

ORIGINAL ARTICLE

The risk for depression conferred by stressful life events is modified by variation at the serotonin transporter 5HTTLPR genotype: evidence from the Spanish PREDICT-Gene cohort

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We report results from the PREDICT-Gene case-control study nested in a prospective cohort designed to identify predictors of the onset of depression among adult primary-care attendees. We tested the potential gene-by-environment interaction between 5HTTLPR genotype at the serotonin transporter gene and previous exposure to threatening life events (TLEs) in depression. A total of 737 consecutively recruited participants were genotyped. Additional information was gathered on exposure to TLEs over a 6-month period, socio-demographic data and family history of psychological problems among first-degree relatives. Diagnoses of depression were ascertained using the Composite International Diagnostic Interview (CIDI) by trained interviewers. Two different depressive outcomes were used (ICD-10 depressive episode and ICD-10 severe depressive episode). Both the s/s genotype and exposure to increasing number of TLEs were significantly associated with depression. Moreover, the 5HTTLPR s/s genotype significantly modified the risk conferred by TLEs for both depressive outcomes. Thus, s/s homozygous participants required minimal exposure to TLE (1 TLE) to acquire a level of risk for depression that was only found among l/s or l/l individuals after significantly higher exposure to TLEs (two or more TLEs). The interaction was more apparent when applied to the diagnosis of ICD-10 severe depressive episode and after adjusting for gender, age and family history of psychological problems. Likelihood ratios tests for the interaction were statistically significant for both depressive outcomes (ICD-10 depressive episode: LR $X^2 = 4.7$, $P = 0.09$ (crude), LR- $X^2 = 6.4$, $P = 0.04$ (adjusted); ICD-10 severe depressive episode: LR $X^2 = 6.9$, $P = 0.032$ (crude), LR- $X^2 = 8.1$, $P = 0.017$ (adjusted)).

Molecular Psychiatry (2007) 12, 748–755; doi:10.1038/sj.mp.4001981; published online 27 March 2007

Keywords: serotonin transporter gene polymorphism; SLC6A4; gene–environment interaction; affective disorders; social stress; primary care

Introduction

The causal processes underlying depression are yet to be identified but, undoubtedly, comprise both genetic and environmental components. One of the environmental risk factors consistently linked to depression is the exposure to stressful life events.^{1–4} From the genetic viewpoint, the serotonin transporter gene

(SLC6A4) that plays a key role in serotonergic neurotransmission is a candidate gene for depression. Moreover, its protein product is the central target for most antidepressant drugs. One of the polymorphisms described in the gene, the 5-HTTLPR, consists of an insertion/deletion polymorphism in the promoter region. Its short variant (s allele) reduces the transcriptional efficiency of the gene, resulting in decreased serotonin transporter expression in the neuron.⁵ Some association studies have reported an increased risk for depression among s/s genotype carriers,^{6–10} although others have reported negative results.^{11–13}

The interplay between genetic and environmental factors in the aetiology of common and complex

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Received 19 September 2006; revised 25 January 2007; accepted 6 February 2007; published online 27 March 2007

diseases has been well recognized,¹⁴ but the technology required to explore this relationship has only become available recently. Research on gene-by-environment interactions can improve our understanding of the aetiology of complex diseases, such as mental disorders by providing a more accurate estimation of population-attributable risks for genetic and environmental risk factors. Such research can also contribute to the design of preventative and therapeutic interventions for depression.¹⁵ Over the past 3 years, the interaction between the 5HTTLPR polymorphism and exposure to stressful life events has been under scrutiny. Animal research has shown a greater likelihood of depressive outcomes in macaques subjected to adverse rearing experiences who carry the risk allele of the rh5HTTLPR polymorphism.¹⁶ Moreover, imaging studies on humans have demonstrated amygdala hyperreactivity in response to fearful stimuli among s allele carriers compared to l/l individuals.¹⁷

Longitudinal data on people with one or two s alleles at the 5HTTLPR locus indicate that they are more vulnerable to depression than non-s allele carriers for the same level of exposure to stressful life situations.¹² Moreover, variations at the same locus modify the risk effect of developing depression in those maltreated in childhood.¹² These findings have been more recently replicated^{18–23} to include: a population-based adult twin study in which people with the s/s genotype were more vulnerable to the depressogenic effects of exposure to stressful life events;²¹ research on 101 children in whom the s/s genotype at the 5HTTLPR locus made them more susceptible to depression when they had experienced maltreatment and/or lack of social support²⁰ and a longitudinal follow-up of 127 people over 25 years in whom the s/s genotype was found to modify the effect of previous exposure to adverse life events as a risk factor for first onset of depression.²³

