



Research report

Childhood adversity interacts separately with 5-HTTLPR and BDNF to predict lifetime depression diagnosis

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ABSTRACT

The serotonin transporter polymorphism (5-HTTLPR) and the brain-derived neurotrophic factor (BDNF) val66met polymorphism have both been linked to depression symptoms and to depression diagnosis (MDD) in interaction with adversity; there have also been failures to find the effects. We reexamined both interactions for lifetime MDD in a college sample. Lifetime MDD was diagnosed by Structured Clinical Interview for DSM-IV in 133 undergraduates; genotypes for 5-HTTLPR and BDNF were assayed from blood, and self-reports were collected concerning childhood adversity (Risk). 5-HTTLPR interacted with Risk such that Risk predicted less likelihood of MDD among ll carriers and tended to predict greater likelihood of MDD among s carriers. BDNF interacted with Risk such that Risk predicted greater likelihood of MDD among met carriers and did not influence val/val carriers. These two interactions were additive; both were significant in a combined model.

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1. Introduction

The potential involvement of specific genes in vulnerability to depression has been under study for some time (e.g., Levinson, 2006). Probably the most frequently examined candidate has been the serotonin transporter polymorphism, 5-HTTLPR. This gene, which appears to regulate uptake of serotonin from the synaptic cleft (Lesch et al., 1996) contains a repetitive sequence with varying numbers of repeats. Although there may be as many as 10 alleles in humans (Nakamura et al., 2000), by far the most common are what are generally termed short (s) and long (l) alleles. Some studies have related the s allele directly to elevated probability of depression (Collier et al., 1996; Hoefgen et al., 2005), others have not (e.g., Anguelova et al., 2003).

Rather than a main effect for depression, Caspi et al. (2003) have argued for a gene × environment interaction, such that the differential vulnerability associated with the two alleles is expressed only under adversity. They reported findings consistent with that view: carriers of the s allele were more likely to have had a major depressive episode by adulthood than those with the l allele, but only if they had experienced relatively high adversity. Successful replications of this interactive effect have been reported, along with failures to replicate it. One recent meta-analysis concluded that the interactive effect is not supported (Risch et al., 2009); others have reached the opposite conclusion (Caspi et al., 2010; Uher and McGuffin, 2008, 2010). There appears to be a need for more data. The study reported here tested the question again.

Vulnerability to depression has also been associated with genes relating to brain-derived neurotrophic factor (BDNF). BDNF is involved in neuroplasticity and protection against stress-induced neural damage. The genetic marker for BDNF that has received the most attention is the single nucleotide

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polymorphism (SNP) at nucleotide 196 (G/A), in which there is an amino acid substitution (valine to methionine) at codon 66, often referred to as val66met (dbSNP number rs6265). This SNP affects processing and secretion of BDNF, with the met allele being associated with lower BDNF activity (Egan et al., 2003). Some studies found carriers of the met allele to have a higher rate of depression, but others have failed to replicate the effect (Levinson, 2006).

As with 5-HTTLPR, recent studies have turned to the gene \times environment hypothesis. Some have reported support for an interaction between BDNF and environment in predicting depressive symptoms (Aguilera et al., 2009; Hosang et al., 2010; Kaufman et al., 2006; Wichers et al., 2008); others have failed to replicate that interaction (Nederhof et al., 2010). One study tested diagnosis of depression (Kim et al., 2007), and another tested diagnosis of depression or dysthymia (Lavebratt et al., 2010). These studies all found support for a gene \times environment hypothesis. Again, however, there appears to be a need for additional information.

Finally, two studies have suggested that there may even be interactive effects between 5-HTTLPR and BDNF in increasing risk for depressive symptoms in the context of adversity. These studies examined effects in children (Kaufman et al., 2006) and in the elderly (Kim et al., 2007). Here we test for such a gene \times gene \times environment interaction in an early adult sample.

2. Method

2.1. Participants

Participants were 133 undergraduates at the University of Miami (98 female), who participated in partial fulfillment of a course requirement. The project was approved by the University of Miami IRB and all participants gave informed consent before participating. A general description of the project was posted on a departmental website, and interested persons made appointments for group sessions. Mean age was 18.71 years. Participants' ethnicities (by self-report) included 57.1% Caucasian, 24.1% Hispanic, 7.5% Asian, 4.5% African American, 2.3% Caribbean, and 4.5% other.

2.2. Procedure

Participants convened in groups of approximately 20, to complete self-report measures and computer-administered laboratory tasks (apart from one self-report measure, these are not discussed further here). Blood was drawn for genotyping as well. At the end of these sessions, individual appointments (for approximately one week later) were made for a subset of persons from the initial session (staffing limitations prevented us from conducting the second session for everyone). An effort was made to oversample participants for the second session whose profiles on self-reports in the group session suggested the possibility of depression vulnerability. In the subsequent individual sessions, participants completed a diagnostic interview, described below.

