Archives of Psychiatry and Psychotherapy, 2014; 4 : 15–26

A comparison of a single genetic factor, two stress factors, and one psychosocial coping factor as predictors of depression in an Australian community sample

Christopher F. Sharpley, Suresh K. A. Palanisamy, Kate Metcalf, Kim A. Jones, Brian Kelly, James R. McFarlane

Summary

Aim. Although both have shown significant effects upon depression in clinical samples, no direct comparison has been reported of the relative power of psychological resilience and the short form of the serotonin transporter gene 5-HTTLPR as predictors of depression in a community sample.

Material and Methods. In a sample set by a priori power analysis, 67 adult females and 59 adult males were used to enable a comparison between a single genetic factor, childhood stressors, recent stressors, psychological resilience and depression.

Results. None of genotype, childhood or recent stressors was significantly associated with depression scores, but resilience was a significant inverse predictor of depression scores and also of the presence of clinically significant depression.

Discussion. These data suggest that measures of an individual's ability to resist or recover from stress may be useful in assessing vulnerability to depression when used with 'at risk' individuals in everyday practice.

depression / genes / resilience / stressors / distal / proximal

Although often seen as being of potential major benefit to clinical treatment settings, the search for a unique and comprehensive genetic biomarker of depression has not been clearly successful as yet [1-3] with a recent mega-analysis of 1.2 million single-nucleotide polymorphisms failing to identify robust and replicable findings for any specific genetic factor [4]. Instead, a great deal of recent research has focussed upon the interaction of genes and environment via the serotonin transporter 5-HTTLPR plus the individual's response to various stressors [5-7]. The short (ss) form of the 5-HTTLPR has been implicated in studies of affective disorders [8] and in depression among individuals who have experienced distal major stressful life events such as childhood adversity, as well as more recent proximal major stressors from financial, relationship, and occupational sources. This led Karg and colleagues [9] to report that, from a meta-analysis of 54 studies, there was "strong evidence" (p. 444) of the ss allele of the 5-HTTLPR being as-

Christopher F Sharpley[1], Suresh KA Palanisamy[2], Kate Metcalf[3], Kim A. Jones[4], Brian Kelly[5], James R McFarlane[6]: [1]Brain-Behaviour Research Group, University of New England, Australia and Centre for Autism Spectrum Disorders, Bond University, Australia; [2]Collaborative Research Network for Mental Health and Well-Being in Rural and Regional Communities, University of New England, Australia, [3]University of New England, Australia; [4]University of New England, Armidale, NSW, Australia; [5]University of Newcastle, Newcastle, Australia, [6]University of New England, Armidale, NSW, Australia. **Correspondence address**: csharpley@onthenet.com.au

Acknowledgements: The authors acknowledge the contribution made by the Collaborative Research Network on Mental Health and Well-being in Rural Communities, supported by the Department of Industry, Innovation, Science, Research and Tertiary Education, Commonwealth Government of Australia.

sociated with an increased risk of individuals developing depression following distal (childhood) major stressors, major medical conditions and (less robust but still statistically significant) more recent proximal stressors. Those initial findings were recently confirmed by a further meta-analysis of 81 studies [10].

However, when considering the applicability of these findings to screening for individuals in clinical practice who are at risk of developing major depression, it is worthy of note that 22 of the 54 studies that Karg et al. (2011) included in their meta-analysis reported data that were "positive" (i.e., that carriers of the ss allele were significantly more likely to have higher depression than carriers of other alleles),13 reported data that were "partially positive", 15 reported no association between the ss, stress and depression, and a further 6 reported that carriers of the long (ll) allele were more likely than ss carriers to become depressed following significant life stress [9, Table 1]. That is, despite the overall strong relationship reported between the ss, stress and depression in 40.7% of the studies reviewed, that relationship was not replicated in 38.8% of the 54 studies. Similar proportions were reported by Sharpley et al [10], who found that nearly 26% of 81 studies failed to show any significant association between the 5-HTTLPR, stress and depression. In addition to these challenges to the overall findings, ll carriers have been reported as displaying significantly greater likelihood than ss carriers of becoming depressed after stress in a wide range of populations such as Australian adolescents aged 17-18 years [11], Chinese patients with Major Depressive Disorder (MDD) [12], and young adult females in the USA , the relationships between the ss, ll and sl (combination) forms of the 5-HTTLPR, recent and past stress, and depression remain valuable targets for research into ways of identifying persons from the community who may be at risk of developing depression following major stressors.

The fact that a considerable number of studies failed to show a significant relationship between any form of the 5-HTTLPR, environmental stress and depression suggests that the gene x environment relationship with depression may not be completely described by the variables used in previous studies that reported significant associations between the ss allele, stress and depression. For example, as noted by Karg, et al.[9] and Sharpley et al [10], the stressors that have been included in previous studies have included chronic disease, Parkinson Disease, stressful life events, myocardial infarction, childhood adversity, trauma, child abuse, care-giving to Alzheimer patients, stroke and hip fracture. Although measures of stressful life events and childhood adversity were taken in many of the studies reviewed by Karg, et al. [9] and Sharpley et al. [10], neither of these types of stressors universally supported the association between the ss allele and depression. In addition, none of the 54 studies reviewed by Karg and colleagues or the 81 studies investigated by Sharpley et al. investigated the effects of any factors which may have ameliorated depression in people who experienced stressors of the types reported. Inclusion of both 'contributors' to depression (which increase the likelihood of depression following stress) and 'buffers' against depression (which decrease the likelihood of depression following stress) may assist in forming a more complete model of the association between genotype, environmental events and depression by adding 'behavioural predisposition' to that equation.

