriers of the short (s) allele who were previously abused showed an increased risk of insecure attachment, as measured by the specific subscale of the Dimensional Assessment of Personality Pathology-Basic Questionnaire. In a sample of 86 participants, Caspers et al. (2009) found an association between the s-allele and increased risk for disorganized attachment, as measured by the Adult Attachment Interview. In contrast with these positive findings, Reiner and Spangler (2010) found no association between 5-HTTLPR genotypes and attachment styles, as measured by the Adult Attachment Interview, in a sample of 167 German adults.

Although the above studies have yielded inconsistent findings, there are several *a priori* reasons to suspect a role for 5-HTTLPR genotype in the etiology of attachment insecurity. First, carriers of the 5-HTTLPR s-allele tend to have higher levels of neuroticism as measured by the NEO family of personality inventories (Lesch et al. 1996, Sen et al. 2004) and/or harm avoidance as measured by the TPQ/TCI family of personality inventories (Munafò et al. 2005). Neuroticism and harm avoidance are correlated with attachment insecurity even though the two sets of measures are not simply redundant (Nofle and Shaver 2006). Second, carriers of the s-allele are at higher risk for depression in the context of environmental adversity (Haberstick et al. 2016) and attachment insecurity confers greater sensitivity to the depressogenic effects of adverse life events (Bifulco et al. 2002). Finally, carriers of the s-allele have increased amygdala reactivity to negative emotional stimuli (Madsen et al. 2016), and recent studies have demonstrated a key role of the amygdala in mediating emotional reactivity associated

with attachment insecurity in healthy adult individuals (Riem et al. 2012, Redlich et al. 2015). We therefore predicted that, in our sample of adult individuals, an insecure attachment style would be associated with the s-allele.

Methods

Participants

The sample of this study consisted of 113 patients (72% women; mean $\pm$ SD age: 36.51 ± 11.11 years) consecutively admitted to the day hospital of the psychiatric clinic at the University of Rome Tor Vergata and 222 healthy volunteers (63% women; mean $\pm$ SD age: 31.49 ± 10.59 years). Experienced clinical psychiatrists used the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-CV) and the Schedule for Interviewing DSM-IV Personality Disorders-IV (SIDP-IV) to formulate the principal diagnosis according to the DSM-IV-TR criteria. Patients with medical or neurological disorders, mental retardation, or psychotic disorders were excluded from the sample. The diagnostic composition of the clinical group was as follows: major depressive disorder, 41%; anxiety disorders, 26%; bipolar disorder, 21%; cluster B personality disorders, 12%. All patients were interviewed when they had partially improved and reached the drug treatment dosage prescribed for maintenance therapy. Healthy volunteers were recruited among students in the medical school, paramedic staff members, and conscripts of the Italian army. Inclusion in the control group required the absence of current or past psychiatric disorders, as confirmed by diagnostic interview.

Sampling strategy was designed to minimize the impact of those confounding factors that might be responsible for the inconsistent findings of previous studies of the association between attachment styles and 5-HTTLPR. Considering that even among European populations there are ethnic differences in the allele frequencies of the 5-HTTLPR (Noskova et al. 2008), all the participants enrolled in the present study were of Italian origin. We enrolled both healthy subjects and psychiatric patients to enable the generalization of the results across various population groups. The selection of psychiatric patients with mood, anxiety, and cluster B personality disorders was based on the findings of previous studies showing that these diagnostic categories are associated with high frequencies of both insecure attachment (Ward et al. 2006) and the s-allele (Gelernter 2014).

All data were obtained under informed written consent and using procedures and protocols approved by the Fondazione Santa Lucia Intramural Ethics Committee.

Psychometric assessment

To measure adult attachment style, we used the Italian version (Troisi et al. 2001) of the Relationship Questionnaire (RQ; Bartholomew and Horowitz 1991). The RQ is a short instrument made up of four short paragraphs, each describing a prototypical attachment pattern as it applies in close adult peer relationships (secure, preoccupied, fearful, and dismissing). The four attachment patterns are defined in terms of two dimensions: anxiety (i.e., a strong need for care and attention from attachment figures coupled with a pervasive uncertainty about the willingness of attachment figures to respond to such needs) and avoidance (i.e.,

discomfort with psychological intimacy and the desire to maintain psychological independence). Participants are asked to rate their degree of correspondence to each prototype on a 7-point scale. The RQ was designed to obtain continuous ratings of each of the four attachment patterns. However, the RQ can also be used to categorize participants into their best fitting attachment pattern. A problem arises when two or more attachment prototypes are rated equally high. To deal with this, we also asked participants to choose a single, best fitting attachment pattern. Even though there are important differences between the preoccupied, fearful, and dismissing styles, all these three categories reflect a pattern of insecure attachment.

