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Association Between the Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Adult Unresolved Attachment

Kristin M. Caspers, Sergio Paradiso, Rebecca Yucuis, and Beth Troutman
University of Iowa

Stephan Arndt
University of Iowa and Iowa Consortium on Substance Abuse Research and Evaluation

Robert Philibert
University of Iowa

Research on antecedents of organized attachment has focused on the quality of caregiving received during childhood. In recent years, research has begun to examine the influence of genetic factors on quality of infant attachment. However, no published studies report on the association between specific genetic factors and adult attachment. This study examined the link between the 5-HTTLPR promoter polymorphism of the serotonin transporter gene and adult unresolved attachment assessed with the Adult Attachment Interview. Genetic material and information on attachment-related loss or trauma were available for 86 participants. Multivariate regression analyses showed an association between the short 5-HTTLPR allele and increased risk for unresolved attachment. Temperament traits and psychological symptoms did not affect the association between 5-HTTLPR and unresolved attachment. The authors hypothesize that the increased susceptibility to unresolved attachment among carriers of the short allele of 5-HTTLPR is consistent with the role of serotonin in modulation of frontal–amygdala circuitry. The findings challenge current thinking by demonstrating significant genetic influences on a phenomenon previously thought to be largely environmentally driven.

Keywords: unresolved attachment, serotonin, emotion regulation, 5-HTTLPR

Research analyzing childhood sibling similarities on attachment largely supports common environmental influences on the organization of attachment (Bakermans-Kranenburg, Van IJzendoorn, Bokhorst, & Schuengel, 2004; Bokhorst et al., 2003; O'Connor & Croft, 2001; Van IJzendoorn et al., 2000). For disorganized infant attachment, common environmental influences have not been demonstrated (Bakermans-Kranenburg et al., 2004; Bokhorst et al., 2003; O'Connor & Croft, 2001; Van IJzendoorn et al., 2000). Recent analyses of adult siblings produced similar findings, with substantial similarities between siblings on organized representations of attachment but not disorganized attachment (Caspers, Yucuis, Troutman, Arndt, & Langbehn, 2007; Constantino et al., 2006). The lack of common environmental influences on disorganized attachment suggests genetic variability as a potential source of influence on adult disorganized attachment. Therefore, this study examines the serotonin transporter promoter polymorphism (5-HTTLPR) as a potential candidate gene in the susceptibility to disorganized attachment in adulthood.

The examination of genetic contributions to disorganized attachment is not without precedence. The long variant of the dopamine D4 receptor (DRD4) gene has been shown to significantly predict infant disorganized attachment (Lakatos et al., 2000), although not all studies have been consistent (Bakermans-Kranenburg & Van IJzendoorn, 2004). The DRD4 polymorphism has also been found to moderate the intergenerational transmission of disorganized attachment whereby maternal unresolved attachment predicts disorganized infant attachment only among carriers of the 7-repeat DRD4 polymorphism (Van IJzendoorn & Bakermans-Kranenburg, 2006). A recent study introduces further complexity to understanding the interplay between genes and environment in attachment (Gervai et al., 2007). Disruptive maternal affective communication has been proposed as a mechanism for intergenerational transmission of disorganized attachment (see Lyons-Ruth, Bronfman, & Parsons, 1999; Madigan, Moran, Schuengel, Pederson, & Otten, 2007). Gervai et al. (2007) examined the interaction between DRD4 gene polymorphisms and disruptive maternal affective communication in the prediction of infant disorganized attachment. Higher rates of infant disorganized attachment were found when the 7-repeat DRD4 allele was absent.

Kristin M. Caspers, Sergio Paradiso, Rebecca Yucuis, Beth Troutman, and Robert Philibert, Department of Psychiatry, Carver College of Medicine, University of Iowa; Stephan Arndt, Department of Psychiatry, Carver College of Medicine, University of Iowa, and the Iowa Consortium for Substance Abuse Research and Evaluation, Iowa City, IA.

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Correspondence concerning this article should be addressed to Kristin M. Caspers, Psychiatry Research/MEB, Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA 52242. E-mail: kristin-caspers@uiowa.edu

The above findings on associations between specific genes and infant disorganized attachment demonstrate the complexity of identifying candidate genes and highlight the necessity for theory-driven hypotheses bridging genetic and attachment research. Determination of adult disorganized attachment, herein referred to as unresolved attachment, relies on expert examination of discourse patterns elicited during recollection of experiences of loss and trauma (Main & Goldwyn, 1998). Shifts in discourse and reasoning patterns may represent lapses in consciousness, undue influence of overwhelming emotions, or inappropriate interference of memories surrounding the event such that speech is no longer actively being monitored (Hesse & Main, 2000; Hesse & Van IJzendoorn, 1999). For example, an individual might speak about a deceased loved one as though the person was still alive (i.e., indicating a lapse in reasoning). Another individual might speak about a traumatic event in such detail that it suggests a loss of awareness of the immediate purpose and context of the discourse (i.e., an indication of lapse in thought). This affective modulation of language suggests that the cognitive processes of adults with disorganized attachment may be influenced by alteration in the neurobiological processes governing emotions (Phillips, Drevets, Rauch, & Lane, 2003). Therefore, we constructed our hypotheses combining evidence about genetic variability influencing susceptibility to environmental stressors and on the neural structures subserving emotional response (Main, 1999).

Serotonin (5-HT) is a major neurotransmitter involved in emotion regulation (Ressler & Nemeroff, 2000). 5-HT removal from the synaptic cleft is largely achieved through the activity of 5-HT transporter. The amount of 5-HT transporter is influenced by the 5′ promoter region regulating the transcription of the 5-HTT gene. The promoter contains a polymorphic region with a variable number of tandem repeats (5-HTTLPR), with the short allele responsible for less efficient production of the 5-HT transporter (Collier et al., 1996). The discovery of the 5-HTTLPR promoter polymorphism has led to important developments on predisposing factors for psychopathology (Canli & Lesch, 2007; Ebstein, 2006; Lesch et al., 1996). For example, profound alterations in the functioning of the 5-HT system (documented as lower cerebrospinal fluid 5-HT metabolite) have been shown in animal and human carriers of the short allele in response to chronic or acute stressful experiences (Bennett et al., 2002; Williams et al., 2003). Greater severity of mood disorder and behavioral phenotypes indicative of psychopathology are reported among carriers of the short allele of 5-HTTLPR who experienced stressful life events (Caspi et al., 2003; Fox et al., 2005; Kaufman et al., 2004; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Wilhelm et al., 2006), although not all findings are consistent. Finally, Suomi and colleagues (Champoux et al., 2002; Suomi, 1999, 2003, 2006) have shown a significantly greater impact of maternal deprivation among infant rhesus monkeys who are carriers of the risk allele.

These data support the role of the 5-HT system in modulating emotional response to environmental stressors. Progress on the neurobiology of arguably one of the most overwhelming of human emotions (i.e., fear) allows the development of specific hypotheses on the genetic mechanisms underlying unresolved–disorganized attachment. The amygdaloid complex is a central station for processing emotionally charged stimuli, especially frightening socially relevant stimuli (Adolphs, Baron-Cohen, & Tranel, 2002; Phelps & LeDoux, 2005). Application of neuroimaging to the study of attachment in humans has highlighted the role of the amygdala during positive and negative attachment experiences (Buchheim et al. 2006; Leibenluft, Gobbini, Harison, & Haxby, 2004; Lemche et al., 2006). Recent studies in humans have helped to show how the association between the 5-HTTLPR short allele and stress-related emotional reactivity may be displayed at the neural level. Carriers of the short allele (s/s or s/l) of 5-HTTLPR exhibit greater amygdala activity in response to salient frightening stimuli compared with individuals homozygous for the long allele (l/l; Hariri et al., 2002, 2005). Similar findings are reported in four separate samples of healthy participants (Bertolino et al., 2005).

The present original research aims to determine the extent to which the short variant of the 5-HTTLPR allele predicts unresolved adult attachment. On the basis of the influences of the 5-HTTLPR polymorphism on amygdala reactivity, we predicted that individuals who are carriers of the short 5-HTTLPR allele would show greater shifts in discourse and reasoning patterns as a result of poor emotion regulation and would thus demonstrate higher rates of unresolved attachment. On the basis of the above-summarized literature, we further hypothesized that carriers of the short 5-HTTLPR allele would only display differential coherence of discourse during discussions of loss or trauma. In addition, short allele carriers were not predicted to be differentially exposed to loss or trauma experiences. Studies have typically converged on dominance of the short 5-HTTLPR allele (Ebstein, 2006; Canli & Lesch, 2007). We chose to directly test the assumption of dominance because some studies have shown "dose-related" effects of the short 5-HTTLPR variant in addition to dominance effects (Caspi et al., 2003; Kaufman et al., 2004; Wilhelm et al., 2006). Because alteration in 5-HT metabolism has been posited to affect personality and mood disorders (Phillips et al., 2003; Ressler & Nemeroff, 2000), we examined the association between 5-HTTLPR genotype and unresolved attachment with mood and personality measures.

