The CREB1-BDNF-NTRK2 Pathway in Depression: Multiple Gene-Cognition-Environment Interactions

Gabriella Juhasz, Jason S. Dunham, Shane McKie, Emma Thomas, Darragh Downey, Diana Chase, Kathryn Lloyd-Williams, Zoltan G. Toth, Hazel Platt, Krisztina Mekli, Antony Payton, Rebecca Elliott, Steve R. Williams, Ian M. Anderson, and J.F. William Deakin

Background: The neuroplastic pathway, which includes cyclic adenosine monophosphate response element-binding protein 1 (CREB1), brain-derived neurotrophic factor (BDNF), and its receptor (neurotrophic tyrosine kinase receptor, type 2 [NTRK2]), plays a crucial role in the adaptation of brain to stress, and thus variations of these genes are plausible risk factors for depression.

Methods: A population-based sample was recruited, subsets of which were interviewed and underwent functional magnetic resonance imaging. We investigated the association of nine polymorphisms throughout the CREB1-BDNF-NTRK2 pathway with lifetime depression, rumination, current depression severity, negative life events, and sad face emotion processing in a three-level design.

Results: In the population study, BDNF-rs6265 and CREB1-rs2253206 major alleles were significantly associated with rumination and through rumination with current depression severity. However, childhood adversity increased the risk of lifetime depression in the minor allele carriers of BDNF-rs6265 and CREB1-rs2253206 and in alleles of six other single nucleotide polymorphisms (SNPs). We validated our findings in the interviewed subjects using structural equation modeling. Finally, using functional magnetic resonance imaging, we found that viewing sad faces evoked greater activity in depression-related areas in healthy control subjects possessing the minor alleles of BDNF-rs6265 and CREB1-rs2253206.

Conclusions: Genetic variation associated with reduced function in the CREB1-BDNF-NTRK2 pathway has multiple, sometimes opposing, influences on risk mechanisms of depression, but almost all the SNPs studied amplified the effect of childhood adversity. The use of cognitive and neural intermediate phenotypes together with a molecular pathway approach may be critical to understanding how genes influence risk of depression.

Key Words: BDNF, childhood trauma, CREB1, depression, NTRK2, rumination

There is increasing evidence that neuroplastic pathways have an important role in the pathophysiology of depression and its response to antidepressant treatment (1–3). Much interest has focused on brain-derived neurotrophic factor (BDNF), its tropomyosin-related kinase B receptor (TrkB), and the transcription factor cyclic adenosine monophosphate (cAMP) response element-binding protein 1 (CREB1), which increases the expression of target genes including the TrkB receptor (neurotrophic tyrosine kinase receptor, type 2 [NTRK2]) and BDNF genes (1–8). Both animal and human data suggest that acute stress decreases the expression of BDNF and thus TrkB signaling in the hippocampus and prefrontal cortex, and this phenomenon is at least partially driven by the cAMP–CREB1 signaling cascade (6,9). Behavioral effects of antidepressant drugs have been associated with their ability to increase BDNF expression and activation of TrkB signaling in hippocampus and prefrontal cortex, in part mediated by CREB1 (1–8). It has been also reported that early-life stress causes a persistent decrease in BDNF expression in the prefrontal cortex of adult rats by long-lasting epigenetic changes (10). However, overexpression or up-regulation of CREB1-BDNF-TrkB signaling in the ventral tegmental area and nucleus accumbens produces a depression-like phenotype (4,6). Thus, it cannot be assumed that simple upregulation or downregulation of the pathway in the entire brain is of etiological relevance for depression or response to treatment; regionally specific effects are likely to be important.

