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Effect of Genetic Variant in *BICC1* on Functional and Structural Brain Changes in Depression

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Genes and early-life adversity (ELA) interactively increase the risk of developing major depressive disorder (MDD). A recent genome-wide association study suggests that the minor T-allele of single-nucleotide polymorphisms in the *bicaudal C homolog 1* gene (*BICC1*) has a protective role against MDD. The aims of the study were to investigate whether the minor T-allele of *BICC1* is protective against hippocampal structural brain changes, whether it is associated with increased functional brain activity in the emotion regulation system, and how ELA would modify this association. Forty-four patients with MDD and 44 healthy controls were investigated using structural magnetic resonance imaging (MRI) and functional MRI with an emotion inhibition task. Analysis of a single-nucleotide polymorphism in the *BICC1-1* (rs999845) gene was performed. Right hippocampal bodies of patients and controls without a history of ELA and who carry the protective T-allele of BICC1 were significantly larger compared with those participants homozygous for the major C-allele of *BICC1*. However, MDD patients with ELA, who carry the T-allele, had smaller hippocampal head volumes compared with MDD patients without ELA. FMRI showed that patients and controls carrying the protective T-allele of *BICC1* activate the emotion regulation system significantly more compared with those participants homozygous for the major C-allele ($p<0.05$, family wise error corrected). These results are suggestive that the minor T-allele of *BICC1* has a protective role against MDD and its known structural and functional brain changes. However, this protective effect seems to be lost in the case of co-occurrence of ELA.
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INTRODUCTION

Major depressive disorder (MDD) is a common disorder that has a lifetime prevalence of 16% and a 12-month prevalence of 6.6% (Kessler *et al*, 2003). The World Health Organization ranks MDD as the third leading cause of disease burden worldwide, accounting for 65.5 million disability-adjusted life years (Mathers *et al*, 2008). Despite its high prevalence and debilitating nature, relatively little is known about the pathophysiology of MDD (Krishnan and Nestler, 2008).

Early-life adversity (ELA) is recognized as an environmental risk factor for developing MDD (Edwards *et al*, 2003; Felitti *et al*, 1998; Heim and Nemeroff, 2001). ELA encompasses physical, emotional, and sexual abuse, and also physical and emotional neglect. The exact way in which ELA increases the risk of developing MDD is not entirely understood but it is thought to involve the dysregulation of the hypothalamic–pituitary–adrenal axis (Mello *et al*, 2003), which is known to be hyperactive in MDD patients (Pariante and Lightman, 2008). Not every individual that experiences ELA will go on to develop MDD even when challenged with further stressors in adulthood. The reason for this is not understood but gene–environment ($G \times E$) interactions are thought to be important as a number of studies have suggested that genetic factors moderate the relationship between ELA and MDD (Heim *et al*, 2009).

Recently, we demonstrated that ELA interacting with the short allele of the 5-HTTLPR is associated with smaller hippocampal volumes in patients with MDD (Frodl *et al*, 2010). The hippocampus is involved in consolidating short-term memory to cortical long-term memory (Campbell and MacQueen, 2004) and has long been recognized as being involved in the pathogenesis of MDD. There are a number of reasons for this implication. First, the hippocampus is a highly stress-sensitive region of the brain and MDD is a stress-sensitive illness; second, the hippocampus is involved in memory, and memory impairment is often a feature of

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MDD (MacQueen and Frodl, 2011). Finally, on a structural level it has been shown that depressed patients have smaller hippocampal volumes in comparison with healthy controls (HCs) (MacQueen and Frodl, 2011; McKinnon *et al*., 2009). Abnormal functional resting-state connectivity and connectivity during emotional and cognitive stimulation have consistently been found in patients with MDD compared with HCs with hyperactivity in midline brain regions, such as the anterior cingulate cortex, the ventromedial prefrontal cortex, basal ganglia, and decreased activity in the dorsolateral prefrontal cortex and posterior cingulate cortex (Hasler and Northoff, 2011). A recent review about the impact of genetic variations on the brain function concluded that genetic variants can be associated with brain function in depression (Scharinger *et al*, 2010). One example is that short allele carriers of the 5-HTTLPR have increased amygdala reactivity to masked emotional faces in 35 patients with MDD (Dannlowski *et al*, 2007). With regard to brain function no studies to date have investigated the interactive effect of ELA and genetic variations in MDD.

