*Psychological Medicine*, 2005, **35**, 101–111. 
DOI: 10.1017/S0033291704002727 Printed in the United Kingdom

# The relationship between stressful life events, the serotonin transporter (5-HTTLPR) genotype and major depression

NATHAN A. GILLESPIE*, JOHN B. WHITFIELD, BEN WILLIAMS, ANDREW C. HEATH AND NICHOLAS G. MARTIN

*Queensland Institute of Medical Research, Brisbane, Australia; Royal Prince Alfred Hospital, Sydney, Australia; Department of Psychiatry, Washington University School of Medicine, St Louis, MO, USA*

## ABSTRACT

**Background.** Serotonin is a good candidate for major depression. We attempted to replicate the study by Caspi and colleagues [*Science* (2003) **301**, 386–389] which reported a significant interaction between serotonin transporter (5-HTTLPR) genotype and stressful life events when predicting major depression.

**Method.** We typed the serotonin promoter 5-HTTLPR gene in 1206 male and female twins aged 19–78 years (mean = 39, S.D. = 11). A DSM-IV diagnosis of major depression was available for 1199 twins. Most of these twins had participated in a 1988–1990 study which included a stressful life events inventory and self-report measure of depression based on the SCL-90 and DSSI/sAD. Complete 5-HTT genotype and life events data, self-report symptoms and major depression diagnoses were available for 1091 subjects. We regressed categorical and ordinal measures of depression onto stressful life events and genotype.

**Results.** There were significant main effects for stressful life events but there was no evidence for any effect of 5-HTT genotype, nor a genotype × stressful life event interaction.

**Conclusions.** Regardless of whether our results were based on binary logistic or ordinal regression analyses we found no evidence to support a main effect of 5-HTTLPR, or an interaction between the 5-HTTLPR genotype and stressful life events on major depression. Only 20% of our subjects were aged below 30 years. It is possible that the effect reported by Caspi and colleagues is specific to young people, in which case our study has much less power in this age group.

## INTRODUCTION

The serotonergic system is a strong genetic candidate for major depression and suicide. In their review, Owens & Nemeroff (1994) highlighted considerable evidence to support the hypothesis that there are significant differences in the serotonergic systems in patients with and without major depression. It is known that the 's' allele of the polymorphism reduces the transcriptional efficiency of this serotonin promoter, resulting in decreased 5-HTT expression and serotonin uptake in lymphoblasts (Lesch *et al*. 1996).

More recently, Caspi *et al*. (2003) have reported that variation in the 5-HTT gene-linked polymorphic region (5-HTTLPR) moderates the influence of stressful life events (SLEs) on major depression. Based on a sample of 1037 adults aged 26 years, the influence of SLEs on the incidence of major depression was significantly greater amongst homozygous and heterozygous carriers of the short ('s') allele in the 5-HTTLPR genotype. The same genotype by environment (G × E) interaction also approached significance for suicidal behaviour.

* Address for correspondence: Nathan Gillespie, Genetic Epidemiology Unit, Queensland Institute of Medical Research, Post Office, Royal Brisbane Hospital, 300 Herston Road, Herston, Qld 4029, Australia.
(Email: nathanG@qimr.edu.au)

### Aim

Using a sample of Australian twins, the aim of this study is to determine whether variation in the serotonin transporter (5-HTTLPR) genotype moderates the influence of SLEs on major depression and suicidal behaviour.

## MATERIALS AND METHOD

### Sample

Subjects were drawn from the Australian National Health and Medical Research Council Twin Register (ATR). The ATR is a volunteer register founded in 1978 with approximately 25 000 twins of all types and ages enrolled at various stages of active participation. This represents approximately 10–20% of living twins in Australia. Numerous analyses have shown that the ATR is typical of the Australian population in many respects including the prevalence of psychiatric symptoms (Kendler *et al.* 1986), although the ATR sample tends to be slightly more middle class and educated than average, particularly for males (Baker *et al.* 1996).

The current project was based on an older cohort of 3808 twin pairs born before 1965 which was first surveyed during 1980–1982 and was then followed up during 1988–1990 to investigate persistence and changes in drinking habits. Mailed questionnaire or telephone interview data were obtained from 2997 complete twin pairs and 334 singles, with an individual response rate of 83%, and a complete pair response rate of 79%. At the same time, the 576 incomplete twin pair respondents (singletons) and their co-twins from the 1980–1982 study were also followed-up with an identical questionnaire. The 1988–1990 study contained a lengthy self-report Health and Lifestyle Questionnaire (HLQ) which incorporated many of the questions sent out to the same twins 8 years previously such as tobacco use, alcohol consumption, personality, sociodemographic variables, psychiatric symptoms, SLEs, social attitudes and numerous other behavioural measures (Heath *et al.* 1994).

Four years later in 1992, 3882 twin pairs, many of whom had been targeted for the 1988–1990 study, were approached and asked to participate in a study which included a semi-structured assessment for the genetics of alcoholism. We set out to obtain blood samples from as many of these twins as possible, selected only on the basis of willingness to cooperate. Samples were obtained from 3347 individual twins (comprising 1383 complete twin pairs and 581 singletons) before funds were exhausted.

### DNA extraction and genotyping

Genomic DNA was extracted (Miller *et al.* 1988) from peripheral venous blood samples. Zygosity of same-sex twins was determined by typing nine independent DNA microsatellite polymorphisms at QIMR using the profiler AmpFLSTRR Profiler PlusT multiplex marker set (Applied Biosystems, Foster City, CA, USA). All twins were also typed for ABO, Rh and MNS blood groups by the Red Cross Blood Service in Brisbane. Genotypes for the 5-HTTLPR polymorphism were determined using the method described by Turker *et al.* (1998), with primers 5′-GGCGTTGCCGCTCTGAATGCC and 5′-CAGGGGAGATCCTGGGAGAGGT. The PCR products were separated on 7% polyacrylamide gel and visualized with ethidium bromide. The product lengths were 221 bp (short allele) and 265 bp (long allele).

