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Brief Research Communication

Relationship of 5-HTTLPR Genotypes and Depression Risk in the Presence of Trauma in a Female Twin Sample

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Several studies have implicated an insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (SLC6A4; 5-HTT) in the development of mood disorders. In the present study of a sample of 247 young adult female twins from Missouri, we examine whether this polymorphism interacts with the effect of adverse life events to increase risk for developing depression. We found a significant interaction between the number of high-activity L_A alleles and exposure to trauma (OR = 1.70, $P < 0.0001$). This differs from previous reports, in that the higher activity genotypes (L_A/L_A, L_A/S, L_A/L_G), rather than the low activity genotypes (S/S, S/L_G, L_G/L_G), are associated with an increased incidence of major depressive disease (MDD) in the presence of environmental trauma.

KEY WORDS: serotonin transporter; depression; trauma; functional polymorphism; gene–environment interaction

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There is mounting evidence implicating alterations in serotonergic (5-HT) systems in the pathophysiology of depression. The serotonin transporter gene (SLC6A4, MIM: 182138) is a prime candidate as its protein product is the target of selective serotonin reuptake inhibitors (SSRIs) which have been found effective in the treatment of major depression [White et al., 2005]. Heils et al. [1996] reported that a 44 bp insertion/deletion polymorphism (5-HTTLPR insertion/deletion polymorphism) in the transcriptional control region upstream of the coding sequence of SLC6A4 affected basal promoter activity levels, expression levels being about threefold higher for the variant with the 44 bp insertion (long or L allele) compared to the variant with the deletion (short or S allele). Several epidemiologic and community-based studies have implicated an interaction of this polymorphism with environmental factors in the etiology of MDD. Relative to homozygous L/L individuals, young adult carriers of the S allele (S/L or S/S individuals) were reported to have increased vulnerability to major depression when exposed to adverse events [Caspi et al., 2003]. Eley et al. [2004] reported significant risk from the S/S genotype, but only for girls in a high environmental risk group, with a nonsignificant trend for overall association of the short allele with low depressive symptoms groups. Another group [Grabe et al., 2005] reported that the S allele of the 5-HTT promoter region showed significant interactions between genotype, unemployment and chronic diseases in females, but not in males; there was no independent association of the genotype with mental and physical health. Kendler et al. [2005] found significantly increased sensitivity in young adults to the depressogenic effects of stressful life events for S/S homozygous individuals when compared to those with one or two long alleles (S/L or L/L), a different outcome for the heterozygous class than reported in Caspi et al. [2003]. Kaufman et al. [2006] also found increased risk for MDD in maltreated children who carry the S/S genotype.

As part of an ongoing study of the roles of genetic and environmental factors and brain morphology on the risk for MDD, we have collected information about MDD and environmental stressors for an epidemiologically ascertained sample of adolescent and young adult female twins in Missouri. The evidence reported by others prompted us to examine whether the interaction between 5-HTTLPR genotype and adverse life events on MDD could be replicated in this sample.

More recently than many of the aforementioned reports, Hu et al. [2004] found a common functional A/G substitution (SNP rs25531) in the first of two extra 22 bp repeats of the L allele which generates two forms (L_A and L_G). Kraft et al. [2005] reported that rs25531 is located 18 bp upstream of a 43 bp ins/del, rather than within the insertion. However, the published frequency of S_G is less than 1% [Wendland et al., 2006] and does not affect analysis of the 5-HTTLPR region. Investigating expression assays in lymphoblastoid cell lines representative of the six 5-HTTLPR genotypes, Hu et al. observed co-dominant allele activity with low, nearly equivalent expression for the S and L_G alleles while the L_A allele was associated with high levels of 5-HTT mRNA expression. This suggests that the S and L_G alleles could be appropriately grouped when testing for genotypic effect. It also suggests that the studies comparing S and L alleles may merit reexamination.