In human genetic association studies, the BDNF gene valine (Val) 66 methionine (Met) (Val66Met) (rs6265) nonsynonymous polymorphism is the most investigated variant in depression, although other variants within the gene may modify its effects (11–13). In vitro studies demonstrated that the Met-allele decreases processing of the pro-BDNF and thus affects activity-dependent secretion of BDNF (14). In line with this finding, structural imaging studies have repeatedly found smaller hippocampal volume in Met-allele carriers, both in healthy control subjects and in depressed subjects (15), suggesting a possible susceptibility factor for mood disorders (5). However, two recent meta-analyses failed to show global association between the Val66Met BDNF polymorphism and major depressive disorder (MDD), although there is a possible gender-dependent effect (16,17). Groves (5) hypothesized that BDNF may be a mediator of depression caused by chronic stress rather than a direct risk factor for depressive symptoms. For example, there is evidence that childhood maltreatment, possibly by promoting rumination on negative mood (18,19), produces more intense depressive symptoms in Met-allele carriers than in Val66Val genotype carriers (20–23). In addition, Met-allele carriers also showed increased amygdala activation in an emotional processing genetic imaging study (24). Other studies that did not take chronic stress into account found that the Val allele is a vulnerability factor for a variety of phenotypes, such as neuroticism (25), anxiety (26), or depressive symptoms in adults (27) and children (19,28).
Much less is known about NTRK2 and its role in human depression. One association study investigated three single nucleotide polymorphisms (SNPs) in childhood-onset mood disorders with negative results (29). Our group recently found two possible functional SNPs (rs1187323, rs1187326) at the 5’ end of this gene whose minor alleles were associated with less hippocampal TrkB protein than the major alleles measured in postmortem human brains (12).

Regarding the CREB1 gene, Zubenko et al. (30) found linkage of the 2q33–35 chromosomal region to mood disorders, which includes the gene for CREB1. A rare functional variant (G↓−656ΔA) in the CREB1 promoter appeared to confer unipolar mood disorders with high penetrance among women, but two subsequent studies using this and other SNPs throughout the CREB1 gene failed to find association with MDD (31,32). Another SNP near to the CREB1 gene, with unknown functional relevance, showed associations with treatment-emergent suicidality in MDD patients and emotional face processing in an imaging study (33). Unpublished data from our postmortem brain study (12) found that another, more prevalent promoter variant (rs2253206) may be functional because it was associated with reduced hippocampal BDNF expression.

We aimed to test the hypothesis that variation in three genes with disparate genomic locations would nevertheless show similar associations with depression by virtue of their sequential role in the same neuroplastic pathway. Specifically, we predicted that variants in the CREB1-BDNF-NTRK2 pathway genes would increase the risk of lifetime depression/depressive symptoms in the presence of childhood/recent life stresses and that rumination, as an intermediate phenotype, would mediate the effect of life stresses onto depression. We also investigated the effect of these genes on brain function using sad emotion processing functional magnetic resonance imaging (fMRI) task, a proposed neurobiological marker for depression (34), and hypothesized that risk genetic variants will be associated with increased blood oxygenation level-dependent (BOLD) signal in depression-related brain areas.

Methods and Materials
We designed a three-level study to test our hypothesis. The study was approved by the local Ethics Committees and was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Level-1 Study (Community Cohort)
Subjects aged 18 to 60 years predominantly from Greater Manchester, United Kingdom, were recruited as a population sample through general practices and a website (http://www.newmood.co.uk). Details of our recruitment strategy and responses have been published previously (35). In summary, a questionnaire pack and a genetic sampling kit were sent to the participants by post. From those who returned our questionnaires, we included in the study 1269 subjects who were of Caucasian origin, provided DNA, and had not reported manic or hypomanic episodes or psychotic or obsessive-compulsive symptoms. A detailed description of the questionnaires can be seen in Supplement 1.

In brief, the level-1 questionnaire booklet included a background questionnaire (BGR) (35,36) and reported lifetime depression (DEP) was derived from this, using questions relating to personal psychiatric disorder history. Depressive symptoms were measured by the 53-item Brief Symptom Inventory (37) using the depression plus additional items subscale (BSI-DEP). Participants completed the Ruminative Responses Scale (RRS) brooding and reflection items (38). The List of Life-Threatening Experiences (LTE) was used to measure negative life events occurring in the last year related to intimate relationships, financial difficulties, illnesses/injuries, and network problems (39). Childhood Adversity (CHA) questions were derived from the Childhood Trauma Questionnaire (CTQ) and covered emotional and physical abuse and emotional and physical neglect (40). An additional question asked about parental loss during childhood.

Level-2 Study (Interviewed Cohort)
A subset of 145 level-1 subjects was invited for a level-2 face-to-face diagnostic interview and further psychological testing. We recruited an additional 119 subjects through advertisements using the same inclusion and exclusion criteria as for level-1. These subjects provided BGR and level-2 questionnaire data and took part in the interview but did not fill out level-1 questionnaires. We used this carefully diagnosed level-2 cohort to validate our level-1 results. Validation data can be found in Supplement 1.