Method

Participants

Participants for this study were enrolled as part of a large adoption study consisting of adoptees separated from their biological parents at birth. The average age at adoption was 2.42 months (SD = 6.39 months) with 71% of the adoptees placed with the adoptive parents before 1 month of age and 82% before 3 months of age. Adoptees were originally selected for participation on the basis of the psychiatric diagnoses of their biological parents. Adoption agency and institutional (e.g., hospital and prison) records were reviewed by board-certified psychiatrists to determine the diagnoses of the biological parents (e.g., alcoholism, antisocial behaviors; for review of the methods, see Yates, Cadoret, & Troughton, 1999). Adoptees were classified as a proband when a diagnosis was present in either biological parent or as a comparison when no diagnosis was present in either biological parent. Interviewers were naive to the psychiatric history of the biological parents of all the participants. Adoptive families were predominantly upper (20%) and middle class (76%). Average adoptee household income was $40,000 to $49,999 per year. Participants were predominantly White, non-Hispanic (n = 81; 91%), with the remainder of the participants African American,

non-Hispanic ($n = 4$; 4.5%); African American, Hispanic ($n = 1$; 1%); Caucasian, Hispanic ($n = 1$; 1%); or mixed race ($n = 2$; 2%).

Procedure

All procedures were approved by the Carver College of Medicine, University of Iowa Internal Review Board. The Adult Attachment Interview (AAI; Main & Goldwyn, 1998) was administered, transcribed, and coded for 217 individuals between the years of 2000 and 2004. Age of participants at the time of interview ranged between 28 and 62 years ($M = 40$ years, $SD = 7.63$), and 52% were women. The interviews were anonymously assigned and coded blindly by raters who were trained to be reliable to the coding standards of the laboratory of Mary Main and Eric Hesse (Rebecca Yucuis and Kristin M. Caspers were trained by D. Jacobvitz, Austin, TX, 2000; Jeanne Frederickson and Beth Troutman were trained by J. Sroufe, Minneapolis, MN, 1999 and 2001). Roughly 50% of all interviews were rated by two coders. If there was disagreement between coders and consensus could not be reached, a third rater was selected. Interrater agreement was 93% for the unresolved–not unresolved classification ($\kappa = .71$, $p < .001$). The intraclass correlation, computed with exact agreement methods, for unresolved loss or trauma was .76 and for coherence of transcript was .77. The frequency distribution of unresolved attachment differed significantly from expected rates, $\chi^2(1, N = 86) = 13.35$, $p = .001$, signifying that in our sample unresolved attachment was overrepresented (Van IJzendoorn & Bakermans-Kranenburg, 2008).

The molecular genetic component of the present study began in June of 2001, at which time newly recruited participants were asked to provide buccal swabs for genetic analysis. A total of 111 participants with coded AAIs were asked to provide consent for genetic analysis, and 89 agreed. The difference in the distribution of unresolved versus not unresolved attachment between individuals who provided consent and those who did not was not statistically significant, $\chi^2(1, N = 111) = 0.21$, $p = .65$. Men and women provided cheek swabs at equal rates, $\chi^2(1, N = 111) = 3.11$, $p = .08$. The present study is based on data of only adoptees who provided genetic data and had completed the AAI ($N = 89$). Among participants who provided cheek swabs, 86 reported experiencing loss or trauma and 3 did not. Because reporting a loss or trauma is a necessary condition for exhibiting characteristics of unresolved attachment on the AAI, data analysis was restricted to those individuals who reported experiencing loss or trauma (final $N = 86$). Race (e.g., Caucasian, non-Hispanic vs. other) was not associated with unresolved attachment, $\chi^2(1, N = 86) = 0.00$, $p = .98$, or 5-HTTLPR genotype, $\chi^2(1, N = 86) = 0.06$, $p = .97$.

Measures

Adult attachment. Adult attachment representations were derived using the AAI (Main & Goldwyn, 1998). Interviews were audiotaped, transcribed verbatim, and scored with the standard AAI classification system (Main & Goldwyn, 1998). Participants are asked to provide five adjectives for their childhood relationship with their mother and father. Participants are then asked to provide experiential support for the descriptors (e.g., a detailed recount of personal events). Questions about parental responses during episodes of emotional upset, illness, and injury are also probed. Participants are asked about experiences of loss or trauma. On the AAI, loss is defined as deaths of individuals who were important to the interviewee (occurring at any point during their lifetime). Trauma is defined as maltreatment by parents (occurring during childhood) or overwhelmingly frightening experiences (occurring at any point during their lifetime). Finally, the individual is asked to describe the degree to which his or her current feelings differ from past feelings toward his or her parents.

Transcripts were scored using 9-point scales. A coherence score was also assigned on the basis of overall narrative consistency. In addition to the coherence score, three primary classifications representing organized attachment were derived: dismissing, autonomous, and preoccupied (see Main, 2000). When significant, albeit brief, lapses in discourse were observed during descriptions of loss or trauma, participants were classified as having unresolved attachment. Examples of speech patterns indicative of unresolved attachment included (a) change to the present tense when describing the dead person and/or indication that the dead person was still playing an active role in the participant's life, (b) excessive detail surrounding the event of death or trauma, (c) identifying the self as causing the death of the loved one, (d) viewing themselves as deserving of abuse, and/or (e) reporting extreme reactions to experiences of loss or trauma.

All but 3 participants with available genetic data reported at least one experience of loss. Although there are several possible reasons why the majority of the individuals in this sample reported experiencing at least one major loss, the most plausible explanation is that the chance of experiencing the death of a loved one increases with age. The average age of individuals in this sample was 40 years at the time of the AAI interview, and their adoptive parents' median age was 30 years at the time of the adoption. Indicators of unresolved speech were evaluated independently for each loss.

Trauma was only scored if the experience met specific criteria (e.g., hitting that is inappropriate or induces pain, leaving bodily marks, overwhelmingly frightening parental rage directed toward the child or in the presence of the child) and sufficient information was available to determine the experience was abusive or overwhelmingly frightening. Sixteen transcripts met these criteria. An unresolved scale score (1–9) was independently assigned to each passage of loss (U_L) and trauma (U_{Tr}). An overall unresolved scale score (U_O) was determined from the highest rating across all loss and trauma. Participants were classified with unresolved attachment when the overall score for unresolved loss or trauma was 6 or above. Transcripts with borderline unresolved scale scores (i.e., 5) were reviewed by at least two coders, and the final classification of unresolved or not unresolved was determined through conference.

Temperament traits. The Schedule for Nonadaptive and Adaptive Personality (Clark, 1995) was used to assess temperament traits. Participants indicated whether each of 375 items accurately described them. We used the following three primary temperament scales: Negative Temperament (28 items), Positive Temperament (27 items), and Disinhibition (35 items). We calculated *T* scores using published norms (Clark, 1995). Cronbach's alphas within each of the three scales were good and ranged from .82 to .92.

Mood disorder symptoms. The Brief Symptom Instrument (Derogatis, 1996) is a short form of the Symptom Checklist-90-Revised and assesses dimensions of psychological health. Partic-

ipants rated on a 5-point Likert scale (0 = *not at all*, 1 = *a little bit*, 2 = *moderately*, 3 = *quite a bit*, 4 = *extremely*) the degree to which they experienced symptoms of depression, anxiety, and interpersonal sensitivity in the previous 7 days. The Brief Symptom Instrument was administered following administration of the AAI as a measure of concurrent mood disturbance. We derived *T* scores for symptoms of depression from published, gender-specific adult nonpatient norms. Cronbach's alphas within each of the scales were adequate (α = .79 to .89).

5-HTTLPR. Buccal swabs were obtained using Cytotech brushes (Medical Packaging Corp, Camarillo, TX). These swabs were assigned a study code, stripped of all other participant identifiers, and stored in a refrigerator at 4 °C until analyzed. DNA from these swabs was prepared using a QIAmp DNA minikit (Qiagen, Inc., Valencia, CA). Genotyping of the 5-HTTLPR locus was carried out using the primers F- GGCGTTGCCGCYCYGAATGC and R-GAGGGACTGAGCTGGACAACCAC (Persico et al., 2000). Vent polymerase was used according to manufacturer's suggestion (New England Biolabs, Ipswich, MA) and 100 μmol/L 7-deaza guanosine triphosphate (Boehringer Mannheim, Indianapolis, IN) was added to aid amplification through this GC-rich region. Cycling parameters were as follows: 98 °C × 15 s, 68 °C × 15 s, and 72 °C × 45 s, with a 7-min final extension at 72 °C. Approximately 3 μL of each of the above polymerase chain reaction products were denatured, then loaded on a standard 6% polyacrylamide sequencing gel and electrophoresed for 2 to 3 hr. The gels were exposed to standard X-ray film and the visualized polymerase chain reaction products sized by comparison to an internal sequencing ladder. Gels were read independently and naively with respect to participant behavioral outcome or genetic background. Frequencies of the 5-HTTLPR alleles (s and l) and genotypes (s/s, s/l, and l/l) are shown in Table 1. Genotypic frequencies were in Hardy–Weinberg equilibrium for the s/s, s/l, and l/l genotypes, $\chi^2(2, N = 86) = 3.56$, $p = .17$.