Our subjects were ascertained from the general European-American population through Missouri birth records [Heath et al., 1999, 2002; Glowinski et al., 2003]. This prospective study assessed female–female twin pairs born from 1975 onwards, with subjects initially assessed at 13, 15, 17, and 19 years of age. A parent (usually the biological mother) and each twin completed separate telephone interviews using a

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modified version of the Child Semi-Structured Assessment for the Genetics of Alcoholism (C-SSAGA) to assess medical and psychiatric history. Twins 18 years old and above completed a 10-item self-report questionnaire about traumatic life events, including involvement in a life-threatening accident or disaster, as well as physical and sexual abuse or neglect [Kessler et al., 1995].

Inclusion criteria for affected twin pairs in this study were as follows: (1) female twin pair, either mono- or dizygotic, aged between 13 and 23 years, (2) both twins right-handed, (3) at least one twin with a lifetime DSM-IV [American Psychiatric Association, 1994] diagnosis of MDD, with at least one episode of four week duration, and (4) age of onset before 18 years of age. For the control twin-pairs neither twin could have a personal lifetime history of MDD, no first degree relative and no more than two second degree relatives could have a lifetime history of a mood disorder. Exclusion criteria for all subjects were as follows: (1) mental retardation, (2) autism, schizophrenia, Tourette's disorder, or eating disorder, (3) major medical illness known or hypothesized to affect the central nervous system, (4) significant neurological illness, (5) pregnancy (temporary exclusion), (6) history of a serious head injury with loss of consciousness for more than five minutes, (7) intraocular metallic objects, cochlear implants, pacemakers, or other electrical, mechanical, or magnetically activated implants, (8) alcohol or drug dependence, or (9) being adopted. The exclusion criteria were designed to eliminate most known causes of changes in brain structure and conditions contraindicated for MRI. Inclusion criteria did not require presence or absence of traumatic events.

Blood samples were available for genetic analysis for 247 female individuals: 14 twin pairs concordant for MDD, 44 discordant pairs, and 63 unaffected control pairs. The average age of onset of MDD was 16 years and the average length of the episode was 12 weeks. Two depressed and three healthy subjects are included without a co-twin. Mean ages at last assessment are similar for case and control subjects (22.1 ± 3.2 years vs. 21.9 ± 3.3 years; $t = -0.43$, $P = 0.67$). Complete data (MDD status, genotype, exposure to trauma) were available on 227 individuals (71 with MDD and 156 unaffected).

Genomic DNA was purified from blood using standard protocols: red blood cells lysis, proteinase K with sodium dodecyl sulfate treatment, and phenol/chloroform extraction using Phase-Lock Gel Light tubes (Eppendorf, Inc.). DNA was precipitated with sodium acetate in ethanol and resuspended in 10 mM Tris, 1 mM EDTA, pH 7.4 (TE buffer). DNA concentration was standardized using absorbance at 260 nm (UV). The available samples were genotyped for the A/G variation in the 43 bp insertion of 5-HTTLPR. To that purpose, we used a PCR assay to determine both the ins/del polymorphism length as well as the genotype at SNP rs25531. We designed a set of primers to amplify a 249 bp region around the insertion (F-GCCAGCACCTAACCCCTAAT; R-AGGGGAGATCCTGGGAGAG). The SNP is part of a recognition site for the *Msp*I restriction endonuclease, which cuts one base 5′ of the SNP when the G nucleotide is present and does not cut when the A nucleotide is present. We used the Invitrogen PCRx Enhancer System kit for PCR amplification. This allowed us to avoid using 7-deazaGTP (otherwise necessary to amplify the GC-rich region), which impairs the ability of *Msp*I to completely cut the PCR product. PCR followed by digestion overnight with *Msp*I yields a 249 bp fragment (uncut L_A allele), two fragments of 148 bp and 101 bp (cut L_G allele), or a 206 bp fragment (S allele). Fragments were separated on 2.5% agarose gels, stained with ethidium bromide and visualized by UV fluorescence. Genotypes were determined by two independent readers without knowledge of affection status.

