Three-way interaction effect of 5-HTTLPR, BDNF Val66Met, and childhood adversity on depression: A replication study

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Abstract
Both the serotonin transporter linked promoter region (5-HTTLPR) and the brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms have been shown to interact with unfavourable environment in relation to depression symptoms and to depression diagnosis. Several attempts have been made to study a three-way interaction effect of these factors on depression, however with contradictory results. We aimed to test the hypothesis of a three-way interaction effect and to attempt at replication in an independent population-based sample. Family maltreatment, sexual abuse and depression were self-reported by an adolescent population-based cohort (N=1393) from the county of Västmanland, Sweden. DNA was isolated from saliva, and used for genotyping of the 5-HTTLPR and BDNF Val66Met polymorphisms. Neither 5-HTTLPR or BDNF genotypes separately, nor in interaction with each other had any relation to depression, however in an environment adjusted model a two-way interaction and a three-way interaction effect was found. Both 5-HTTLPR and BDNF Val66Met interacted with unfavourable environment in relation to depressive symptoms (Adj $R^2=0.19$). Depressive symptoms and depression were more common among carriers of either the ss/sl+Val/Val or the ll+Met genotypes in the presence of early-life adversities. This three-way effect was more pronounced among girls. The current study, with a virtually similar set-up compared to previous studies, can partially confirm previous findings and their generalizability. The study also shows the importance of genetic plasticity in individuals with different environmental exposure, for different phenotypic expression.

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1. Introduction

Duncan and Keller (2011) recently reviewed the literature on candidate gene-by-environment interaction (cG X E) studies in...
psychiatry over a decade (2000-2009); their analysis of 103 studies suggested that “well-powered direct replications deserve more attention than novel C6 × E findings and indirect replications”.

To date, six studies on Caucasians have focused on the three-way interaction effect of 5-HTTLPR × BDNFVal66Met × childhood adversity on depression. The first evidence of such an effect was shown in a relatively extreme case-control sample (Kaufman et al., 2006), and attempts have been made to replicate it in population-based samples (Wichers et al., 2008; Aguilera et al., 2009; Nederhof et al., 2010; Buchmann et al., 2012; Grabe et al., 2012) with contradictory results (Table 1).

Neurobiological evidence supports an interaction effect between the serotonin (5-HT) and brain-derived neurotrophic factor (BDNF) systems. Synergistic effects have been reported for the regulation of the development and plasticity of neural circuits, which are of importance for affective behaviours (Martinowich and Lu, 2008). With regard to antidepressant responses, selective serotonin reuptake inhibitors have been shown to enhance the BDNF gene expression (Martinowich and Lu, 2008), and an epistatic effect between 5-HTTLPR and BDNF Val66Met has been shown in response to lithium treatment (Rybakowski et al., 2007), and on anterior cingulate anatomy and its structural connectivity with the amygdala (Pezawas et al., 2008).

The current study aims to test the hypothesis of a three-way interaction effect and to attempt to replicate it in an independent population-based sample.

2. Experimental procedures

For a further description of the experimental procedures, see Supplementary Material.

2.1. Participants

The study included 1819 adolescents (17-18 years old) who participated in a population-based study in 2006, which was based on a self-reported questionnaire on adolescent life (Aslund et al., 2009). The study was approved by the Regional Ethical Review Board of Uppsala University.

2.2. Genotyping

The serotonin transporter-linked promoter region (5-HTTLPR) polymorphism, consisting of an insertion/deletion that creates a long (l) or a short (s) allele, and the brain-derived neurotrophic factor (BDNF) polymorphism, consisting of a single-nucleotide polymorphism (SNP) G/A substitution at codon 66 (Val66Met), were analysed using DNA isolated from saliva as previously described (Aslund et al., 2009; Comasco et al., 2011).

2.3. Psychosocial measures

Family relations were assessed using six questions on exposure to negative psychological or physical experiences within the family. Self-reported answers were rated dichotomously and a family relations index variable with a range of 0-6 points was created by summing the scores (Nilsson et al., 2011).

Sexual abuse was assessed using three questions on the experience of sexual abuse, both actual or attempted, and rated as frequency of occurrence, from never to more than four times. A sexual abuse summation index of the three questions was created using a range of 0-9 points (Nilsson et al., 2011).