Statistical Analyses

Univariate associations between study variables were tested. Nonparametric tests (e.g., Kruskal–Wallis, Mann–Whitney *U*, Spearman rho correlations) were used for nonnormally distributed variables. The association of unresolved attachment with 5-HTTLPR genotype was tested using the dichotomous classification (i.e., not unresolved, unresolved) and the 9-point scales to avoid spurious effects (Eaves, 2006). A secondary set of analyses explored individual characteristics (temperament, psychological symptoms) that may be associated with 5-HTTLPR genotype or influence its association with unresolved attachment. Finally, we used regression analyses to examine the influence of control variables (including sex, age, age at adoption, and biological parent diagnosis) on the association between 5-HTTLPR genotype and unresolved attachment. For the logistic regression analyses, 5-HTTLPR was entered as three-level categorical variable (s/s, s/l, or l/l), with the l/l genotype designated as the indicator variable. For the linear regression analyses, we tested the association between unresolved attachment scale scores and 5-HTTLPR genotype using two dummy coded vectors. The first vector, hereafter referred to as homozygous short, coded the s/s genotype as 1 and the s/l or l/l genotypes as 0. The second vector, hereafter referred to as heterozygous short, coded the s/l genotype as 1 and the s/s or l/l genotypes as 0. The two 5-HTTLPR dummy variables were entered simultaneously in the regression model. For the logistic and linear regression analyses, we compared the s/s and s/l 5-HTTLPR genotypes using a second dummy variable (s/s = 0 and s/l = 1). Finally, we tested dominance of the short 5-HTTLPR allele by constructing a third dummy variable where s/s or s/l 5-HTTLPR genotypes were coded 1 and the l/l 5-HTTLPR genotype was coded 0. The final dominance contrast was tested using both logistic and linear regression.

Table 1
Frequency of 5-HTTLPR Alleles and Genotypes

	Allele		Genotype			
Measure	s	l	s/s	s/l	l/l	Total
Frequency	72	100	21	30	35	86
Percentage	42	58	24	35	41	100

Results

Sample Characteristics

Descriptive statistics for study variables are presented in Table 2. The types of losses ranged from loss of a parent (n = 24; 28%), grandparent (n = 63; 73%), other family member (e.g., aunt, uncle, cousin; n = 27; 31%), and/or a nonfamily member (e.g., friend, coworker; n = 27; 31%). The average ages at the time of each type of loss were as follows: parent (M = 31.63 years, SD = 8.48; minimum, maximum = 20, 50), grandparent (M = 14.89 years, SD = 8.49; minimum, maximum = 2, 39), other family member (M = 20.26 years, SD = 9.26; minimum, maximum = 6, 36), and nonfamily member (M = 19.41 years, SD = 9.36; minimum, maximum = 8, 44). There were no participants reporting loss of a parent in childhood.

Types of traumas, as defined by the AAI manual, consisted primarily of excessive physical punishment (e.g., leaving welts or bruises; n = 14; 16%). Four participants were rated for the following overwhelmingly frightening events with no corresponding parental abuse: burglary (n = 1), car accident (n = 2), and combat (n = 1). The average age at the time of trauma was 10.17 years (SD = 5.30). Participants reported an average of 3.24 losses (SD = 1.40; minimum, maximum = 1, 7; n = 86) and an average of 1.28 traumatic events (SD = 0.54; minimum, maximum = 1, 3; n = 16).

Univariate Associations Between Participant Characteristics and Unresolved Attachment and 5-HTTLPR Genotype

Means and standard deviations are presented for continuous variables, and cell frequencies (percentages) are presented for nominal variables. For comparisons involving unresolved attachment, *t* tests were used for normally distributed continuous variables, Mann–Whitney *U* comparisons for variables not normally distributed, and chi-square analyses for nominal data. For tests of 5-HTTLPR genotype associations, we used overall *F* tests for normally distributed variables, Kruskal–Wallis tests of association

Table 2
Study Variables for Total Sample

Variable	*N*	*M* (*SD*) or %	Skewness (*SE*)	Kurtosis (*SE*)
Age	86	36.89 (7.03)	0.91 (0.26)	0.63 (0.51)
No. of loss–trauma events	86	3.23 (1.39)	0.28 (0.26)	−0.29 (0.51)
AAI unresolved attachment (present)	25	29		
AAI scale scores	86			
Overall unresolved		3.58 (2.15)	0.21 (0.26)	−1.28 (0.51)
Unresolved loss	84	3.45 (2.17)	0.30 (0.26)	−1.28 (0.52)
Unresolved trauma	16	3.22 (2.10)	0.50 (0.56)	−1.04 (1.09)
Coherence of transcript	86	4.76 (1.68)	−0.16 (0.26)	−1.15 (0.51)
Sex				
Male	39	45		
Female	47	55		
Biological parent diagnosis				
Control	51	59		
Proband	35	41		
Age at adoption				
Less than 1 month	59	70		
1 to 3 months	10	12		
3 to 6 months	8	10		
Greater than 6 months	7	8		
SNAP				
Negative Temperament	82	44.19 (11.48)	0.81 (0.27)	−0.43 (0.53)
Positive Temperament	82	47.95 (10.60)	−0.72 (0.27)	−0.43 (0.53)
Disinhibited Temperament	82	42.39 (8.48)	0.65 (0.27)	1.20 (0.53)
BSI				
Interpersonal sensitivity	81	50.63 (10.17)	0.93 (0.27)	−0.19 (0.53)
Depression	81	52.73 (10.17)	0.66 (0.27)	−0.57 (0.53)
Anxiety	81	49.04 (10.47)	0.82 (0.27)	−0.25 (0.53)
Hostility	81	51.32 (10.02)	0.35 (0.27)	−0.49 (0.53)

Note. Biological parent diagnosis includes alcohol problems and/or antisocial behaviors. AAI = Adult Attachment Interview; SNAP = Schedule for Nonadaptive and Adaptive Personality; BSI = Brief Symptom Instrument.

for variables not normally distributed, and chi-square tests for nominal data. Finally, nonparametric tests were used for comparisons of the 9-point unresolved scale scores and included Mann–Whitney *U*, Kruskal–Wallis, and Spearman correlations.

AAI unresolved attachment classification. Descriptives for study variables by unresolved and not unresolved attachment are presented in Table 3. Overall coherence of transcript was lower for unresolved attachment (Cohen's $d = 1.07$). Women were nearly three times more likely to be classified as unresolved than were men. No other associations between study variables, including temperament traits and psychological symptoms, and unresolved attachment reached significance (see Table 3).

5-HTTLPR genotype. Descriptive statistics for the study variables by 5-HTTLPR genotypes are presented in Table 4. None of the associations were found to be significant. Furthermore, there were no significant effects of 5-HTTLPR genotype on any of the temperament traits or psychological symptoms (see Table 4). The absence of significant associations suggests that these characteristics do not account for the association between 5-HTTLPR genotype and unresolved attachment.

AAI unresolved attachment scale scores. Further examination of the data revealed that 92% (23/25) of unresolved attachment cases were classified as such based on examination of speech during description of a loss. Therefore, we conducted two sets of univariate analyses involving the unresolved scale scores. First, we analyzed the 9-point scale score representing the maximum score assigned to any loss and/or trauma (U_O). Second, we analyzed the unresolved 9-point scale score specific to descriptions of loss (U_L). Similar analyses were not conducted for the unresolved scale score specific to trauma (U_{Tr}) because of insufficient sample size.

Descriptive statistics for associations between the study variables and unresolved attachment scores are presented in Table 5. Sex, biological parent diagnosis, age at adoption, and temperament traits were not significantly associated with unresolved scale scores. Higher U_L scores were associated with a higher number of reported losses or traumas, lower overall coherence of transcript, and higher symptoms of depression and interpersonal sensitivity. Temperament traits or psychological symptoms were not found to be associated with the U_O scale scores.

In summary, examination of the study variables by unresolved attachment and 5-HTTLPR showed significantly higher unresolved attachment among women and among individuals reporting a greater number of loss–trauma events. Higher symptoms of depression and interpersonal sensitivity were found with higher U_L scores. 5-HTTLPR genotype was not associated with any study variables including overall coherence of transcript.