### Imputation of missing genotypes

There were 15 monozygotic twins with missing 5-HTTLPR genotypes whose values were imputed using their co-twin's genotype. A total of 1206 subjects had complete 5-HTTLPR genotype information. The age range for this sample was 19–78 years (mean = 39 years, S.D. = 11 years). The sample was then split into three groups on the basis of genotype. The frequencies for the 'ss', 'sl' and 'll' genotypes were 22% ($n=262$), 48% ($n=577$) and 30% ($n=367$) respectively. These were not significantly different from Hardy–Weinberg equilibrium ($\chi^2_1=1{\cdot}81$), although this was tested without regard to the relatedness of twins. In addition, there was no significant difference in genotype frequencies between the sexes ($\chi^2_2=0{\cdot}82$, $p=0{\cdot}66$). Population stratification was unlikely given that more than 95% of the subjects' great-grandparents were reported as being of northern European ancestry, mainly from Britain and Ireland.

### 1988–1990 General Health Questionnaire

Details of the SLEs and self-report measures of depression which formed part of the 1988–1990 questionnaire are described below.

### SLEs

Originally identified by Brown & Harris (1978) and Bebbington *et al.* (1981), the SLEs were adapted from the List of Threatening Experiences (LTE) by Brugha *et al.* (1985). In this study, items were divided into two categories based on previous research which has examined these events in terms of (i) personal events which primarily happen to the subject and (ii) network events which happen to an individual in his or her social network (Kendler & Karkowski-Shuman, 1997; Kendler *et al.* 2001*b*).

The 12-item personal SLE inventory in the 1988–1990 study was very similar to that used by Caspi and colleagues. Subjects were asked if during the past 12 months any of the following events had occurred: divorce; marital separation; broken engagement or steady relationship; separation from other loved one or close friend; serious illness or injury; serious accident (not involving personal injury); being burgled or robbed; laid off or sacked from job; other serious difficulties at work; major financial problems; legal troubles or involvement with police; and living in unpleasant surroundings. We expanded the study to also include network SLEs which were assessed with a 28-item inventory similar to that used by Kendler and colleagues (1995, 1997, 2001*b*). The first 21 items asked whether, during the last 12 months, a spouse, child, mother or father, twin, sibling, relative, or someone close had died, suffered a serious illness/injury, or suffered a serious personal crisis. An additional seven items asked subjects whether, during the last 12 months, they had any serious problems getting along with their spouse, other family members, a close friend, a neighbour, someone living with them (e.g. child or elderly parent), their twin, or a workmate or co-worker.

In order to correct for positive skewness as well as maintain consistency with Caspi and colleagues, SLE scores were re-coded onto a 5-point ordinal scale using the same categories as Caspi and colleagues, namely 0, 1, 2, 3, 4+ life events. When based on the entire 1988–1990 sample, there were no significant sex differences in the distribution of personal life events ($\chi^2_4 = 5{\cdot}95$, $p = 0{\cdot}20$). There was, however, a significant sex difference for network life events ($\chi^2_4 = 12{\cdot}58$, $p < 0{\cdot}05$). Females reported a greater number of network life events than males. When based on the genotyped sample, this difference was no longer significant. Complete personal ($n = 1099$) and network ($n = 1102$) SLE data were available from 90% of the genotyped sample. There were no significant sex differences for personal ($\chi^2_4 = 1{\cdot}27$, $p = 0{\cdot}87$) or network ($\chi^2_4 = 1{\cdot}32$, $p = 0{\cdot}86$) events.

### Depression – Self-report questionnaire

Nineteen items from the SCL-90 (Derogatis *et al.* 1973) were combined with the 14 similarly worded items from the DSSI/sAD (Bedford *et al.* 1976) scales and all 33 items were included in the 1988–1990 questionnaire. On the basis of previous factor analyses (Gillespie *et al.* 1999), a self-report measure of depression was calculated based on nine items. Each item had been rephrased to conform to the DSSI/sAD format of inquiry, 'Recently I have had …' rather than use the SCL-90 format, 'In the past two weeks …'. The response set was also changed from a 5-point scale of distress from 'not at all' (0) to 'extremely' (4) (Derogatis *et al.* 1973) to the DSSI/sAD 4-point distress scale: (1) 'not at all', (2) 'a little', (3) 'a lot', (4) 'unbearably'. Complete depression scores were available from 1090 subjects or 89% of the genotyped sample. In order to correct for positive skewness, self-report depression scores were recoded to a 3-point ordinal scale (0 = 0; 1–4 = 1; 5–27 = 2). When based on the entire 1988–1990 sample ($n = 5775$) there was a highly significant association between sex and the recoded depression scores ($\chi^2_2 = 20{\cdot}75$, $p < 0{\cdot}001$), with females having higher scores of moderate and severe depression (35%, 13%) than males (33%, 9%). However, when based on the genotyped subjects with complete self-report depression data ($n = 1090$), the sex difference was no longer significant ($\chi^2_2 = 1{\cdot}58$, $p = 0{\cdot}46$).