Symptoms of depression were estimated using an adolescent version of the Depression Self-Rating Scale (DSRS), based on the Dsm-IV A and C criteria for depression (APA, 2000; Svanborg and Ekselius, 2003). Dichotomous answers to 16 questions on depressive symptoms occurring during the last two weeks were used to sum the symptoms reported on the DSRS, and to calculate a depression index (Sjöberg et al., 2006). Subjects fulfilling the DSM-IV A and C criteria were classified as screening positively for depression (referred to as symptoms of depression in the present study).

2.4. Statistical analyses

In total, psychosocial and genetic data of interest for this study were available for 1393 participants, 704 boys and 689 girls. The 5-HTTLPR genotypes were grouped by homozygosity for the long versus short variant, and BDNF Val66Met genotypes were grouped into Val/Val carriers versus Met carriers. Differences between groups in depression scores were analysed by the Mann-Whitney test. The adjusted multivariate gene × environment interaction models were analysed by general linear models using the symptoms of depression as a scale variable. In the final models (Tables 2-4) only significant main and interaction effects are reported. A p-value of <0.05 was considered significant for main effects, and p<0.10 was considered significant for interaction effects (Fleiss, 1986).

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk factors</th>
<th>Sex effect</th>
<th>Subjects</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaufman et al. (2006)</td>
<td>SS × Met/Val × Maltreatment</td>
<td>-</td>
<td>Children</td>
<td>196</td>
</tr>
<tr>
<td>Wichers et al. (2008)</td>
<td>LS × Met × Adversity</td>
<td></td>
<td>Adult (females)</td>
<td>394</td>
</tr>
<tr>
<td>Aguilera et al. (2009)</td>
<td></td>
<td>-</td>
<td>Young adults</td>
<td>534</td>
</tr>
<tr>
<td>Nederhof et al. (2010)</td>
<td></td>
<td>-</td>
<td>Adolescents</td>
<td>1096</td>
</tr>
<tr>
<td>Grabe et al. (2012)</td>
<td>SS × Val/Val × Maltreatment; L × Met × Maltreatment</td>
<td>Females</td>
<td>Adults</td>
<td>2035</td>
</tr>
<tr>
<td>Present study</td>
<td>SS × Val/Val × Maltreatment; L × Met × Maltreatment</td>
<td>More evident among females</td>
<td>Adolescents</td>
<td>1393</td>
</tr>
</tbody>
</table>
Among the adolescents, 223 boys (26%) and 244 girls (33%) described their families as having poor relationships, and 86 boys (10%) and 160 girls (22%) had experienced some kind of sexual abuse. There were 481 boys (68%) and 459 girls (67%) who carried the short 5-HTTLPR allele and 208 boys (29%) and 200 girls (29%) were Met-carriers of the BDNF Val66Met genotype.

Boys had a lower mean depression score than girls (mean 2.2 ± 2.5 versus 3.3 ± 2.7; Z = 9.18, p < 0.001). There was also a sex difference in the proportion of depressed individuals (χ² = 33.70, p < 0.001), with 56 of the boys (8%) and 126 of the girls (18%) fulfilling the DSM-IV A and C criteria for depression.

Both boys and girls showed higher depression scores if they came from families with poor relationships (boys: χ² = 9.52, p < 0.001; girls: χ² = 8.61, p < 0.001), as well as if they reported sexual abuse experiences (boys: χ² = 4.71, p < 0.001; girls: χ² = 7.16, p < 0.001). There were no differences between 5-HTTLPR or BDNF Val66Met genotypes in relation to depression scores, or to family relations or experiences of sexual abuse.

Table 2 displays a multivariate general linear model (GLM) on the total sample. Both BDNF Val66Met and 5-HTTLPR genotypes showed a main effect on symptoms of depression when adjusted for family relations and sexual abuse. Furthermore, there was a significant gene × gene interaction effect, as well as gene × environment interaction effects and a gene × gene × environment interaction effect in relation to depression scores. The observed power was 0.58-1.0 for the main effects, 0.65-0.99 for the two-way interactions, and 0.41-0.86 for the three-way interaction.