Univariate Associations Between 5-HTTLPR and Unresolved Attachment Associations

Data showing univariate associations between unresolved attachment and 5-HTTLPR genotype are shown in Table 6. The

Table 3
Study Variables by Unresolved Attachment Classification

Variable	Not unresolved		Unresolved		Test statistic
	N	*M* (*SD*) or %	*N*	*M* (*SD*) or %	
Age	61	37.85 (6.11)	25	40.72 (8.69)	$t(84) = -1.74$
No. of loss–trauma events	61	3.08 (1.33)	25	3.60 (1.47)	$t(84) = -1.59$
AAI coherence of transcript	61	5.21 (1.65)	25	3.66 (1.21)	$t(84) = 4.24^{***}$
Sex					$\chi^2(1) = 4.28^{*}$
Male	32	52	7	28	
Female	29	48	18	72	
Biological parent diagnosis					$\chi^2(1) = 0.78$
Control	38	62	13	52	
Proband	23	38	12	48	
Age at adoption					$\chi^2(3) = 3.55$
Less than 1 month	44	73	15	63	
1 to 3 months	5	8	5	21	
3 to 6 months	5	8	3	13	
Greater than 6 months	6	10	1	4	
SNAP					
Negative Temperament	58	43.62 (12.04)	24	45.82 (10.68)	$z = -1.25$
Positive Temperament	58	47.97 (10.27)	24	47.68 (11.84)	$z = -0.27$
Disinhibited Temperament	58	41.65 (8.87)	24	44.54 (7.67)	$z = -1.63$
BSI					
Interpersonal sensitivity	57	49.58 (92.00)	24	53.17 (10.66)	$z = -1.27$
Depression	57	51.74 (10.39)	24	55.63 (9.60)	$z = -1.59$
Anxiety	57	48.39 (10.35)	24	50.50 (11.17)	$z = -0.65$
Hostility	57	50.81 (10.18)	24	52.42 (9.89)	$z = -0.72$

Note. Biological parent diagnosis includes alcohol problems and/or antisocial behaviors. The *z* statistic is from the Mann–Whitney *U* test. AAI = Adult Attachment Interview; SNAP = Schedule for Nonadaptive and Adaptive Personality; BSI = Brief Symptom Instrument.
$^{*}p < .05$. $^{***}p < .001$.

overall chi-square statistic was significant for the association between unresolved versus not unresolved attachment and 5-HTTLPR genotype. A significant overall association between 5-HTTLPR genotype was also found for the U_L scale scores (see Table 6). In contrast, the association between 5-HTTLPR genotype and U_O scale scores did not reach statistical significance. Despite the nonsignificant overall effect of 5-HTTLPR genotype and the U_O scale score, we present effect sizes for both scale scores. For U_O, medium effect sizes were observed for the homozygous short (Cohen's $d = 0.41$) and heterozygous short (Cohen's $d = 0.50$) genotypes. In comparison, effect sizes were medium to large for the U_L scale scores (Cohen's $d = 0.61$ and 0.68, respectively).

In summary, univariate analyses suggest that carriers of the short variant of 5-HTTLPR show higher rates of unresolved attachment. Multivariate analyses presented below test associations between 5-HTTLPR and unresolved attachment after adjusting for significant control variables. These analyses also directly test dominance of the short 5-HTTLPR allele.

Multivariate Analysis of Unresolved Attachment Classification and 5-HTTLPR

Unresolved attachment classification. Logistic regression tested multivariate associations between 5-HTTLPR genotype and the unresolved attachment classification (see below and see Table 7). We constructed a dichotomous trauma variable (0 = no trauma, 1 = trauma reported) because the experience of trauma was associated with degree of unresolved attachment. We present findings for three unnested models. In Model 1, we determined which control variables significantly predicted unresolved attachment. In Model 2, we retained the significant control variables from Model 1 and added the presence of reported trauma and 5-HTTLPR genotype. Model 3 presents the dominance model of 5-HTLLPR genotype.

The interaction between sex and 5-HTTLPR genotype did not reach statistical significance, Wald(2, $N = 86$) = 4.63, $p = .10$. Therefore, we present findings for the three main effects models (see Table 7). None of the control variables were significant in Model 1. Sex approached significance and was therefore included in Model 2 because of significant univariate associations. Women were more likely to be classified as unresolved (Model 2). The *p* values associated with the homozygous and heterozygous 5-HTTLPR contrasts were both significant (see Model 2, Table 7). The chi-square for the log-likelihood ratios associated with Model 2 was also significant (−2 log likelihood = 86.91), $\chi^2(4, N = 86) = 16.77$, $p < .01$. The dummy variable comparing the homozygous or heterozygous genotypes ($B = -0.18$), Wald(1, $N = 86$) = 0.09, $p = .77$, odds ratio = 0.84, 95% confidence interval = 0.26, 2.70, and the log-likelihood ratio for the model (−2 log likelihood = 66.44), $\chi^2(3, N = 86) = 2.67$, $p = .45$, did not reach statistical significance. The test for dominance of the short 5-HTTLPR allele with the s/s or s/l genotypes (coded as 1) and the l/l 5-HTTLPR genotype (coded 0) are presented in Table 7 (see Model 3, Table 7). The contrast and the overall model (−2 log likelihood = 86.97), $\chi^2(3, N = 86) = 16.70$, $p < .001$, were significant (see Model 3, Table 7). Predicted probabilities estimated from Model 3 were .03 for men reporting no trauma and

Table 4
Study Variables by 5-HTTLPR Genotype

	5-HTTLPR genotype						
	s/s		s/l		l/l		
Variable	*N*	*M* (*SD*) or %	*N*	*M* (*SD*) or %	*N*	*M* (*SD*) or %	Test statistic
Age	21	39.05 (6.44)	30	39.50 (7.49)	35	37.77 (7.05)	$F(2, 83) = 0.52$
No. of loss–trauma events	21	3.48 (1.72)	30	3.17 (1.15)	35	3.14 (1.38)	$F(2, 83) = 0.43$
AAI coherence of transcript	21	4.92 (1.65)	30	4.25 (1.78)	35	5.10 (1.55)	$F(2, 83) = 2.42$
Sex							$\chi^2(2) = 0.54$
Male	10	48	12	40	17	49	
Female	11	52	18	60	18	51	
Biological parent diagnosis							$\chi^2(2) = 2.11$
Control	11	52	16	53	24	69	
Proband	10	48	14	47	11	31	
Age at adoption							$\chi^2(6) = 5.95$
Less than 1 month	12	60	20	67	27	81	
1 to 3 months	3	15	6	19	1	3	
3 to 6 months	3	15	2	~7	3	8	
Greater than 6 months	2	10	2	~7	3	8	
SNAP							
Negative Temperament	20	45.24 (12.54)	28	44.31 (11.44)	34	43.65 (11.56)	K-W: $\chi^2(2) = 0.27$
Positive Temperament	20	49.25 (10.48)	28	46.76 (11.80)	34	48.00 (10.03)	K-W: $\chi^2(2) = 0.59$
Disinhibited Temperament	20	45.57 (10.71)	28	41.79 (7.72)	34	41.26 (7.67)	K-W: $\chi^2(2) = 3.02$
BSI							
Interpersonal sensitivity	20	50.65 (11.09)	28	51.96 (10.18)	33	49.52 (9.88)	K-W: $\chi^2(2) = 0.57$
Depression	20	55.25 (11.35)	28	52.64 (9.75)	33	51.67 (10.06)	K-W: $\chi^2(2) = 1.25$
Anxiety	20	51.90 (11.76)	28	47.89 (10.01)	33	48.21 (10.28)	K-W: $\chi^2(2) = 1.58$
Hostility	20	52.35 (10.05)	28	50.18 (9.89)	33	51.58 (10.43)	K-W: $\chi^2(2) = 0.67$

Note. Biological parent diagnosis includes alcohol problems and/or antisocial behaviors. AAI = Adult Attachment Interview; SNAP = Schedule for Nonadaptive and Adaptive Personality; K-W = Kruskal–Wallis test; BSI = Brief Symptom Instrument.

who had the homozygous long 5-HTTLPR genotype, compared with .75 for female carriers of the short 5-HTTLPR allele who reported trauma.

Overall scale scores for unresolved loss and/or trauma. Examination of the 9-point unresolved scale scores revealed a bimodal distribution. For this reason, we constructed the following dummy variable from the overall unresolved scale score: Unresolved scale scores of 1 were coded as 0 and scale scores above 1 were coded 1. The significance of the dummy variable is not of theoretical importance and allows interpretation of significant associations with the scale scores as predicting degree of unresolved attachment given lack of resolution. To minimize the importance of the inflated significance of the model attributable to the categorical variable, we also present overall model fit attributable solely to the 5-HTTLPR genotypes. We tested the three models described above using linear regression with contrasts. Because of asymmetrical distributions, the unresolved scale scores were ranked prior to being submitted to the regression analysis to approximate a nonparametric test (Conover, 1980, 1999; Conover & Iman, 1981).