### Depression – clinical interview

Many of the subjects who participated in the 1988–1990 survey were followed up approximately 4 years later in 1992 with a psychiatric interview by telephone. The interview included an adaptation of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA, 2004) which was modified for administration by telephone interview. The SSAGA was originally designed to assess the physical, psychological

Table 1. *Prevalence of DSM-IV major depression (MD) diagnoses by sex based on the genotyped subjects*

| | Males | | Females | | | | |
|---|---|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | $\chi^2$ | df | *p* |
| DSM-IV[a] | 84% (378) | 16% (73) | 78% (575) | 22% (165) | 6·45 | 1 | * |
| DSM-IV[b] | 91% (408) | 10% (43) | 87% (643) | 13% (97) | 3·45 | 1 | 0·06 |
| DSM-IV[c] | 93% (421) | 7% (30) | 91% (672) | 9% (68) | 2·39 | 1 | 0·12 |
| DSM-IV[d] | 94% (425) | 6% (26) | 92% (683) | 8% (57) | 1·62 | 1 | 0·20 |

[a] Based on lifetime DSM-IV MD.
[b] Based on DSM-IV MD first diagnosed before SLE reporting period.
[c] Based on DSM-IV MD first diagnosed during or after SLE reporting period.
[d] Based on DSM-IV MD first diagnosed exclusively after SLE reporting period.
* $p < 0{\cdot}05$.

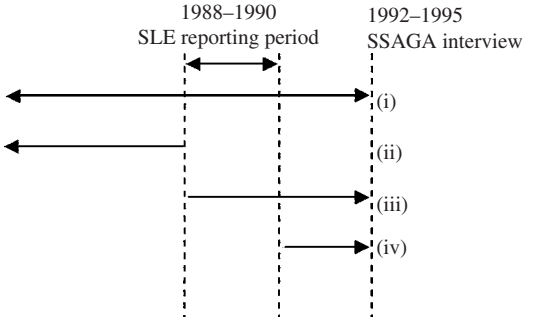


FIG. 1. DSM-IV major depression diagnostic time-frames: (i) lifetime diagnoses, (ii) diagnoses made before the reporting period of the stressful life events, (iii) diagnoses made either during or after the reporting period and (iv) diagnoses made exclusively after the reporting period.

and social manifestations of alcohol abuse or dependence and other psychiatric disorders according to DSM-III-R criteria. The scoring algorithm was modified in order to obtain DSM-IV diagnosis of major depression. In addition, the interview ascertained general demographic information, medical history, information about tobacco use, suicide attempts, and included a psychosis screener to identify individuals requiring clinical follow-up.

Ninety-eight per cent ($n = 1191$) of the genotyped sample completed the SSAGA interview. The average time between the 1988–1990 questionnaire return date and SSAGA interview was 46 months (S.D. = 7 months, range = 2–60 months). As illustrated in Fig. 1, the SSAGA DSM-IV major depression was variously redefined based on age of first onset: (i) lifetime diagnoses, (ii) diagnoses made before the reporting of the SLEs (< 1988–1990), (iii) diagnoses made either during or after the reporting period (⩾ 1988–1990), and (iv) diagnoses made exclusively after the reporting period (> 1988–1990). Apart from a lifetime diagnosis, any cases with a date of onset outside the diagnostic time-frame were given a non-positive diagnosis.

Lifetime prevalence of DSM-IV major depression was significantly higher among females than males (see Table 1) and approached significance for diagnoses made before the 12-month reporting period for SLEs. For diagnoses made during or after the reporting period, the sex difference in the prevalence of major depression were not significant. When attempting to replicate the G × E interaction reported by Caspi *et al.* (2003), a total of 1091 subjects with 5-HTTLPR and personal SLE data, had also completed the psychiatric interview for DSM-IV major depression.

## Suicide

The SSAGA interview also included an assessment of suicide behaviour based on lifetime history of suicide planning, suicide attempts and persistent thoughts of suicide (Statham *et al.* 1998). Complete data were available from 1190 subjects of whom 13% ($n = 96$) and 12% ($n = 54$) of males and females respectively indicated that at some stage in their lives they had persistent thoughts, had planned or attempted suicide. There was no association between sex and suicidal behaviour ($\chi^2_1 = 0{\cdot}26$, $p = 0{\cdot}61$).

## Regression analysis

### *Binary logistic regression*

Binary logistic regression analyses were used to estimate the association between a diagnosis for major depression and (*a*) the 5-HTTLPR

Table 2. *Binary logistic and ordinal regressions. The regression of sex, age, genotype, stressful life events (SLEs) and the genotype × environment interaction on depression*