Some research has been conducted exclusively in women. Eley *et al.*¹⁸ compared 377 adolescents girls categorized by scores on the self-reported short form of the Mood and Feelings Questionnaire, and found that the risk of social environmental factors was higher among carriers of short (s) alleles. Sjöberg *et al.*²² reported similar findings in female adolescents, but showed the opposite effects in male adolescents (i.e., male s allele carriers were less likely to develop depression after being exposed to risky environmental factors). There have also been two larger studies that have failed to replicate the gene \times environment interaction. The first study was conducted on an adult cohort of 1206 twins,²⁴ and the other was a 1-year follow-up of 4175 people.²⁵

Overall, the strongest evidence is in favour of the effect of an interaction between 5HTTLPR and social distress.²⁶ However, to date research has been limited by selective sample studies (i.e., children, adolescents or twins) and the use of non-standard depressive outcomes. There is, hence, an urgent need to replicate these findings in large representative adult popula-

tions on whom validated measures of depression have been used. The PREDICT-Gene study tests the hypothesis that polymorphic variation at the 5HTTLPR locus interacts with social adversity (exposure to stressful life events), modifying the risk for depression in a Spanish population of primary-care attendees.

Materials and methods

Design

The PREDICT-Gene study⁹ is a case-control association study nested in a cohort of Spanish participants who were part of a larger study on prediction of onset of depression in European primary-care attendees (PREDICT study). A detailed description of the PREDICT study design and its method has been reported elsewhere.²⁷ In brief, the PREDICT study is a 1-year prospective study assessing consecutive general practice attendees at 0 (time-1), 6 (time-2) and 12 months (time-3). Only cross-sectional (time-1) data are used in this analysis. Both PREDICT and the PREDICT-Gene studies were approved by the relevant research ethics committees.

Sample

Consecutive attendees to nine (two rural and seven urban) primary care centres in the area of Málaga (Spain), aged 18–75, were asked to participate between April 2003 and September 2004. The participant's family doctor asked his/her patient to take part, and time-1 interviews were undertaken by three trained researchers within 2 weeks of informed consent being provided. Attendees over 75 were excluded because of higher prevalence of cognitive impairment after that age. Participants unable to understand Spanish, as well as those with an organic mental disorder and/or any terminal illness, were also excluded. This genetic study was not a part of the original PREDICT study protocol, that aimed to construct a predictive model of depression for use by general practitioners. Consequently, at time-3, further informed consent was requested to obtain a biological sample for genetic analysis consisting of 10 cm³ of blood and/or up to 4-mouth swabs for saliva collection.

Independent measures

The PREDICT risk factor assessment was shown to have adequate test-retest reliability.²⁷ In brief, the risk factors for depression were either based on previously validated measures, concerned exposures (such as socio-demographic data) that are likely to be reported with a high degree of reliability, or (where new questions were developed, e.g., family history of psychological problems and living arrangements) were subjected to reliability testing at the outset of the study.

Social distress was measured using the List of Threatening Events.²⁸ This is a list including serious events shown to carry high degrees of contextual

threat. The list includes serious life-events, such as the death of a parent, spouse or child, the death of another relative, the onset of a serious illness or accident affecting a relative, a marital separation, the ending of a friendship or relationship, a serious problem with a close friend, neighbour or relative, a financial crisis, the theft or loss of an item of personal value, having troubles with the police or courts, loss of work through redundancy and loss of work through dismissal. Subjects were asked whether any of these events had occurred within the 6 months before the interview. For the purposes of the analysis, we divided participants into three levels of exposure to threatening life events (TLEs): Having had no TLE, having had just one TLE or having had two or more TLEs, over the 6-month period before the interview.

5HTTLPR genotype assays

DNA from both blood and saliva was obtained by standard procedures. The 5-HTTLPR polymorphism at SLC6A4 was genotyped in all samples. Amplification of genomic DNA was performed using 50 ng of DNA, 0.25 μM of each primer (forward: 5'-GGCGTTGCCGCT CTG AAT GCC-3' and reverse: 5'-CAGGGGAGATCC TGG GAG AGG T-3'), 250 μM each of dATP, dCTP, dGTP and dTTP, 1.5 mM MgCl_2 , 50 mM KCl, 10 mM Tris-HCl and 0.3 units of DNA polymerase in a total volume of 25 μl . Samples were amplified for an initial cycle of 8 min at 95°C followed by 35 cycles each consisting of 30 s at 95°C, 30 s at 62°C and 1 min at 72°C. After amplification, genotypes were resolved by a 2% agarose gel electrophoresis and ethidium bromide staining.

