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Interaction between the *5-HTTLPR* serotonin transporter polymorphism and environmental adversity for mood and anxiety psychopathology: evidence from a high-risk community sample of young adults

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Abstract

Previous research examining gene–environment interaction (G × E) with regard to vulnerability to depression and anxiety has yielded conflicting results. The present study was designed to further investigate G × E between *5-HTTLPR* and exposure to environmental adversity, using different phenotypic and genotypic characterizations as well as different types of adversity within a prospective study design. Data were available from an ongoing epidemiological cohort study following the outcome of early risk factors from birth to adulthood. At age 19 yr, 309 participants (142 males, 167 females) were characterized on measures of depression and anxiety through interview and questionnaire (DSM-IV diagnosis, Beck Depression Inventory, Harm Avoidance). Environmental adversity was assessed at birth (family adversity), and at age 19 yr (stressful life events). Bi- and tri-allelic *5-HTTLPR* genotypes were obtained from genomic DNA. Results indicated that depression and anxiety in 19-yr-olds were strongly associated with both family adversity and stressful life events. Individuals with the LL genotype of *5-HTTLPR* who were exposed to high family adversity displayed significantly higher rates of depressive or anxiety disorders and had more depressive symptoms than those without either condition. This G × E replicates recent findings from an epidemiological cohort study of adolescents but is in contrast to many previous reports suggesting an interaction with the S allele. No evidence for G × E was obtained with regard to current stressful life events and trait anxiety. One possible source for the conflicting findings might be attributed to heterogeneity in depression phenotypes and environmental adversity.

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Introduction

Mood and anxiety psychopathology comprises highly prevalent psychiatric disorders with a lifetime incidence of >20% in Western countries. Many of these disorders are associated with significant morbidity and increased mortality and co-occurrence of other clinical syndromes (such as substance use disorder) is common. The social and economic consequences of these conditions are huge; major depression is currently the leading cause of disability in individuals aged 15–44 yr, and it is estimated that it will rank first in the global burden of disease by 2030 (Mathers & Loncar, 2006). The comorbidity of these disorders is high, with prospective studies indicating that anxiety

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disorders often precede the onset of depression and identify individuals at risk for developing depression (Beesdo *et al*. 2007). Despite intense research efforts, progress in understanding the aetiology of these disorders is limited. Genetic epidemiological studies reported moderate heritability, with an estimated genetic contribution of ~40–50% for depression and 30–40% for anxiety disorders, although the specific genes that confer risk are largely unidentified (Hettema *et al*. 2001; McGuffin *et al*. 2003).

Of the brain systems suggested to be involved in the aetiology of these disorders, much attention has been placed on serotonergic (5-HT) neurotransmission. A critical role in the regulation of serotonin function in the brain pertains to the serotonin transporter (5-HTT), making the gene encoding this protein a prominent candidate for genetic association studies. This locus contains a well-studied bi-allelic polymorphism in the promoter region (*5-HTTLPR*) consisting of two common alleles, which appear to result in differential 5-HTT expression and function (Heils *et al*. 1996; Lesch *et al*. 1996). Compared with the 'long' (L) variant, the 'short' (S) allele was found to exhibit significantly lower transcriptional activity of the 5-HTT gene *in vitro*. Recently, a third functional allele has been described, resulting from an A>G substitution in the L allele, which was reported to be equivalent in expression to the S allele (Nakamura *et al*. 2000). Failure to distinguish between these alleles may be one reason for the inconsistency in previous studies investigating the effects of *5-HTTLPR*.

Since the first reports by Lesch *et al*. (1996), numerous studies have examined a potential role of *5-HTTLPR* in determining a variety of personality traits and psychiatric disorders, including depression and anxiety. However, a consistent picture of the contribution of this polymorphism has not yet emerged. In a meta-analysis surveying 17 independent samples, evidence for a significant, albeit weak association of *5-HTTLPR* with bipolar depression was found, indicating that the S allele increased the risk (OR 1.13, 95% CI 1.05–1.22). While no association with unipolar depression was detected in this meta-analysis which was comprised of samples of small sizes (Lasky-Su *et al*. 2005), a subsequent analysis conducted in a large and homogeneous sample did find association (OR 1.26, 95% CI 1.07–1.48) (Hoefgen *et al*. 2005). A meta-analysis of the association between *5-HTTLPR* and trait anxiety based on 26 studies failed to provide support for a relationship between anxiety and the presence of the S allele (Schinka *et al*. 2004). Recently, in a meta-analysis of 51 studies, the negative findings of previous surveys was confirmed with regard to two measures of trait anxiety [Harm Avoidance (HA) and EPQ Neuroticism], suggesting, however, a possible association with NEO Neuroticism (Munafo *et al*. 2008*c*).