One such buffer against depression is psychological resilience [13]. Resilience refers to an individual's capacity to cope with stressors and resist the harmful effects of future negative events [14], possibly by an active physiological process that reduces autonomic responses to stressors [15]. Various definitions of resilience have been offered, including it being a personal trait or attribute that promotes rebounding from disappointments [16], positive adjustment in adverse circumstances [17], or simply successful adaptation to challenging life stressors [18]. Although once considered to be similar to recovery from trauma, resilience has been shown to follow a distinct trajectory to recovery when individuals experience a traumatic event [19]. As well as having been shown to intervene between the experience of traumatic events and the individual's later return to optimism in the face of such occurrences as old age [20], terrorist attacks [19] and chronic pain [21], resilience assists individuals to overcome the experience of trauma during early childhood and to progress to normal and satisfying lives [22] and this has particular rele-

vance to studies of the interaction between distal and proximal stressors, 5-HTTLPR and depression. Initially investigated as a psychological variable, resilience also has a biological basis that relies upon plasticity of the reward and fear circuits in the brain [23, 24], with several possible neurological mediators of the resilient response to extreme stress having been identified to date [15]. This has led to the suggestion that resilience may be analysed at various levels [25] so that preventative as well as treatment modalities should be considered [26].

Several recent studies have examined the association between 5-HTTLPR and resilience. Stein, Campbell-Sills and Genernter [27] noted that carriers of the ss allele had significantly lower resilience scores than carriers of the ll allele among 423 undergraduate students with a median age of 19 yr. However, Carli and colleagues [28] found that the opposite effect, i.e., that the ll allele was significantly associated with lower resilience and higher depression among participants who had experienced high levels of childhood traumas in their sample of 763 male prisoners. By contrast, O'Hara, et al. [29] found no significant association between either the ss or ll alleles of the 5-HTTLPR and resilience in their sample of 99 healthy older adults (Mean age = 71.5yr). Thus, the association between the 5-HTTLPR, resilience, previous adversity and depression remains to be described, although the apparent lack of a clear definition of resilience (see above) may play a part in that as yet unsuccessful outcome.

Finally, the great majority of studies on the 5-HTTLPR have been conducted with MDD patients, although some recent reports have focussed upon community samples to determine if effects generalise to non-MDD participants [e.g., 30]. This is particularly relevant to the translation of research outcomes into clinical settings in which patients may present with minor depression or subsyndromal depression (SSD), particularly following chronic minor stress (rather than a single major stressor). Judd et al., reported that there were no large consistent differences in impairment between patients with MDD and those with SSD across eight domains of functioning [31], with both depressive groups suffering significantly more than participants with no symptoms of MDD [32]. Patients who meet the criteria for SSD have a 5.5-fold chance of developing MDD within one year compared to people who have none of the symptoms of MDD at all [33], and show significantly greater levels of psychological disability, hopelessness and death ideation [34]. Other data suggest that SSD patients "are as ill as those with minor or major depression (in terms of) medical burden" [35, p. 214], and that SSD significantly lowers patients' quality of life [36]. The investigation of the association between the 5-HTTLPR, stress and depression in patients who may present with milder symptomatology than MDD is of direct clinical relevance to everyday clinical practice, and data collected from community samples (where the prevalence of severe depression is by definition less than in samples wholly composed of patients suffering from MDD) can help clarify this association.

Therefore, this study focussed upon the comparative effects of distal stressors (adverse childhood events), proximal stressors (recent life stress) and their interaction with the three forms of the 5-HTTLPR that have been previously reported (ss, sl, ll). To determine if the relationship between genotype, stress and depression might be buffered by a "positive" factor, psychological resilience was also included in data collection. In order to provide results which might be of general use in clinical settings where many patients present with minor or subsyndromal depression, a community sample of limited size was recruited rather than a very large sample of diagnosed MDD cases. Further, one of the explanations of previous nonsignificant findings between genetic factors and MDD has been limited sample size and statistical power used in genome-wide association studies with very many genetic factors. To avoid this limitation in power to detect the presence of a statistically significant relationship, we used only a restricted genetic variable rather than a genome-wide range of variables, and the sample size was verified a priori via power analysis as being sufficient to detect significant relations between depression, resilience, distal and proximal stressors and a single genetic factor (5-HTTLPR). These decisions avoided the limitations of statistical power that apply for studies of very large numbers of genetic factors.

METHOD

Participants

The sample consisted of 67 female and 59 male volunteers for a study about "how you think about stress", who were aged between 18 and 69 years (M = 32.53yr, SD = 13.49 yr). Participants were recruited from the general population of a large regional city of about 22,000 people in rural New South Wales, Australia. Following initial contact from the second, third or fourth authors, participants were invited to complete the questionnaire described below and provide a mouthwash sample for genotyping for the 5-HTTLPR. To maximise generalisability to the population, no attempt was made to screen participants apart from ensuring they were at least 18 years of age. In this way, the generalisability of the sample to the overall community, of whom a proportion will seek psychiatric assistance for minor or major depression, was maximised.