The Sex × 5-HTTLPR Genotype interactions were not significant for the U_O or U_L scale scores, $\Delta F(2, 72) = 1.03$, $p = .36$, $\Delta R_{adj}^2 = .02$, and $\Delta F(2, 70) = 0.95$, $p = .39$, $\Delta R_{adj}^2 = .03$, respectively. The final models are presented in Table 8. The overall F statistic for Model 1 did not reach statistical significance for the U_O scale scores, $F(8, 70) = 1.99$, $p = .08$, $R_{adj}^2 = .07$ (see Table 7). The overall F test for Model 2, which included sex, current age, presence of trauma, the dummy unresolved scale score variable, and 5-HTTLPR genotype, was significant, $F(6, 79) = 32.08$, $p < .001$, $R_{adj}^2 = .69$. The estimated increase in R^2 due to 5-HTTLPR genotype in Model 2 was also significant, $\Delta F(2, 79) = 3.66$, $p = .03$, $\Delta R_{adj}^2 = .03$, with significance for the heterozygous short contrast. The comparison of the homozygous short and heterozygous short 5-HTTLPR genotypes did not reach statistical significance ($\beta = 0.11$), $t = 1.34$, $p = .19$. Finally, the dominance contrast, where s/s or s/l 5-HTTLPR genotypes were coded 1 and the l/l genotype was coded 0, was significant in Model 3, $\Delta F(1, 80) = 5.31$, $p < .05$, $\Delta R_{adj}^2 = .02$.

The overall control model (Model 1) was significant for the U_L scale score, $F(8, 68) = 2.71$, $p = .02$, $R_{adj}^2 = .15$ (see Table 8). Women and older participants received higher unresolved loss scale scores. The model testing the significance of 5-HTTLPR genotype after controlling for covariates was significant, $F(6, 77) = 31.74$, $p = .001$, $R_{adj}^2 = .69$, as was the increase in R^2 specific to 5-HTTLPR genotype, $\Delta F(2, 77) = 6.97$, $p < .01$, $\Delta R_{adj}^2 = .05$. The comparison of the homozygous short and heterozygous short 5-HTTLPR genotypes did not reach statistical significance ($\beta = 0.11$), $t = 1.35$, $p = .19$. The overall F test for the dominance model was significant, $\Delta F(5, 78) = 37.38$, $p < .001$, $\Delta R_{adj}^2 = .69$, with significant and unique contributions of the short 5-HTTLPR allele to U_L, $\Delta F(1, 78) = 12.07$, $p = .001$, $\Delta R_{adj}^2 = .05$.

In summary, multivariate analyses tested the association between 5-HTTLPR genotype and unresolved attachment after controlling for significant control variables. Biological parent diagnosis and adoptee age at time of adoption did not reach statistical

Table 5
Study Variables by Unresolved Attachment Scale Scores

	Unresolved scale score					
	U_O			U_L		
Variable	*N*	*M* (*SD*)	Test statistic	*N*	*M* (*SD*)	Test statistic
Age	86		$r = .15$	84		$r = .21$
No. of loss–trauma events	86		$r = .24^{*}$	84		$r = .32^{**}$
AAI coherence of transcript	86		$r = -.28^{**}$	84		$r = -.24^{*}$
Sex			$z = -1.92$			$z = -1.80$
Male	39	3.09 (1.89)		38	2.97 (1.92)	
Female	47	3.99 (2.28)		46	3.85 (2.30)	
Biological parent diagnosis			$z = -0.05$			$z = -0.04$
Control	51	3.57 (2.14)		51	3.44 (2.11)	
Proband	35	3.60 (2.20)		33	3.47 (2.29)	
Age at adoption			K-W: $\chi^2(3) = 2.22$			K-W: $\chi^2(3) = 2.42$
Less than 1 month	59	3.52 (2.20)		58	3.34 (2.20)	
1 to 3 months	10	4.30 (2.43)		10	4.30 (2.43)	
3 to 6 months	8	3.81 (1.69)		7	3.79 (1.82)	
Greater than 6 months	7	2.86 (1.68)		7	2.86 (1.68)	
SNAP						
Negative Temperament	82		$r = .17$	80		$r = .21$
Positive Temperament	82		$r = -.05$	80		$r = -.05$
Disinhibited Temperament	82		$r = .19$	80		$r = .16$
BSI						
Depression	81		$r = .21$	79		$r = .24^{*}$
Interpersonal sensitivity	81		$r = .21$	79		$r = .24^{*}$
Anxiety	81		$r = .11$	79		$r = .12$
Hostility	81		$r = .15$	79		$r = .12$

Note. The *r*s are Spearman rho correlations, and the *z* statistics are from Mann–Whitney *U* tests. U_O = unresolved loss or trauma scale score; U_L = unresolved loss scale score; AAI = Adult Attachment Interview; K-W = Kruskal–Wallis; SNAP = Schedule for Nonadaptive and Adaptive Personality; BSI = Brief Symptom Instrument.
$^{*}p < .05$. $^{**}p < .01$.

significance. Significant control variables included sex and current age (for scale scores). Unresolved attachment was greater among women and older participants. There was not a statistically significant interaction between sex and 5-HTTLPR, suggesting the association with unresolved attachment was invariant. Finally, support for dominance of the short variant of 5-HTTLPR in unresolved attachment was found.

Discussion

Incorporating specific genetic markers into models predicting psychosocial outcomes has enriched our understanding of the complex interplay between genes and environment. The present study examined the association between 5-HTTLPR genotype and unresolved attachment. We found a strong dominant effect of the

Table 6
Univariate Associations Between 5-HTTLPR Genotype and Unresolved Attachment

	5-HTTLPR genotype						
	s/s		s/l		l/l		
AAI measure	*N*	*M* (*SD*) or %	*N*	*M* (*SD*) or %	*N*	*M* (*SD*) or %	Test statistic
Unresolved classification							$\chi^2(2) = 9.07^{*}$
Not Unresolved	13	21	17	28	31	51	
Unresolved	8	32	13	52	4	16	
AAI scale scores							
Overall unresolved	21	3.81 (2.05)	30	4.09 (2.43)	35	3.01 (1.85)	K-W: $\chi^2(2) = 4.08$
Unresolved loss	21	3.81 (2.05)	30	4.09 (2.43)	33	2.64 (1.75)	K-W: $\chi^2(2) = 7.33^{*}$
Unresolved trauma	3	3.33 (2.52)	4	3.13 (2.39)	9	3.22 (2.12)	K-W: $\chi^2(2) = 0.02$
Coherence of transcript	21	4.92 (1.65)	30	4.25 (1.78)	35	5.10 (1.55)	K-W: $\chi^2(2) = 4.00$

Note. AAI = Adult Attachment Interview; K-W = Kruskal–Wallis.
$^{*}p < .05$.

Table 7
Multivariate Logistic Regression Predicting Unresolved Attachment Classification

Source of variation	*B*	Wald	*df*	*p*	OR	95% CI
Model 1: Control variables						
Current age	0.07	2.45	1	.12	1.07	0.98, 1.16
Sex	−1.17	3.48	1	.06	0.31	0.09, 1.06
Biological parent diagnosis	−0.85	1.93	1	.16	0.43	0.13, 1.42
Age adopted		1.77	3	.62		
1 to 3 months	1.03	1.73	1	.19	2.81	0.60, 13.10
3 to 6 months	0.49	0.23	1	.63	1.63	0.22, 11.98
Greater than 6 months	−21.03	0.00	1	1.00	0.00	0.00
Depression	0.04	0.83	1	.36	1.04	0.96, 1.12
Interpersonal sensitivity	0.04	0.85	1	.36	1.04	0.96, 1.12
Model 2: 5-HTTLPR genotype						
Sex	−1.21	4.57	1	.03	0.30	0.10, 0.91
Trauma reported	1.17	2.78	1	.10	3.21	0.82, 12.61
5-HTTLPR contrasts[a]		8.79	2	.01		
s/s genotype	1.85	6.05	1	.02	6.34	1.46, 27.64
s/l genotype	2.01	8.15	1	<.01	7.43	1.88, 29.41
Model 3: 5-HTTLPR dominance effect						
Sex	−1.21	4.64	1	.03	0.30	0.10, 0.90
Trauma reported	−1.16	2.77	1	.10	0.31	0.08, 1.23
5-HTTLPR dominance[b]	1.94	8.73	1	<.01	6.97	1.92, 25.24

Note. $N = 86$. Biological parent diagnosis includes alcohol problems and/or antisocial behaviors. OR = odds ratio; CI = confidence interval.
[a] The contrast option available in the logistic regression command was used. The l/l 5-HTTLPR genotype was designated as the reference group. [b] Dummy coding was used to construct the dominance contrast: s/s or s/l = 1 and l/l = 0.

short allele of 5-HTTLPR on unresolved loss. The magnitude of the effect was equivalent to that found for anxiety-related traits (~3–9%; Lesch et al., 1996), with the short variant of the 5-HTTLPR allele predicting increased risk of unresolved attachment. The effect of 5-HTTLPR genotype was found for both the dichotomous classification and the continuous scale score for unresolved loss. This suggests that our findings are not a statistical artifact (Eaves, 2006). In addition, significant associations between temperament traits or psychological symptoms and 5-HTTLPR genotype were not statistically significant, signifying that these characteristics may not act as mediators. Finally, the attachment–5-HTTLPR genotype association was found for speech related to loss but not overall coherence.