Epidemiological studies have amply demonstrated that stress constitutes a major risk factor for the development and persistence of depression and anxiety. However, little is known as to why certain individuals exposed to specific environmental pathogens actually develop a disorder while others remain unaffected. Only very recently have researchers started to investigate the importance of interactions between genetic and environmental factors (G × E) in the development of these disorders. In a seminal study, Caspi *et al*. (2003) reported an interaction between *5-HTTLPR* and depression, demonstrating that the S allele was associated with depression only in individuals exposed to stressful life events (SLE). These findings have attracted a large number of replication attempts, the majority of which provided results largely consistent with the initial reports (Uher & McGuffin, 2008). While a similar relationship was confirmed for alcohol use, no evidence was found for G × E in anxiety.

Although the majority of previous positive G × E findings showed that individuals exposed to stress had an increased risk of depression only when carrying the S allele of *5-HTTLPR*, a few studies revealed the opposite effect suggesting an interaction with the L allele (Eley *et al*. 2004; Gillespie *et al*. 2005; Surtees *et al*. 2006). In a recent publication reporting the results from two large Australian community surveys of adolescents and young adults, Chipman *et al*. (2007) failed to replicate a significant G × E between *5-HTTLPR* and recent SLE or childhood adversity on symptoms of depression. However, using data from the Australian Temperament Project (ATP), a longitudinal study following child development since early infancy, Chipman *et al*. found evidence that adolescents aged 17–18 yr with the LL genotype of *5-HTTLPR* who had experienced persistently high levels of family adversity were at a greater risk of depression than those without either condition. Based on these findings, the authors proposed that the duration of exposure to stress might be a critical condition which may account for the conflicting results.

A similar finding was reported by Olsson *et al*. (2005) with regard to anxiety in adolescents, indicating an increase of persistent ruminative anxiety in risk settings with each additional copy of the L allele. Recently, a study by Sjoberg *et al*. (2006) in an adolescent sample suggested that the interaction between *5-HTTLPR* and environmental stress factors on depression might be sex-specific. While females with the

SS genotype displayed a significant increase in depressive symptoms when exposed to psychosocial adversity, the opposite effect was found in males, with higher scores only in those who were carriers of the LL genotype and had experienced adversity. Considering the current evidence, in a most recent meta-analysis (Munafo *et al*. 2008*b*) concluded that the effects of *5-HTTLPR* and its interaction with SLE on risk of depression were negligible and positive results were compatible with chance findings.

Given the discrepant evidence regarding the moderating effect of *5-HTTLPR* on vulnerability to adverse environments, the present study aims to further investigate G × E between *5-HTTLPR* and exposure to environmental adversity on depression and anxiety in a high-risk community sample of young adults. Particular attention is given to variation in genotypic and phenotypic characteristics as well as to the duration of stressors, considering both persistent family adversity and current SLE as potential environmental pathogens.

Method

Participants

This investigation was conducted as part of the Mannheim Study of Children at Risk, an ongoing epidemiological cohort study following the outcome of early risk factors from infancy into adulthood (Laucht *et al*. 2000). The initial sample comprised 384 children born between 1986 and 1988, of predominantly (>99.0%) European descent. Infants were recruited from two obstetric and six children's hospitals of the Rhine-Neckar region of Germany and were included consecutively into the sample according to a two-factorial design intended to enrich and control the status of the sample regarding obstetric and psychosocial risks (for more details, see Laucht *et al*. 1997). Only first-born children with singleton births and German-speaking parents were enrolled in the study. Furthermore, children with severe physical handicaps, obvious genetic defects, or metabolic diseases were excluded. Assessments were conducted at regular intervals throughout development, most recently at age 19 yr. Of the initial sample of 384 participants, 18 (4.7%) were excluded because of severe handicaps (IQ or MQ<70 or neurological disorder), 39 (10.2%) were dropouts or had incomplete data, and 18 (4.7%) refused to participate in blood sampling. The final sample on which complete data were available consisted of 309 young adults (142 males, 167 females). Loss of subjects was not selective with regard to sex and obstetric or psychosocial risks. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all participants.