2.3. Measures

2.3.1. Structured Clinical Interview for DSM-IV (SCID)

The SCID (First et al., 1997) was administered to determine whether participants met criteria for lifetime major depressive disorder (MDD; American Psychiatric Association, 2000). SCIDs were conducted by graduate students in clinical psychology who had completed extensive training in diagnostic interviewing, including didactic material, role-playing sessions, practice interviews, and weekly reliability meetings. The SCID has good test–retest reliability among trained interviewers (Williams et al., 1992). In this study, all interviews were audiotaped, and a random sample of 10 interviews was reviewed for reliability. Inter-rater reliability, assessed with intra-class correlations using SPSS reliability analyses to assess absolute agreement on dichotomous variables, was high for lifetime diagnoses of MDD, $r_i = .87$. Cases were defined as those who met criteria for a major depressive episode (MDE) at any point in their lifetime ($n = 35$). Controls were defined as those who had never met criteria for a MDE ($n = 98$).

2.3.2. Risky families

To assess early adversity, we gathered a self-report measure called Risky Families (Taylor et al., 2004), which has been found in one previous study to interact with the 5-HTTLPR polymorphism in predicting current depression symptoms (Taylor et al., 2006). This 13-item questionnaire was adapted from a scale developed by Felitti et al. (1998) to assess the relation of family stress to mental and physical health outcomes in adulthood. Taylor et al. (2004) validated this questionnaire against clinical interviews conducted and coded by trained clinical interviewers. Participants rated 13 aspects of their early family environment on 5-point scales ranging from 1 ("not at all") to 5 ("very often" or "very much"). Items assessed whether the respondent had felt loved and cared for; was insulted, put down, sworn at, or made to feel threatened; was shown physical affection; was pushed, grabbed, shoved, or slapped; was verbally abused; was physically abused; observed quarreling or shouting between parents; observed violence or aggression between family members; lived with a substance abuser; lived in a well-organized, well-managed household; and whether family members knew what the child was doing. Positively framed items were reverse-coded, and responses were averaged. Alpha in this sample was .82. Average scores ranged from 1.00 to 3.85, $M = 1.80$, $SD = 0.52$, with higher values representing a more adverse early family environment. The Risky Families scale was treated as a continuous variable, which was centered before analysis.

2.4. Genotyping

Genotyping was performed at the laboratories of the Hussman Institute of Human Genomics, University of Miami Miller School of Medicine. SNP genotyping is conducted using Taqman allelic discrimination assays from Applied Biosystems (ABI). Three ng of genomic DNA, extracted from whole blood according to established protocols, were used in the amplification reaction. Cycling was performed on GeneAmp PCR Systems 9700 thermocyclers. End-point fluorescence

was measured on the ABI 7900 HT system. Genotype discrimination of results was then conducted using ABI's 7900 HT Sequence Detection Systems version 2.3 analysis software. To ensure genotyping accuracy, 32 quality control samples per 384 plates that match within and across plates, were included. Sample call rates across both polymorphisms were >99.7%.

2.4.1. Serotonin transporter promoter region polymorphism

The assay is a modification of the method described by Lesch et al. (1996). The primers used to amplify this polymorphism were 5'FAM TGGCGTTGCCGCTCTGAATGC 3' (forward) and 5'-AGGGACTGAGCTGGACAACCA-3' (reverse); the forward primer was labeled with FAM fluorescent dye. 40 ng of DNA was used to set up 20ul PCR reactions. Thermocycling was done using the ABI Veriti™ 96-Well Fast Thermal Cycler with the following parameters: 95 °C for 5 min; 37 cycles of [95 °C for 30 s; 61 °C for 30 s; 72 °C for 1 min]; 72 °C for 5 min; Hold at 15 °C. PCR quality was confirmed using 2% agarose gel electrophoresis. After denaturation for 5 min at 95 °C, followed by ice for 2 min, each sample was analyzed by capillary electrophoresis on the ABI 3730xl DNA Analyzer with a 36 cm, 96-capillary array following manufacturer's protocol. Data were analyzed with GeneMapper® Software v4.0 [Applied Biosystems, Foster City, California, USA].

The 5-HTTLPR genotype frequencies were as follows: l/l 28%, s/l 53%, and s/s 19%. Genotype frequencies were in Hardy–Weinberg equilibrium. Consistent with most previous studies, carriers of one or more s allele were combined in one group and compared in analyses against those with two l alleles.

2.4.2. BDNF

Genotyping was done for the GrA (valinermethionine) variation at position 758 of the BDNF coding sequence (rs6265). SNP genotyping was conducted using Taqman allelic discrimination assays from Applied Biosystems (ABI). Cycling was performed on GeneAmp PCR Systems 9700 thermocyclers, with conditions recommended by ABI. End-point fluorescence was measured on the ABI 7900 HT system. Genotype discrimination of experimental results was then conducted using ABI's 7900 HT Sequence detection Systems version 2.3 analysis software.