| | Sex | | | Age | | | 5-HTTLPR | | | Personal SLEs | | | 5-HTTLPR × SLEs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *β* | S.E. | *p* | *β* | S.E. | *p* | *β* | S.E. | *p* | *β* | S.E. | *p* | *β* | S.E. | *p* |
| DSM-IV MD[a] | 0·36 | 0·17 | * | 0·11 | 0·12 | 0·33 | −0·09 | 0·14 | 0·51 | 0·43 | 0·13 | ** | 0·08 | 0·10 | 0·43 |
| DSM-IV MD[b] | 0·32 | 0·21 | 0·12 | 0·34 | 0·14 | * | −0·15 | 0·17 | 0·39 | 0·41 | 0·15 | ** | 0·02 | 0·12 | 0·88 |
| DSM-IV MD[c] | 0·28 | 0·24 | 0·24 | −0·23 | 0·17 | 0·18 | 0·03 | 0·20 | 0·88 | 0·32 | 0·18 | 0·08 | 0·10 | 0·13 | 0·47 |
| DSM-IV MD[d] | 0·24 | 0·26 | 0·34 | −0·20 | 0·18 | 0·26 | −0·07 | 0·21 | 0·75 | 0·22 | 0·20 | 0·26 | 0·11 | 0·15 | 0·43 |
| Suicide[e] | 0·08 | 0·20 | 0·69 | −0·06 | 0·14 | 0·69 | 0·09 | 0·17 | 0·62 | 0·58 | 0·15 | *** | 0·01 | 0·11 | 0·96 |
| Depression[f] | −0·02 | 0·13 | 0·87 | −0·09 | 0·09 | 0·32 | 0·10 | 0·11 | 0·36 | 0·71 | 0·12 | *** | 0·04 | 0·10 | 0·66 |
| | Sex | | | Age | | | 5-HTTLPR | | | Network SLEs | | | 5-HTTLPR × SLEs | | |
| | *β* | S.E. | *p* | *β* | S.E. | *p* | *β* | S.E. | *p* | *β* | S.E. | *p* | *β* | S.E. | *p* |
| DSM-IV MD[a] | 0·32 | 0·17 | 0·05 | 0·04 | 0·11 | 0·71 | −0·23 | 0·20 | 0·25 | 0·18 | 0·10 | 0·06 | 0·11 | 0·08 | 0·13 |
| DSM-IV MD[b] | 0·29 | 0·21 | 0·16 | 0·28 | 0·14 | * | −0·30 | 0·25 | 0·23 | 0·17 | 0·12 | 0·14 | 0·09 | 0·09 | 0·31 |
| DSM-IV MD[c] | 0·27 | 0·24 | 0·26 | −0·29 | 0·17 | 0·08 | −0·07 | 0·30 | 0·82 | 0·15 | 0·15 | 0·30 | 0·10 | 0·11 | 0·36 |
| DSM-IV MD[d] | 0·24 | 0·26 | 0·35 | −0·27 | 0·18 | 0·13 | −0·02 | 0·30 | 0·95 | 0·12 | 0·15 | 0·43 | 0·04 | 0·11 | 0·74 |
| Suicide[e] | 0·04 | 0·19 | 0·84 | −0·14 | 0·14 | 0·31 | −0·01 | 0·25 | 0·96 | 0·22 | 0·12 | 0·07 | 0·06 | 0·09 | 0·53 |
| Depression[f] | 0·01 | 0·13 | 0·96 | −0·16 | 0·09 | 0·07 | −0·02 | 0·16 | 0·92 | 0·34 | 0·10 | ** | 0·11 | 0·08 | 0·15 |

[a] Based on lifetime DSM-IV MD.
[b] Based on DSM-IV MD first diagnosed before SLE reporting period.
[c] Based on DSM-IV MD first diagnosed during or after SLE reporting period.
[d] Based on DSM-IV MD first diagnosed exclusively after SLE reporting period.
[e] Persistent thoughts, planning or attempts at suicide.
[f] Based on recoded self-report depression scores from the 1988–1990 Health and Lifestyle Survey, contemporaneous with the SLE reporting period: 0 symptoms = 0; 1–4 symptoms = 1; 5–27 symptoms = 2.
SLEs: 0–4 + events.
5-HTTLPR: 0 = 'ss'; 1 = 'sl'; 2 = 'll'.
Sex: 0 = male; 1 = female.
* $p < 0{\cdot}05$, ** $p < 0{\cdot}01$, *** $p < 0{\cdot}001$.

genotype, (*b*) SLEs, and (*c*) the G × E interaction. Sex and age were also included in the model,

$$\begin{aligned}\text{Depression} = {} & \beta_0 + \beta_1(\text{sex}) + \beta_2(\text{age}) \\ & + \beta_3(\text{5-HTTLPR}) + \beta_4(\text{SLE}) \\ & + \beta_5(\text{5-HTTLPR} \times \text{SLEs}),\end{aligned}$$

where $\beta_0$ is the intercept, $\beta_1$ is the male deviation (female = 0, male = 1), $\beta_2$ is the age regression coefficient (19–30 years = 0; 31–45 years = 1; 46–78 years = 2), $\beta_3$ is the 5-HTTLPR regression coefficient ('s' = 0, 'sl' = 1, 'll' = 2), and $\beta_3$ is the SLE regression coefficient (0 events = 0; 1 event = 1; 2 events = 2; 3 events = 3; $\geqslant 4$ events = 4).

*Ordinal regression*

We fit an ordinal data regression model in SPSS (version 11.0.1) to the recoded depression scores. Tests of parallel lines were also run to ensure that the slopes across all levels of the dependent measure were equal.

The current analyses ignored the relatedness of twins by treating all subjects as unrelated individuals. Although this will not produce biased estimates, it will underestimate their variance and hence overestimate their significance. Normally, if significant estimates are obtained, then analyses ought to be repeated using more elaborate methods which take into account the degree of biological relatedness (e.g. STATA or Mx). As will be seen, this is unnecessary here.

## RESULTS

### Binary logistic regression

Results for the binary logistic regression analyses based on personal and network SLEs are shown in Table 2. An increase in the total number of personal events was associated with increased liability to lifetime DSM-IV major depression, as well as DSM-IV major depression first diagnosed before the 12-month reporting period. The association between personal life events and a first episode of DSM-IV major depression

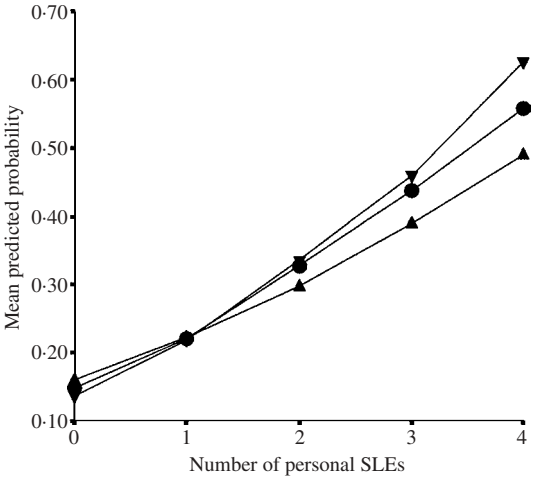


FIG. 2. Mean predicted probability of lifetime DSM-IV major depression by number of personal stressful life events (SLEs). Based on binary logistic regression. Genotype: –▲–, ss; –●–, sl; –▼–, ll.