A genome-wide study (GWAS) identified suggestive association between genetic variants of the *bicaudal C homolog 1* gene (BICC1) and MDD, however, did not achieve genome-wide significance (Lewis *et al*, 2010). Two directly genotyped variants in high linkage disequilibrium (LD; rs9416742 and rs999845) approached significance ($p = 3.12 \times 10^{-7}$ for rs999845) and were nominally significant in a meta-analysis of three studies. Imputed SNPs at this locus achieved significance ($p = 5.7 \times 10^{-9}$ for rs7903712). The study suggests that the minor allele of these high-LD SNPs at *BICC1* has a protective role against depression. *BICC1* is also highly interesting, as a role of the *BICC*1 gene in plasticity and cell-to-cell communication has been shown in experimental studies (Mahone *et al*, 1995; Snee and Macdonald, 2009) in line with the neuroplasticity hypothesis of depression. The newest GWAS again stimulated discussion, as no SNP with genome-wide significance could be identified. However, some interesting associations between MDD and adenylate cyclase-3, galanin, and CACNA1C, however, not with *BICC1*, were detected (Wray *et al*, 2012). Thus, it is likely that specific genetic variants individually make very small contributions to the etiology of MDD, however, they could be relevant in association with environmental factors and their effect on the brain structure and function could be much stronger.

The overall objective of our study was to determine whether the *BICC1* gene and ELA interactively affect hippocampal volumes and brain function in patients with MDD and HCs. Magnetic resonance imaging (MRI) was used in the first part of this study to examine differences in hippocampal volume between the participants. We expected that carriers of the 'protective' T-allele (TT plus TC subjects) have larger hippocampal volumes compared with homozygous CC subjects, whereas the presence of ELA would interact with the effect of BICC1. Functional MRI (fMRI) was used in the second part of this study to investigate whether the protective T-allele might be associated with higher BOLD activity in areas implicated in emotion control and how this would be influenced by ELA.

PARTICIPANTS AND METHODS

Participants

The study included 44 adult patients with MDD from the mental health services of the Adelaide and Meath Hospital, incorporating the National Children's Hospital, Dublin or St James's Hospital, Dublin. The diagnosis of these patients with MDD was a clinical diagnosis based on DSM-IV criteria and confirmed by an independent psychiatrist using the SCID interview. Forty-four HC subjects from the local community were recruited and the groups were balanced for age and sex (Table 1). Exclusion criteria were age <18 or >65, history of neurological or comorbid psychiatric disorders (Axis I or Axis II), other severe medical illness, head injury, or substance abuse. Demographic variables, inclusion and exclusion criteria, were documented using a standardized questionnaire and through a structured interview by a psychiatrist. The same participants as described above took part in the fMRI task. However, fMRI data were not available for three patients therefore leaving a total of 85 participants.

Written informed consent was obtained from all participants after being given detailed description of the study which was designed and performed in accordance to the ethical standards laid out by the Declaration of Helsinki, and was approved by the ethics committee of St James and the Adelaide and Meath Hospitals, Dublin.

Rating Instruments

Self- and observer-rated scales were also filled out for all participants included in the study. The rating scales that were used comprised: the Hamilton Rating Scale for Depression (Hamilton, 1969), Beck's Depression Inventory (BDI-II) (Beck *et al*, 1996), Childhood Trauma Questionnaire (CTQ) (Bernstein *et al*, 1994), and the Structured Clinical Interview for DSM-IV (SCID-II) personality questionnaire. CTQ is a standardized self-report instrument that assesses five types of childhood maltreatment: emotional, physical and sexual abuse, and emotional and physical neglect. Reliability and validity of the CTQ have been established, including measures of convergent and discriminative validity from structured interviews, stability over time, and corroboration (Bernstein *et al*, 2003).

Genetic Methods

rs999845 was genotyped in this sample using a Taqman SNP Genotyping Assay on a 7900HT Sequence Detection System (Applied Biosystems). The call rate for the Taqman genotyping was $>95\%$ and all samples were in Hardy–Weinberg equilibrium ($p > 0.05$). Along with the test samples, a number of HapMap CEU DNA sample-positive controls (www.hapmap.org) and non-template-negative controls were genotyped for quality control purposes. For positive controls, all genotypes were found to be concordant with available online HapMapdata. All non-template samples returned a negative result. rs999845 is in Hardy–Weinberg equilibrium ($p = 0.526$) in this sample. Our test SNP at *BICC1* has a minor allele frequency of ~ 0.2. T is the minor allele and because homozygous TT samples are rare in our sample, we grouped them with heterozygous TC samples for analysis.

Table 1 Demographic and Clinical Data for Patients and Controls Stratified by BICC1