The Italian version of the Temperament and Character Inventory (TCI) was used to assess harm avoidance. The TCI, one of the most widely used inventories in molecular genetics studies of personality, is a 240-item, true-false, self-report questionnaire that measures seven dimensions of personality, including the original three Tridimensional Personality Questionnaire scales (or temperaments) of novelty seeking, harm avoidance, and reward dependence. A large body of research has demonstrated that the TCI personality dimension of harm avoidance (defined as a tendency to respond intensely to signals of aversive stimuli, thereby learning to avoid punishment and novelty) has trait-like properties (Farmer et al. 2003).

The Italian version of the depression subscale of the Profile of Mood States (POMS-D) was used to assess the presence and severity of depressive symptoms. Participants were asked to carefully read each of 15 items, then respond to a 5-point Likert scale ranging from 1 (Not at All) to 5 (Extremely) based on how they were feeling the day they completed the inventory. Correlations with other measures of depression (i.e., the Beck Depression Inventory, the Hopkins Symptom Distress Scale and the MMPI-2 Depression Scale) confirm the concurrent validity of the POMS-D (Griffith et al. 2005).

The subsample of 271 participants for whom TCI and POMS data were available did not differ from the total sample in terms of age ($t = 0.44$, $p = 0.66$), gender ratio (chi square = 0.48, $p = 0.49$), and relative percentage of psychiatric patients (chi square = 0.61, $p = 0.80$).

DNA extraction and genotyping

For all participants DNA was obtained from buccal samples (Freeman et al. 2003) and DNA was extracted using standard procedures. The 5-HTTLPR was genotyped as described in Caspi et al. (2003) and Gelernter et al. (1997) using a forward primer having the sequence (5'- ATGCCAGCACCTAACCCCTAATGT-3') and a reverse primer having the sequence (5'- GGACCG-CAAGGTGGGCGGGA-3'). PCR was carried out using the following conditions: initial 5-min denaturing step at 95.0°C, followed by 35 cycles at 94.0°C for 30 sec, 66.0°C for 30 sec and 72.0°C for 40 sec, and a final extension phase at 72.0°C for 15 min. Reactions were

performed in 25 µl total volume with 50 ng genomic DNA, 1.5 mM MgCl₂, 5 pmoles of each primer, 0.3 mM dNTPs, and 1 U of native Taq (Promega, Madison, WI). The primers used yielded a 419 base pair product for the 16 repeat ('l') allele and a 375 base pair product for the 14 repeat ('s') allele. PCR products were assayed on a 2.5% agarose gel and stained with ethidium bromide.

Statistical analysis

Comparisons between groups were performed using the t-test on continuous measures and the chi-square test on categorical measures. The effect of 5-HTTLPR was examined across the entire sample and separately within the two subcohorts of healthy subjects and psychiatric patients by using the chi-square test, with phi as the measure of the strength of association between categorical variables and the odds ratio (OR) as the index of comparative risk. Binary logistic regression was used to predict subjects' membership in the two allele categories (l/l vs. s/l + s/s). Effects are reported as odds ratios (OR) (i.e. $\exp(\beta)$) with 95% confidence intervals (CI) and p-values against the hypothesis of no association. Analysis was performed on a personal computer using SPSS for Windows, version 10.0.5 (SPSS, Chicago, IL).