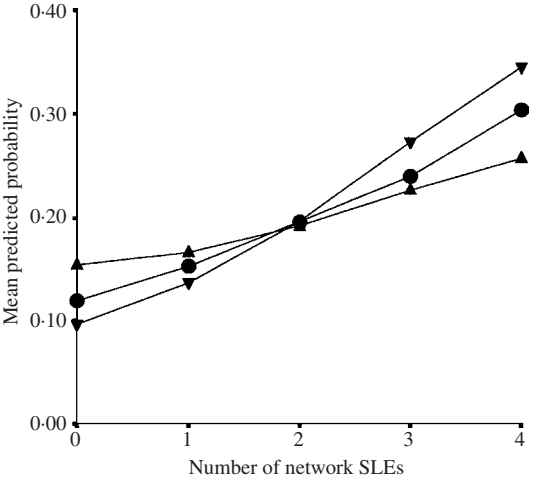


FIG. 3. Mean predicted probability of lifetime DSM-IV major depression by number of network stressful life events (SLEs). Based on binary logistic regression. Genotype: –▲–, ss; –●–, sl; –▼–, ll.

diagnosed during or after the reporting period also approached significance. There was a significant effect of age when predicting DSM-IV major depression first diagnosed before the reporting period, but not for lifetime diagnoses. There was no evidence to support a 5-HTTLPR × personal life events interaction (see Fig. 2).

The analyses were repeated using network life events. The main effects of sex and network life events approached significance for lifetime major depression. There was a significant effect for age for diagnoses made before the 12-month reporting period. There was no evidence to support a 5-HTTLPR × network life events interaction (see Fig. 3).

The number of personal SLEs was also significantly associated with persistent thinking, planning or attempting suicides. The association between network events and suicidal behaviour approached significance at the 0·05 level.

### Ordinal data regression

In the test of parallel lines, a model which constrained the regression slopes to be equal across all levels of the recoded self-report depression scores provide a good fit to the personal ($\Delta\chi^2_5 = 6{\cdot}79, p = 0{\cdot}24$) and network ($\Delta\chi^2_5 = 5{\cdot}27, p = 0{\cdot}38$) life-event models. As is also shown in Table 2, the ordinal regression analyses revealed significant main effects for both personal and network life events. Consistent with results based on the binary regression analyses, there were no main effects for genotype at any level, nor was there any evidence to a support G × E interaction.

## DISCUSSION

We found no evidence to support either a genotypic main effect or a G × E interaction. This conclusion was consistent across the categorical and ordinal measures of depression and regardless of whether or not the results were based on psychiatric diagnoses made before, during or after the reporting period of personal and network SLEs. Moreover, the signs of the non-significant terms were all opposite to those reported by Caspi *et al.* (2003).

In addition to age and sex, there were main effects for SLEs on depression. For instance, a lifetime diagnosis of DSM-IV major depression was significantly predicted by personal events as well as self-reports of thinking, planning and attempted suicides. Network events approached significance for DSM-IV major depression. The regression of personal events onto diagnoses of major depression made during or after the 1988–1990 reporting period also approached significance.

Because we did not have lifetime assessments of SLEs we cannot conclude from our data that depression 'causes' SLEs. Nonetheless, there is evidence to suggest that the relationship

between SLEs and major depression is in part causal (Kendler *et al*. 1993*a*, 1999, 2000). Indeed, some of the strongest predictors of DSM-III-R major depression are SLEs that are followed by genetic factors, previous history of major depression and neuroticism (Kendler *et al*. 1993*a*, 2004).

The depressogenic impact of SLEs has been shown to decline markedly over time (Kendler *et al*. 1999). The fact that our results reveal main effects for recently experienced SLEs in the prediction of lifetime depression therefore suggests that these items are indexing a persistent liability to encounter adverse events which in turn increases the risk of depression. This possibility is supported by findings from previous twin studies, as well as preliminary analyses of the current data, which have shown that familial aggregation for the total number of SLEs is significant, and is best explained by aspects of the shared environment and genetic effects (Kendler *et al*. 1993*b*; Foley *et al*. 1996; Kendler & Karkowski-Shuman, 1997; Bolinskey *et al*. 2004).

Why we do not see stronger main effects for stressors when regressed onto major depression diagnosed during and after the reporting period for life events is uncertain. Apart from a smaller number of positive diagnoses which reduce power, one explanation is that the relationship between the number of life events and major depression is diminished by the number of previous depressive episodes. One previous study has shown that for every additional previous episode of major depression there is an increased risk of further episodes (Kendler *et al*. 2000). This risk increases until the total number of previous episodes reaches nine, after which no additional risk is seen. This is further complicated by interactions between the number of previous episodes of major depression, putative effect of particular SLEs as well as the degree of genetic risk (see Kendler & Eaves, 1986; Kendler & Karkowski-Shuman, 1997; Kendler *et al*. 2000, 2001*a*). For example, as the number of previous episodes of depression rises from 0 to 9, the strength of the association between stressful events and depression reportedly declines by 13% (Kendler *et al*. 2000). This decline in association is consistent with a 'kindling' hypothesis which predicts that SLEs are more likely to precede the onset of major depression in individuals experiencing their first episode of major depression (Kendler *et al*. 2000). In other words, as the number of previous episodes increases, individuals require less provocation; they become increasingly desensitized to stressful events and require fewer events to trigger depression. This might also suggest that early onsets of depression are more likely to be influenced by $G \times E$ interactions than late-onset episodes.

As previously mentioned, the impact of genetic risk can also alter the association between SLEs and depression. Even after controlling for number of previous depressive episodes, Kendler & Karkowski-Shuman (1997) have shown that genetic risk factors alone, increase the probability of experiencing SLEs and selection into high-risk environments. An increased genetic risk can also influence the 'speed of kindling' as well as determine the levels of 'pre-kindling' prior to a stressful event (Kendler *et al*. 2001*a*).