Notably, there were no differences in depression scores by either the BDNF Val66Met or the 5-HTTLPR genotypes separately, or in combination without adjusting for environment. The most evident gene × gene interaction was found among individuals with no family relation problems or sexual abuse experience, where individuals who were homozygous for the long 5-HTTLPR allele and the BDNF Met allele had the lowest depression scores, whereas carriers of the short 5-HTTLPR allele and the BDNF Met allele had the highest depression scores. However, among individuals who experienced one or both types of environmental exposure, those who were homozygous for the long 5-HTTLPR allele and the BDNF Met allele had the highest depression scores.

We also performed analyses including sex as a covariate (not shown in tables); sex had a main effect in relation to...
depression ($F=34.88$, $p<0.001$). When sex × genotypes and sex × environment were entered into the model, the main effect of sex was non-significant, as were both of the sex × genotype interaction effects. However, the sex × family relations and sex × sexual abuse interaction effects were significant ($F=9.62$, $p=0.002$, and $F=5.34$, $p=0.02$, respectively) in relation to depression scores (full model adjusted $R^2=21.6$). Among individuals who were not exposed to environmental risk factors, girls had higher depression scores, whereas among the more environmentally exposed adolescents, the difference in depression scores between the sexes was smaller. Therefore, we also further investigated the models separately for each sex.

Among boys, there were no significant main effects for any of the genotypes or sexual abuse, respectively; however there was a significant main effect of family relations, a gene × gene interaction effect, and a gene × environment interaction effect of both the BDNF Val66Met and the 5-HTTLPR genotypes with family relations (Table 3). There was a gene × gene × environment interaction effect of the BDNF Val66Met and the 5-HTTLPR genotypes and sexual abuse on depression. There was also a dose response effect for family maltreatment and sexual abuse. Among boys, individuals with no family relation problems or sexual abuse experience and the short variant of the 5-HTTLPR genotype showed different directions of depression symptoms depending on the BDNF Val66Met genotype. The lowest depression scores were found among those who had not been maltreated, had the short variant of the 5-HTTLPR genotype and were BDNF Met carriers.

Among girls, there was no main effect for the BDNF Val66Met genotype. However, there were significant main effects of the 5-HTTLPR genotype, family relations, and sexual abuse, a gene × gene interaction effect, and a gene × environment interaction effect of the 5-HTTLPR genotype with family relations and sexual abuse (Table 4). Finally,
there was a gene × gene × environment interaction effect of the BDNF Val66Met and the 5-HTTLPR genotypes with sexual abuse on depression. The lowest depression scores were found among those who had not been maltreated, had the short variant of the 5-HTTLPR genotype and were BDNF Met carriers.

The explained variance (adjusted $R^2$) in the total sample was 19.2% using a GLM (16.4% accounted for by environment). Among boys, the explained variance was 17.9% (15.6% accounted for by environment), and among girls, it was 16.6% (14.8% accounted for by environment). The gene × gene × environment interaction effect for depressive symptoms in the total sample of adolescents is illustrated in Figure 1.

4. Discussion

In the present study, we found robust evidence for a 5-HTTLPR × BDNF Val66Met × childhood adversity interaction effect in relation to symptoms of depression in a population-based sample of adolescents, especially among girls. The present results were confirmed in the total sample and among boys and girls separately. Even though two- and three-way interaction models need large samples, the power of the present study was acceptable. However, if a design with the three groups of 5-HTTLPR genotypes, SS, LS, and LL, were used, the subgroups in the three-way interaction would be too small, leading to insufficient power in the present sample.