The level-2 BGR questionnaire was the same as that used for level-1 and the answers were elaborated during the face-to-face interview. To assess the participants’ lifetime psychiatric history, we used the Structured Clinical Interview for DSM-IV (SCID; lifetime history of major depressive disorder, MDD) (41). Depressive symptom severity was rated by trained interviewers using the Montgomery Asberg Depression Rating Scale (MADRS) (42) and also measured by self-reported BSI-DEP. Participants completed the full version of the RRS and a rumination score was calculated from the brooding and reflection items as in level-1. The Life Events Questionnaire (LEQ) was devised for New Molecules for Mood Disorders (NewMood) level-2 by adapting validated questionnaires (39,43–45). The CTQ was used to measure childhood adversity (40).

Level-3 Study (Imaging Cohort)
From the level-2 interviewed cohort, we recruited 37 control subjects for the third fMRI level. These participants had no current or past psychiatric disorders according to the SCID and no family history. The genotyping for two subjects was not successful and another two subjects’ data were not suitable for analysis because of movement artefacts. Therefore, we report results from 33 level-3 subjects.

Participants performed an emotional face processing task (46,47) in the scanner. Neutral, happy, sad, and fearful faces were used and participants pressed a button to indicate the sex of each face. We were specifically interested in the comparison between sad and neutral faces, which has been proposed as a neurobiological marker for depression (34,48). For a detailed description of the scanning procedure, see Supplement 1.

Genotyping
For BDNF, we genotyped the previously identified nonsynonymous Val66Met (rs6265) functional polymorphism (14) and another five haplotype-tagging SNPs (htSNPs) (rs12273363, rs962369, rs988748, rs7127507, and rs1519480) to cover the whole gene; of these, rs12273363 and rs7127507 had been found in our previous study to influence the hippocampal proBDNF density (12). For CREB1, we genotyped the rs2253206 promoter htSNP that was associated with reduced hippocampal BDNF expression in unpublished data from our previous study (12). For NTRK2, we genotyped two htSNPs at the 5’ end of the gene (rs1187323 and rs1187326), both of which significantly influenced the TrkB receptor density in the hippocampus (12). For the laboratory method of genotyping, see Supplement 1.

Statistical Analysis
PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) was used for testing association of different genetic models (dominant [DOM], recessive [REC], and additive [ADD]; linear and logistic re-
formatting and ensuring natural language coherence. Extracting tables and figures as necessary.
regions showing significant gene \times CTQ (rank) interaction. In all analyses, age, sex, and rumination scores were used as covariants.

Results

Table 1 shows the demographic and clinical composition of the three study populations. In each of these cohorts, all the genotyped SNPs were in Hardy-Weinberg equilibrium both in control subjects and in depressed patients (DEP/MDD) (Table S1 in Supplement 1). In the level-1 population, the selected SNPs in the BDNF were in significant linkage disequilibrium, as were those in the NTRK2 (Table S2 in Supplement 1).

Level-1 Genetic Association Results

Rumination was associated with genetic variations in the CREB1-BDNF-NTRK2 pathway: minor allele carriers of the BDNF-rs6265 (A, 66Met) and CREB1-rs2253206 (A) polymorphisms were significantly less likely to ruminate and this effect remained significant after multiple testing (Figure 1, Table 2). A similar trend can be seen in the BDNF-rs988748 C allele carriers. The CREB1-rs2253206 A allele carriers had fewer BSI-DEP symptoms (beta = −.152, \( P_{\text{DOM}} = .016 \)). The NTRK2-rs1187326 C allele significantly interacted with CHA (beta = −.076, \( P_{\text{REC}} = .030 \)) and with LTE (beta = .214, \( P_{\text{REC}} = .015 \)) on BSI-DEP (Table S3 in Supplement 1). These associations became nonsignificant after covariation with rumination, which suggests that rumination mediated these genetic effects. However, these associations did not survive correction for multiple testing.