Measures of depression

Six months prevalence of ICD-10 depressive episode (mild, moderate or severe) was ascertained using the depression section of the Composite International Diagnostic Interview (CIDI).²⁹ The CIDI was administered by trained lay interviewers. We tested our hypothesis by performing two sets of analyses. In the first, we used ICD-10³⁰ depressive episode of any severity as our depression outcome, and in the second we repeated the analyses only in those with an ICD-10 depressive episode of severe intensity.

Data quality control

Data quality was monitored to ensure that the project yielded data of the highest validity and reliability. The Spanish version of the PREDICT protocol was translated from English and then back translated by professional translators before the coordinating centre in London finally verified no major discrepancy in any back-translation. At a local level, each interview was checked for completion by each interviewer, all of whom had previously undergone a standardized training on administering the CIDI and the risk factor questionnaire, recruitment and interviewing of patients and data management. A Spanish research coordinator made two assessments of each interviewer during the time-1 baseline interviews to

monitor the adequacy of the interview and tackle any problems as they arose. Before transferring data to the coordinating centre, data quality control sheets were used and progress reports were submitted every 6 months to allow critical assessment by the PREDICT study steering group during regular project management meetings. Ten per cent of data were double entered which revealed an error rate of only 0.13%.

Statistical analyses

The data were analysed using the STATA 9.0 statistical package.³¹ An initial exploratory analysis was performed to study the distribution of both independent and dependent variables. Univariable associations were explored, using parametric or non-parametric significance tests as appropriate. Bivariable risks were estimated using classical stratified analysis. Using a multivariable logistic regression analysis, odds ratios with 95% confidence intervals for depression were calculated across 5HTTLPR genotype categories (s/s vs l/s and/or l/l) and also across the three levels of exposure to previous TLE. Finally, using a logistic regression model, we tested the interaction between the genetic (5HTTLPR genotype) and the environmental (exposure to TLEs) factors, both crudely and then after adjustment for sex, age and family history of psychological problems among first-degree relatives. We calculated probabilities for depression across all combinations of levels of exposure to TLE by 5HTTLPR genotype (s/s vs l/s or l/l). Crude and adjusted probabilities for depression across strata were also calculated. Finally, likelihood ratios tests for both differences of probabilities between such strata and for the genetic by environment interaction were also estimated.

Results

The sample

A total of 737 (80%) out of 922 participants at time-3 gave informed consent to be included in the PREDICT-Gene genetic study and provided a biological sample for genetic testing ($n=737$). The sample's mean age was 49 years (s.d. = 15.2). Five hundred and twenty-nine participants were women (71.8%) and 208 were men (21.2%). Most participants were married or living with a partner (71%), had primary (60%) or secondary (33.6%) schooling as their highest educational level and were working either in (30%) or away (30.3%) from home, whereas 15.9% were retired and 5.2% were unemployed. 36.6% of the sample had a positive family history of any psychological disorder amongst at least one first-degree relative. Participants who agreed to take part in the genetic analysis did not vary systematically, in terms of sex (female gender: 74 vs 71%, $X^2=0.47$, $P=0.49$), mean age (49.18 vs 50 years, Student's $t=0.87$, $P=0.38$), marital status (unmarried 33 vs 29%, $X^2=4.37$, $P=0.49$) or prevalence of ICD-10 depressive episode (35.4 vs 34.7%, $X^2=0.021$, $P=0.88$), from those who refused to give a genetic sample. Nor were there any

significant differences on these variables between participants in the genetic study and those who participated in the initial baseline assessments (mean age 49.18 vs 49.02 years, Student's $t=0.18$, $P=0.85$; female gender 74 vs 71.8%, $X^2=1.59$, $P=0.020$; being unmarried 33 vs 31%, $X^2=4.37$, $P=0.49$; prevalence of ICD-10 depressive episode (35.4 vs 33.4%, $X^2=0.72$, $P=0.39$).

Independent variables frequencies

Demographic, genotypic and phenotypic data on the sample are provided in Table 1. Summarizing, one in four participants had not experienced any TLE in the previous 6 months, and of the rest, about half had reported at least one TLE and the other half at least two or more TLEs. Just over a half of participants had the l allele, whereas the rest had the s allele. Approximately half the participants had the s/l genotype, a quarter had the l/l genotype and the remaining quarter had the s/s genotype (see Table 1 for details). Genotype frequencies were in Hardy-Weinberg equilibrium, both in cases and controls.