What mechanisms underlie the risk to develop unresolved attachment when having the short 5-HTTLPR allele? We propose that unresolved attachment is indicative of an emotional regulatory system that has short circuited. The ventral and medial prefrontal cortexes participate in regulation of the amygdaloid complex (Blair et al., 2007; Drevets et al., 1997). These networks are influenced by the 5-HTTLPR genotype (Heinz et al., 2005; Pezawas et al., 2005) and regulate subjective appreciation of emotional experiences, thereby playing a role in the recall and inhibition of emotional memories (Grimm et al., 2006; Phan et al., 2004; Phan, Wager, Taylor, & Liberzon, 2004; Sierra-Mercado, Corcoran, Lebron-Milad, & Quirk, 2006). The presence of the 5-HTTLPR short allele may influence the interconnectivity between these brain regions (Bertolino et al., 2005), thereby increasing susceptibility to the disorganizing effects of elevated affective intensity experienced during discussions of loss (Fearon & Mansell, 2001; Hariri et al., 2005; Heinz et al., 2005; Hesse & Main, 2006). Impaired interconnectivity may result in a reduced ability to effectively regulate heightened emotion and ultimately interfere with active monitoring of speech. This interpretation is consistent with several indexes of unresolved attachment. For example, "dead–not dead" is an example of a lapse of reasoning in which a deceased person is spoken about as though the person was still alive and is indicated by shifts to present tense. Heightened affectivity associated with talking about the deceased, coupled with impaired connection of memories surrounding the events (e.g., date, time), may result in the momentary and unmonitored shift to present tense. Excessive attention to detail when describing a traumatic event might arise because of heightened emotional reactivity and a simultaneous inability to regulate orientation.

In addition to understanding potential underlying physiological mechanisms by which 5-HT regulation affects attachment, we must recognize the plausibility that multiple neurotransmitter systems and neural circuitries are involved in emotion regulation (Luciana, Collins, & Depue, 1998; Meyer-Lindenberg et al., 2005; Ressler & Nemeroff, 2000). In this context, comparison of the findings in this study with the findings on the neurobiology of attachment in childhood is warranted. Research on the genetic susceptibility for childhood disorganized attachment has focused on the dopaminergic system (i.e., DRD4 gene; Bakermans-Kranenburg & Van IJzendoorn, 2004; Gervai et al., 2005; Lakatos et al., 2000, 2002; Van IJzendoorn & Bakermans-Kranenburg, 2006). Disorganized infant attachment is viewed as a breakdown in the infant's behavioral and attentional regulation of fear. Infants may demonstrate sequential and contradictory approach and avoidance behaviors, freezing, stereotypies, disoriented wandering, or movement without direction or finality. The amygdala and associated neural circuits are also influenced by dopamine (Phelps & LeDoux, 2005; Rosenkranz & Grace, 2001, 2003; Sesack, Carr,

Table 8
Multivariate Linear Regressions Predicting Ranked Unresolved Scale Scores

	Ranked unresolved scale scores					
	U_O			U_L		
Source of variation	β	*t*	*p*	β	*t*	*p*
Model 1: Control variables						
Sex	.19	1.67	.10	.20	1.77	.08
Current age	.26	2.17	.03	.33	2.66	.01
Biological parent diagnosis	.07	0.66	.51	.05	0.47	.64
Age adopted contrasts						
1 to 3 months	.05	0.48	.64	.06	0.57	.57
3 to 6 months	−.02	−0.18	.86	−.06	−0.51	.61
Greater than 6 months	−.31	−2.45	.02	−.29	−2.37	.02
Depression	.13	0.84	.41	.16	1.03	.31
Interpersonal sensitivity	.19	1.21	.23	.18	1.14	.26
Model 2: 5-HTTLPR genotype						
Sex	.15	2.42	.02	.14	2.18	.03
Current age	.06	0.86	.39	.11	1.67	.10
Trauma reported	−.03	−0.50	.62	−.11	−1.66	.10
Dummy unresolved present[a]	.79	12.44	.001	.78	11.79	.001
5-HTTLPR contrasts[b]						
Homozygous short	.07	1.02	.31	.14	2.03	.05
Heterozygous short	.18	2.70	.01	.26	3.71	.001
Model 3: 5-HTTLPR dominance effect						
Sex	.16	2.51	.01	.14	2.28	.03
Current age	.06	0.92	.36	.11	1.73	.09
Trauma reported	−.03	−0.42	.67	−.10	−1.58	.12
Dummy unresolved present[a]	.78	12.30	.001	.75	11.67	.001
5-HTTLPR dominance[c]	.14	2.30	.02	.22	3.47	.001

Note. Biological parent diagnosis includes alcohol problems and/or antisocial behaviors. U_O = scale score for unresolved loss or trauma ($N = 86$); U_L = scale score for unresolved loss ($N = 84$).
[a] Unresolved scale score recoded into dummy variable (scale score of 1 = 0; scale scores > 1 = 1). [b] Dummy coding was used to construct the 5-HTTLPR contrasts; two vectors were created: the homozygous short vector (s/s = 1, s/l = 0, l/l = 0) and the heterozygous short vector (s/s = 0, s/l = 1, l/l = 0). [c] Dummy coding was used to construct the dominance contrast: s/s or s/l = 1 and l/l = 0.

Omelchenko, & Pinto, 2003), as are brain regions that play a functional role in emotion regulation and nonsocial regulatory abilities (e.g., attention; Chamberlain, Muller, Robbins, & Sahakian, 2006). Therefore, exploring the role of dopaminergic regulation in infant disorganized attachment, and possibly adult unresolved attachment, appears warranted.

Finally, studies in molecular psychiatry have shown phenotypic specificity when exploring genetic susceptibility for a wide variety of behaviors (Rhee & Waldman, 2002; Roisman & Fraley, 2006). Similarly, our findings suggest that the origins of unresolved attachment in adulthood may have etiologic specificity that is experientially and possibly genetically moderated (Hughes, Turton, Hopper, McGauley, & Fonagy, 2004; Jacobvitz, Leon, & Hazen, 2006; Lyons-Ruth, Yellin, Melnick, & Atwood, 2003). Although conclusions drawn from our data are limited, we showed significant 5-HTTLPR genotype associations specific to unresolved loss. All but 2 of our participants were classified unresolved with regard to loss experiences, thus the specificity of the association with the scale scores for unresolved loss is not surprising. However, the specific influence of the 5-HTTLPR genotype is also consistent with differences in the behavioral correlates among parents classified as unresolved because of loss versus trauma (Abrams, Rifkin, & Hesse, 2006; Hesse & Main, 2006; Jacobvitz et al., 2006; Lyons-Ruth, Yellin, Melnick, & Atwood, 2005). The results of the present study may offer a basis for further hypothesis testing aimed at explaining incomplete prediction of infant disorganized attachment from parental unresolved attachment (Gervai et al., 2007; Madigan et al., 2006; Schuengel, Bakermans-Kranenburg, & Van IJzendoorn, 1999; Van IJzendoorn & Bakermans-Kranenburg, 2006; Van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999).

Some caveats need to be addressed. Because our sample was composed of adoptees, the results of this study need to be interpreted with caution. In the present study, rates of unresolved attachment were higher than expected. The loss of a child by miscarriage or perceived loss of a child because of infertility by the adoptive parent(s) may influence parental behavior toward future children, placing an individual at greater risk for unresolved status in adulthood (Bakermans-Kranenburg, Schuengel, & Van IJzendoorn, 1999; Hughes, Turton, McGauley, & Fonagy, 2006). Furthermore, adopted individuals may be more susceptible to experiences of loss, given possible feelings of loss associated with being adopted (Borders, Penny, & Portnoy, 2000; Feeney, Passmore, & Peterson, 2007). Our study is also limited by the examination of a single genetic polymorphism. Evidence of gene–gene interactions (e.g., the DRD4 gene and the functional −521 C/T promoter polymorphism) have been reported, albeit inconsistently, which further complicates interpretation of isolated genetic effects

(Bakermans-Kranenburg & Van IJzendoorn, 2004; Gervai et al., 2005; Lakatos et al., 2002; Van IJzendoorn & Bakermans-Kranenburg, 2006). Finally, it is standard among researchers using the adoption paradigm to assume that passive gene–environment correlations are absent because of the lack of biological relatedness between parent and child. Passive gene–environment correlation refers to the inheritance of genes that are also associated with characteristics of the environment. We acknowledge that passive gene–environment correlations are possible in these data. The adoptive parents and their biologically unrelated offspring could have common genotypes for a specific genetic marker (e.g., 5-HTTLPR polymorphism) that might also be correlated with environmental factors associated with unresolved attachment (e.g., frightened–frightening maternal behavior). Unfortunately, direct testing of passive gene–environment correlations is not possible with these data because of the absence of genetic information on the adoptive parents (or a comparable biologically intact comparison group). Therefore, we can only tentatively argue that passive gene–environment correlations do not contribute to our findings.

In summary, this study provides preliminary evidence of an underlying genetic susceptibility to unresolved attachment in adulthood. The findings of the present research are intriguing, but replication is necessary and needs to be extended to samples closely matched to the characteristics of ours as well as to more generally defined populations. We hope that this study fosters research aimed at uncovering the exact mechanisms and specificity by which the 5-HTTLPR genotype regulates emotions (Fox, Hane, & Pine, 2007).