	Patients T-carriers ($N=13$)	Patients CC ($N=31$)	Controls T-carriers ($N=18$)	Controls CC ($N=26$)	Statistics df = 3/84
Age (years)[a]	45.8 (9.3)	40.0 (10.3)	36.6 (11.8)	35.3 (13.4)	$F=2.7$, $p=0.05$
Gender (female/male)	6/7	22/9	9/9	18/8	$\chi^2=4.1$, $p=0.25$
Height (cm)	170.9 (5.9)	171.6 (8.6)	176.4 (11.4)	169.1 (8.4)	$F=2.5$, $p=0.07$
Weight (kg)	75.3 (11.0)	76.6 (16.6)	75.7 (16.7)	66.6 (14.8)	$F=2.3$, $p=0.08$
Alcohol (units)	3.6 (6.0)	3.6 (5.8)	5.3 (7.0)	4.7 (5.2)	$F=0.4$, $p=0.8$
Cigarettes (day)	6.2 (9.6)	3.2 (6.2)	1.4 (4.8)	2.3 (6.0)	$F=1.5$, $p=0.23$
Hamilton depression score[b]	31.3 (7.4)	28.4 (5.5)	3.1 (3.8)	2.5 (2.1)	$F=223.8$, $p<0.001$
Beck depression index[b]	36.8 (11.4)	32.9 (10.5)	2.7 (3.8)	2.5 (3.4)	$F=113.8$, $p<0.001$
Emotional abuse[b]	10.7 (6.7)	10.1 (5.3)	5.8 (1.2)	6.7 (2.1)	$F=6.6$, $p<0.001$
Physical abuse[a]	10.4 (7.4)	7.8 (4.5)	5.9 (1.7)	5.7 (1.5)	$F=4.7$, $p=0.005$
Sexual abuse[b]	9.2 (6.9)	7.8 (5.5)	5.8 (1.5)	5.3 (1.0)	$F=3.4$, $p=0.02$
Emotional neglect[b]	11.1 (5.6)	12.2 (5.7)	7.3 (2.6)	7.0 (2.5)	$F=8.5$, $p>0.001$
Physical neglect[b]	8.3 (4.4)	8.3 (3.3)	6.3 (1.8)	5.9 (1.4)	$F=4.8$, $p=0.004$
ELA total[b]	49.7 (26.6)	46.2 (18.7)	31.1 (5.9)	30.6 (5.2)	$F=8.3$, $p<0.001$
					df = 1/42
Age of onset	28.2 (15.4)	24.1 (12.5)			$F=0.9$, $p=0.35$
Cumulative illness duration	13.8 (13.3)	7.6 (8.0)			$F=3.6$, $p=0.07$
Medication (free/SSRI/dual acting/valdoxan)	4/5/4/0	9/10/11/1			$\chi^2=0.6$, $p=0.9$, df = 3

There was no significant difference between BICC1 T-allele frequency (T/C) ($\chi^2=1.2$, $p=0.2$). ANOVA was used to test for group differences. *Post-hoc* tests were carried out with least square difference analysis.
[a]Higher in patients carrying the minor T-allele compared with both groups of healthy controls.
[b]Both patients groups differed from both control groups.

MRI T1 Data Acquisition

Magnetic resonance images were obtained with a Philips Achieva MRI scanner (Philips Medical System, Netherland B.V., Veenphuis 4–6, 5684 PC Best, The Netherlands) operating at 3 T. A sagittal T1 three-dimensional turbo field echo was used to scan all participants (TR user defined of 8.5 ms; TE user defined of 3.9 ms; total acquisition time of 7 min; field of view of FH (foot to head): 256 mm, AP (anterior to posterior): 256 mm, RL (right to left): 160 mm; and a matrix of 256×256). Slice thickness was 1 mm and voxel size was $1 \times 1 \times 1$ mm. All data sets were realigned and resampled three dimensionally in the anterior commissure to posterior commissure (AC-PC) line, according to the coordinates of Talairach, with the software program BRAINS2 (Brain Research: Analysis of Images, Networks and Systems). The program BRAINS2 allowed the regions of interest to be simultaneously controlled on sagittal, coronal, and transverse sections simultaneously.

Definition of Hippocampal Formation

We used manual tracing of the bilateral hippocampus with the help of the software BRAINS2 as we have previously described (Carballedo *et al*, 2012). The evaluating and tracing researcher (A.C. for hippocampus) were blind to participant status. The hippocampus was outlined manually using a mouse-driven cursor. To determine inter-rater reliability, 10 brains were randomly chosen and ROIs determined independently by two raters. The intraclass correlation for both the inter-rater reliability and subregions was high (left hippocampus: ricc = 0.92, right hippocampus: ricc = 0.88, left head: ricc = 0.98, right head: ricc = 0.88, left body: ricc = 0.87, right body: ricc = 0.90, left tail: ricc = 0.78, right tail: ricc = 0.79).

Statistical Analysis for Hippocampal Data

All statistical analyses were considered to be significant if $p<0.05$. Morphometric measurements in both groups were normally distributed (using Kolmogorov Smirnov test) and their variances were homogenous (using Levene's test). Hippocampal volumes were subjected to an omnibus analysis of covariance (ANCOVA) to assess the main and interaction effects of the within-subjects factor hemisphere (left, right) and region (head, body, tail), and the between-subjects factor group (MDD, HC), genetic polymorphism (minor T-Allele carriers, homozygous for C), and ELA using age, gender, medication status, and total intracranial volume as covariates. For significant interactions, *post-hoc* ANCOVA analysis was carried out using SIDAK Bonferroni tests. ELA was used according to accepted cut-off values (yes, no) (Bernstein *et al*, 1994).