Associations with depression

The 6-month prevalence of an ICD-10 depressive episode was 35.4% (262) and that of ICD-10 severe depressive episode was 25.4% (183) (see Table 1). Table 2 shows that depression was associated with the 5HTTLPR s/s genotype, as reported in detail elsewhere.⁹ In brief, the association between the s/s genotype and depression was independent of age, sex, family history of psychological problems among first-degree relatives and GAD, but these associations were stronger for more severe depressive episodes. Both outcomes of depression were strongly and independently associated with previous exposure to TLE with initial crude associations remaining robust after adjusting for age, gender, marital status, education and family history of psychological problems (Table 2). Conversely, depression was not associated with marital status, professional situation, living arrangement or educational level in this sample.

5HTTLPR genotype interaction with threatening life experiences

The 5HTTLPR polymorphism significantly modified the risk effect for depression conferred by an increasing level of exposure to TLE (Table 3 and Figure 1). The interaction reached a higher level of significance when the TLE effect on depression in s/s genotype carriers was compared with the other two genotypes combined (l/l or l/s) (Table 3) and when only severe depression was considered. On adjustment, age did not modify the results and was hence excluded from the explanatory models. Finally, the interaction was stronger for both depressive outcomes after adjusting for gender, age and family history of psychological problems amongst first-degree relatives (Table 3 and Figure 1).

Table 1 Summarized frequencies of independent variables and depressive outcomes

Socio-demographic variables			
<i>Gender</i>			
	Female	529	(71.8%)
	Male	208	(28.2%)
<i>Mean age</i>			
		49.05 years	(s.d. 15.21)
<i>Education</i>			
	Illiterate	24	(3.3%)
	Primary	443	(60.1%)
	Secondary or higher	270	(36.6%)
<i>Marital status</i>			
	Married/couple	522	(70.8%)
	Single	126	(17.1%)
	Other	89	(12.1%)
<i>Profession</i>			
	Housekeeping	221	(29.9%)
	Working	223	(30.2%)
	Disabled/retired	220	(29.8%)
	Other	73	(10.1%)
<i>Living arrangements</i>			
	Alone	38	(5.2%)
	Other	699	(94.8%)
Frequencies of depression outcomes			
<i>ICD-10 depressive episode</i>			
	Depressed	262	(35.4%)
	Not depressed	475	(64.6%)
<i>ICD-10 severe depressive episode</i>			
	Depressed	183	(24.8%)
	Not depressed	475	(64.5%)
	Excluded from the analyses	79	(10.7%)
Independent variables			
<i>5HTTLPR genotypes</i>			
	<i>l/l</i>	192	(26%)
	<i>l/s</i>	367	(50%)
	<i>s/s</i>	178	(24%)
<i>Exposure to threatening experiences</i>			
	<i>No TLE</i>	191	(26%)
	<i>1 TLE</i>	266	(36%)
	<i>2 TLEs</i>	280	(38%)
<i>Family history of psychological problems amongst first-degree relatives</i>			
	<i>FH+</i>	270	(36.6%)
	<i>FH-</i>	467	(63.4%)

Abbreviations: FH, family history; s.d., standard deviation; TLE, threatening life events.

Discussion

Summary of results

Our main findings are that the 5HTTLPR s/s genotype and exposure to increasing numbers of TLEs were independently associated with depression, and that

Table 2 Associations between depression and genetic or environmental factors

	ICD-10 depressive episode			ICD-10 severe depressive episode		
	Cases	Controls	Adjusted* OR (95% CI), P	Cases	Controls	Adjusted* OR ^a (95% CI), P
<i>Genotypes</i>						
l/l	64 (24)	129 (27)	1.0 (reference)	42 (23)	129 (27)	1.0 (reference)
l/s	120 (46)	246 (52)	0.8 (0.6–1.4), <i>P</i> =0.8	85 (46)	246 (52)	1.0 (0.6–1.6), <i>P</i> =0.9
s/s	77 (30)	101 (21)	1.5 (0.9–2.3), <i>P</i> =0.06	56 (31)	101 (21)	1.7 (1.0–2.7), <i>P</i> =0.03
<i>Homozygous s/s</i>						
l/*	185 (70)	374 (79)	1.0 (reference)	127 (69)	375 (79)	1.0 (reference)
s/s	77 (30)	101 (21)	1.5 (1.1–2.2), <i>P</i> =0.015	56 (31)	101 (21)	1.6 (1.1–2.4), <i>P</i> =0.011
<i>Alleles</i>						
l	248 (47.5)	503 (52.8)	1.0 (reference)	169 (46)	484 (53)	1.0 (reference)
s	274 (52.5)	449 (47.2)	1.24 (1.0–1.5), <i>P</i> =0.05	197 (54)	432 (47)	1.3 (1.0–1.7), <i>P</i> =0.031
<i>Family history</i>						
Negative	140 (53)	327 (69)	1.0 (reference)	89 (49)	148 (31)	1.0 (reference)
Positive	122 (47)	148 (31)	1.9 (1.4–2.6), <i>P</i> =0.0001	94 (51)	327 (69)	2.1 (1.4–2.6), <i>P</i> =0.0001
<i>Threatening life events</i>						
No	50 (19)	141 (30)	1.0 (reference)	31 (17)	141 (30)	1.0 (reference)
1	80 (31)	186 (39)	1.2 (0.8–1.8)	51 (28)	186 (39)	1.2 (0.7–2)
2 or more	132 (50)	148 (31)	2.5 (1.6–3.7), <i>P</i> =0.0001 ^b	101 (55)	148 (31)	3.1 (1.9–4.9), <i>P</i> =0.0001 ^b