References

Abrams, K. Y., Rifkin, A., & Hesse, E. (2006). Examining the role of parental frightened/frightening subtypes in predicting disorganized attachment within a brief observational procedure. *Developmental Psychopathology, 18,* 345–361.

Adolphs, R., Baron-Cohen, S., & Tranel, D. (2002). Impaired recognition of social emotions following amygdala damage. *Journal of Cognitive Neuroscience, 14,* 1264–1274.

Bakermans-Kranenburg, M. J., Schuengel, C., & Van IJzendoorn, M. H. (1999). Unresolved loss due to miscarriage: An addition to the Adult Attachment Interview. *Attachment and Human Development, 1,* 157–170.

Bakermans-Kranenburg, M. J., & Van IJzendoorn, M. H. (2004). No association of the dopamine D4 receptor (DRD4) and −521 C/T promoter polymorphisms with infant attachment disorganization. *Attachment and Human Development, 6,* 211–222.

Bakermans-Kranenburg, M. J., Van IJzendoorn, M. H., Bokhorst, C. L., & Schuengel, C. (2004). The importance of shared environment in infant–father attachment: A behavioral genetic study of the Attachment Q-Sort. *Journal of Family Psychology, 18,* 545–549.

Bennett, A., Lesch, K., Heils, A., Long, J., Lorenz, J., Shoaf, S., et al. (2002). Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Molecular Psychiatry, 7,* 118–122.

Bertolino, A., Arciero, G., Rubino, V., Latorre, V., De Candia, M., Mazzola, V., et al. (2005). Variation of human amygdala response during threatening stimuli as a function of 5′HTTLPR genotype and personality style. *Biological Psychiatry, 57,* 1517–1525.

Blair, K. S., Smith, B. W., Mitchell, D. G., Morton, J., Vythilingam, M., Pessoa, L., et al. (2007). Modulation of emotion by cognition and cognition by emotion. *NeuroImage, 35,* 430–440.

Bokhorst, C. L., Bakermans-Kranenburg, M. J., Fearon, R. M., Van IJzendoorn, M. H., Fonagy, P., & Schuengel, C. (2003). The importance of shared environment in mother–infant attachment security: A behavioral genetic study. *Child Development, 74,* 1769–1782.

Borders, L. D., Penny, J. M., & Portnoy, F. (2000). Adult adoptees and their friends: Current functioning and psychosocial well-being. *Family Relations, 49,* 407–418.

Buchheim, A., Erk, S., George, C., Kachele, H., Ruchsow, M., Spitzer, M., et al. (2006). Measuring attachment representation in an fMRI environment: A pilot study. *Psychopathology, 39,* 144–152.

Canli, T., & Lesch, K. P. (2007). Long story short: The serotonin transporter in emotion regulation and social cognition. *Nature Neuroscience, 10,* 1103–1109.

Caspers, K., Yucuis, R., Troutman, B., Arndt, S., & Langbehn, D. (2007). A sibling adoption study of adult attachment: The influence of shared environment on attachment states of mind. *Attachment and Human Development, 9,* 375–391.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003, July 18). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science, 301,* 386–389.

Chamberlain, S. R., Muller, U., Robbins, T. W., & Sahakian, B. J. (2006). Neuropharmacological modulation of cognition. *Current Opinions in Neurology, 19,* 607–612.

Champoux, M., Bennett, A., Shannon, C., Higley, J. D., Lesch, K. P., & Suomi, S. J. (2002). Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Molecular Psychiatry, 7,* 1058–1063.

Clark, L. (1995). *SNAP: Schedule for Nonadaptive and Adaptive Personality—Schedule for administration, scoring, and interpretation.* Minneapolis, MN: University of Minnesota Press.

Collier, D. A., Stober, G., Li, T., Heils, A., Catalano, M., Di Bella, D., et al. (1996). A novel functional polymorphism within the promoter of the serotonin transporter gene: Possible role in susceptibility to affective disorders. *Molecular Psychiatry, 1,* 453–460.

Conover, W. J. (1980). *Practical nonparametric statistics* (2nd ed.). New York: Wiley.

Conover, W. J. (1999). *Practical nonparametric statistics* (3rd ed.). New York: Wiley.

Conover, W. J., & Iman, R. L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician, 35,* 124–133.

Constantino, J. N., Chackes, L. M., Wartner, U. G., Gross, M., Brophy, S. L., Vitale, J., et al. (2006). Mental representations of attachment in identical female twins with and without conduct problems. *Child Psychiatry and Human Development, 37,* 65–72.

Derogatis, L. (1996). *Brief Symptom Instrument.* Minneapolis, MN: National Computer Systems.

Drevets, W. C., Price, J. L., Simpson, J. R., Jr., Todd, R. D., Reich, T., Vannier, M., et al. (1997, April 24). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature, 386,* 824–827.

Eaves, L. (2006). Genotype × Environment interaction in psychopathology: Fact or artifact? *Twin Research and Human Genetics, 9,* 1–8.

Ebstein, R. P. (2006). The molecular genetic architecture of human personality: Beyond self-report questionnaires. *Molecular Psychiatry, 11,* 427–445.

Fearon, R. M., & Mansell, W. (2001). Cognitive perspectives on unresolved loss: Insights from the study of PTSD. *Bulletin of the Menninger Clinic, 65,* 380–396.

Feeney, J. A., Passmore, N. L., & Peterson, C. C. (2007). Adoption, attachment, and relationship concerns: A study of adult adoptees. *Personal Relationships, 14,* 129–147.

Fox, N., Hane, A., & Pine, D. (2007). Plasticity for affective neurocircuitry: How the environments affects gene expression. *Current Directions in Psychological Science, 16,* 1–5.

Fox, N. A., Nichols, K. E., Henderson, H. A., Rubin, K., Schmidt, L.,

Hamer, D., et al. (2005). Evidence for a gene–environment interaction in predicting behavioral inhibition in middle childhood. *Psychological Science, 16,* 921–926.

Gervai, J., Nemoda, Z., Lakatos, K., Ronai, Z., Toth, I., Ney, K., et al. (2005). Transmission disequilibrium tests confirm the link between DRD4 gene polymorphism and infant attachment. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 132,* 126–130.

Gervai, J., Novak, A., Lakatos, K., Toth, I., Danis, I., Ronai, Z., et al. (2007). Infant genotype may moderate sensitivity to maternal affective communications: Attachment disorganization, quality of care, and the DRD4 polymorphism. *Social Neuroscience, 2,* 307–319.

Grimm, S., Schmidt, C. F., Bermpohl, F., Heinzel, A., Dahlem, Y., Wyss, M., et al. (2006). Segregated neural representation of distinct emotion dimensions in the prefrontal cortex—An fMRI study. *NeuroImage, 30,* 325–340.

Hariri, A. R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., et al. (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry, 62,* 146–152.

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., et al. (2002, July 19). Serotonin transporter genetic variation and the response of the human amygdala. *Science, 297,* 400–403.

Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D., et al. (2005). Amygdala–prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience, 8,* 20–21.

Hesse, E., & Main, M. (2000). Disorganized infant, child, and adult attachment: Collapse in behavioral and attentional strategies. *Journal of the American Psychoanalytic Association, 48,* 1097–1127.

Hesse, E., & Main, M. (2006). Frightened, threatening, and dissociative parental behavior in low-risk samples: Description, discussion, and interpretations. *Developmental Psychopathology, 18,* 309–343.

Hesse, E., & Van IJzendoorn, M. H. (1999). Propensities towards absorption are related to lapses in the monitoring of reasoning or discourse during the Adult Attachment Interview: A preliminary investigation. *Attachment and Human Development, 1,* 67–91.

Hughes, P., Turton, P., Hopper, E., McGauley, G. A., & Fonagy, P. (2004). Factors associated with the unresolved classification of the Adult Attachment Interview in women who have suffered stillbirth. *Developmental Psychopathology, 16,* 215–230.

Hughes, P., Turton, P., McGauley, G. A., & Fonagy, P. (2006). Factors that predict infant disorganization in mothers classified as U in pregnancy. *Attachment and Human Development, 8,* 113–122.

Jacobvitz, D., Leon, K., & Hazen, N. (2006). Does expectant mothers' unresolved trauma predict frightened/frightening maternal behavior? Risk and protective factors. *Developmental Psychopathology, 18,* 363–379.

Kaufman, J., Yang, B. Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J. H., et al. (2004). Social supports and serotonin transporter gene moderate depression in maltreated children. *Proceedings of the National Academy of Sciences of the USA, 101,* 17316–17321.

Kendler, K. S., Kuhn, J. W., Vittum, J., Prescott, C. A., & Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: A replication. *Archives of General Psychiatry, 62,* 529–535.