The BDNF genotype frequencies were as follows: val/val 63%, val/met 29%, and met/met 7%. Genotype frequencies were in Hardy–Weinberg equilibrium. In line with previous studies, carriers of one or more met allele were combined in one group and compared in analyses against val/val.

2.5. Data analytic strategy

Statistical analysis was performed using SPSS version 17.0. We began by testing gene effects and gene × environment interactions separately for 5-HTTLPR and BDNF, given that each of these genetic variables has its own literature. Then we ran a model that tested them jointly. Because the outcome was dichotomous (positive diagnosis of lifetime MDE versus all others), analysis was by logistic regression. Interaction terms were created by multiplying centered Risk by the relevant genetic variable. Ethnicity and Sex were both entered as covariates. 5-HTTLPR, BDNF, ethnicity, and sex were dummy coded with $K - 1$ variables.

3. Results

3.1. Descriptive analysis

Table 1 shows allele frequency for 5-HTTLPR and BDNF genotypes by case versus control status and ethnicity. We examined whether the 5-HRRLPR (ll, ss + sl) and BDNF (val/val, val/met + met/met) genotypes differed by ethnic group; no significant differences were observed.

3.2. 5-HTTLPR

Main effects of 5-HTTLPR and Risk, along with Sex and Ethnicity (as control variables), were entered as Step 1; the interaction between 5-HTTLPR and Risk was entered as Step 2. The first step did not yield a significant effect, $\chi^2(8) = 6.90$, $p = .55$. Entry of the interaction term caused a significant increase in the prediction of group membership, $\chi^2(1) = 8.85$, $p = .003$, overall Nagelkerke $R^2 = .16$. The interaction term was the only variable that made a significant contribution to predicting group membership, $B = 3.73$, Wald = 5.49, $p = .02$, (Fig. 1). Simple slope analyses determined that higher Risk predicted lower probability of having a diagnosis of lifetime MDD for those in the ll group, $B = -3.64$, Wald = 3.80, $p = .05$, OR = 0.03. The tendency of higher risk to predict higher probability of lifetime MDD in the s carriers did not attain significance, however, $B = 0.83$, Wald = 3.00, $p = .08$, OR = 2.29.

3.3. BDNF

Main effects of BDNF and Risk, along with Sex and Ethnicity as control variables, were entered as Step 1, and the interaction was entered as Step 2. Again the first step did not yield a significant effect, $\chi^2(8) = 7.09$, $p = .53$. Entry of

Table 1
Allele frequency by ethnicity and case/control status.

	5-HTTLPR			BDNF		
	ll	sl	ss	val/val	val/met	met/met
<i>Non-Hispanic Caucasian (n = 75)</i>						
No past MDE	14	32	7	34	15	3
Past MDE	6	11	6	11	12	0
<i>Hispanic (n = 32)</i>						
No past MDE	8	11	8	19	6	2
Past MDE	0	5	0	4	1	0
<i>Asian (n = 10)</i>						
No past MDE	3	4	1	3	3	2
Past MDE	0	1	1	0	0	2
<i>African American (n = 6)</i>						
No past MDE	3	2	0	5	0	0
Past MDE	1	0	0	1	0	0
<i>Caribbean (n = 3)</i>						
No past MDE	0	1	1	1	1	0
Past MDE	0	1	0	1	0	0
<i>Other (n = 6)</i>						
No past MDE	2	1	0	3	0	0
Past MDE	0	2	1	2	1	0

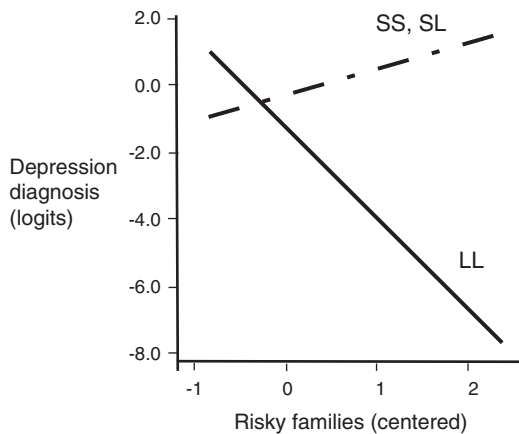


Fig. 1. Logit probability lifetime major depressive disorder as a function of 5-HTTLPR genotype and early adversity.

the interaction term caused a significant increase in prediction of group membership, $\chi^2(1) = 6.95$, $p = .008$, overall Nagelkerke $R^2 = .15$. The interaction term made a significant unique contribution to prediction of group membership, $B = 2.49$, Wald = 5.75, $p = .02$ (Fig. 2). Simple slope analyses determined that Risk significantly increased the probability of having a diagnosis of lifetime MDD among those who were met carriers, $B = 1.68$, Wald = 4.08, $p = .04$, OR = 5.37, but did not have a significant effect among those homozygous for the val allele, $B = -1.23$, Wald = 2.60, $p = .11$, OR = 0.29.