The CREB1-rs2253206 A allele showed a significant protective effect (odds ratio = .74, \( P_{\text{DOM}} = .029 \)) on DEP, which did not survive correction for multiple testing. Eight of the nine SNPs showed significant interaction with CHA on DEP after correction for multiple testing (Figure 2; Table S4 in Supplement 1). The association became more significant between CREB1-BDNF-NTRK2 pathway and DEP after covariation for rumination, suggesting that rumination does not mediate this genetic effect on DEP (Table 2).

None of the SNPs showed significant interaction with sex on BSI-DEP or DEP.

We also created BDNF haplotypes and found that T,A,C,T,A,T haplotype, which carries the A/66Met allele and has a frequency 18.5% in our population, was significantly negatively associated with rumination and showed significant interaction with CHA on DEP after covariation for age, sex, and rumination (Table S6 in Supplement 1). These results suggest that the diplotypic effect was driven by the functional BDNF-rs6265 effect or by other variants in the BDNF gene that are in strong linkage disequilibrium with BDNF-rs6265.

---

**Table 2.** Genetic Association of the CREB1-BDNF-NTRK2 Pathway with Ruminative and Genetic Interaction with Childhood Adversity on Reported Depression in the Level-1 Population (n = 1269)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>MA</th>
<th>Test</th>
<th>Beta (SE)</th>
<th>t</th>
<th>p</th>
<th>q</th>
<th>OR (95% CI)</th>
<th>( \chi^2 )</th>
<th>( p )</th>
<th>( q )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREB1</td>
<td>rs2253206</td>
<td>A</td>
<td>ADD</td>
<td>−.078 (.030)</td>
<td>2.613</td>
<td>.009b</td>
<td>.048b</td>
<td>1.15 (1.06–1.26)</td>
<td>3.232</td>
<td>.001b</td>
<td>.004b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DOM</td>
<td>−.091 (.036)</td>
<td>2.529</td>
<td>.01b</td>
<td>.048b</td>
<td>1.18 (1.06–1.31)</td>
<td>3.030</td>
<td>.002b</td>
<td>.007b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>REC</td>
<td>−.118 (.082)</td>
<td>1.372</td>
<td>.15</td>
<td>.17</td>
<td>1.31 (1.03–1.67)</td>
<td>2.199</td>
<td>.03b</td>
<td>.02</td>
</tr>
<tr>
<td>NTRK2</td>
<td>rs1187326</td>
<td>C</td>
<td>ADD</td>
<td>−.029 (.026)</td>
<td>1.116</td>
<td>.27</td>
<td>.21</td>
<td>.92 (0.87–0.97)</td>
<td>2.342</td>
<td>.02b</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DOM</td>
<td>−.052 (.034)</td>
<td>1.552</td>
<td>.12</td>
<td>.17</td>
<td>.88 (0.80–0.96)</td>
<td>2.834</td>
<td>.005b</td>
<td>.005b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>REC</td>
<td>−.035 (.055)</td>
<td>−.123</td>
<td>.09</td>
<td>.41</td>
<td>.97 (0.84–1.31)</td>
<td>3.61</td>
<td>.02</td>
<td>.32</td>
</tr>
</tbody>
</table>

Data covaried for age and sex in all analyses.

ADD, additive; BDNF, brain-derived neurotrophic factor; CHA, Childhood Adversity; \( \chi^2 \), chi-squared test; CI, confidence interval; CREB1, cyclic adenosine monophosphate response element-binding protein 1; DEP, depression; DOM, dominant; MA, minor allele; NTRK2, neurotrophic tyrosine kinase receptor, type 2; OR, odd ratio; q, false discovery rate corrected significance value for multiple testing; REC, recessive; RRS, Ruminative Responses Scale; SE, standard error; SNP, single nucleotide polymorphism; t, t test.

*a*After covariation of RRS.

**Significant results (\( p < .05 \)).

**Trends (\( 1 < p < .05 \)).
Validation of Level-1 Findings with Level-2 Data

We tested whether the level-1 best fit model fitted the level-2 data in which interview ratings and diagnosis and full questionnaire self-reports were substituted for level-1 booklet self-ratings. Complete data were available for \( n = 238 \) level-2 subjects.