Abbreviations: CI, confidence interval; OR, odds ratio.

*Adjusted by age, gender, family history of psychological problems and presence of generalized anxiety disorder.

^aOdds ratio for each increasing level of exposure.

^bCrude associations that remained robust after adjusting for age, gender, marital status, education and family history of psychological problems.

the 5HTTLPR s/s genotype significantly modified the risk conferred by TLEs for both depressive outcomes. Thus, s/s homozygous participants required minimal exposure to TLE (1 TLE) to acquire a level of risk for depression, whereas l/s or l/l individuals required higher exposure to two or more TLEs. This interaction was more apparent for people with an ICD-10 diagnosis of severe depression and after adjustment for gender, age and family history of psychological problems.

Study design and limitations

A case-control study nested in a cohort study with retrospective environmental and genetic data is an appropriate design to examine the gene–environment interaction hypothesis¹⁵ However, such studies may be limited by selection, recall and/or survivor bias. In this study, these biases were minimized by sampling a representative population of general practice attendees. In addition, no significant differences were found on socio-demographic factors, and the level of depression between the participants included in our genetic analyses with those who refused a genetic specimen or were lost to follow-up at time-3.⁹ We did not use the newly reported 5HTTLPR reclassification procedure by additionally genotyping the sample for the so-called A/G variant,³² which may imply a potential limitation to this study. However, some

authors have posed that reclassification of subjects using such new polymorphism seem to render comparable results to the well-established method used by us.³³

Novelty and interest

We aimed to replicate previous findings in which variation at the 5HTTLPR locus modified the risk effect for depression conferred by previous exposure to stressful life events.^{12,18–23} It was important to do this as although, the earliest report on the gene–environment interaction was conducted on an adult sample,¹² most of the other studies were restricted to populations, such as women but not men,^{18,19,22} younger people,^{18,20,22} twins²¹ or people with affective disorders.³⁴ Our study used consecutive primary-care adult attendees and hence constitutes the first representative population-based replication of the earliest research. To our knowledge, it is also the first study to examine genetic–environment interaction in a homogeneous Spanish population in whom genotype frequencies^{7,10} and prevalences of exposure to TLEs^{3,28} were similar to most other European populations. Lastly, it is the first study to take account of potential confounders, such as age, gender and family history of psychological problems among first-degree relatives.

Depressive phenotypes

ICD-10 research criteria³⁰ do not consider the impact of depressive symptoms on daily living activities

Table 3 5HTTLPR genotype interaction with threatening life experiences

	Adjusted* probability (s.e.)	Adjusted* OR (95% CI), P
<i>ICD-10 depressive episode</i>		
<i>l/l or l/s</i>		
No TLE	0.22 (0.19)	1.0 (reference)
1 TLE	0.23 (0.17)	1.0 (0.6–1.7), P=0.8
2*TLE	0.46 (0.14)	3.0 (1.8–4.8), P=0.001
<i>s/s</i>		
No TLE	0.30 (0.33)	1.0 (reference)
1 TLE	0.46 (0.25)	2.0 (0.8–4.5), P=0.10
2*TLE	0.46 (0.25)	2.0 (0.8–4.5), P=0.09
LR test for interaction: LR $X^2=6.4$, P=0.04 (adjusted)*		
<i>ICD-10 severe depressive episode</i>		
<i>l/l or l/s</i>		
No TLE	0.14 (0.24)	1.0 (reference)
1 TLE	0.14 (0.21)	0.9 (0.5–1.8), P=0.9
2*TLE	0.39 (0.15)	3.9 (2.2–6.7), P=0.001
<i>s/s</i>		
No TLE	0.21 (0.38)	1.0 (reference)
1 TLE	0.39 (0.27)	2.4 (0.93–6.0), P=0.068
2*TLE	0.39 (0.27)	2.4 (0.9–6.2), P=0.06
LR test for interaction: LR $X^2=8.1$, P=0.017 (adjusted)*		

Abbreviations: LR, likelihood ratio; TLE, threatening life events.