The 'speed of kindling' hypothesis predicts that everyone begins with the same degree of association between stressful events and risk of developing major depression, but that the rate of kindling is positively correlated with genetic risk (Kendler *et al*. 2001*a*). In other words, people who are genetically disposed towards depression, require a shorter time-interval before significantly fewer stressful events are needed to induce depression.

In terms of 'pre-kindling', people who are at high genetic risk are already pre-kindled and do not require much in the way of stressful events to trigger depression. As a result, the association between stressful events and depression is weaker for genetically at-risk individuals. Evidence for this hypothesis is stronger. Based on a sample of 2395 twins who were interviewed up to four times, Kendler *et al*. (2001*a*) reported that the decline in association between stressful events and major depression with increasing number of previous episodes of depression was strongest in low genetic risk individuals as opposed to weak in individuals at high genetic risk.

In terms of causal models, Kendler & Karkowski-Shuman (1997) have proposed two pathways. The first is a direct pathway from genetic risk, to major depression, to SLEs, in what it is more commonly referred to as a 'scar' effect. The second is a diathesis model, whereby liability to major depression and exposure to SLEs

are caused by a third underlying trait, such as neuroticism. Based on a community sample of 2150 sib pairs, 410 trios and 81 quads, Rijsdijk *et al.* (2001) have found that correlations between neuroticism and adverse life events are low and that there appears to be little interaction between neuroticism and adverse life events in the prediction mood and affect as measured by the General Health Questionnaire (Rijsdijk *et al.* 2001). Recently, however, Kendler *et al.* (2004) examined data from 7500 individual twins and found an interaction between neuroticism and adversity, such that individuals with high neuroticism are at greater overall risk for major depression, and are more sensitive to the depressogenic effects of SLEs. The same study also found an interaction between adversity and sex, such that the excess risk for major depression in women was confined to individuals with low stress exposure.

Unlike Caspi *et al.* (2003), we found no significant sex differences in terms of the overall number of personal and network SLEs. This is consistent with previous findings ( Kendler *et al.* 2001*b*). When based on the entire 1988–1990 sample, we again found no sex differences in the overall distribution of personal events ($\chi^2_4 = 5{\cdot}95$, $p = 0{\cdot}20$). It was only at the item level that sex differences emerged. Women were more likely to endorse items such as separation from loved ones or close friends. Men, on the other hand, were more likely to experience frequent difficulties at work. For network events, the sex difference was significant with women reporting a greater number ($\chi^2_4 = 12{\cdot}58$, $p < 0{\cdot}05$) and at the item level, women more frequently endorsed network events relating to serious illness/injury of children, mother or father dying, co-twins or other relatives with a serious illness/injury/crisis, and someone close experiencing a personal crisis.

## Strengths and limitations

It is important to interpret our study in the context of its strengths and limitations. In terms of strengths, the 1099 subjects with complete genotyping, SLE data and psychiatric assessments was slightly larger than the sample of 1037 subjects reported by Caspi *et al.* (2003). The SSAGA was administered by trained lay interviewers and is itself a highly reliable and validated instrument for diagnosing psychiatric disorders (Bucholz *et al.* 1994; Hesselbrock *et al.* 1999). We also assessed a wide range of SLEs which were largely identical to those used by Caspi and colleagues.

Eight limitations are noteworthy. First, the age range of our sample was much broader than that reported by Caspi and colleagues. Their subjects were all aged 26 years. Although the regression of age onto the clinical and ordinal measures of depression revealed no significant main effects, the current age distribution may have diminished our power to detect an interaction, including important age differences in the aetiology of depression. As mentioned previously, early onsets of depression may be more influenced by G × E interactions than late-onset episodes. If, for comparability with Caspi *et al.* (2003), we limited our analyses to subjects below the age of 30 years, the sample size was greatly reduced ($n = 244$) and no G × E interaction was observed. G × E interactions are notoriously hard to detect in any free-living species. Even with the specification of a measured genotype (Martin & Oakeshott, 1983; Eaves & Sullivan, 2001), a measured environment (Heath *et al.* 1998; Boomsma *et al.* 1999), or both (Martin *et al.* 1987; Caspi *et al.* 2003), the power remains poor. In order to increase power to detect a G × E interaction we plan to genotype an additional 2600 subjects for the 5-HTTLPR genotype.

Second, Caspi *et al.* (2003) reported on life events over a 5-year period. The distribution of personal stressors in our sample was markedly different from that reported by Caspi *et al.* Our data relied on life events reported over a 12-month period. Consequently, it is not surprising that they found a higher prevalence of subjects who reported four or more SLEs, whereas the majority of subjects in our sample reported no stressful events. Although there are no comparable data to determine whether our sample of twins was representative of the general population in terms of the prevalence of SLEs, a comparison with a previous study of life events over 12 months based on the Virginia Twin Registry, based on a sample of 4630 twins, found that the distribution of item responses was, in fact, similar (Kendler *et al.* 1993*b*). Dividing our sample into three age cohorts shows a similar distribution of total life events in each age group (results not shown).

Third, we were unable to derive a continuous symptom count based on the nine diagnostic items for major depression used in the 1992 SSAGA interview. The protocol of the structured telephone interview skipped over the criterion A symptoms 3–9 if symptoms 1 and 2 were not endorsed. As an alternative, we used the 9-item self-report depression scale administered as part of the 1988–1990 survey. It was inappropriate to run OLS regressions because of the severe skewness. Therefore, ordinal regressions were run instead, and again there was no evidence of a G × E interaction.

Fourth, our regression analyses failed to take account of the non-independence of twin subjects. This can lead to overestimation of significance of regression terms. However, since none of the regression terms of interest even approached significance on the assumption of independence, this refinement, which is readily implemented in STATA (StataCorp, 1999) or Mx was uncalled for.