Although the genetic association results for level-2 data were no longer significant after correction for multiple testing (Table S5 in Supplement 1), the level-1 best fit model showed very good fit for the level-2 dataset (CMIN = 11.556, df = 12, CMIN/df = .963, CFI = 1.000, RMSEA < .001), suggesting that our findings based on the level-1 population study are generalizable to a clinically evaluated population (Figure 3). The model in this population explained 14\% of the variance (\( R^2 \)) in MADRS, 56\% in MDD, and 35\% in RRS.

fMRI Study: Effects of BDNF-rs6265 and CREB1-rs2253206 on Brain Responses to Sad Emotion Processing

The effects of significant genotypes in the SEM model were examined in prespecified ROI. The BDNF-rs6265 A(Met66) carriers showed significantly (\( p_{\text{FDR}} < .05 \)) increased BOLD response to sad faces in the right parahippocampal gyrus (Figure 4A), left globus pallidus, and inferior temporal gyrus (Table 3), while CREB1-rs2253206 A carriers showed significantly (\( p_{\text{FDR}} < .05 \)) enhanced BOLD response in the middle cingulate gyrus (Figure 4A), precuneus, left cuneus, right thalamus, and the right middle and superior temporal gyrus (Table 3). Post hoc analysis did not show increased BOLD response in BDNF-rs6265 or CREB1-rs2253206 GG carriers in the selected ROI during sad emotion processing or BDNF-rs6265 A(Met66) and CREB1-rs2253206 A allele effect on happy-neutral and fear-neutral contrasts, at the \( p_{\text{FDR}} < .05 \) threshold.

At \( p_{\text{unc}} \leq .001 \), significance threshold genotype \( \times \) CHA interaction was seen in a single cluster in the hypothalamus, which showed enhanced brain activation in BDNF-rs6265 A(Met66) carriers relative to GG(Val66Val) carriers with increasing CTQ score (Figure 4B, Table 3). The CREB1-rs2253206 did not show significant genotype \( \times \) CHA interactions at \( p_{\text{unc}} \leq .001 \) level.

Discussion

Our study is a strong test of the risky prediction that if the BDNF pathway is an important mechanism of stress and depression, then functional variants in three different genes on the same pathway, CREB1, BDNF, and NTRK2, should show similar influences on the processes of depression. Indeed, we found that these genetically nonlinked regions, which code functionally interconnected proteins, showed similar association patterns with rumination, depressive symptom scores, and reported lifetime depression. Specifically, the carriers of the less frequent, and functionally less active, alleles of the BDNF (rs6265 A/Met66 or the T,A,C,T,A,T haplotype) and CREB1 (rs2253206 A) genes decreased ruminative response style and through reduced rumination were associated with fewer current depressive symptoms. In contrast, the same BDNF and CREB1 genotypes and NTRK2-rs1187326 T-allele carriers showed increased risk for lifetime depression if childhood adversity was present, and this effect was independent of rumination. Thus, our results emphasize that the same allele of a gene can exert different effects on depression: the variant that promotes a well-established cognitive risk factor for depression (rumination) seems to be protective against depression when another environmental risk factor (childhood adversity) is acting (40\% of our sample). The net genetic effect of this complex interplay is very difficult to understand without taking into account the mediating and interacting factors that result in symptoms and diagnosis.
Rumination

Although there is good evidence that rumination represents a trait vulnerability factor to depression (18,19,38), genetic influences on this trait are unknown. Two studies with small sample sizes investigated the role of the BDNF Val66Met polymorphism with conflicting results (19,51). Our results demonstrated that BDNF Val66Val carriers—and also CREB1-rs2253206 GG carriers—ruminate more than the minor allele carriers.

Previous studies established that BDNF 66Met carriers have reduced gray matter volumes in several brain areas, especially in the hippocampus and prefrontal cortex (52,53). In line with this finding, BDNF 66Met carriers have poorer long-term memory (14,54) and showed poorer performance on cognitive tasks (55–57). The CREB1 may also have a very important and complex role in memory through experience-based neuroadaptations (6,58).

Rumination is associated with enhanced retrieval of negative memories (59) and overgeneralized autobiographical memories (60) that make demands on cognitive resources. These processes might be facilitated in individuals with genes that enhance cognitive processes, which through increased rumination lead to depressive symptoms. Indeed, our model revealed a direct positive effect of rumination on depressive symptoms (Figure 3), especially on self-rated symptom scores but not directly on reported lifetime depression or on MDD diagnosis. Any genetic influence of the major alleles on depression via their influence on rumination is evidently overridden by the direct effect of the minor alleles on depression when childhood adversity is present.