Functional Magnetic Resonance Imaging

A cognitive–emotional inhibition task was used in the fMRI experiment, where participants were asked to process visual stimuli. The task, fMRI pre-processing and primary data analysis, was described in detail in Lisiecka *et al* (2011) and

can also be seen in Supplementary Methods. The task was event-related and consisted of 180 pseudo-randomized trials. Each trial in the task was 4-s long and consisted of a viewing stage and a response stage where participants answered a question about the emotional valence or the shape of the pictures from the International Affective Picture System database.

The MRI protocol consisted of the acquisition of a high-resolution 3D T1-weighted structural dataset (SPGR sequence with TR/TE = 8.5/3.9 ms and 1 mm^3 = spatial resolution), followed by an fMRI experiment (SE-EPI sequence with TR/TE = 2000/35 ms, in place resolution = 3 × 3 mm^2, 4.8-mm slice thickness, 550 dynamic scans each with 2 s duration).

Preprocessing steps included realignment to correct for motion. Participants were excluded when movement parameters exceeded one slice thickness (4.8 mm). Then co-registration of each participant's structural image to the mean of the motion-corrected functional images, slice time correction, spatial normalization, and smoothing using a 8-mm full width, half maximum (FWHM) Gaussian kernel were applied. Data were analyzed with Statistical Parametric Mapping (SPM8). Motion correction values were added as a covariate. In first-level analyses, three *t*-test contrasts were calculated comparing emotional trials (judging the emotional content) with geometrical trials (judging the geometry of the images) for each emotional valence separately. In consequence, a set of three subsequent contrasts was acquired for each individual: (1) neutral emotion trial > neutral geometrical trial, (2) negative emotion trial > negative geometrical trial, and (3) positive emotion trial > positive geometrical trial. Mean activation images are depicted in Supplementary Figure 2.

2 × 2 × 2 ANCOVA was performed with SPM8 on the contrasts where the first factor was group (MDD, HC), the second ELA (yes, no), and the third BICC1 gene (minor T-Allele carriers, homozygous for C), while age, gender and medication were used as covariates. Whole-brain voxel level family wise error (FWE) correction with $p < 0.05$ was used in all comparisons to ensure statistical significance of our findings. Only clusters formed of 15 or more significant voxels would be taken into account in the final conclusions. The automated anatomical labeling atlas was used to localize the significant areas in a standard stereotactic space (template from the Montreal Neurological Institute).

RESULTS

Depression scores derived from standard questionnaires were significantly higher in MDD patients compared with HCs (Table 1). MDD patients did not differ in demographic variables gender, heights, and weight from HCs. No significant differences were detected between subgroups stratified by diagnosis and genetic variant in terms of height, weight, cigarettes smoked per day, and alcohol units consumed per week. Age was marginally higher in patients carrying the T-allele compared with the controls. Medication status (none, SSRI, dual acting substances in monotherapy) was not significantly different between patients carrying the T-allele compared with those homozygous for the C-allele ($\chi^2 = 0.6$, df = 3, $p = 0.9$). Moreover, medication status did not significantly influence these hippocampus or fMRI results, when taken into account as a covariate. Age, gender, and medication were added as covariates in all subsequent analyses.

Hippocampus Results

BICC1, ELA, or diagnosis did not show a significant main effect on hippocampal volumes. No significant medication effect was found on hippocampal volumes. However, there was a significant four-way interaction between *BICC1*, ELA, diagnosis and region (F = 6.0, df = 1, 76, $p = 0.017$). Therefore, we analyzed the effects of *BICC1*, ELA, and diagnosis in the hippocampal subregions separately to explore this significant interactive effect further. There was a significant three-way interaction between *BICC1*, ELA, and diagnosis on hippocampal head volumes (F = 4.7, df = 1, 76, $p = 0.034$). Patients with ELA had smaller hippocampal head volumes when they carried the T-allele of *BICC1* compared with those patients with ELA homozygous for the C-allele (F = 7.9, df = 1, 20, $p = 0.01$, sidak corrected $p = 0.047$). Moreover, there was a marginal significant effect for patients without ELA carrying the T-allele of *BICC1* of having larger right hippocampal head volumes compared with patients with ELA carrying the T-allele of *BICC1* (F = 4.8, df = 1, 9, $p = 0.05$) (Figure 1).