We employed logistic regression to test the relationship between genotypes, environmental history, and risk for depression. Statistical analyses were done with Stata 9.2 (StataCorp LP). Data were analyzed hierarchically. Since few covariates are considered in these analyses, we fit all possible models (all combinations of main effects and interaction terms) and selected the model with the lowest associated Aikaike Information Criterion (AIC). The cluster option and robust SE were used to take into account the correlations that may exist between twins, with standard errors adjusted for within-family correlation.

Trauma data were available for 236 individuals, with 109 (46.2%) reporting at least one traumatic event. In preliminary analyses, we first confirmed the effect of trauma on depression risk in this sample, finding significant associations with trauma analyzed as either presence/absence (OR = 1.72; 95% CI: 1.25–2.36; $P < 0.001$) or by number of traumatic events (OR = 2.03; 95% CI: 1.39–2.96; $P < 0.001$).

The allelic distribution in the 238 individuals who were genotyped (S: 40%; L_A: 52%; L_G: 8%, Table I) is similar to that previously observed in US and Finnish Caucasians [Hu et al., 2006]. We did not find any S_G alleles in our sample. Observed allele frequencies are in Hardy-Weinberg equilibrium in the whole sample ($\chi^2 = 0.81$; df = 4; $P = 0.94$) as well as in the affected and unaffected groups separately ($P = 0.75$ and 0.89, respectively) [http://wbiomed.curtin.edu.au/genepop/genepop_op1.html].

We examined the relationship between genotype, trauma and depression using, first, the original allele classification (S/L) to allow comparison with previous reports that used this classification, and, second, taking into account the similarity in activity levels for the S and L_G alleles. In the later analyses, genotypes were recoded as low (SS, SL_G, L_GL_G), medium (L_AL_G, L_AS), or high (L_AL_A) activity (Table II).

With the first genotype classification, we found neither association of MDD with 5-HTTLPR S/L (OR = 1.20; 95% CI: 0.82–1.93; $P = 0.280$) nor strong evidence for interaction between exposure to trauma and number or presence of S alleles (OR = 1.27; 95% CI: 0.93–1.73; $P = 0.127$). On the other hand, when contrasting the S and L_G alleles to the L_A allele, there is a marginal association between the number of L_A alleles and MDD (OR = 1.41; 95% CI: 0.97–2.05; $P = 0.071$) and strong evidence for an interaction effect between exposure to trauma and the number of L_A alleles (OR = 1.70; 95% CI: 1.29–2.23; $P < 0.0001$). Association between MDD risk and the number of L_A alleles was significant in the sub-sample exposed to trauma (OR = 1.79; 95% CI: 1.11–2.89; $P = 0.017$; on 105 observations), but not in the absence of trauma (OR = 1.21; 95% CI: 0.65–2.25; $P = 0.540$; on 122 observations). We checked for a multicollinearity effect between genotype and adverse life events, but found no significant correlation (OR = 0.82; 95% CI: 0.58–1.17; $P = 0.281$). Whether analyzed as an interval scale (one, two, or more life events) or as multiple groups, we found an interaction effect of genotype and environment when experiencing one or more adverse events

TABLE I. Genotype Distribution: Comparison of Old and New Classifications

Genotypes	Old (S vs. L) classification			
New classification	SS	SL	LL	Total
SS	34			34
SL_G		18		18
L_GL_G			1	1
L_AL_G			17	17
L_AS		103		103
L_AL_A			65	65
Total	34	121	83	238

TABLE II. Percent of MDD by Genotype and Traumatic Exposure (Sample Sizes in Parentheses)