In our imaging study, BDNF-rs6265 Val66Val or CREB1-rs2253206 GG carriers did not show increased response in brain areas that are reportedly more active in depressed subjects during a sad face emotion task (34,48); this further suggests that the BDNF-rs6265 Val66Val or (CREB1-rs2253206 GG) genotype is not a direct risk factor for MDD, even though it promoted rumination.

Recent Negative Life Events

Both animal and human studies demonstrate that genes influence the impact of stressful experiences in triggering the development of depressive symptoms and behaviors (61–65). The first such report in humans was the influence of the serotonin transporter gene promoter region short allele in amplifying the risk of depression following negative life events (66–68).

In our genetic association study, variations in the BDNF or CREB1 genes did not show significant interaction with recent negative life events. However, using structural equation modeling, rumination, which was associated with BDNF-rs6265 Val66Val or CREB1-rs2253206 GG genotypes, showed a positive effect on the number of reported negative life events, and negative life events increased depression symptom scores. Indeed, it has been demonstrated that rumination predicts mood reactivity to life stresses in a nonclinical sample (69).

It is interesting to note that NTRK2 SNPs were not associated with rumination but they showed a weak interaction with negative life events on Brief Symptom Inventory depression scores and lifetime depression with minor alleles being the risk variants. It can be
speculated that the decreased TrkB availability may increase stress sensitivity, as has been found in TrkB knockout animals (70). However, because of the complexity of the NTRK2 polymorphisms should be included in future studies to better describe the function of this gene.

**Childhood Adversity**

Our data add to the evidence that the BDNF-rs6265 66Met allele has a major role in the effect of childhood adversity on risk of depression (10,20–23). In addition, this is the first study that demonstrates a significant gene × childhood adversity interaction occurs with several variants in the CREB1-BDNF-NTRK2 neuroplastic pathway. It is tempting to assume that neuroplastic gene variants interact with childhood adversity at the time of adversity to modify brain development and further that the opposing direct neuroplastic gene effects on rumination occur in the adult brain. However, the opposite is equally possible. Studies in BDNF knockout animals have successfully modeled the interaction with maternal environment (71) but further studies using, for example, inducible knockouts are needed to help identify when and where in the brain neuroplastic genes and environment interact. One study suggests that adverse early rearing causes long-lasting epigenetic changes in the region of the CREB1 binding site of the BDNF gene (10). Further studies such as these could help unravel the molecular mechanisms of the associations we have described.

It is not clear which intermediate phenotypes lead to depression in maltreated children. Our imaging results suggest that risk allele carriers (BDNF-rs6265 66Met or CREB1-rs2253206 A) show enhanced responses to sad faces in brain areas that are also enhanced in depressed patients during the same task. These results are similar to previous findings (24,33) and suggest that the risk neurotrophin genotypes act to enhance emotion processing biases associated with vulnerability in depression in key emotion processing regions, possibly by influencing their development.

The main effects of genotype on emotion processing were not enhanced in those with greater childhood trauma scores. However, BDNF-rs6265 66Met allele carriers showed greater activation in the hypothalamus in relation to childhood trauma (Figure 4B), albeit at an exploratory level of statistical significance. This finding is intriguing in the light of evidence that in depressed patients BDNF-rs6265 66Met allele carriers show more disinhibition of hypothalamic-pituitary-adrenal activity in relation to history of childhood adversity than noncarriers (72). In animal studies, early stress can induce a long-lasting epigenetic impairment of hippocampal negative feedback control of the hypothalamic-pituitary-adrenal area (73). In those with a less active neuroplastic pathway, childhood adversity might be especially potent in weakening the development of hippocampal restraint of the hypothalamus, resulting in greater hypothalamic responsiveness to sad faces in relation to childhood trauma score.

**Limitations**

Although we used a pathway approach, we limited our analysis to apparently functional variants relying on our prior findings (12). Our genetic association results based on a mainly female population did not always survive correction for multiple testing; thus, further replication studies are required. In addition, our structural equation model involved only three phenotypic variables (childhood adversity, recent negative life events, and rumination) that are risk factors for depression, and we have not investigated other factors, for example, the role of social support that might modulate the effect of adversities (74). As questionnaire measures of life events are prone to recall bias, prospective studies need to support our findings. And finally, our imaging findings are preliminary and need further replication with sample sizes preselected for genetic and environmental risk factors.