A trend for a significant interaction was seen between *BICC1* and ELA on hippocampal body volumes (F = 2.8, df = 1,76, $p = 0.09$). Exploring this interaction further showed that the right hippocampal body of patients and controls carrying the minor T-allele of *BICC1* without ELA was significantly larger compared with patients and controls homozygous for the C-allele of *BICC1* without ELA (F = 11.5, df = 1, 47, $p = 0.001$, sidak corrected $p = 0.002$) and with ELA (F = 7.7, df = 1, 38, $p = 0.008$, sidak corrected $p = 0.045$) (Figure 2). The left hippocampal body did not survive sidak Bonferroni correction: Patients and controls carrying the minor T-allele of *BICC1* without ELA had larger left hippocampal bodies compared with subjects

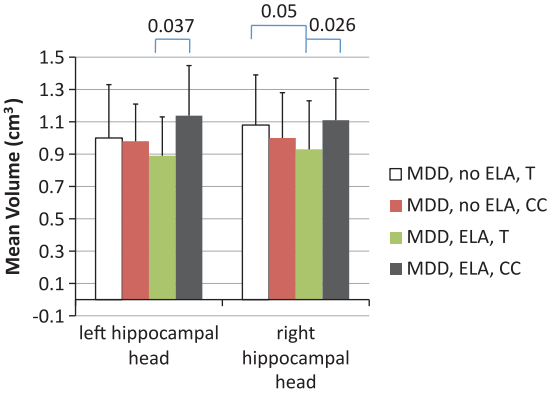

Figure 1 Smaller hippocampal head volumes, left and right, in patients with major depressive disorder (MDD) and early-life adversity (ELA), when they carry the T-allele of BICC1 compared with when they are homozygous for the C-allele. Larger right hippocampal head volumes in those patients carrying the T-allele of BICC1 when they do not have ELA compared with those with the same genotype when they have a history of ELA.

homozygous for the C-allele of *BICC1* without ELA ($F = 5.1$, $df = 1, 47$, $p = 0.029$, sidak corrected $p = 0.10$) and with ELA ($F = 3.5$, $df = 1, 38$, $p = 0.068$, sidak corrected $p = 0.28$).

Functional MRI Results

Positive stimuli. Results from ANCOVA analysis of neural responses to judging positive emotional content fully corrected for multiple comparison across all voxels in the brain ($p < 0.05$, FWE corrected) are shown in Supplementary Table 1. There was a significant interaction between diagnosis x *BICC 1* in the right and left middle cingulate cortex, as well as right dorsolateral frontal cortex (Figure 3a). There was also a significant interaction between diagnosis × ELA and *BICC1* in the right precuneus. Interestingly, patients carrying the T-allele of *BICC1* had significantly more activity in the right middle cingulate cortex compared with HCs, who carry the T-allele (Supplementary Table 1).

Patients carrying the T-allele of *BICC1* also had significantly increased neuronal responses while judging positive stimuli in the dorsomedial, dorsolateral, and middle cingulate cortex compared with those patients homozygous for the C-allele (Figure 3b). Patients with MDD and without ELA had significantly higher activities in the left dorsomedial prefrontal cortex and superior motor area, when they carry the T-allele compared with those patients homozygous for the C-allele (Figure 3c). Interestingly, HCs carrying the T-allele without a history of ELA showed significantly increased responses in the left inferior frontal cortex and insula compared with those HCs homozygous for the C-allele and without history of ELA.

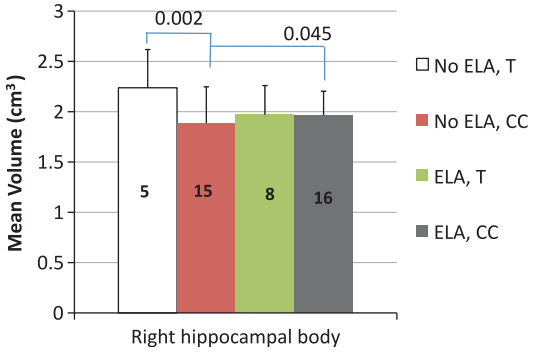

Figure 2 Larger right hippocampal head volumes in those subjects carrying the T-allele of BICC1 when they do not have early-life adversity (ELA) compared with those homozygous for the C-allele when they have a history and also when they do not have a history of ELA. Sidak corrected values. Values inset indicate number of subjects in each group.

Negative stimuli. Results from ANCOVA analysis of neural responses to judging negative stimuli are shown in Supplementary Table 2. There was a significant overall effect of *BICC1*. When patients and controls judged a negative image, it was observed that T-allele carriers of *BICC1* have significantly larger BOLD responses than those homozygous for the C-allele in the inferior frontal cortex. When the HCs' results are taken alone T-allele carriers of *BICC1* had significantly increased neural activity in the frontal inferior cortex left, insula left, precentral left, caudate left, and middle cingulate cortex left, compared with those homozygous for the C-allele of BICC1 (Supplementary Figure 1).

Neutral stimuli. No significant activity differences were recorded for neural responses to the neutral stimuli.