Assessment

To obtain psychiatric diagnoses for the period between ages 15 yr and 19 yr, i.e. between prior and current assessment, the Structured Clinical Interview for DSM-IV (SCID; APA, 1994; German version by Wittchen *et al*. 1997) was administered to the 19-yr-olds. The SCID is a widely used diagnostic interview, for which a considerable body of reliability and validity data has been published. Twenty-four (7.8%) of the young adults met criteria for any depressive disorder, and 19 (6.8%) met criteria for any anxiety disorder. Due to the low number of clinical diagnoses in this epidemiological study, a broad phenotype was utilized for the present evaluation, defined as diagnosis of any anxiety or depressive disorder ($n=39$, 12.6%). In addition, symptoms of depression and trait anxiety at age 19 yr were assessed by the Beck Depression Inventory (BDI; Beck & Steer, 1987; German version by Hautzinger *et al*. 1994) and the Harm Avoidance subscale of the Temperament and Character Inventory (TCI; Cloninger *et al*. 1994; German version by Richter *et al*. 2000), respectively. Both self-report instruments have been used extensively in clinical and epidemiological research and have excellent psychometric properties.

Measurement of family adversity according to an 'enriched' index as proposed by Rutter & Quinton (1977) was derived from a standardized parent interview conducted at the 3-month assessment. The index assesses the presence or absence of 11 adverse family factors (Table 1), covering characteristics of the parents, the partnership, and the family environment during a period of 1 yr prior to birth (mean = 1.93, S.D. = 2.06, range 0–7). Assessment of stability over a period of >10 yr revealed coefficients of about $r=0.70$.

Current SLE were assessed using a modified and shortened version of the Munich Events List (MEL; Maier-Diewald *et al*. 1983). The 53-item questionnaire asked about occurrence and threat of severe life events and chronic difficulties in the period between the 15-yr and 19-yr assessments. The items addressed all areas of young adults' lives from school and job to partner, family, parents, living conditions, legal troubles, and health problems. Several indices can be derived from the MEL. For the current analysis, a total life event score was computed which counted the number of life events throughout the past 4 yr (mean = 7.74,

Table 1. Definition of the family adversity items and characteristics of the group exposed to high adversity ($n = 144$)

	Item	Definition	%
1	Low educational level of a parent	Parent without completed school education or without skilled job training	31.4
2	Overcrowding	More than 1.0 person per room or size of housing $\leqslant 50$ m²	16.0
3	Parental psychiatric disorder	Moderate to severe Axis I or Axis II disorder according to DSM-III-R criteria (interviewer rating, kappa = 0.98)	41.7
4	History of parental broken home or delinquency	Institutional care of a parent/more than two changes of parental figures until the age of 18 yr or history of parental delinquency	35.4
5	Marital discord	Low quality of partnership in two out of three areas (harmony, communication, emotional warmth) (interviewer rating, kappa = 1.00)	22.2
6	Early parenthood	Age of a parent $\leqslant 18$ yr at childbirth or relationship between parents lasting <6 months at time of conception	48.6
7	One-parent family	At childbirth	20.1
8	Unwanted pregnancy	An abortion was seriously considered	25.7
9	Poor social integration and support of parents	Lack of friends and lack of help in child care (interviewer rating, kappa = 0.71)	9.0
10	Severe chronic difficulties	Affecting a parent lasting more than 1 yr (interviewer rating, kappa = 0.93)	53.5
11	Poor coping skills of a parent	Inadequate coping with stressful events of the past year (interviewer rating, kappa = 0.67)	72.9

S.D. = 5.04, range 0–28). Several studies have confirmed the psychometric characteristics of the MEL (Wittchen *et al*. 1989).