*Adjusted by gender and family history of psychological problems amongst first-degree relatives.

in arriving at a diagnose of a depressive episode. Consequently, we performed parallel tests using both a broader depressive phenotype (i.e., ICD-10 depressive episode of any severity) and a narrower phenotype (i.e., only ICD-10 severe depressive episode). The results for the gene–environment interaction are more apparent when using the latter construct. This may indicate that ICD-10 severe depressive episode is a more specific depressive phenotype. It may also suggest that there could be a linear tendency for the reported gene–environment interaction to influence increasingly more intense depressive states. The definition of the depressive phenotype is crucial in tests of the gene–environment interactions and has been one of the major limitations of previous research on this topic.^{18,22,24,25}

The gene–environment interaction

Both crude and adjusted gene–environment interactions, on both depressive outcomes, show a non-linear effect of the risk conferred by TLEs for depression as a function of the 5HTTLPR genotype. Hence, among s/s individuals minimal levels of exposure to TLEs (from one onwards) confer a degree of risk for depression that is only reached by non-s/s individuals who have had higher levels of exposure (two or more). Thus, our results show an interaction that follows a step-wise pattern with an abrupt change, when comparing genotypes, at moderate levels (1 TLE) of exposure to TLEs (see Figure 1). We believe this may be partially owing to the intense threatening nature of the stressful life-events measured by the scale we used, where only seriously threatening stressful life events are recorded.²⁸ Caspi *et al.*¹² reported an interaction of 5HTTLPR genotype with a linear progression of exposure to life events, possibly because their measure of stressful life-events included a wide range of situations, including those with a lower severity and contextual threat than those used in our measure.³⁵ Our results are most similar to Kendler *et al.*²¹ and Wilhelm *et al.*,²³ who demonstrated a step-wise pattern for the interaction according to the

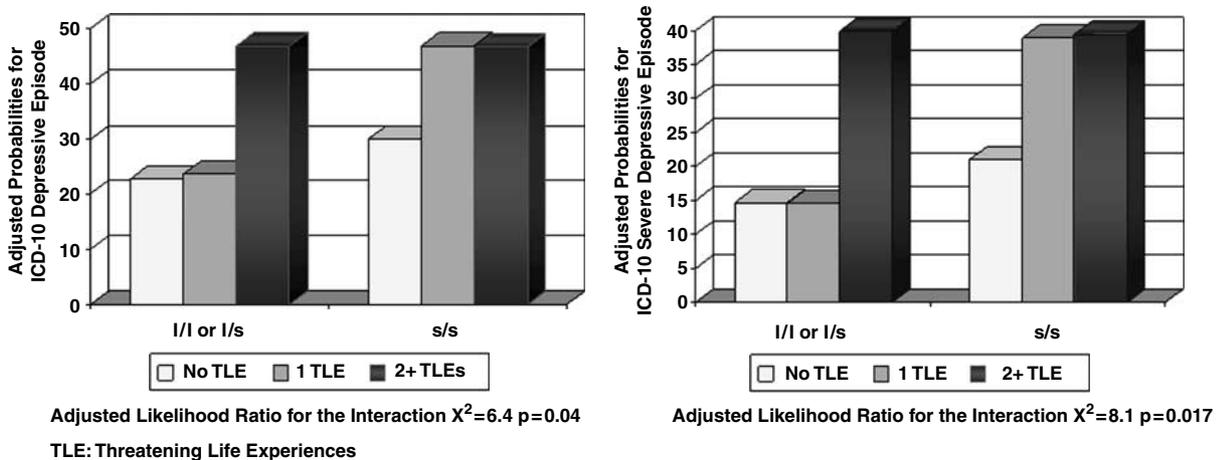


Figure 1 Adjusted s/s genotype by TLEs interaction effect on probabilities for depression.