DISCUSSION

This study is novel in the way that we investigated the role of *BICC1* on the brain structure and function in MDD and

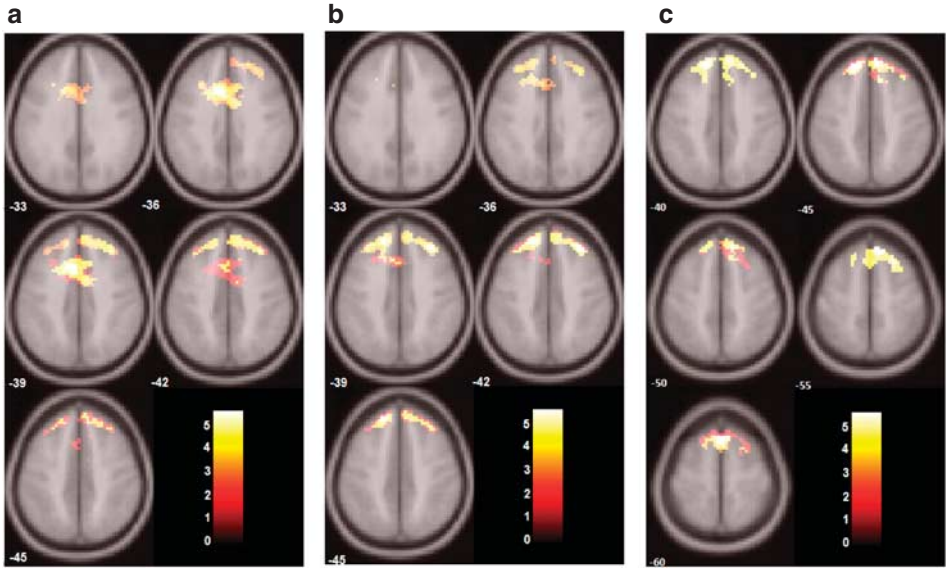

Figure 3 Positive stimuli. Axial slice presenting areas statistically significant for: (a) the interaction between diagnosis × BICC1, (b) differences in patients neural activity between minor T-allele carriers compared with homozygous C-allele carriers, and (c) differences in patients neural activity between minor T-allele carriers without ELA compared with homozygous C-allele carriers without ELA. The bar under the picture presents T values scale with the values matching colors representing activation. All significance is on the level of $p < 0.05$ after whole-brain voxel family wise error correction.

that we found biological evidence for a protective role of *BICC1* in MDD—thus supporting a recent genetic study, which indicated that the minor T-allele of *BICC1* is less frequent in patients with MDD (Lewis *et al*, 2010).

Most interestingly, we found, as hypothesized, a significant interactive effect between factors *BICC1*, ELA, diagnosis, and subregion on hippocampal volumes. Patients and controls had larger hippocampal body volumes when carrying the protective T-allele of *BICC1* and having no history of ELA compared with patients and controls carrying the homozygous C-allele both with and without a history of ELA. Either ELA or being homozygous for the C-allele of *BICC1* was then associated with a smaller volume of the hippocampal body compared with carrying a T-allele and this is reflected in the smaller hippocampal body volumes between our patients and controls. On a biological level these results support evidence that carrying the T-allele might increase protection against structural brain changes and against depression as shown in the above mentioned genetic study (Lewis *et al*, 2010). However, we also demonstrated that this protection can be lost due to ELA. This finding is also interesting because it suggests a general mechanism irrespective of diagnosis: the minor T-allele results in larger hippocampal volumes when everything goes well during childhood development, but not in the case of childhood adversity.

Interestingly, patients with MDD, who have a history of ELA and carry the minor T-allele, show smaller right hippocampal head volumes compared with patients with the same genotype without a history of ELA. Also patients with MDD, who have a history of ELA and carry the minor T-allele, show smaller left and right hippocampal head volumes compared with patients carrying the homozygous C-allele and who have a history of ELA. These results might suggest that those subjects who carry the protective minor T-allele, but develop smaller hippocampal head volumes due to ELA, are at a higher risk to develop depression.

This result is based on a significant three-way interaction between the factors *BICC1*, ELA and diagnosis, and should be explored further in future studies. Within the hippocampus the DG is thought to have an important role in hippocampal neurogenesis and as a result is highly adaptive, however, stress has been found to suppress this neurogenesis and cause atrophy of the CA subfields in animal studies (McEwen and Magarinos, 2001). Subregional structural characteristics and molecular expression profiles are presented along the long axis of the hippocampus (Small *et al*, 2011). A limitation of our study is that our whole-brain T1 MRI sequence did not allow investigation of these subregions within the hippocampus. This would have required high-resolution T2 imaging of just the hippocampal area. Whether the decrease of hippocampal head volume with ELA is associated with changes in the CA fields, which were found to be altered by experimental stress in animal studies (Sapolsky, 2001), thus, needs further exploration with detailed subregional analyses. Interestingly, within this sample of subjects we could find that the hippocampus is reduced in those patients with a reduced expression of glucocorticoid inducible genes, ie, GILZ mRNA, suggesting association between the stress system and hippocampal volumes (Frodl *et al*, 2012). As the hippocampal head contains relatively more CA subfields than the body (Malykhin *et al*, 2010), such a hypothesis might be promising.