A candidate gene × gene × environment interaction effect of 5-HTTLPR × BDNF Val66Met × childhood adversity on depression has been investigated among Caucasians in six studies (see Table 1). The findings have been inconsistent regarding both the presence of a three-way interaction effect and the genetic risk variants in the presence of childhood adversity. Four studies found evidence of a three-way interaction effect with the following genetic risk variations: the SS genotype and Met allele (Kaufman et al., 2006), the LS genotype and Met allele (Wichers et al., 2008), and the SS genotype and Val/Val genotype, and LL genotype and Met allele (Buchmann et al., 2012; Grabe et al., 2012). Two other studies did not find such an effect (Aguilera et al., 2009; Nederhof et al., 2010). The present results virtually confirm Grabe et al.’s results, with the presence of early-life adversity being associated with increased depression vulnerability among carriers of either the SS/LS+Val/Val or the LL+Met genotypes, as shown in Figure 1. This is also in line with previous neuroimaging research, which show a protective effect of the Met allele on the short allele of the 5-HTTLPR in relation to anterior cingulate anatomy and its structural connectivity with the amygdala, and an abnormal ‘wiring’ between these structures among carriers of the 5-HTTLPR S allele and the BDNF Val/Val genotype, the high-risk group associated with depression (Pezawas et al., 2008).

The current study showed two-way gene × environment effects as previously demonstrated (Åslund et al., 2009), as well as gene × gene interaction effects, but only after adjustment for environmental exposure. Therefore, it is essential to investigate the individual’s environment and adjust genetic models of phenotypic expression according to adequate environmental factors. Moreover, the environmental effect was a dose-response effect, which it was essential to adjust for to detect the genetic interaction effects. This could lead to different results in different populations, depending on the distribution of environmental exposure. The present study also shows the importance of population-based research to draw generalizations to the population. If an extreme sample with traumatized individuals was investigated, the gene × gene or gene × environment effects would have been very different from a sample of predominantly non-traumatized individuals.

There were no major differences in the gene × gene, gene × environment or gene × gene × environment effects between boys and girls in the present study. However, girls, on average, had higher depression scores and a somewhat broader spread of scores, and more girls had experienced poor family relations and sexual abuse. Consequently, the effects were more easily detected, and seemingly more apparent among girls. Moreover, the most evident gene × gene effects were among non-environmentally exposed individuals, and among those, girls had significantly higher depression scores.

Furthermore, the results may be interpreted in relation to the differential susceptibility hypothesis, also called the hypothesis of biological sensitivity to context (Boyce and Ellis, 2005; Belsky and Pluess, 2009; Beaver and Belsky, 2012). In the present study, individuals homozygous for the long 5-HTTLPR allele who were also carriers of the BDNF Met allele seemed to show the highest sensitivity to environmental context, as they had the lowest depression scores when no family relation problems or sexual abuse were reported, whereas they had the highest depression scores in the presence of one or both types of environmental exposure.

The inconsistency or failure of attempts to replicate evidence of Cg × E associations can be explained by the differences in: the design of the studies (e.g., cross-sectional/longitudinal, sample size, self-report/interview data); the statistical methodology used (e.g., transformation, dichotomous/continuous variables); the phenotypic outcome variable (e.g., self-rating scale of symptoms/clinical diagnosis, measurement scale); and the nature of the environmental factors (e.g., acute/repeated/chronic stressful life events, single/several stressful life events) (Duncan and Keller, 2011). There are also several issues regarding publication bias of findings within the area of biopsychiatric research (e.g., false positive results, bias versus significant results), as recently pointed out by Duncan and Keller (2011). To this list, we would like to add the importance of differences in the environmental exposure, according the susceptibility hypothesis (Boyce and Ellis, 2005; Belsky and Pluess, 2009; Beaver and Belsky, 2012), which predicts very different results of genetic plasticity, depending on different environment.

The current study attempted to test a previous hypothesis, and to replicate previous findings in a relatively large population-based independent sample. Our study may assist in explaining the findings of these previous attempts at replication. We closely replicated the Kaufman et al. (2006) study with regard to the
phenotypic variable, genetic polymorphisms, statistical models, environmental moderator, and inclusion of both sexes. Indeed, this represents a major strength of the current study, which will be of importance in drawing clearer conclusions in the context of future meta-analytical studies.

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Contributors

KWN and LO designed the study and wrote the protocol.
CÅ managed the data collection and preparation of data in accordance with the protocol.
EC and LO managed the genetic analyses.
KWN undertook the statistical analyses.
EC performed the literature search.
EC and KWN wrote the first draft of the manuscript.
All authors contributed to and have approved the final manuscript.

Conflict of interest

None declared by any of the authors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2013.01.010.

References