Fifth, our failure to replicate a G × E interaction may be related to the analyses used. Kendler *et al.* (1995) used a logistic regression and found no evidence of an interaction between SLEs and genetic risk when predicting the onset of major depression. This was despite the fact that the depressogenic effect of SLEs was much greater for persons already at high genetic risk for depression which is consistent with a model for genetic control of sensitivity to the environment (Kendler *et al.* 1995). When the authors reanalysed their data using regressions based on the scale of simple probabilities (*versus* logarithmic) they found a significant G × E interaction.

The sixth limitation concerns the sampling procedure. One of the goals of the SSAGA survey was to acquire blood samples from as many of these twins as possible. Although the genotyped twins were primarily selected on the basis of willingness to cooperate, there was preferential selection for complete pairs. Given the study's primary objectives, some effort was also made to enrich this sample for cases with alcohol dependence, but this was only moderately successful. The prevalence of DSM-IV alcohol dependence was higher amongst the genotyped female respondents ($\chi^2_1 = 7{\cdot}10, p < 0{\cdot}01$), whereas for males, there were no significant differences between the genotyped and ungenotyped samples ($\chi^2_1 = 0{\cdot}66, p = 0{\cdot}42$).

Seventh, there were also small but significant age differences between the genotyped and ungenotyped samples. Of the 5999 psychiatric interviews conducted as part of the SSAGA study, date of birth was available from 5876 subjects. The genotyped male and female subjects were approximately $1\frac{1}{2}$ years younger than the ungenotyped SSAGA subjects. This is not surprising, since to be bled they had to healthy and alive.

If the non-significant sex differences in self-report depression are related to the genotyping selection procedure, we are unable to explain why the sex differences for major depression persist. As shown in Table 1, the lifetime prevalence of DSM-IV major depression remained significantly higher among females compared to males, and approached significance for diagnoses made before the 12-month reporting period for SLEs.

The final limitation concerns our use of the total number of life events. Future replications will also need to pay closer attention to the contextual aspects and putative independence of life events. Contextual threat can be rated in terms of how most people would be expected to feel about a stressful event, whereas independence measures the probability that a life event is caused by an individual's own behaviour (Kendler *et al.* 1999). When event severity is controlled for, dependent events appear to be more strongly associated with major depression than independent events (Kendler *et al.* 1999). Another study has found that the severity of SLEs is positively and significantly correlated with the number of previous depressive episodes (Kendler *et al.* 2000). In a more recent study of life events rated in terms of contextual threat, diagnoses of major depression and mixed depression and generalized anxiety are predicted by higher ratings of loss and humiliation (Kendler *et al.* 2003). This is in contrast to diagnoses of generalized anxiety which are better predicted by higher ratings of loss and danger (Kendler *et al.* 2003).

## CONCLUSION

The number of SLEs was significantly associated with continuous and categorical measures of depression. Regardless of whether our results were based on binary logistic or ordinal regression analyses, we found no evidence to support a main effect of 5-HTTLPR or an

interaction between the 5-HTTLPR genotype and SLEs on major depression. It is possible that the effect reported by Caspi and colleagues is specific to young people (in their twenties), in which case our study has much less power in this age group. Our results do not support such an interaction as a lifelong phenomenon.

## ACKNOWLEDGEMENTS

This work as supported by NIH grants AA07535, AA07728, AA10249, AA11998, MH-31302 and NHMRC (Australia) grant nos. 941177 and 971232, and from 'beyondblue: the national depression initiative' (Australia). We thank the staff from QIMR Genetic Epidemiology Unit for administering the mail-out, the telephone interview and for data management, and the twins from the Australian NHMRC Twin Registry for their cooperation.

## DECLARATION OF INTEREST

None.

## REFERENCES

**Baker, L. A., Treloar, S. A., Reynolds, C. A., Heath, A. C. & Martin, N. G.** (1996). Genetics of educational attainment in Australian twins: sex differences and secular changes. *Behavior Genetics* **26**, 89–102.

**Bebbington, P. E., Tennant, C. & Hurry, J.** (1981). Adversity and the nature of psychiatric disorder in the community. *Journal of Affective Disorders* **3**, 345–366.

**Bedford, A., Foulds, G. A. & Sheffield, B. F.** (1976). A new personal disturbance scale (DSSI/sAD). *British Journal of Social and Clinical Psychology* **15**, 387–394.

**Bolinskey, P. K., Neale, M. C., Jacobson, K. C., Prescott, C. A. & Kendler, K. S.** (2004). Sources of individual differences in stressful life event exposure in male and female twins. *Twin Research* **7**, 33–38.

**Boomsma, D. I., de Geus, E. J., van Baal, G. C. & Koopmans, J. R.** (1999). A religious upbringing reduces the influence of genetic factors on disinhibition: evidence for interaction between genotype and environment on personality. *Twin Research* **2**, 115–125.

**Brown, G. W. & Harris, T. O.** (1978). *Social Origins of Depression: A Study of Psychiatric Disorder in Women*. Free Press: New York.

**Brugha, T., Bebbington, P., Tennant, C. & Hurry, J.** (1985). The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat. *Psychological Medicine* **15**, 189–194.

**Bucholz, K. K., Cadoret, R., Cloninger, C. R., Dinwiddie, S. H., Hesselbrock, V. M., Nurnberger Jr., J. I., Reich, T., Schmidt, I. & Schuckit, M. A.** (1994). A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *Journal of Studies on Alcohol* **55**, 149–158.

**Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. & Poulton, R.** (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389.

**Derogatis, L. R., Lipman, R. S. & Covi, L.** (1973). SCL-90: an outpatient psychiatric rating scale – preliminary report. *Psychopharmacology Bulletin* **9**, 13–28.