Genotype classification	No trauma	One traumatic event	Two or more traumatic events
SS	23.5 (17)	30.0 (10)	20.0 (5)
SL (SL_A, SL_G)	21.7 (60)	23.1 (26)	56.7 (30)
LL (L_AL_A, L_AL_G, L_GL_G)	22.2 (45)	43.5 (23)	63.6 (11)
Low (SS, SL_G, L_GL_G)	16.0 (25)	21.4 (14)	45.5 (11)
Medium (SL_A, L_GL_A)	23.7 (59)	28.6 (28)	53.9 (26)
High (L_AL_A)	23.7 (38)	47.1 (17)	66.7 (9)

($P = 0.03$), but marginal evidence that additional trauma further increases risk ($P = 0.054$). In direct comparisons, using the number of L_A alleles in the analyses (assuming co-dominance for the L_A allele) provided a better fit to the data than alternatives (dominant: carrier of L_A vs. non-carriers; recessive: carriers of two alleles vs. others; comparisons done with the Akaike Information Criterion).

The influence of a single adverse life event on the risk for major depression is elevated for individuals who have one copy of the L_A allele and is significantly greater for those with two copies. Unlike the previous reports described above, the low-activity genotypes (S/S, S/L_G, L_G/L_G) are not associated with increased MDD risk in the presence of environmental trauma. Subjects who reported two or more stressful life events were all at very high risk for developing MDD. Although there is a higher prevalence of MDD for the high- and medium-activity genotypes in this high-environmental-risk group (67% and 54% compared to 46% for the low-activity genotypes, *P*-values 0.007, 0.020 and 0.157, respectively), the genotype effect itself is not statistically significant ($P = 0.355$).

The results we obtain from our analysis of this sample of young adult female twins differ from previous reports [Caspi et al., 2003; Eley et al., 2004; Grabe et al., 2005; Kendler et al., 2005; Kaufman et al., 2006] where it is the low-activity S allele that is associated with increased risk for depression in adverse environments. In a biological sense, it seems puzzling that lower serotonin uptake resulting from a genetic predisposition (the 5-HTTLPR S or L_G allele) would be associated with higher depression risk. Lowering serotonin uptake by treatment with serotonin selective reuptake inhibitors relieves depression in adults, and augmenting serotonergic neurotransmission by SSRI administration or tryptophan supplementation increases positive mood, while an acute decrease in central serotonergic activity via depletion of the serotonin precursor tryptophan decreases positive mood [Flory et al., 2004]. Therefore, one might expect that the high-activity allele (L_A), with its increased number of 5-HTT transporter proteins and concomitant decrease in serotonin levels, would decrease positive mood. However, in all of the reports cited above, the old L versus S allele classification is used, which does not correctly distinguish low (L_G) and high (L_A) activity alleles based on the levels of transcription described by Hu et al. [2004]. It would be interesting to know the results of a re-analysis of their samples using the L_G and L_A allele classification.

In contrast, our results suggest that the high-activity L_A allele of 5-HTTLPR is associated with elevated risk for depression in the presence of moderate or severe trauma. While it seems logical that higher gene expression by the L_A allele would reduce synaptic 5-HT levels and depress mood, "sensible" biological explanations of the moment are often overcome as a result of the complexity revealed by additional discoveries of interacting factors and developmental effects. In addition to its role as neurotransmitter, serotonin acts as a trophic factor modulating developmental processes such as neuronal division, differentiation, migration and synaptogenesis [Sikich et al., 1990; Gaspar et al., 2003]. Transient inhibition of 5-HTT in new-born mice treated with fluoxetine, a commonly used SSRI, produces abnormal emotional behaviors in adult mice, similar to those that lack the 5-HTT gene [Ansorge et al., 2004]. Disrupting this fine tuning of 5-HT during development, either by genes, substances, epigenetic or environmental factors, may lead to unexpected emotional conditions. Given the direction of findings in the current study of adolescent onset MDD, which is contrary to previous reports with a different allele classification, we note the limitations due to the moderate sample size and realize that these findings require confirmation in larger population-based adolescent samples.

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