Lakatos, K., Nemoda, Z., Toth, I., Ronai, Z., Ney, K., Sasvari-Szekely, M., et al. (2002). Further evidence for the role of the dopamine D4 receptor (DRD4) gene in attachment disorganization: Interaction of the exon III 48-bp repeat and the −521 C/T promoter polymorphisms. *Molecular Psychiatry, 7,* 27–31.

Lakatos, K., Toth, I., Nemoda, Z., Ney, K., Sasvari-Szekely, M., & Gervai, J. (2000). Dopamine D4 receptor (DRD4) gene polymorphism is associated with attachment disorganization in infants. *Molecular Psychiatry, 5,* 633–637.

Leibenluft, E., Gobbini, M. I., Harrison, T., & Haxby, J. V. (2004). Mothers' neural activation in response to pictures of their children and other children. *Biological Psychiatry, 56,* 225–232.

Lemche, E., Giampietro, V. P., Surguladze, S. A., Amaro, E. J., Andrew, C. M., Williams, S. C., et al. (2006). Human attachment security is mediated by the amygdala: Evidence from combined fMRI and psychophysiological measures. *Human Brain Mapping, 27,* 623–635.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996, November 29). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274,* 1527–1531.

Luciana, M., Collins, P. F., & Depue, R. A. (1998). Opposing roles for dopamine and serotonin in the modulation of human spatial working memory functions. *Cerebral Cortex, 8,* 218–226.

Lyons-Ruth, K., Bronfman, E., & Parsons, E. (1999). Atypical attachment in infancy and early childhood among children at developmental risk—IV: Maternal frightened, frightening, or atypical behavior and disorganized infant attachment patterns. *Monograph of the Society for Research in Child Development, 64,* 67–96, 213–220.

Lyons-Ruth, K., Yellin, C., Melnick, S., & Atwood, G. (2003). Childhood experiences of trauma and loss have different relations to maternal unresolved and hostile–helpless states of mind on the AAI. *Attachment and Human Development, 5,* 330–414.

Lyons-Ruth, K., Yellin, C., Melnick, S., & Atwood, G. (2005). Expanding the concept of unresolved mental states: Hostile/helpless states of mind on the Adult Attachment Interview are associated with disrupted mother–infant communication and infant disorganization. *Development and Psychopathology, 17,* 1–23.

Madigan, S., Bakermans-Kranenburg, M. J., Van IJzendoorn, M. H., Moran, G., Pederson, D. R., & Benoit, D. (2006). Unresolved states of mind, anomalous parental behavior, and disorganized attachment: A review and meta-analysis of a transmission gap. *Attachment and Human Development, 8,* 89–111.

Madigan, S., Moran, G., Schuengel, C., Pederson, D. R., & Otten, R. (2007). Unresolved maternal attachment representations, disrupted maternal behavior and disorganized attachment in infancy: Links to toddler behavior problems. *Journal of Child Psychology and Psychiatry, 48,* 1042–1050.

Main, M. (1999). Epilogue: Attachment theory—Eighteen points with suggestions for future studies. In J. Cassidy & P. R. Shaver (Eds.), *Handbook of attachment: Theory, research, and clinical applications* (pp. 845–888). New York: Guilford.

Main, M. (2000). The organized categories of infant, child, and adult attachment: Flexible vs. inflexible attention under attachment-related stress. *Journal of the American Psychoanalytic Association, 48,* 1055–1096.

Main, M., & Goldwyn, R. (1998). *Adult attachment scoring and classification system*. Berkeley, CA: University of California at Berkeley.

Meyer-Lindenberg, A., Hariri, A. R., Munoz, K. E., Mervis, C. B., Mattay, V. S., Morris, C. A., et al. (2005). Neural correlates of genetically abnormal social cognition in Williams syndrome. *Nature Neuroscience, 8,* 991–993.

O'Connor, T. G., & Croft, C. M. (2001). A twin study of attachment in preschool children. *Child Development, 72,* 1501–1511.

Persico, A. M., Militerni, R., Bravaccio, C., Schneider, C., Melmed, R., Conciatori, M., et al. (2000). Lack of association between serotonin transporter gene promoter variants and autistic disorder in two ethnically distinct samples. *American Journal of Medical Genetics, 96,* 123–127.

Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., et al. (2005). 5-HTTLPR polymorphism impacts human cingulated–amygdala interactions: A genetic susceptibility mechanism for depression. *Nature Neuroscience, 8,* 828–834.

Phan, K. L., Taylor, S. F., Welsh, R. C., Ho, S. H., Britton, J. C., & Liberzon, I. (2004). Neural correlates of individual ratings of emotional salience: A trial-related fMRI study. *NeuroImage, 21,* 768–780.

Phan, K. L., Wager, T. D., Taylor, S. F., & Liberzon, I. (2004). Functional neuroimaging studies of human emotions. *CNS Spectrums, 9,* 258–266.

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: From animal models to human behavior. *Neuron, 48,* 175–187.

Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003). Neurobiology of emotion perception I: The neural basis of normal emotion perception. *Biological Psychiatry, 54,* 504–514.

Ressler, K. J., & Nemeroff, C. B. (2000). Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depression and Anxiety, 12*(Suppl. 1), 2–19.

Rhee, S. H., & Waldman, I. D. (2002). Genetic and environmental influences on antisocial behavior: A meta-analysis of twin and adoption studies. *Psychological Bulletin, 128,* 490–529.

Roisman, G. I., & Fraley, R. C. (2006). The limits of genetic influence: A behavior-genetic analysis of infant–caregiver relationship quality and temperament. *Child Development, 77,* 1656–1667.

Rosenkranz, J. A., & Grace, A. A. (2001). Dopamine attenuates prefrontal cortical suppression of sensory inputs to the basolateral amygdala of rats. *Journal of Neuroscience, 21,* 4090–4103.

Rosenkranz, J. A., & Grace, A. A. (2003). Affective conditioning in the basolateral amygdala of anesthetized rats is modulated by dopamine and prefrontal cortical inputs. *Annals of the New York Academy of Sciences, 985,* 488–491.

Schuengel, C., Bakermans-Kranenburg, M. J., & Van IJzendoorn, M. H. (1999). Frightening maternal behavior linking unresolved loss and disorganized infant attachment. *Journal of Consulting and Clinical Psychology, 67,* 54–63.

Sesack, S. R., Carr, D. B., Omelchenko, N., & Pinto, A. (2003). Anatomical substrates for glutamate-dopamine interactions: Evidence for specificity of connections and extrasynaptic actions. *Annals of the New York Academy of Sciences, 1003,* 36–52.

Sierra-Mercado, D., Jr., Corcoran, K. A., Lebron-Milad, K., & Quirk, G. J. (2006). Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction. *European Journal of Neuroscience, 24,* 1751–1758.

Suomi, S. J. (1999). Attachment in rhesus monkeys. In J. Cassidy & P. R. Shaver (Eds.), *Handbook of attachment: Theory, research, and clinical applications* (pp. 181–197). New York: Guilford.

Suomi, S. J. (2003). Gene–environment interactions and the neurobiology of social conflict. *Annals of the New York Academy of Sciences, 1008,* 132–139.

Suomi, S. J. (2006). Risk, resilience, and Gene × Environment interactions in rhesus monkeys. *Annals of the New York Academy of Sciences, 1094,* 52–62.

Van IJzendoorn, M. H., & Bakermans-Kranenburg, M. J. (2006). DRD4 7-repeat polymorphism moderates the association between maternal unresolved loss or trauma and infant disorganization. *Attachment and Human Development, 8,* 291–307.

Van IJzendoorn, M. H., & Bakermans-Kranenburg, M. J. (2008). The distribution of adult attachment representations in clinical groups: Meta-analytic search for patterns of attachment. In H. Steele & M. Steele (Eds.), *Clinical applications of the Adult Attachment Interview* (pp. 69–98). New York: Guilford.

Van IJzendoorn, M. H., Moran, G., Belsky, J., Pederson, D., Bakermans-Kranenburg, M. J., & Kneppers, K. (2000). The similarity of siblings' attachments to their mother. *Child Development, 71,* 1086–1098.

Van IJzendoorn, M. H., Schuengel, C., & Bakermans-Kranenburg, M. J. (1999). Disorganized attachment in early childhood: Meta-analysis of precursors, concomitants, and sequelae. *Developmental Psychopathology, 11,* 225–249.

Wilhelm, K., Mitchell, P. B., Niven, H., Finch, A., Wedgwood, L., Scimone, A., et al. (2006). Life events, first depression onset and the serotonin transporter gene. *British Journal of Psychiatry, 188,* 210–215.

Williams, R. B., Marchuk, D. A., Gadde, K. M., Barefoot, J. C., Grichnik, K., Helms, M. J., et al. (2003). Serotonin-related gene polymorphisms and central nervous system serotonin function. *Neuropsychopharmacology, 28,* 533–541.

Yates, W., Cadoret, R., & Troughton, E. (1999). The Iowa Adoption Studies: Methods and results. In M. LaBuda & E. Grigorenko (Eds.), *On the way to individuality: Current methodological issues in behavioral genetics* (pp. 95–121). Commack, NY: Nova Science Publishers.

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