Another strong finding in depression research is the emotional–cognitive dysbalance and related functional brain changes in limbic and prefrontal brain regions (Frodl *et al*, 2011). Most studies like ours exploring emotional tasks do not show alterations in hippocampal activity, because of limitations of sensitivity of BOLD fMRI in ventral, anterior brain regions such as the anterior hippocampus and because the tasks do not primarily involve hippocampus functions. Here, we used functional MRI of cognitive–emotional interplay to investigate the effects that diagnosis, *BICC1*, and ELA have on the well-known changes in neural activity during emotion regulation both individually and in interaction with each other. Observed is a significant interactive effect between diagnosis and *BICC1* in terms of judging positive stimuli. Judging positive stimuli generates greater activation in patients carrying the T-allele compared with HCs with the same genotype in the right middle cingulate cortex. Moreover, patients with MDD carrying the T-allele of BICC1 showed significantly increased activity in the left and right dorsolateral prefrontal cortex and left middle cingulate cortex compared with those patients homozygous for the C-allele. These regions are involved in emotion regulation (Esslen *et al*, 2004) underpinning the importance of the finding for MDD.

Also observed is a significant interactive effect between diagnosis, *BICC1,* and ELA. Patients with MDD, who do not have a history of ELA had significantly higher activity in the left and right dorsomedial prefrontal cortex and superior motor areas when they carry the T-allele compared with those homozygous for the C-allele. Healthy subjects homozygous for the C-allele of *BICC1* show higher activity in the caudate and ACC, when positive for ELA compared with negative. This higher activity associated with ELA in subjects who stay healthy needs further exploration in future studies as it might be associated with resilience. In line with this finding healthy first-degree relatives of MDD patients have higher neural activities in the cingulate cortex than healthy subjects without family history, which might be related strategies they build up for staying healthy (Lisiecka *et al*, 2011). Whether the higher brain activity in patients and controls carrying the minor T-allele is advantageous in terms of being able to react more to positive stimuli needs further exploration.

In response to negative images, there was also significantly more activation in the left frontal cortex in T-allele carriers compared with those subjects homozygous for the C-allele. However, there were no significant differences between patients and controls, and no significant interaction between diagnosis and *BICC1*, suggesting that tasks with positive stimuli may trigger patients with depression more than those with negative stimuli and thus might show more specific findings associated with genetic vulnerability for depression. One possibility for this is that greater response to positive stimuli corresponds to a higher level of arousal in depressed patients. This is consistent with previous studies that have found increased activity, for example, in the amygdala in response to positive stimuli in patients with MDD (Davey *et al*, 2011).

Generalization of the study results is limited by a number of factors, including the characteristics of the participants and the constructs that were assessed. First, CTQ to assess ELA was filled out in retrospect, which could lead to inaccurate recalling of events affecting CTQ score. Investigation of a study population that has already been investigated for childhood maltreatment during childhood may overcome this issue. Differences in medication of some of the participants, with one-third of the patients being medication free and two-thirds of depressed patients being on a monotherapy with antidepressants might also limit the generalization. However, there was no difference in frequency of antidepressant treatment between depressed patients with the T-allele compared with those homozygous for the C-allele and using medication as a covariate did not affect the results. Our results have significant statistical power, however, in particular with respect to the significant three-way interactions between diagnosis, ELA, and *BICC1* future studies should recruit larger samples to explore this interaction further. The present results are valuable, as *post-hoc* tests we carried out were corrected for multiple comparisons and in particular the differences between T-allele carriers without ELA and the other groups were strong enough to survive these corrections.

The results of this study shed some further light on the role *BICC1* has in MDD both alone and in interaction with diagnosis and ELA. On a structural level results suggest possessing the minor T-allele is protective against MDD. However, the presence of a history of ELA changes and might even block this protective role of the gene leading to structural changes in the hippocampus becoming more prominent. Patients carrying the protective T-allele also show increased activation in brain regions involved in emotion processing. More research is needed to understand both the function of *BICC1* and its relevance in psychiatric disorders.

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DISCLOSURE

The authors declare that over the past 3 year TF received compensation for presentations from Shire and Eli Lilly. The remaining authors declare no conflict of interest.

REFERENCES

Beck AT, Steer RA, Brown GK (1996). *BDI-II Manual*. Harcourt Brace and Company: San Antonio, TX.

Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K *et al* (1994). Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry* **151**: 1132–1136.

Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T *et al* (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl* **27**: 169–190.

Campbell S, MacQueen G (2004). The role of the hippocampus in the pathophysiology of major depression. *J Psychiatry Neurosci* **29**: 417.

Carballedo A, Lisiecka D, Fagan A, Saleh K, Ferguson Y, Connolly G *et al* (2012). Early life adversity is associated with brain changes in subjects at family risk for depression. *World J Biol Psychiatry;* e-pub ahead of print.