In summary, we found that several allelic variants that reduce function in the neuroplastic pathway amplified the effects of childhood adversity on risk of depression. However, the same alleles were associated, in some cases significantly, with reduced rumination scores, which, in turn, decreased reporting of life events and current symptoms and via this indirect route lessened life-time depression. These countervailing influences acting through different intervening processes would be difficult to establish or replicate in genetic association studies that do not address intermediate phenotypes or environmental risk factors.

The study was supported by the Sixth Framework Program of the European Union, NewMood, LSHM-CT-2004-503474, by the National...
Table 3. Effects of the BDNF rs6265 and CREB1 rs2253206 Polymorphisms on the Brain Basis of Sad Emotion Processing

<table>
<thead>
<tr>
<th>BDNF Met(A) &gt; Val/Val(GG)</th>
<th>CREB1 A &gt; G*</th>
<th>MNI Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hemisphere</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>Parahippocampal gyrus</td>
<td>R</td>
<td>27/30</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>L</td>
<td>37</td>
</tr>
<tr>
<td>Middle cingulate gyrus</td>
<td>R</td>
<td>24/32</td>
</tr>
<tr>
<td>Precuneus</td>
<td>L</td>
<td>−11</td>
</tr>
<tr>
<td>Middle cingulate gyrus</td>
<td>R</td>
<td>7</td>
</tr>
<tr>
<td>Precuneus</td>
<td>L</td>
<td>−18</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>R</td>
<td>37</td>
</tr>
<tr>
<td>Thalamus</td>
<td>R</td>
<td>22</td>
</tr>
<tr>
<td>Cuneus</td>
<td>L</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BDNF Met(A) &gt; Val/Val(GG) with increasing CTQ^b</th>
<th>Hypothalamus</th>
<th>MNI Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>−7</td>
</tr>
</tbody>
</table>

BA, Brodmann area; BDNF, brain-derived neurotrophic factor; CREB1, cyclic adenosine monophosphate response element-binding protein 1; CTQ, Childhood Trauma Questionnaire; L, left hemisphere; Met, methionine; MNI, Montreal Neurological Institute; NA, not applicable; P_{FDR}, false discovery rate significance value; p_{unc}, uncorrected significance value; R, right hemisphere; Val, valine; Z, Z scores.

^aRegion of interest analysis used regions that showed significant effect (F-contrast, p_{FDR} < .05) of sad-neutral contrast in either of the genetic groups. Minor allele carriers (BDNF-rs6265 Met66, n = 9; CREB1-rs2253206 A, n = 25) showed increased brain activation compared with major genotype carriers (BDNF-rs6265 Val/Val, n = 23; CREB1-rs2253206 GG, n = 8) at p_{unc} < .05 significance level in healthy control subjects.

^bFurthermore, in an exploratory brain analysis, hypothalami showed increased activation with increasing CTQ ranks in BDNF-rs6265 Met66 carriers compared with Val/Val carriers at p_{unc} < .001 significance threshold. The hypothalamus defined by the WFU PickAtlas (75–77) was used for small volume correction at p_{FDR} < .05 significance level.

Institute for Health Research Manchester Biomedical Research Centre, and by the TAMOP-4.2.1.B-09/1/KMR-2010-0001, Hungary.

We are grateful to Heaton Mersey Medical Practice and Cheadle Medical Practice for their assistance in the recruitment.

Professor Deakin has carried out consultancy and speaking engagements for Bristol-Myers Squibb, AstraZeneca, Eli Lilly, Schering Plough, Janssen-Cilag, and Servier. All fees are paid to the University of Manchester to reimburse them for the time taken. He has share options in P Vital, Professor Anderson has received grant support from AstraZeneca and consultancy fees/honoraria for speaking/support to attend conferences from Wyeth, Servier, Eli Lilly, Lundbeck, Cephalon, and Bristol-Myers Squibb. Professor Williams has received grant support from AstraZeneca. All other authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.


www.sobp.org/journal


www.sobp.org/journal