Genotype analysis

Genomic DNA was extracted from whole blood or saliva with the Qiamp (Qiagen, USA) kit. The bi-allelic LS polymorphism was amplified by polymerase chain reaction (PCR), as previously described (Heils *et al*. 1996). The 484-bp fragment was designed as S and the 528-bp fragment as L, respectively. The functional rs25531 variant which is located on this locus defines a tri-allelic polymorphism. This is comprised of the L_A, L_G, and S_A alleles (the S_G allele is extremely rare). The L_G and S alleles have comparable levels of 5-HTT expression which are lower than that of the L_A allele. The *5-HTTLPR* locus was amplified by PCR as outlined by Wendland *et al*. (2006), without multiplexing. In a total volume of 20 μl, $\sim$25 ng genomic DNA was amplified in the presence of 1 × Promega PCR Master Mix (www.promega.com) with oligonucleotide primers '*5-HTTLPR* and rs25531 forw.'/'*5-HTTLPR* and rs25531 rev.' PCR conditions were: 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 90 s at 70 °C, 60 s at 72 °C, and a final extension of 10 min at 72 °C. Restriction was performed with 10 U *Hpa*II for 4 h, according to the manufacturer's instructions (www.neb.com). Genotypes were scored according to the tri-allelic polymorphism L_A, L_G, and S alleles. To check *5-HTTLPR* amplifications for dropout of the L allele, the genotypes produced by two different sets of primers and amplification conditions were compared. Comparison between the protocols of Heils *et al*. (1996) and Wendland *et al*. (2006) yielded an error rate <0.01 in 95% of the total sample genotyped in comparison.

Data analysis

t tests or analyses of variance and χ^2 tests, respectively, were performed to test differences in scores and frequencies between sex and genotype groups. Linear and logistic regression analyses were conducted, as appropriate, to examine whether *5-HTTLPR* genotypes moderated the effect of environmental adversity on continuous and categorical outcome measures. For each analysis, sex was controlled for in the first step, the main effects of genotype and adversity were entered in the second step, followed by their interaction in the third step. To assess potential sex-specific G × E effects, a three-way interaction with sex was entered in the final step. Family adversity and current SLE were examined in separate models. Results are reported for the traditional LS classification and, additionally, for a re-classification, based on the tri-allelic genotypes. Therefore, the tri-allelic genotypes were transformed into a bi-allelic model according to their level of expression as follows: L_GS, L_GL_G, and SS were designated as S'S', L_AS and L_AL_G as L'S', and L_AL_A as L'L'. The distribution of genotypes (LL, 34.0%; LS, 50.8%; SS, 15.2%; and L'L', 25.6%; L'S', 55.7%; S'S', 18.8%) did not deviate from Hardy–Weinberg equilibrium.

Table 2. Multiple (linear and logistic) regression models testing the effects of *5-HTTLPR* genotype, environmental adversity and their interaction on measures of depression and anxiety in young adults

Genotype …	Bi-allelic LS polymorphism			Bi-allelic L'S' polymorphism		
	5-HTTLPR genotype	Environmental adversity	*5-HTTLPR* × adversity	*5-HTTLPR* genotype	Environmental adversity	*5-HTTLPR* × adversity
Family adversity						
Any DSM-IV depressive or anxiety disorder[a]	0.080 (0.267)	0.257 (0.079)***	0.289 (0.132)*	0.166 (0.269)	0.257 (0.079)***	0.265 (0.128)*
BDI score[b]	0.538 (0.494)	0.786 (0.162)***	0.639 (0.254)*	0.232 (0.504)	0.794 (0.162)***	0.304 (0.242)
HA score[b]	0.073 (1.097)	0.878 (0.361)***	−0.287 (0.573)	0.628 (1.119)	0.870 (0.360)***	−0.445 (0.540)
Current stressful life events						
Any DSM-IV depressive or anxiety disorder[a]	−0.083 (0.280)	0.186 (0.035)***	−0.066 (0.055)	0.132 (0.277)	0.184 (0.034)***	−0.045 (0.051)
BDI score[b]	0.133 (0.430)	0.667 (0.058)***	0.086 (0.088)	0.197 (0.436)	0.668 (0.057)***	−0.023 (0.086)
HA score[b]	−0.254 (1.086)	0.579 (0.146)***	−0.164 (0.222)	0.609 (1.101)	0.572 (0.145)***	−0.134 (0.218)