Material and Procedure

Background questionnaire. This measured age, gender and whether participants were currently taking antidepressant medication.

Depression. The Zung Self-Rating Depression Scale (ZSDS) [37] is a standardised paper and pencil test of depression that was developed on the basis of factor analytic studies of the syndrome of depression which underlie the DSM definition [38]. The ZSDS includes items for all of the current DSM-IV-TR criteria for Major Depressive Episode (MDE) [37], and has 20 items on which respondents are asked to indicate the frequency of occurrence to them "during the last two weeks" by answering in one of four possible ways: "None or a little of the time", "Some of the time", "Good part of the time", or "Most or all of the time". Raw scores range from 20 to 80, with higher scores being indicative of more severe depression. The ZSDS has demonstrated split-half reliability of .81 [37], .79 [39] and .94 [40]. Internal consistency (alpha) has been reported as .88 for depressed patients and .93 for non-depressed patients [41], and as .84 for a previous Australian community sample [42]. The ZSDS has been shown to be superior to the MMPI Depression Scale and the Beck Depression Inventory for assessing depression in male psychiatric inpatients [41] and has sensitivity of 93% in predicting depression validated via clinical interview [43]. ZSDS raw scores of 40 or above indicate the presence of "clinically significant depression" [44, p. 335] and raw scores were used in this study.

Negative Childhood Events. The Adverse Childhood Events (ACE) questionnaire is a retrospective self-report inventory consisting of 30 statements relating to emotional, physical and sexual abuse as well as emotional and physical neglect which occurred during childhood. The ACE questionnaire was developed by the Centers for Disease Control and Prevention (CDC) and Kaiser Permanente in San Diego, using 17,000 participants [45]. Items were drawn from the Conflict Tactics Scale [46] the Childhood Trauma Questionnaire [46, 47], and Wyatt [48] The ACE questionnaire has good reliability (Cronbach alpha = .711) and validity with interview data from children who have been neglected [49].

Recent Life Stressors. In order to determine if participants had experienced major recent life stressors during the last two weeks, 9 items were drawn from the Effects of Life Events Inventory (ELEI) [50], which has satisfactory validity and reliability (.741). ELEI items were derived from Sarason et al. [51] from items used in Paykel et al.'s Distress Scale [52] and Holmes and Rahe's Social Readjustment Rating Scale [53] and were amended to suit to the Australian setting [54]. The items used here were about health, bereavement, family relationships, social interactions, educational demands, work issues, moving house, and legal challenges.

Psychological Resilience. The Connor-Davidson Resilience Scale (CD-RISC) (Connor & Davidson, 2003) includes 25 items such as "I like a challenge", "When things look hopeless I don't give up", "I bounce back after illness or hardship", and "I am able to adapt to change". The CDRISC has been found to have five factors that measure "Personal competence, high standards and tenacity", "Trust in one's instincts, tolerance of negative affect, strengthening effects of stress", "Positive acceptance of change and secure relationships with others", "Control", and "Spiritual influences" (Connor & Davidson,

2003). Total scores on the CD-RISC are significantly correlated (.83) with total scores on the Kobasa Hardiness Measure (KHM: Kobasa, 1979) and negatively correlated (-.76) with total scores on the Perceived Stress Scale (PSS-10; Cohen, Kamarck, & Mermelstein, 1983), indicating high concurrent validity. The CD-RISC has acceptable reliability, ranging from 0.89 (Cronbach's alpha) to 0.87 (test-retest reliability) (Conner & Davidson, 2003).

Genotyping. Genomic DNA was isolated from buccal cells collected from participants vigorously rinsing their mouths with 15ml of commercial alcohol-free mouthwash for 1 minute. The resulting mouthwash samples were stored at room temperature and generally processed within two weeks but the DNA remained intact for more than 2 months (data not shown). The genomic DNA was isolated using a modified method previously described by Heam and Arblaster [55], which included centrifuging of the mouthwash sample for 1 minute at 10,000 rpm, discarding of the supernatant, adding 1.0mL of Lysis buffer to the pellet and vortexing for 20 seconds. Proteinase K (10µl of 10 mg/ml) was then added and incubated at 60°C for 10 minutes. The samples were centrifuged briefly for 10-30 seconds and the supernatant was transferred to sterile 2ml sterile tubes. Genomic DNA was precipitated by adding 100µl of 2.5M NaCl to the supernatant followed by one volume of 100% ethanol. After gentle mixing it was centrifuged at 10,000 rpm for 10 minutes. The pellet was then washed with 70% ethanol. The DNA was resuspended in 50 µl of nuclease-free water and the DNA integrity checked on 1% agarose gel. The resultant DNA samples were genotyped for HTTLPR short (s) and long (l) polymorphisms using the PCR procedure and primers described by Wendland et al. (56]. All genotyping was performed in duplicate.