Dannlowski U, Ohrmann P, Bauer J, Deckert J, Hohoff C, Kugel H *et al* (2007). 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. *Neuropsychopharmacology* **33**: 418–424.

Davey CG, Allen NB, Harrison BJ, Yucel M (2011). Increased amygdala response to positive social feedback in young people with major depressive disorder. *Biol Psychiatry* **69**: 734–741.

Edwards VJ, Holden GW, Felitti VJ, Anda RF (2003). Relationship between multiple forms of childhood maltreatment and adult mental health in community respondents: results from the adverse childhood experiences study. *Am J Psychiatry* **160**: 1453–1460.

Esslen M, Pascual-Marqui R, Hell D, Kochi K, Lehmann D (2004). Brain areas and time course of emotional processing. *NeuroImage* **21**: 1189–1203.

Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V *et al* (1998). Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med* **14**: 245–258.

Frodl T, Carballedo A, Hughes M, Saleh K, Fagan A, Skokauskas N *et al* (2012). Reduced expression of glucocorticoid-inducible genes GILZ and SGK-1: high IL-6 levels are associated with reduced hippocampal volumes in major depressive disorder. *Translational Psychiatry* **2**: e88.

Frodl T, Reinhold E, Koutsouleris N, Donohoe G, Bondy B, Reiser M *et al* (2010). Childhood stress, serotonin transporter gene and brain structures in major depression. *Neuropsychopharmacology* **35**: 1383–1390.

Frodl T, Scheuerecker J, Schoepf V, Linn J, Koutsouleris N, Bokde ALW *et al* (2011). Different effects of mirtazapine and venlafaxine on brain activation: an open randomized controlled fMRI study. *J Clin Psychiatry* **72**: 448–457.

Hamilton M (1969). Standardised assessment and recording of depressive symptoms. *Psychiatr Neurol Neurochir* **72**: 201–205.

Hasler G, Northoff G (2011). Discovering imaging endophenotypes for major depression. *Mol Psychiatry* **16**: 604–619.

Heim C, Bradley B, Mletzko T, Deveau TC, Musselmann DL, Nemeroff CB *et al* (2009). Effect of childhood trauma on adult depression and neuroendocrine function: sex-specific moderation by CRH receptor 1 gene. *Front Behav Neurosci* **3**: 41.

Heim C, Nemeroff CB (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* **49**: 1023–1039.

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR *et al* (2003). The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMAt* 289(23): 3095–3105.

Krishnan V, Nestler EJ (2008). The molecular neurobiology of depression. *Nature* **455**: 894–902.

Lewis CM, Ng MY, Butler AW, Cohen-Woods S, Uher R, Pirlo K *et al* (2010). Genome-wide association study of major recurrent depression in the UK population. *Am J Psychiatry* **167**: 949–957.

Lisiecka DM, Carballedo A, Fagan AJ, Connolly G, Meaney J, Frodl T (2011). Altered inhibition of negative emotions in subjects at

family risk of major depressive disorder. *J Psychiatr Res* **46**: 181–188.
MacQueen G, Frodl T (2011). The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol Psychiatry* **16**: 252–264.
Mahone M, Saffman EE, Lasko PF (1995). Localized Bicaudal-C RNA encodes a protein containing a KH domain, the RNA binding motif of FMR1. *EMBO J* **14**: 2043–2055.
Malykhin NV, Carter R, Seres P, Coupland NJ (2010). Structural changes in the hippocampus in major depressive disorder: contributions of disease and treatment. *J Psychiatry Neurosci* **35**: 337–343.
Mathers C, Fat DM, Boerma J (2008). *The Global Burden of Disease: 2004 Update*. World Health Organization: Geneva.
McEwen BS, Magarinos AM (2001). Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. *Hum Psychopharmacol* **16**: S7–S19.
McKinnon MC, Yucel K, Nazarov A, MacQueen GM (2009). A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. *J Psychiatry Neurosci* **34**: 41–54.
Mello AF, Mello MF, Carpenter LL, Price LH (2003). Update on stress and depression: the role of the hypothalamic-pituitary-adrenal (HPA) axis. *Revista Brasileira de Psiquiatria (Sao Paulo, Brazil: 1999)* **25**: 231–238.
Pariante CM, Lightman SL (2008). The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* **31**: 464–468.
Sapolsky RM (2001). Atrophy of the hippocampus in posttraumatic stress disorder: how and when? *Hippocampus* **11**: 90–91.
Scharinger C, Rabl U, Sitte HH, Pezawas L (2010). Imaging genetics of mood disorders. *NeuroImage* **53**: 810–821.
Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA (2011). A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci* **12**: 585–601.
Snee MJ, Macdonald PM (2009). Bicaudal C and trailer hitch have similar roles in gurken mRNA localization and cytoskel*etal* organization. *Dev Biol* **328**: 434–444.
Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR *et al* (2012). Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry* **17**: 36–48.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)