BDI, Beck Depression Inventory; HA, Harm Avoidance.
The table presents unstandardized regression coefficients *b* (S.E.) for the main effects (second step), and for the interaction effects (third step) adjusted for sex;
[a] Coefficients from logistic regression; [b] coefficients from linear regression.
* $p<0.05$, *** $p<0.001$.

Results

There was a significant effect of sex on measures of depression and anxiety, indicating higher BDI ($p<0.001$) and HA scores ($p<0.002$) as well as higher rates of DSM-IV diagnoses related to anxiety or depression ($p<0.002$) in females than in males. Genotype groups did not differ significantly with regard to sex, age, IQ, family adversity, or number of SLE (data not shown).

Table 2 summarizes the findings of linear regression models testing the effect of *5-HTTLPR* genotypes and environmental adversity on measures of depression and anxiety in young adults. When *family adversity* was examined, a significant interaction was observed for diagnosis of depression or anxiety with regard to both the *5-HTTLPR* LS and L'S' polymorphisms, such that higher adversity was associated with increased rates of disorder in LL and L'L' homozygotes, respectively (LL: OR 1.64, 95% CI 1.21–2.22; L'L': OR 1.64, 95% CI 1.16–2.33) but not in S allele carriers (LL: OR 1.16, 95% CI 0.96–1.40; L'L': OR 1.20, 95% CI 0.99–1.44). Similar results were obtained for the BDI score and the *5-HTTLPR* LS polymorphism but not the L'S' polymorphism. Figure 1 illustrates the interaction, indicating that, when exposed to high adversity, LL homozygotes scored significantly higher on the BDI than all other groups except for SS homozygotes. For this analysis, individuals were grouped according to a

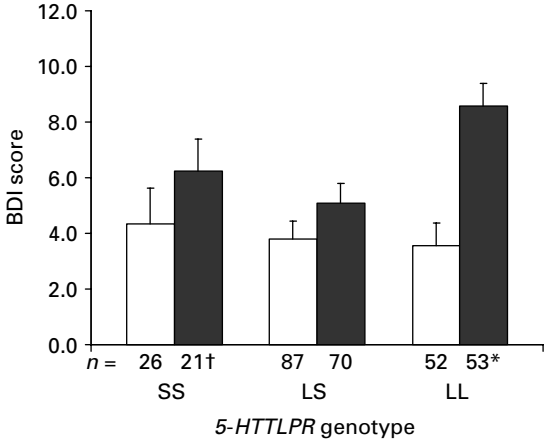

Fig. 1. Mean Beck Depression Inventory (BDI) scores (S.E.), adjusted for sex, in young adults grouped by *5-HTTLPR* genotype and exposure to family adversity (□, low; ■, high). * Significantly different from all other groups (except †) according to Fisher's least significant difference test.

median split on the family adversity index. No significant G × E emerged between *5-HTTLPR* genotypes and family adversity on the HA score. Furthermore, there was a significant main effect of family adversity on all outcome measures, with higher scores and rates of depression or anxiety associated with increasing adversity, while no significant main effect of *5-HTTLPR* genotypes and no three-way interaction with sex were found (data not shown).

With regard to current SLE, Table 2 confirms the presence of a significant main effect of SLE on all outcome measures, indicating rising scores or rates of depression and anxiety when the number of life events increased, and the absence of any G × E with *5-HTTLPR* genotypes.

Discussion

The present study aimed at further investigating G × E between *5-HTTLPR* and exposure to environmental adversity, using different phenotypic and genotypic characterizations as well as different types of adversity. Based on data from a 19-yr longitudinal study of a high-risk community sample, our results demonstrate that *5-HTTLPR* and adverse psychosocial conditions interact to predict patterns of mood and anxiety psychopathology in young adults. Specifically, we found that individuals homozygous for the L allele of *5-HTTLPR* displayed higher rates of depressive or anxiety disorders and reported more depressive symptoms when exposed to adversity, while no such relationship emerged in individuals carrying the S allele. This G × E was demonstrated for (i) exposure to family adversity, but not to current SLE, and (ii) depressive symptoms, but not trait anxiety.