Statistical analysis

Data were analysed via IBM SPSS version 20. Descriptive analysis was undertaken by Frequencies and Explore to obtain means, standard deviations and 5% means to test for the effects of outliers, skewness and kurtosis. Distribution of variables was analysed by the Kolmogorov-Smirnov test plus inspection of Normal Q-Q plots. Cronbach alpha was obtained to test the internal consistency of scales used. Participants were formed into subgroups on the basis of mean scores on scales, and MANOVA was used to test for the presence of significantly different ZSDS scores in high vs low subgroups of CDRISC, Negative Childhood Events and Recent Life Stressors, plus genotype. ANOVA plus Scheffe post hoc contrasts were performed to test for differences in ZSDS scores across the three allele subgroups. Linear regression was used to examine the relative contribution that CDRISC, Negative Childhood Events and Recent Life Stressors made to ZSDS score; Logistic regression was used to test these variables against ZSDS clinical status; Hierarchical regression and change in R square was used to test for the effect of adding variables into the regression equation. Alpha was set at .05 and observed power was determined to test for the presence of Type II errors. A priori power analysis (G Power 3) was undertaken for both the planned MANOVA and regression analyses. For the MANOVA, the required sample size (Effect size = .2, alpha = .05, Power (1-B) = .95, number of groups = 9) was 126 (which we set as our sample size). For the regression, using a similarly small ES, the required sample size was 121.

All procedures were approved by the University of New England Human Research Ethics Committee.

RESULTS

Table 1 shows the mean, SD, median, 5% trimmed mean, maximum and minimum scores for each of the variables except gender and genotype, plus the Cronbach alpha internal consistency values for the scales measuring psychological variables. The 5% trimmed means were all relatively close to the actual means, indicating minimal effects from outliers. Internal consistency values (Cronbach's alpha) were acceptable. Kolmogorov-Smirnov statistics were non-significant for the ZSDS and CDRISC. Although there was some minor skewness in scales collecting data on childhood events and recent stressors, inspection of the Normal Q-Q plots for these scales indicated the presence of fairly straight lines, suggesting acceptable normali-

ty for all variables and justifying the use of parametric analyses. Six participants (4.8% of the sample) were currently taking antidepressants, and 21 participants (16.67%) met Zung's criteria for clinically significant depression, including 2 of those participants who reported taking antidepressant medication.

Table 1. Descriptive data (n = 126)

Variable	Mean	SD	Median	5% trimmed mean	Maximum	Minimum	Cronbach alpha
CDRISC	73.29	14.35	73.0	73.67	100	34	.928
Child negative	7.66	8.99	5.0	6.49	43	0	.673
Recent stressors	3.08	4.22	0.00	2.62	17	0	.653
ZSDS	32.92	7.62	32.0	32.64	53	20	.812

Table 2. ANOVA results for genotype, resilience, negative childhood events an decent life stressors on ZSDS total score (df = 1, 119)

Variable	F	p[1]	Observed power
Genotype	3.971	.022	.700
CDRISC	19.603	.000	.992
Negative childhood events	.203	.653	.073
Recent life stressors	2.934	.090	.396
Genotype x Negative Childhood Events x Recent Life Stressors	3.329	.040	.618

1 corrected *p* = .0125

There were 29 ss, 53 sl and 44 ll carriers of these subtypes of the 5-HTTLPR polymorphism (Hardy-Weinberg Equilibrium: X2 = 2.709, p > .05). There was no significant difference in the distribution of the three allele types according to gender. Prior to further exploration of relationships between variables, MANOVA indicated that there were no significant differences on any of the dependent variables shown in Table 1 according to gender, thus allowing all participants' data to be examined in a single data set.

As an initial exploration of the relationships between genotype and the psychological variables, orthogonal contrasts were undertaken by splitting the sample according to the mean scores for the CDRISC, Negative Childhood Events and Recent Life Stressors, plus coding the three genotypes (ss, sl, ll) separately. A 3 (genotype) x 2 (high vs low resilience) x 2 (high vs low negative childhood events) x 2 (high vs low recent life stressors) ANOVA with ZSDS total score as the dependent variable was conducted and results are shown in Table 2. There was a significant effect for CDRISC, with participants with CDRISC scores above the mean having significantly lower ZSDS scores (M = 29.111, SD = 5.809) than participants with CDRISC scores below the mean (ZSDS M = 36.582, SD = 7.536). Other effects were non-significant when the appropriate Bonferroni correction was applied (.05/4 = .0125). The observed power (shown in Table 2) for the comparisons made between genotypes largely excludes the presence of a Type II error in that result.

Comparisons represent a robust form of data analysis, one which describes major effects. As suggested by the non-significant trends observed in the ANOVA reported in Table 2 when appropriate corrections were applied for multiple comparisons, there may be more subtle relationships between these variables which warrant further investigation. Therefore, in order to more fully explore the inter-relationships between recent and distant stressor events, resilience, genotype and depression, data were analysed via a regression model. As a first step in that process, Pearson correlations were performed and Table 3 shows the correlation matrix between age and psychological variables (resilience, childhood negative events, recent stressors) and total ZSDS depression score. Although the frequency of reported Negative Childhood Events increased with age, that relationship was not significant at the Bonferroni-corrected level of p =

.0125. The significant direct relationship between depression and Negative Childhood Events and also between depression and recent stressors fits with the hypothesis that both proximal and distal stress can be associated with increased depression. The inverse relationship between depression and resilience is also congruent with previous data that suggest the latter factor may have a buffering effect upon depression.