**Eaves, L. J. & Sullivan, P.** (2001). Genotype-environment interaction in transmission disequilibrium tests. *Advances in Genetics* **42**, 223–240.

**Foley, D. L., Neale, M. C. & Kendler, K. S.** (1996). A longitudinal study of stressful life events assessed at interview with an epidemiological sample of adult twins: the basis of individual variation in event exposure. *Psychological Medicine* **26**, 1239–1252.

**Gillespie, N., Kirk, K. M., Heath, A. C., Martin, N. G. & Hickie, I.** (1999). Somatic distress as a distinct psychological dimension. *Social Psychiatry and Psychiatric Epidemiology* **34**, 451–458.

**Heath, A. C., Cloninger, C. R. & Martin, N. G.** (1994). Testing a model for the genetic structure of personality: a comparison of the personality systems of Cloninger and Eysenck. *Journal of Personality and Social Psychology* **66**, 762–775.

**Heath, A. C., Eaves, L. J. & Martin, N. G.** (1998). Interaction of marital status and genetic risk for symptoms of depression. *Twin Research* **1**, 119–122.

**Hesselbrock, M., Easton, C., Bucholz, K. K., Schuckit, M. & Hesselbrock, V.** (1999). A validity study of the SSAGA – a comparison with the SCAN. *Addiction* **94**, 1361–1370.

**Kendler, K. S. & Eaves, L. J.** (1986). Models for the joint effect of genotype and environment on liability to psychiatric illness. *American Journal of Psychiatry* **143**, 279–289.

**Kendler, K. S., Heath, A., Martin, N. G. & Eaves, L. J.** (1986). Symptoms of anxiety and depression in a volunteer twin population. The etiologic role of genetic and environmental factors. *Archives of General Psychiatry* **43**, 213–221.

**Kendler, K. S., Hettema, J. M., Butera, F., Gardner, C. O. & Prescott, C. A.** (2003). Life event dimensions of loss, humiliation, entrapment, and danger in the prediction of onsets of major depression and generalized anxiety. *Archives of General Psychiatry* **60**, 789–796.

**Kendler, K. S., Karkowski, L. M. & Prescott, C. A.** (1999). Causal relationship between stressful life events and the onset of major depression. *American Journal of Psychiatry* **156**, 837–841.

**Kendler, K. S. & Karkowski-Shuman, L.** (1997). Stressful life events and genetic liability to major depression: genetic control of exposure to the environment? *Psychological Medicine* **27**, 539–547.

**Kendler, K. S., Kessler, R. C., Neale, M. C., Heath, A. C. & Eaves, L. J.** (1993*a*). The prediction of major depression in women: toward an integrated etiologic model. *American Journal of Psychiatry* **150**, 1139–1148.

**Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C. & Eaves, L. J.** (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. *American Journal of Psychiatry* **152**, 833–842.

**Kendler, K. S., Kuhn, J. & Prescott, C. A.** (2004). The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. *American Journal of Psychiatry* **161**, 631–636.

**Kendler, K. S., Neale, M., Kessler, R., Heath, A. & Eaves, L.** (1993*b*). A twin study of recent life events and difficulties. *Archives of General Psychiatry* **50**, 789–796.

**Kendler, K. S., Thornton, L. M. & Gardner, C. O.** (2000). Stressful life events and previous episodes in the etiology of major depression in women: an evaluation of the 'kindling' hypothesis. *American Journal of Psychiatry* **157**, 1243–1251.

**Kendler, K. S., Thornton, L. M. & Gardner, C. O.** (2001*a*). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *American Journal of Psychiatry* **158**, 582–586.

**Kendler, K. S., Thornton, L. M. & Prescott, C. A.** (2001*b*). Gender differences in the rates of exposure to stressful life events and sensitivity to their depressogenic effects. *American Journal of Psychiatry* **158**, 587–593.

**Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H. & Murphy,**

**D. L.** (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527–1531.

**Martin, N. G., Eaves, L. J. & Heath, A. C.** (1987). Prospects for detecting genotype × environment interactions in twins with breast cancer. *Acta Geneticae Medicae et Gemellologiae* (*Roma*) **36**, 5–20.

**Martin, N. G. & Oakeshott, J. G.** (1983). Is Pi a selectively balanced polymorphism? *Human Heredity* **33**, 24–28.

**Miller, S. A., Dykes, D. D. & Polesky, H. F.** (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**, 1215.

**Owens, M. J. & Nemeroff, C. B.** (1994). Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clinical Chemistry* **40**, 288–295.

**Rijsdijk, F. V., Sham, P. C., Sterne, A., Purcell, S., McGuffin, P., Farmer, A., Goldberg, D., Mann, A., Cherny, S. S., Webster, M., Ball, D., Eley, T. C. & Plomin, R.** (2001). Life events and depression in a community sample of siblings. *Psychological Medicine* **31**, 401–410.

**SSAGA** (2004). Semi-Structured Assessment for the Genetics of Alcoholism (http://www.niaaa.nih.gov/publications/ssaga-text.htm).

**StataCorp** (1999). Stata Statistical Software: Release 6.0. Stata Corporation: College Station, TX.

**Statham, D. J., Heath, A. C., Madden, P. A., Bucholz, K. K., Bierut, L., Dinwiddie, S. H., Slutske, W. S., Dunne, M. P. & Martin, N. G.** (1998). Suicidal behaviour: an epidemiological and genetic study. *Psychological Medicine* **28**, 839–855.

**Turker, T., Sodmann, R., Goebel, U., Jatzke, S., Knapp, M., Lesch, K. P., Schuster, R., Schutz, H., Weiler, G. & Stober, G.** (1998). High ethanol tolerance in young adults is associated with the low-activity variant of the promoter of the human serotonin transporter gene. *Neuroscience Letters* **248**, 147–150.