The results outlined above are at variance with those from numerous previous studies indicating *5-HTTLPR* by adversity interaction in which higher rates of depression were observed in carriers of the S allele who experienced SLE (Uher & McGuffin, 2008). However, they are, in agreement with a recent report from the ATP study (Chipman *et al*. 2007) which demonstrated that adolescents homozygous for the L allele were at greater risk of depression when exposed to persistently high levels of family adversity, while no evidence for G × E was obtained in individuals exposed to recent SLE or recent family adversity. The present study can be considered as a direct replication of the ATP findings, as it shares a number of important features with that study. Both the ATP and our study (i) rely on data collected within an epidemiological cohort design following children during the course of their development, (ii) use similar measures of psychosocial adversity (in particular, of family adversity) and differentiate between different types of adversity, i.e. between current (episodic) life stress *vs*. persistent family adversity, and (iii) examine G × E with regard to depression in young age cohorts at the transition from adolescence to adulthood.

The current findings parallel recent evidence of a moderating effect of *5-HTTLPR* on the relationship between SLE and alcohol use, revealing a similar inconsistency. While Covault *et al*. (2007) and Kaufman *et al*. (2007) found evidence for earlier and heavier alcohol use only in carriers of the S allele following SLE, Olsson *et al*. (2005) reported an increase in binge drinking with each additional copy of the L allele in individuals exposed to childhood adversity. The latter observation corresponds to a number of more recent reports indicating that carriers of the LL genotype exhibit higher drinking activity (Bleich *et al*. 2007; Hinckers *et al*. 2006; Hu *et al*. 2006; Kweon *et al*. 2005). Given the high comorbidity of depression with alcohol dependence, and of adolescent behaviour disorders with depression and alcohol abuse, a moderating effect of *5-HTTLPR* on the relationship of these disorders with stress seems plausible.

A number of reasons may explain why some studies offer support for a potential *5-HTTLPR* × adversity interaction implicating the S allele as a risk allele, while others found the opposite effect. One aspect that has been largely overlooked in previous research is the importance of a developmental perspective. The majority of studies conducted so far have neglected developmental issues, using subjects whose ages spanned a wide range. Given the dynamics of genetic influences across the lifespan, the impact of genetic factors is likely to depend on developmental stages (Reiss & Neiderhiser, 2000). Research findings observed across the life cycle suggest that adolescent- and adult-onset depressive and anxiety disorders may represent different subtypes. For example, most adults with depression were found not to have been depressed as adolescents (Klein *et al*. 1999). Moreover, depressed adolescents were reported to differ from depressed adults with regard to various neurobiological correlates and treatment response, such as basal cortisol secretion, response to serotonergic probes, and efficacy of tricyclic medication (Kaufman *et al*. 2001). Furthermore, compared to individuals with adult onset, those with adolescent onset were more likely to have experienced unique childhood risks, such as neurodevelopmental problems, psychopathology and instability in their family of origin, and behavioural problems, in particular of the externalizing spectrum, such as antisocial and hyperactive behaviour (Jaffee

et al. 2002). Another feature specific to adolescent onset is the high comorbidity with other psychiatric disorders, particularly with conduct disorder (Angold *et al*. 1999). Following this line of evidence, it could be hypothesized that depression and anxiety in young adulthood may represent a heterogeneous phenotype, which should be differentiated into developmentally specific subtypes, with the adolescent-onset subtype being characterized by a particularly high psychosocial load and more externalizing disorders.