Although the correlation matrix shown in Table 3 suggests the presence of several statistically significant relationships between variables, the predictive power of the genotype and psychological variables upon depression is more clearly depicted via linear regression. Tolerance values were all greater than .5, and VIF values were less than 2.0, indicating that multicollinearity was not a problem with these data. Inspection of the Normal Probability Plot (P-P) and scat-

Table 3. Correlation matrix for age and psychological variables

	CDRISC	Child negative events	Recent stressors	ZSDS
Age	.098	.201	-.046	-.147
CDRISC		-.079	-.151	-.596*
Negative Childhood Events			.162	.284*
Recent Life Stressors				.356*

** p < .0125*

Table 4. Linear regression of predictive power for significant correlates of ZSDS total score

Variable	Beta	t
Genotype	.026	.396
CDRISC	-.553	-8.177*
Negative Childhood Events	.208	3.063*
Recent Life Stressors	.230	3.355*

** p < .0125*

Table 5. Logistic regression predicting likelihood of clinically significant depression

		B	S.E.	Wald	df	p[1]	Odds Ratio	95% C.I. for Odds Ratio	
								Lower	Upper
	CDRISC	-.084	.022	14.140	1	.000	.919	.880	.961
	Childhood negative events	.057	.029	3.934	1	.047	1.059	1.001	1.120
	Recent Stressors	.118	.058	4.208	1	.040	1.126	1.005	1.261
	Constant	3.100	1.455	4.540	1	.033	22.193		

[1]Corrected p = .0125

terplot showed a straight diagonal line for the former and an approximate rectangle for the latter, with no outliers. Cook's Distance maximum was 0.92. These data indicate that normality, linearity, homoscedasticity and independence of residuals were satisfactory.

When total ZSDS score was the dependent variable and genotype and the psychological predictor variables which showed significant correlations with ZSDS total score (Table 3) were entered together, R square was .474, indicating that the model explained almost half of the variance, and this result was significant ($F(4,122) = 26.558$, $p < .001$). Beta weights (standardised coefficients), t values, and significance are shown in Table 4, and indicate that (as expected from the ANOVA results) participants' resilience scores were the strongest (inverse) predictor of their depression scores, followed by frequency of recent major stressor events and negative childhood events. Genotype failed to significantly predict depressive symptom scores.

To further explain the relationship between these three predictor variables and depression, hierarchical multiple regression was applied, entering only the significant psychological variables (CDRISC, Negative Childhood Events and Recent Life Stressors) in separate blocks, with CDRISC as the first block, Negative Childhood Events as the second block and Recent Life Stressors as the third block. The R square for the first block was .365 ($F(1,122) = 69.461$, $p < .001$), .423 for the second block with an R Square change of .059 ($F = 12.198$, $p < .005$), and .473 for the third block with an R square change of .050 ($F = 11.233$, $p < .005$. ANOVA on ZSDS by high vs low (divided according to the mean values for each scale) subgroups for CDRISC, Recent Life Stressors and Negative Childhood Events showed no significant interactions. Thus, all three predictor variables made separate and significant additional contributions to ZSDS scores and must be considered as independent predictors of depression.

Logistic regression was used to test for the effects of resilience, frequency of negative childhood events and frequency of recent stressors as predictors of whether participants met Zung's cut off for 'clinically significant depression' as described above. Although only 21 participants met this criteria, the full model containing all three psychological predictors shown in Table 5 was statistically significant ($\chi 2$ (3, N = 126) = 30.816, $p < .001$), indicating that the model could distinguish between participants with clinically significant depression and those who did not meet this cutoff. The whole model explained between 22.3% (Cox and Snell R square) and 37.5% (Nagelkerke R Square) of the variance in depression status and correctly classified 83.6% of the cases. As shown in Table 5, only CDRISC significantly contributed to depression clinical status, with the odds ratio indicating that participants with high resilience had a lower than even (91.9%) chance of also experiencing clinically significant depression.

These data indicate that psychological resilience alone significantly predicted depression clinical status but that resilience and distal and proximal stressors significantly predicted total depression scores. However, those analyses were performed using each variable as a discrete entity, and it is of further interest to investigate the interactions between the stressor and resilience variables and depression. Because genotype might also be significantly involved in those relationships via interaction with stressors and resilience, dummy genotype variables were created and then fed into a hierarchical regression model with the existing psychological variables described above, using ZSDS score as the outcome variable. The ss form of genotype was coded as zero (should this be 0 for consistency?), sl as 1 and ll as 0 for a new dummy variable called 'sl'; the ss and sl were coded as 0 and ll as 1 for another dummy variable called 'll'. Three new interaction variables were created using the 'sl' x Negative Childhood Events, 'sl' x Recent Life Stressors, and 'sl' x Resilience; three additional interaction variables were also created similarly using the 'll' dummy variable x each of the three psychological variables. Using these genotype dummy variables, a three-step hierarchical regression was run, using the 'sl' and 'll' dummy variables as the first block, Negative Childhood Events, Recent Life Stressors and Resilience as the second block, and the 6 interaction variables as the third block. The R Square for the first block was .037 (ns); when the second block was added the R square rose to .488 ($F(3,117) = 34.291$, $p < .001$), but did not change significantly when the third block was added (R square =

.516, ns), indicating that genotype effects were not significant predictors of ZSDS scores, either alone or when in association with the stressors and resilience data.