level of threat conveyed by the stressful experiences. Although two independent, large studies have failed to replicate previous reports of this particular gene–environment interaction,^{24,25} their definitions of the depressive phenotypes examined may explain their findings. The study with the largest sample²⁵ used a self-report, potentially non-specific measure of both depression and life events. Moreover, the study was not designed to measure risk factors for depression, but was based on a cohort developed to investigate cancer and nutritional problems. The second study was also based on a self-report instrument for depression in a study of alcoholism that was adapted to identify cases of DSM-IV depression.²⁴ On the whole, our independent findings add to other positive studies that support the notion of a true 5HTTLPR by stressful life-events interaction first reported by Caspi *et al.*¹² and replicated by others.^{18–23,34}

Accounting for gender, age and family history

Our results show a somewhat better model fit after adjustment for potential confounders, such as gender and family history. Age had little impact. The relationship between gender and this particular gene–environment interaction is puzzling as some studies have reported it as valid for both sexes,^{20,21,23,34} whereas others suggest an effect only in women^{18,19} or even an inverse effect in men.²² We found no statistically significant differences in the reported gene–environment interaction when women were compared to men. Family history of psychological problems has been associated with both exposure³⁶ and outcome,³⁷ and thus should remain in the model. The independence from family history of our reported gene–environment interaction may suggest that there could be some specific role for the 5HTTLPR genotype (or the serotonin transporter gene) in its modification of the risk effect for depression conferred by previous TLEs. Nevertheless, there is a report for a different candidate gene for depression also interacting with stressful life experiences, although the sample studied was one of affective disorders sufferers with no controls.³⁴

In conclusion, our findings add further evidence, from a case-control study nested in a Spanish cohort of adult primary-care attendees, in favour of an effect modification by the 5HTTLPR genotype on the risk of depression conferred by previous exposure to stressful life-events.

Acknowledgments

We thank the PREDICT study Core Group members (Miguel Xavier, Igor Slav, Heidi-Ingrid Maaros, Jan Neelman, Francisco Torres-González, Irwing Nazareth and Michael King) for agreeing to include a genetic sub-study to their ongoing study. We also thank the three interviewers (Francisca Vidal, Nuria López and Ana Álvarez) and all nurses and general practitioners at all nine primary care centres in Málaga (Spain) for their collaboration and hard work in collecting most

of the data. This study was co-funded by the fifth Framework Program of the European Commission, a grant from the Ministry of Education and Science (SAF-2004-01310) and by I+D+I Grant from the Ministry of Education and Science SAF2006-07192.

References

- 1 Bebbington PE, Brugha T, MacCarthy B, Potter J, Sturt E, Wykes T *et al.* The Camberwell Collaborative Depression Study. I. Depressed probands: adversity and the form of depression. *Br J Psychiatry* 1988; **152**: 754–765.
- 2 Brown GW, Andrews B, Harris T, Adler Z, Bridge L. Social support, self-esteem and depression. *Psychol Med* 1986; **16**: 813–831.
- 3 Cervilla JA, Prince MJ. Cognitive impairment and social distress as different pathways to depression in the elderly: a cross-sectional study. *Int J Geriatr Psychiatry* 1997; **12**: 995–1000.
- 4 Farmer A, Harris T, Redman K, Sadler S, Mahmood A, McGuffin P. Cardiff depression study. A sib-pair study of life events and familiarity in major depression. *Br J Psychiatry* 2000; **176**: 150–155.
- 5 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1531.
- 6 Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES *et al.* Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. *Am J Med Genet* 1998; **81**: 58–63.
- 7 Gutierrez B, Pintor L, Gasto C, Rosa A, Bertranpetit J, Vieta E *et al.* Variability in the serotonin transporter gene and increased risk for major depression with melancholia. *Hum Genet* 1998; **103**: 319–322.
- 8 Lotrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatr Genet* 2004; **14**: 121–129.
- 9 Cervilla JA, Rivera M, Molina E, Torres-Gonzalez F, Bellón J, Moreno B *et al.* The 5HTTLPR genotype at the serotonin transporter gene (SLC6A4) increases the risk for depression in a large cohort of primary care attendees: The PREDICT-Gene Study. *Am J Med Genet B Neuropsychiatr Genet* 2006; **141B**: 912–917.
- 10 Collier DA, Stober G, Li T, Heils A, Catalano M, DiBella D *et al.* A novel functional polymorphism within the promoter of the serotonin transporter gene: Possible role in susceptibility to affective disorders. *Mol Psychiatry* 1996; **1**: 453–460.
- 11 Anguelova M, Benkelfat C, Turecki G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. *Mol Psychiatry* 2003; **8**: 574–591.
- 12 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**: 386–389.
- 13 Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. *Am J Med Genet B-Neuropsychiatr Genet* 2005; **133B**: 110–115.
- 14 Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. 1902 [classical article]. *Yale J Biol Med* 2002; **75**: 221–231.
- 15 Hunter DJ. Gene–environment interactions in human diseases. *Nat Rev Genet* 2005; **6**: 287–298.
- 16 Barr CS, Newman TK, Shannon C, Parker C, Dvoskin RL, Becker ML *et al.* Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biol Psychiatry* 2004; **55**: 733–738.
- 17 Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D *et al.* Serotonin transporter genetic variation and the response of the human amygdala. *Science* 2002; **297**: 400–403.
- 18 Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P *et al.* Gene–environment interaction analysis of serotonin system markers with adolescent depression. *Mol Psychiatry* 2004; **9**: 908–915.
- 19 Grabe HJ, Lange M, Wolff B, Volzke H, Lucht M, Freyberger HJ *et al.* Mental and physical distress is modulated by a polymorphism