Consistent with this hypothesis, our findings revealed a strong main effect of family adversity on measures of depression and anxiety. Furthermore, according to additional analyses of our data, individuals with depressive or anxiety disorders scored significantly higher on externalizing problems [aggressive behaviour and delinquent behaviour according to the Young Adult Self Report (YASR; Achenbach, 1991)] and were more likely to have a history of externalizing disorders, such as conduct disorder, oppositional-defiant disorder or attention deficit hyperactivity disorder (ADHD). Isolating a phenotype of adolescent-onset depression characterized by high comorbidity with externalizing disorders is of particular importance, as several studies have provided evidence for an association of conduct problems, aggressive behaviour and ADHD in childhood with the LL genotype of *5-HTTLPR* both alone or in interaction with environmental adversity (Cadoret *et al*. 2003; Kent *et al*. 2002; Nobile *et al*. 2007; Seeger *et al*. 2001; Twitchell *et al*. 2001).

Further support for differentiating developmentally specific subtypes of depression and anxiety is provided by two recent studies investigating the association with *5-HTTLPR* in younger samples. In accordance with the findings of the present study, in a sample of 247 young adult twins, Chorbov *et al*. (2007) found a significant interaction between the number of L_A alleles and exposure to traumatic life events with regard to adolescent onset major depression. In a fMRI study assessing amygdala function, Lau *et al*. (2008) demonstrated that adolescents with current anxiety or major depressive disorder who were carriers of the high-functioning L_AL_A genotype of *5-HTTLPR* exhibited higher amygdala activation to fearful faces than patients with the low-functioning S or L_G alleles. This finding is at odds with those reported from affected adults, indicating greater amygdala response in S allele carriers (Munafo *et al*. 2008*a*).

Another possible factor contributing to inconsistency may be heterogeneity in measures and characteristics of environmental adversity investigated in the different studies. Several aspects deserve discussion in this context, one of which is the timing and duration of exposure to adversity. Both animal and human studies have underscored the predisposing effect of exposure to stress during early childhood for the development of later mood and anxiety disorders (Heim *et al*. 2004). Epidemiological studies have provided ample evidence that exposure to recent SLE is likely to precede the onset of episodes of mood and anxiety disorders (Kendler *et al*. 1999). Both early and recent adversity have been studied in previous G × E research in humans, with the majority of studies having examined *5-HTTLPR* as a moderator of the impact of SLE. Far fewer studies have focused on early childhood adversity as an environmental pathogen. Interestingly, several studies reporting association or interaction with the LL genotype such as those outlined above used indices of family adversity as measures of early adversity. However, caution must be exercised in the interpretation of such composite measures, as it is difficult to separate different aspects of exposure to adversity. In particular, several family adversity factors, such as low educational level or psychiatric disorder of a parent are proxies for persistent adverse conditions, as reflected by the high stability found for this index. Thus, measures of family adversity may well confound early exposure to adversity with the duration of exposure, a characteristic found to be salient in the ATP study. Research on individual differences in biological reactivity to environmental stress has highlighted the duration of a stressor as an important determinant of the phenomenon of 'hypocortisolism', characterized by a suppression of the activity of the hypothalamic–pituitary–adrenal (HPA) axis under conditions of stress (Fries *et al*. 2005). This paradoxical down-regulation of the HPA axis which has been noted in both animal and human research is suggested to occur after a prolonged period of hyperactivity of the HPA axis due to chronic stress. Whether the differentiation between acute and chronic stress may contribute to explaining the controversial findings regarding the association between *5-HTTLPR*, stress and depression, remains an interesting question to be addressed in future research.

Sex is another variable to be taken into consideration in light of the conflicting findings. Given the marked differences in the extent to which adult males and females develop anxiety and mood disorders, one possible hypothesis could be that sex-specific G × E may contribute to this pattern. Support for this hypothesis comes from research indicating differences in HPA axis reactivity in males and females which may be directly responsible for higher stress vulnerability in women (Kirschbaum *et al*. 1999). Accordingly, women

are more likely than men to develop depression following SLE (Cyranowski *et al*. 2000), particularly with low stress exposure (Kendler *et al*. 2004). First evidence for a moderating role of sex in G × E findings was reported by a study in non-human primates, suggesting that female animals carrying the S allele in particular exhibited increased stress reactivity following early stress exposure (Barr *et al*. 2004). Consistent with this finding, two human studies in adolescents found an interaction with the S allele in females only and an opposite effect, i.e. higher rates of depression in carriers of the LL genotype, in males (Eley *et al*. 2004; Sjoberg *et al*. 2006). However, in the present study no significant sex differences with regard to *5-HTTLPR* G × E on depression were observed, thus failing to provide further support for this hypothesis.