Results

The frequency distribution of the 5-HTTLPR genotypes was as follows: 32% l/l, 47% l/s, 21% s/s. Genotype frequencies were in Hardy-Weinberg equilibrium and did not significantly diverge from those reported in other Caucasian populations (chi-square = 0.81, $df = 2$, $p = 0.37$). Genotype was associated with neither mental health status (healthy volunteers vs. psychiatric patients; chi-square = 0.64, $df = 1$, $p = 0.42$) nor psychiatric diagnosis (major depressive disorder vs. anxiety disorders vs. bipolar disorder vs. cluster B personality disorders; chi-square = 2.63, $df = 3$, $p = 0.47$). As expected, there was a significant association between insecure attachment and mental health status: 77% of the psychiatric patients, but only 40% of the healthy subjects, had an insecure style of adult attachment (chi-square = 39.93, $df = 1$, $p = 0.000$).

In the entire sample, there was a significant association between genotype and insecure attachment. The percentage of participants with insecure attachment was significantly higher among carriers of the s-allele (chi-square = 7.33, $df = 1$, $p = 0.007$, $\phi = 0.15$, OR = 1.89, CI = 1.19-3.00). The significant association between the s-allele and insecure attachment was confirmed by separate analyses in the subgroups of healthy subjects (chi-square = 5.55, $df = 1$, $p = 0.02$, $\phi = 0.16$, OR = 2.07, CI = 1.12-3.80) and psychiatric patients (chi-square = 5.07, $df = 1$, $p = 0.02$, $\phi = 0.21$, OR = 2.74, CI = 1.12-6.71) (table 1). This analysis estimated that 5-HTTLPR genotype contributed 2.2-4.4% (ϕ^2) of the variance in attachment style in our cohort.

Table 1. Distribution of 5-HTTLPR genotypes across participants with RQ secure or insecure attachment styles

	Entire sample (N = 335, 66% women)		Healthy subjects (N = 222, 63% women)		Psychiatric patients (N = 113, 72% women)	
	Secure	Insecure	Secure	Insecure	Secure	Insecure
l/l	63 (57.8%)	46 (42.2%)	49 (71.0%)	20 (29.0%)	14 (35.0%)	28 (65.0%)
l/s + s/s	95 (42.0%)	131 (58.0%)	83 (54.2%)	70 (45.8%)	12 (16.4%)	61 (83.6%)

In a subsample of 271 participants, we used multiple regression analysis to exclude that the association between the s-allele and insecure attachment was a spurious finding due to inter-correlations between insecure attachment and those mood symptoms and/or personality traits that previous molecular genetics studies have associated with the serotonin transporter polymorphism. RQ attachment style (secure vs. insecure), mental health status (healthy volunteers vs. psychiatric patients), TCI harm avoidance and POMS-D were entered as independent variables into a binary logistic model with 5-HTTLPR genotype (l/l vs. l/s + s/s) as the dependent variable. Neither TCI harm avoidance nor POMS-D entered the equation as significant predictors of the s-allele. In contrast, multiple regression analysis confirmed the significant association between insecure attachment and the s-allele (**table 2**).

insecurity. Currently, the prevalent model based on neuroimaging research points to a higher sensitivity to negative social clues, and enhanced recruitment of social aversion or threat systems in relation to insecure-anxious attachment, in agreement with previous work suggesting higher vigilance to social emotional cues and hyper-activating of secondary attachment-related strategies in these individuals (Vrticka et al. 2012). An alternative hypothesis could be that the l-allele carriers are better able to engage brain circuits that provide coping mechanisms to successfully mitigate the long-term sequelae of attachment-threatening events. Canli et al. (2006) found that both amygdala and hippocampal resting neural activity and neural activation in response to threatening words were negatively correlated with life stress events in l-allele carriers, but positively correlated in s-allele carriers. Moreover, several brain regions known to be recruited during imitative and

Table 2. Multivariate associations between and the 5-HTTLPR short allele and RQ insecure attachment, TCI harm avoidance and POMS depression scale. Results of binary logistic regression

	Beta	P value	OR	95% CI
RQ attachment	- 0.82	0.006	0.44	0.24-0.79
Mental health status	0.29	0.42	1.33	0.67-2.67
TCI harm avoidance	0.01	0.55	1.01	0.97-1.06
POMS-D	- 0.01	0.23	0.98	0.96-1.01

Discussion

In the study reported here and conducted on a sample of 335 adults (222 healthy subjects and 113 psychiatric patients), we found a significant association between insecure attachment and the 5-HTTLPR short allele. Separate analyses in the subcohorts of healthy subjects and psychiatric patients confirmed the association between attachment style and the serotonin transporter polymorphism.