DISCUSSION

Similar to many of the studies reviewed by Karg et al., [9] and Sharpley et al. [10], this study did not find a significant direct association between the ss allele of the 5-HTTLPR and depression, measured either as total scores on the ZSDS or when participants were classified according to their clinical status. Nor were any significant interactions found between that allele, stress and depression. While the current null finding does not challenge the overall robust relationship between the ss and depression that emerged from Sharpley et al.'s meta-analysis of 81 studies, it does add to the previous non-significant findings reported by those authors for a substantial portion of the studies they reviewed, and suggests that there may be some other as yet unidentified gene or genes or other intervening variables in the gene x environmental stress equation which could be further investigated to elucidate the inconsistency across studies found for the ss, stress and depression.

One of those potential intervening variables could be psychological resilience. The data from this study showed that resilience was the most powerful (inverse) predictor of ZSDS total score, followed by Recent Life Stressors and then Negative Childhood events in this sample. Thus, although both proximal and distal stressors contributed to participants' total depression scores on the ZSDS, resilience was a powerful buffer against the presence of elevated ZSDS scores. In addition, only resilience was associated with a reduced likelihood of being included in the clinically significant category for depression. Each of the three environmental/psychological variables may thus be described as making separate and significant contributions to depression when viewed as a continuous variable, with distal and proximal stressors being associated with elevated depression, and resilience being associated with lowered depression. When described as a categorical variable (i.e., presence/absence of clinically significant depression), only resilience was a significant predictor variable.

When investigated for their interaction effects upon depression, no significant results were present for any of the psychological variables when they were paired with genotype via dummy variable procedures. This suggests that the addition of genotype to the three psychological variables did not significantly add to the relative predictive power of those psychological variables, and also suggests that genotype did not compete well with stressors or resilience in predicting depression in this study. Taken alone, these specific findings argue that past and recent stressors, plus resilience, may be more powerful predictors of depression when used alone without the 5-HTTLPR.

This study has several limitations. First, the sample was not overly large, but of sufficient size and statistical power (as demonstrated by a priori power analysis and observed power) to detect the presence of several significant relationships between variables. This is an important point, since the problem of restricted sample size has been suggested as explaining null effects from studies which used genome-wide factors. That is, when a very large number of variables is used (i.e., some hundreds of thousands of genetic factors) to detect significant predictor effects on depression, the required sample size also needs to be commensurately very large. However, in studies of a restricted number of variables (such as this one), statistical power is not an issue when power analysis verifies the satisfactory nature of smaller samples.

Although it was not a limitation per se, the sample was restricted to one geographical locale, and cross-cultural and national factors might be a target for future investigations. The test of depression used in this study is adequate and has satisfactory validity and reliability, but measurement of depression in further studies might use a standardised clinical interview as an alternative, although the presence of significant relationships between some of the psychological factors and ZSDS scores and clinical status supports the use of that instrument here. The study was cross-sectional, and results need to be compared to a longitudinal study, which would also address possible recall bias regarding childhood adverse events. Finally, there are some recent

data suggesting that some patients who have elevated MDD scores on screening instruments may be comorbid Post-Traumatic Stress Disorder patients also [57]. Delineation of these patients from those who are suffering from MDD alone would further clarify the relationship between the 5-HTTLPR, stress and depression.

In conclusion, these results add to those from several studies to support the extension of the gene x environment interaction equation of 5-HTTLPR, distal and proximal stressors to include another factor—that of psychological resilience. None of the studies which Karg, et al. [9] and Sharpley et al. [10] reviewed and which supported the ss allele x stress as significant predictors of depression also demonstrated that all participants with the ss allele and who experienced stress became depressed. That is, as in most studies of such phenomena, although the majority of participants follow the hypothesised causal relationship, several do not. It may be that further exploration of the influence of psychological resilience in those participants might clarify the ways in which the gene x environment interaction occurs. Although resilience appears to have genetic indicators [24, 58], no study to date has used that data to investigate how resilience might interact with 5-HTTLPR and stress to influence depressive status, and that is a potential fruitful avenue of future research into ways of identifying those people who are most at-risk of developing depression following either recent or past stressors, and for identifying them in everyday clinical practice settings. As well as being of potential value to patients with MDD themselves, resilience may also be a factor in the well-being of the families of those patients, who suffer from considerable burden themselves [59].