- in the 5-HT transporter gene interacting with social stressors and chronic disease burden. *Mol Psychiatry* 2005; **10**: 220–224.
- 20 Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal JH *et al*. Social supports and serotonin transporter gene moderate depression in maltreated children. *Proc Natl Acad Sci USA* 2004; **101**: 17316–17321.
 - 21 Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch Gen Psychiatry* 2005; **62**: 529–535.
 - 22 Sjoberg RL, Nilsson KW, Nordquist N, Ohrvik J, Leppert J, Lindstrom L *et al*. Development of depression: sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. *Int J Neuropsychopharmacol* 2006; **9**: 1–7.
 - 23 Wilhelm K, Mitchell PB, Niven H, Finch A, Wedgwood L, Scimone A *et al*. Life events, first depression onset and the serotonin transporter gene. *Br J Psychiatry* 2006; **188**: 210–215.
 - 24 Gillespie NA, Whitfield JB, Williams B, Heath AC, Martin NG. The relationship between stressful life events, the serotonin transporter (5-HTTLPR) genotype and major depression. *Psychol Med* 2005; **35**: 101–111.
 - 25 Surtees PG, Wainwright NW, Willis-Owen SA, Luben R, Day NE, Flint J. Social adversity, the serotonin transporter (5-HTTLPR) polymorphism and major depressive disorder. *Biol Psychiatry* 2006; **59**: 224–229.
 - 26 Zammit S, Owen MJ. Stressful life events, 5-HTT genotype and risk of depression. *Br J Psychiatry* 2006; **188**: 199–201.
 - 27 King M, Weich S, Torres F, Svab I, Maaroos H, Neeleman J *et al*. Prediction of depression in European general practice attendees: the PREDICT study. *BMC Public Health* 2006; **6**: 6.
 - 28 Brugha TS, Cragg D. The List of Threatening Experiences: the reliability and validity of a brief life events questionnaire. *Acta Psychiatr Scand* 1990; **82**: 77–81.
 - 29 Robins LN, Wing J, Wittchen HU, Helzer JE, Babor TF, Burke J *et al*. The Composite International Diagnostic Interview. An epidemiologic Instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Arch Gen Psychiatry* 1988; **45**: 1069–1077.
 - 30 World Health Organisation. *ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research*, 1st edn. World Health Organisation: Geneva, 1993.
 - 31 STATA Statistical Software. *Release 9*. Stata Corporation: College Station, TX, 2006.
 - 32 Hu XZ, Lipsky RH, Zhu GS, Akhtar LA, Taubman J, Greenberg BD *et al*. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* 2006; **78**: 815–826.
 - 33 Parsey RV, Hastings RS, Oquendo MA, Hu XZ, Goldman D, Huang YY *et al*. Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. *Am J Psychiatry* 2006; **163**: 48–51.
 - 34 Mandelli L, Serretti A, Marino E, Pirovano A, Calati R, Colombo C. Interaction between serotonin transporter gene, catechol-O-methyltransferase gene and stressful life events in mood disorders. *Int J Neuropsychopharmacol* 2006; **7**: 1–11.
 - 35 Caspi A, Moffitt TE, Thornton A, Freedman D, Amell JW, Harrington H *et al*. The life history calendar: A research and clinical assessment method for collecting retrospective event-history data. *Int J Methods Psychiatr Res* 1996; **6**: 101–114.
 - 36 Jones I. Genetics of Affective Disorder. In: McGuffin P (ed). *Psychiatr Genet Genomics*. Cambridge University Press: London, 2004, pp 223–224.
 - 37 McGuffin P, Katz R, Bebbington P. The Camberwell Collaborative Depression Study. 3. Depression and Adversity in the Relatives of Depressed Proband. *Br J Psychiatr* 1988; **152**: 775–782.