The most plausible hypothesis to explain our findings is that 5-HTTLPR genotype modulates emotional reactivity or attention to threatening stimuli that activate the attachment behavioral system. Because a basic component of the dynamics of the attachment behavioral system involves the monitoring and appraisal of early experiences, it is possible that genetic variations of the serotonin transporter increase the risk for attachment insecurity via altering emotional reactivity to insensitive parental behavior. Alternatively, genetic variation of the serotonin transporter might induce the development of information-processing biases that consistently favor negative interpretations of early experiences with parents.

Previous studies have demonstrated that carriers of the s-allele have increased limbic neural activity at rest (Canli et al. 2006, Rao et al. 2007) and while processing aversive stimuli (Dannlowski et al. 2008, Heinz et al. 2007). Increased reactivity in emotion-related brain regions has also been shown to correlate with attachment insecurity. In an fMRI experiment, Gillath et al. (2005) found that, when thinking about aversive relationship scenarios, women with higher levels of attachment anxiety showed greater activation in the anterior cingulate cortex, hippocampus, and anterior temporal lobe. Combining fMRI and psychophysiological measures, Lemche et al. (2006) found that, during an experimental task evoking separation distress, autonomic response and amygdala reactivity were positively correlated with the level of attachment

other empathetic behaviors showed increased activation to threatening words in l-allele, but not s-allele, subjects reporting life stress events. These complex epigenetic interactions between life stress and 5-HTTLPR genotype may imbue persons carrying two copies of the l allele with a protection against early trauma.

As is well known, the choice of behavioral phenotype is a crucial problem in molecular genetics studies (Wojczynski and Tiwari 2008). Our aim in this study was to examine the association of 5-HTTLPR genotype with psychometric measures that reflect biologically adaptive endophenotypes in order to test the assumption that such measures may be better objects of molecular genetics studies than commonly used psychological measures. Because the effect size seen in our study (Cramer's phi = 0.15-0.21 approximately corresponding to Cohen's d = 0.30-0.40) (Kline 2004) compares favorably to those reported in previous association studies with the 5-HTTLPR polymorphism (Ebstein 2006), we cautiously interpret our findings to support this assumption. However, this conclusion rests importantly on the independence of our phenotypic measures from those previously associated with 5-HTTLPR genotype. In our sample, insecure attachment, but not the harm avoidance scale of the TCI, was associated with the short variant of the 5-HTTLPR, and the inclusion of harm avoidance and depressive symptoms into a multiple regression model did not weaken the significant correlation between the 5-HTTLPR and insecure attachment. These data confirm that self-reports of attachment style, although correlating with a broad network of anxiety-related personality traits, are not redundant with these constructs (Shaver and Mikulincer 2004).

Although based on large samples, 5-HTTLPR association studies using either NEO neuroticism or TCI harm avoidance as dependent variables have yielded mixed findings (Sen et al. 2004, Munafò et al. 2005). Similarly, we were also not able to confirm an association between TCI-harm avoidance and

5-HTTLPR genotype in our cohort. We hypothesize that, due to its role as an evolved behavioral system that plays a crucial role in modulating emotion regulation and stress sensitivity (Insel and Young 2001), attachment organization may represent a phenotype more robustly linked to 5-HTT function than psychometric constructs derived from traditional personality inventories (Kas et al. 2007).

Finally, we must underline that the association between the 5-HTTLPR short allele and insecure attachment explained only 2.2-4.4% of the variance in attachment style in our cohort. This may not be surprising considering that specific attachment patterns are likely to be affected by a broad array of contextual factors including childhood and adolescent experiences and recent experiences in adult relationships (Shaver and Mikulincer 2004). In addition, considering the complex neurochemical bases of the attachment behavioral system, it is unlikely that 5-HTTLPR is the only or even primary genetic factor influencing adult attachment style (Gillath et al. 2016).

In conclusion, the present study suggests that research into genetic polymorphisms may complement traditional studies of attachment focused on environmental factors and may be a fruitful approach to understanding the neurochemical pathways supporting attachment behaviors. Further studies combining molecular genetics approaches with measurements of environmental factors may shed light on the interplay between genes and environment in the development of attachment style.

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