REFERENCES

1. Bosker F, Hartman C, Nolte I, Prins B, Terpstra P, Posthuma D, et al. Poor replication of candidate genes for major depressive disorder using genome-wide association data. Molecular Psychiatry. 2011; 16: 516-532.
2. Shyn S, Shi J, Kraft J, Potash J, Knowles J, Weissman M, et al. Novel loci for major depression identified by genome-wide association study of sequenced treatment alternatives to releive depression and meta-analysis of three studies. Molecular Psychiatry. 2011; 16: 202-215.
3. Shi J, Potash J, Knowles J, Weissman M, Coryell W, Scheftner W, et al. Genome-wide association study of recurrent early-onset major depressive disorder. Molecular Psychiatry. 2011; 16: 193-201.
4. Consortium MDDWGotPG. A mega-analysis of genome-wide association studies for major depressive disorder. Molecular Psychiatry. 2012.
5. Grabe H, Schwahn C, Appel K, Mahler J, Schulz A, Spitzer C, et al. Update on the 2005 paper: moderation of mental and physical distress by polymorphisms in the 5-HT transporter gene by interacting with social stressors and chronic disease burden. Molecular Psychiatry. 2011; 16: 354-361.
6. Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic Sensitivity to the Environment: The Case of the Serotonin Transporter Gene and Its Implications for Studying Complex Diseases and Traits. American Journal of Psychiatry. 2010; 167(5): 509-527.
7. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene. Science. 2003; 301(5631): 386-389.
8. Hauser J, Leszczynska A, Samochowiec J, Czerski P, Ostapowicz A, Chliopocka M, et al. Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. European Psychiatry. 2003; 18: 129-132.
9. Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited. Archives of General Psychiatry. 2011; 68: 444-454.
10. Sharpley C, Palanisamy S, Glyde N, Dillingham P, Agnew L. An update of the interaction between the serotonic transporter promoter variant (5-HTTLPR), stress and depression, plus an exploration of non-confirming findings. Behavioural Brain Research. 2014; 273: 89-105.
11. Chipman P, Jorm A, Prior M, Sanson A, Smart D, Tan X, et al. No interaction between the serotonin transporter polymorphism (5-HTTLPR) and childhood adversity or recent stressful life events on symptoms of depression. American Journal of Medical Genetics. 2007;144B: 561-565.
12. Zhang K, Xu Q, Xu Y, Yang H, Luo J, Sun Y, et al. The combined effects of the 5-HTTLPR and 5-HTR1A genes modulates the relationship between negative life events and major depressive disorder in a Chinese population. Journal of Affective Disorders. 2009; 114: 224-231.
13. Fredrickson B, Tugade M, Waugh C, Larkin G. A prospective study of resilience and emotions following the terrorist attacks on the United States on September 11th, 2002. Journal of Personality and Social Psychology. 2003; 84: 365-376.
14. Luthar S, Cicchetti D. The construct of resilience: Implications for interventions and social policies. Development and Psychopathology. 2000; 12: 857-885.

15. Charney DS. Psychobiological Mechanisms of resilience and Vulnerability: Implications for Successful Adaptation to Extreme Stress. Focus. 2004; 2(3): 368-391.
16. Brooks R. The power of parenting. In: Brooks R, Goldstein S, editors. Handbook of resilience in children New York: Kluwer Academic/Plenum; 2005. p. 297-314.
17. Tedeschi R, Kilmer R. Assessing strengths, resilience, and growth to guide clinical interventions. Professional Psychology: Research and Practice. 2005; 36: 230-237.
18. Alvord M, Grados J. Enhancing resilience in children: A proactive approach. Professional Psychology: Research and Practice. 2005; 36: 238-245.
19. Bonanno GA, Galea S, Bucciarelli A, Vlahoz D. What predicts psychological resilience after disaster? The role of demographics, resources, and life stress. Journal of Consulting and Clinical Psychology. 2007; 75: 671-682.
20. Jopp D, Rott C. Adaptation in Very Old Age: Exploring the Role of Resources, Beliefs, and Attitudes for Centenarians' Happiness. Psychology and Aging. 2006; 21(2): 266-280.
21. Karoly P, Reuhlman L. Psychological resilience and its correlates in chronic pain. Pain. 2006; 123: 90-97.
22. Watt N, David J, Ladd K, Shamos S. The life course of psychological resilience: A phenomenological perspective on deflecting life's slings and arrows. The Journal of Primary Prevention. 1995; 15: 209-246.
23. Bergstrom A, Jayatissa M, Thykjaer T, Wiborg O. Molecular Pathways Associated with Stress Resilience and Drug Resistance in the Chronic Mild Stress Rat Model of Depression - a Gene Expression Study. J Mol Neurosci. 2007; 33(2): 201-215.
24. Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. Nature Reviews Neuroscience. 2009; 10: 446-457.
25. Cicchetti D, Blender JA. A Multiple-Levels-of-Analysis Perspective on Resilience. Annals of the New York Academy of Sciences. 2006; 1094: 248-258.
26. Haglund MEM, Nestadt PS, Cooper NS, Southwick SM, Charney DS. Psychobiological mechanisms of resilience: Relevance to prevention and treatment of stress-related psychopathology. Development and Psychopathology. 2007; 19: 889-920.
27. Stein M, Campbell-Sills L, Gelernter J. Genetic variation in 5HTTLPR is associated with emotional resilience. American Journal of Medical Genetics B: Neuropsychiatric Genetics. 2009; 150B: 900-906.
28. Carli V, Mandelli L, Zaninotti L, Roy A, Recchia L, Stoppia L, et al. A protective genetic variant for adverse environments? The role of childhood traumas and serotonic transporter gene on resilience and depressive severity in a high-risk population. European Psychiatry. 2011; 26: 471-478.
29. O'Hara R, Marcus P, Thompson W, Flourney J, Vahia I, Lin X, et al. 5-HTTLPR short allele, resilience, and successful aging in older adults. The American Journal of Geriatric Psychiatry. 2012; 20: 452-456.
30. Hovens J, Giltay E, Wiersma J, Spinhoven P, Penninx B, Zitman F. Impact of childhood life events and trauma on the course of depressive and anxiety disorders. Acta Psychiatrica Scandinavia. 2012; 126: 198-207.
31. Judd L, Paulus M, Wells K, Rapaport M. Socioeconomic burden of subsyndromal depressive symptoms and major depression in a sample of the general population. American Journal of Psychiatry. 1996; 163: 1411-1417.
32. Judd L, Akiskal H, Maser J, Zeller P, Emdicott J, Coryell W, et al. A prospective 12-yar study of subsyndromal and syndromal depressive symptoms in unipolar major depressive disorders. Archives of General Psychiatry. 1998; 55: 694-700.
33. Lyness J, Heo M, Datto C, Ten Have T, Katz I, Drayer R, et al. Outcomes of Minor and Subsyndromal Depression among Elderly Patients in Primary Care Settings. Annals of Internal Medicine. 2006; 144(7): 496-504.
34. Chopra M, Zubritsky C, Knott K, Have T, Hadley T, Coyne J, et al. Importance of subsyndromal symptoms of depression in elderly patients. The American Journal of Geriatric Psychiatry. 2005; 13: 597-606.
35. Lyness J, Kim J, Tang W, Tu X, Conwell Y, King D, et al. The clinical significance of subsyndromal depression in oldre primary care patients. American Journal of Geriatric Psychiatry. 2007; 15: 214-223.
36. da Silva Lima A, de Almeida Fleck M. Subsyndromal depression: An impact on quality of life? Journal of Affective Disorders. 2007; 100: 163-169.
37. Zung W. A self-rating depression scale. Archives of General Psychiatry 1965; 12: 63-70.
38. APA. Diagnostic and Statistical Manual of Mental Disorders (4th ed, Text Revision). Washington, DC: American Psychiatric Association; 2000.
39. DeJonge J, Baneke J. The Zung Self-rating Depression Scale: A replication study on reliability, validity and prediction. Psychological Reports. 1989; 833-834.
40. Gabrys J, Peters K. Reliability, discriminant and predictive validity of the Zung Self-Rating Depression Scale. Psychological Reports. 1985; 57: 1091-1096.
41. Schaefer A, Brown J, Watson C, Plenel D, DeMotts J, Howard M, et al. Comparison of the validities of the Beck, Zung and MMPI depression scales. Journal of Consulting and Clinical Psychology. 1985; 53: 415-418.
42. Sharpley C, Rogers H. Naïve versus sophisticated item-writers for the assessment of anxiety. Journal of Clinical Psychology. 1985; 41: 58-62.
43. Agrell B, Dehlin O. Comparison of six depression scales in geriatric stroke patients. Stroke. 1989; 20: 1190-1194.
44. Zung W. From art to science: The diagnosis and treatment of depression. Archives of General Psychiatry. 1973; 29: 328-337.

45. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. American journal of preventive medicine. 1998; 14(4): 245-258.
46. Straus MA, Gelles RJ, Smith C. Physical violence in American families: Risk factors and adaptations to violence in 8,145 families: Transaction Pub; 1995.
47. Bernstein DP, Fink L, Handelsman L, Foote J. Initial reliability and validity of a new retrospective measure of child abuse and neglect. The American Journal of Psychiatry; The American Journal of Psychiatry. 1994.
48. Wyatt GE. The sexual abuse of Afro-American and white-American women in childhood. Child abuse & neglect. 1985; 9(4): 507-519.
49. Wingenfeld K, Schäfer I, Terfehr K, Grabski H, Driessen M, Grabe H, et al. [The reliable, valid and economic assessment of early traumatization: first psychometric characteristics of the German version of the Adverse Childhood Experiences Questionnaire (ACE)]. Psychotherapie, Psychosomatik, medizinische Psychologie. 2011;61(1):e10.
50. Sharpley C, Tanti A, Stone J, Lothian P. The 'Effects of Life Events' Inventory. Counselling Psychology Quarterly. 2004; 17: 45-52.
51. Sarason IG, Johnson JH, Siegel JM. Assessing the impact of life changes: development of the Life Experiences Survey. Journal of Consulting and Clinical Psychology; Journal of Consulting and Clinical Psychology. 1978; 46(5): 932.
52. Paykel ES, Myers JK, Dienelt MN, Klerman GL, Lindenthal JJ, Pepper MP. Life events and depression: A controlled study. Archives of General Psychiatry. 1969; 21(6): 753.
53. Holmes TH, Rahe RH. The social readjustment rating scale. Journal of psychosomatic research. 1967.
54. Tennant C, Andrews G. A scale to measure the stress of life events. Australasian Psychiatry. 1976;10(1): 27-32.
55. Heam R, Arlbaster K. DNA extraction techniques for use in education. Biochemistry and Molecular Biology Education. 2010;38: 161-166.
56. Wendland J, Martin B, Kruse M, Lesch K-P, Murphy D. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Molecular Psychiatry. 2006; 11: 224-226.
57. Bossini L, Casolaro I, Caterini C, Koukouna D, Fortini V, Cecchini F, et al. Diagnostic mistakes in post-traumatic sterss disorder. The problem of symptom overlap with depression. Psychiatr Pol. 2013; 47: 1051-1063.
58. Folsom T, Fatemi H. The involvement of Reelin in neurodevelopmental disorders. Neuropharmacology. 2012.
59. Borowieka-Kluza J, Miernik-Jaeschke M, Jaeschke R, Siwek M, Dudek D. The affective disorder-related burden imposed on the family environment: an overview. Psychiatr Pol. 2013;XLVII: 635-644.