3.4. Joint analysis

Main effects of 5-HTTLPR, BDNF, and Risk, along with Sex and Ethnicity were entered as Step 1, and the interactions between 5-HTTLPR and Risk, BDNF and Risk, and 5-HTTLPR and BDNF were entered as Step 2. Step 1 failed to yield a significant effect, $\chi^2(9) = 8.87$, $p = .45$. Entry of these interaction terms, however, caused a significant increase in prediction of group membership, $\chi^2(3) = 14.80$, $p = .002$, overall Nagelkerke $R^2 = .24$. A significant main effect of Risk emerged in this model, $B = -3.92$, Wald = 4.77, $p = .03$, OR = 0.02. Of greatest importance, however, both the interaction of 5-HTTLPR with Risk ($B = 3.79$, Wald = 4.29, $p = .04$) and the interaction of BDNF with Risk ($B = 2.60$, Wald = 4.52, $p = .03$) continued to make significant unique contributions. The interaction between 5-HTTLPR and BDNF did not contribute to prediction of diagnosis, $B = 0.29$, Wald = 0.06, $p = .82$.

Finally, the interaction of Risk \times 5-HTTLPR \times BDNF was entered in a third step. Entry of this interaction did not provide significantly better prediction of lifetime MDE diagnosis than the model in Step 2, $\chi^2(1) = 0.70$, $p = .40$, and the interaction term itself was not significant, $B = 3.19$, Wald = 0.62, $p = .43$.

4. Discussion

The findings of this study support the position that interactions of early-life adversity with 5-HTTLPR and BDNF polymorphisms both predict lifetime diagnoses of MDD. The study has important limitations. It was conducted with a convenience sample that was relatively small, well-educated,

and young. The relative youth of the sample likely means that some participants whose SCIDs were negative for MDD will later experience depressive episodes. Another limitation is that the measure of early adversity was paper and pencil self-report, which may be subject to recall bias. Finally, the levels of early adversity experienced by this sample were relatively low.

Despite these limitations, the results provided clear support for the gene \times environment interaction involving BDNF and somewhat more ambiguous support for the gene \times environment interaction involving 5-HTTLPR. That is, the latter interaction was significant, but its form was not fully as expected. Among persons reporting relatively high childhood adversity, s-allele carriers were indeed more likely to have a diagnosis for MDD than those with the ll genotype. However, the effect of greater adversity was to reduce the incidence of MDD among the ll genotype, with the increase among s carriers being nonsignificant.

When considering the genotypes together, three further points should be made. First, the gene \times environment interactions were almost completely independent of one another. Including both in a single statistical model revealed that both made independent contributions to prediction of diagnostic status. Second, the overall effect of this model was substantial (Nagelkerke $R^2 = .24$). This leads us to agree with Caspi et al. (2010) that it is premature to discount the possibility that important contributions may be made by such interactions. Third, unlike the pattern reported in some studies (Kaufman et al., 2006; Kim et al., 2007), we found no evidence of a gene \times gene \times environment interaction. This study was clearly underpowered for such an interaction, however, and the negative outcome must be viewed in that light.

Overall, these findings suggest that both BDNF and 5-HTTLPR polymorphisms are related to risk of depression in the context of early adversity. Our findings also highlight the importance of considering gene by environment interactions in exploring genetic influences on risk of disorder (Caspi et al., 2010). Further research is needed to understand how these genetic risk variables relate to endophenotypes that ultimately lead to depression.

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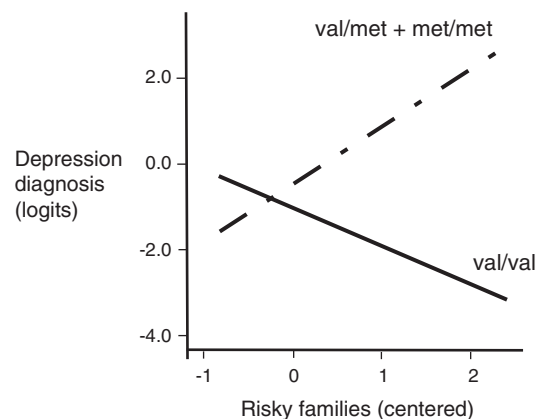


Fig. 2. Logit probability lifetime major depressive disorder as a function of BDNF genotype and early adversity.

Conflict of interest

The authors all declare that they have no conflict of interest concerning the manuscript.

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