Furthermore, two methodological issues should be considered when interpreting the discrepant findings. Recently, an A > G single nucleotide polymorphism within the L allele of *5-HTTLPR* has been described as a potential modulator of 5-HTT function (Hu *et al*. 2006), where the L_G allele was associated with reduced 5-HTT expression making it functionally similar to the S allele. Unrecognized L_G alleles have been suggested as one reason for inconsistency in previous studies. As our G × E findings were, at least in part, dependent on how the L allele was classified, this explanation cannot be ruled out. However, another possible reason for the discrepant results can be excluded by the present study. *5-HTTLPR* is known to be extremely difficult to genotype (Kaiser *et al*. 2002); in a recent study, Yonan *et al*. (2006) demonstrated that levels of magnesium concentrations in the PCR reported in previous studies caused allele-dependent non-random genotyping errors, resulting in clearly different association findings. In our study, typing of the bi-allelic genotype of *5-HTTLPR* was replicated almost completely using two different methods yielding a high degree of agreement.

Finally, the present findings have to be viewed in the light of a number of difficulties inherent in detecting 'true' gene–environment interactions. Major issues of criticism relate to the multiple testing, low statistical power, and the lack of criteria for replication. Multiple testing has long been a serious problem in genetical research. The availability of datasets which afford large numbers of subdivisions (due to different ways of defining genotype and environmental characteristics) multiplies the potential of multiple testing by offering numerous additional possibilities for data mining (Flint & Munafo, 2008). Another difficulty in genetic association research is that most studies are underpowered. Since statistical tests for examining interaction are less powerful than tests of main effects, this problem applies particularly to studies of G × E. The power to detect an interaction depends on a number of conditions, including the distribution of genotypes and environmental exposures in the sample and the sample size. The relationship between these conditions is complex, providing another source of heterogeneity between results in the literature attributable to methodological reasons. Given the probable small effects of any single G × E and the associated risk of false-positive results, this implies a critical need for replication. However, differences in the measurement instruments in assessing genotype, phenotype and environmental variables between studies may produce further heterogeneity. As long as rigorous criteria for replication studies are lacking, the G × E literature is at risk of being flooded with false-positive results, which are broadly described as 'replications' when, in fact, they are not.

Several additional limitations to our study should be noted. First, the sample size of the present investigation is relatively small for a genetic association study examining G × E. Since association studies are prone to false-positive results, the results reported here require further validation in independent samples of adequate size. However, as the present study can be considered a close replication of the study by Chipman *et al*. (2007), this limitation may be mitigated. Second, it is noteworthy that exposure to environmental adversity, such as family adversity or SLE may be under genetic control. Thus, the G × E observed in this study might well be due to interactions between *5-HTTLPR* and other genes that were not identified (Plomin *et al*. 1994). However, as there were no significant differences between genotypes regarding environmental adversity, this is unlikely to be a confounder in the present study. A third limitation involves the effects of population stratification, such that true associations may be hidden by the population substructure. However, the potential impact of this effect is likely to be minimal here, because all probands were selected from an epidemiological cohort sample of a well-defined region, where *5-HTTLPR* allele frequencies in different phenotypes were largely unbiased by geographical variation in proband characteristics. Another point of criticism may refer to population-specific variation in linkage disequilibrium (LD). However, in view of the robust evidence of a physiological impact of *5-HTTLPR* detected *in vivo* (Munafo *et al*. 2008*a*) which strongly supports a functional role of this variant, it is implausible to assume that variation in LD may account for the reported differences.

To summarize, the present study provides further evidence for G × E in the association of *5-HTTLPR* with depression and anxiety in young adults. The finding that individuals with the LL genotype displayed more psychopathology when exposed to family adversity confirms recent reports but is in the opposite direction to those of various previous studies, which demonstrated elevated rates of depression in carriers of the S allele. Potential explanations for the conflicting findings pertain to the need for a developmentally specific phenotype definition and to differences in characterizing environmental adversity.

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Statement of Interest

None.

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