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Do 5HTTLPR and Stress Interact in Risk for Depression and Suicidality? Item Response Analyses of a Large Sample

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The reported interaction between the length polymorphism (5HTTLPR) in the serotonin transporter gene (SLC6A4) and stressful life events on depression has led to many attempts to replicate but with inconsistent results. This inconsistency may reflect, in part, small sample size and the unknown contribution of the long allele SNP, rs25531. Using a large twin sample of 3,243 individuals from 2,230 families aged 18–95 years (mean = 32.3, SD = 13.6) we investigate the interaction between 5HTTLPR (subtyped with SNP rs25531) and stressful events on risk of depression and suicidality using both ordinal regressions and item response theory analyses. Participants reported via mailed questionnaire (82% response rate) both stressful events in the preceeding 12 months and symptoms of depression. Stressful events were defined as "personal" (affecting the individual), or "network" (affecting close family or friends). One to 10 years later (mean = 4.2 years), participants completed a comprehensive clinical psychiatric telephone interview (83% response rate) which assessed DSM-IV major depression and ideation of suicidality. Self-reports of depression and an increase in depression/suicidality assessed by clinical interview are significantly associated with prior personal events ($P < 0.001$) after controlling for age and sex. However, they are inconsistently associated with prior network events (ranging, ns to $P < 0.01$) and are not significantly associated with any of the genotype main effects (5HTTLPR, 5HTTLPR + rs25531) or interactions (stress × genotype). We find no evidence to support the hypothesis of any 5HTTLPR genotype by stress interaction.

Key words: rs25531; serotonin transporter; life events; suicidality; item response theory

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INTRODUCTION

Major depression (MD) is projected to become the world's second leading cause of disability by 2020 [Murray and Lopez, 1996]. Despite heritability estimates of MD ranging from 31% to 42% [Sullivan et al., 2000], few genetic variants have been discovered [Lopez-Leon et al., 2008]: one of the most studied is the length polymorphism repeat (LPR) in the promotor region of the serotonin transporter gene (5HTT renamed SLC6A4). The 5HTTLPR polymorphism comprises a 43-bp insertion or deletion (long, "L," or short, "S," alleles, respectively) [Nakamura et al., 2000; Hu et al., 2005, 2006; Kraft et al., 2005; Wendland et al., 2006]. The S allele reduces transcriptional efficiency, resulting in decreased SLC6A4 expression and 5HTT uptake in lymphoblasts [Lesch et al., 1996]. Meta-analyses show a small association between suicide and

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5HTTLPR [Li and He, 2007] and an inconsistent association between MD and 5HTTLPR with effects observed by some [Serretti et al., 2007; Lopez-Leon et al., 2008] but not others [Anguelova et al., 2003; Lasky-Su et al., 2005; Levinson, 2005].

Caspi et al. [2003] report that individuals who experience stressful life events (SLEs) have an increased risk of depression with each additional S allele, whereas for individuals who had never experienced SLEs, the S allele is not associated with depression. Despite support from animal studies [reviewed in Uher and McGuffin, 2008] attempts to replicate this G × E are also inconsistent. Large-scale studies that measure SLE and depression and collect DNA are costly in both time and money and their limited availability means most studies of this reported interaction are underpowered [Munafo et al., 2008]. Moreover, reviews of the same primary reports also reach different conclusions [Uher and McGuffin, 2008; Munafo et al., 2008]. Reported interactions comprise inconsistent modes of action (additive, S-dominant, and even L-dominant), crossover interactions and show evidence of publication bias [see also Willis-Owen et al., 2005; Uher and McGuffin, 2008; Munafo et al., 2008]. Munafo et al. [2008] conducted a meta-analysis of published results, but only five studies reported data in a format that allowed inclusion: they conclude "some claims of replication seem not to be justified" with the interactions observed compatible with chance findings. More convincingly, a meta-analysis of 14 studies to March 2009 using the primary data, where available, found no evidence of an interaction [Risch et al., 2009]. Of all the studies to date, only one [Tsai et al., 2003] has considered the a/g single-nucleotide polymorphism (SNP) rs25531 in G × E studies of 5HTTLPR. The SNP lies within the L allele of 5HTTLPR, and L alleles with the rarer "g" allele at rs25531 are functionally equivalent to the S allele because of changes to the transcription factor-binding site altered by this SNP [Hu et al., 2006; Wendland et al., 2006].

Statistical interaction is dependent on the scale of measurement and may not equate to biological interaction [Rothman et al., 1980] so that G × E can exist on the dichotomous disease scale even when none is present on the latent scale of disease risk [Eaves, 2006]. Item response theory (IRT) in combination with Markov Chain Monte Carlo (MCMC) estimation provides a flexible and efficient framework for modeling the underlying continuous liability to disease for behavioral phenotypes based on responses to multiple binary items in an interview framework [Reise and Waller, 2009]. Hence, IRT models the underlying scale, thereby controlling for interactions that are artefacts of scale [Kang and Waller, 2005], adding understanding to the interactions detected on the disease scale. In an earlier article, we used IRT analyses of 824 monozygotic (MZ) twin pairs to investigate the relationship between SLE and 5HTTLPR under the hypothesis that within pair variance would be greater for MZ twins homozygous for the S allele compared to those homozygous for the L allele if the interaction were real [Wray et al., 2008]. This novel design and analysis showed no evidence for an interaction, but if anything the trend in within pair variance was in the opposite direction to that expected.

Here we explore the association of 5HTTLPR genotype and its interaction with SLE on risk of depression or suicidality in a large sample of 3,243 individuals from 2,230 twin families, which include the 824 MZ pairs previously analyzed. Among the 14 attempts to replicate the original report [Munafo et al., 2008], only 1 [Surtees et al., 2006] has been larger (N = 4,175) than ours. The remainder had fewer than 1,100 subjects while five studies had fewer than 200. Munafo et al. [2008] investigated the power to detect 5HTTLPR interaction effects using simulation, varying the sizes of main and interaction effects, and concluded that samples of several thousand are needed. Our analyses are conducted (1) for the full data set and (2) for a data set restricted to replicate, as closely as possible, the data structure and analyses of Caspi et al. [2003]. In addition, we genotyped the rs25531 SNP to assess whether it further refines any association with 5HTTLPR. In human studies of this polymorphism's association with neurological activity both S-dominant [Lesch et al., 1996; Hranilovic et al., 2004; Hariri et al., 2005; Heinz et al., 2005] and additive [Bradley et al., 2005; Hu et al., 2006] models are observed, so we consider both here.

Analyses from a subsample (N = 1,091) of the present study have previously been reported [Gillespie et al., 2005]. Differences between the studies are detailed in the supplementary information but briefly, we have, (a) invested substantial effort to improve the genotyping (discussed in Materials and Methods Section), (b) have subtyped the L allele by rs25531, (c) increased the sample size threefold, and (d) improved the models and methods of analysis.

In criticizing the null finding from the earlier article from this group [Gillespie et al., 2005], Uher and McGuffin [2008] argue that our sample was recruited and selected for alcohol addiction: in fact, it is a volunteer population sample assessed on a range of indicators of physical and mental health that included addiction, depression, and SLE, and does not differ in these respects from the larger-base sample from which it was drawn [Heath et al., 1997]. However, Uher and McGuffin [2008] noted other valid limitations applicable to our study sample: that the interaction (i) may be age-dependent, occurring in younger adults, and (ii) may occur more in females. Further, the strongest effects of SLE on depression occur in the month immediately following the SLE, with the effect then decaying over several months [Kendler et al., 1998; Surtees and Wainwright, 1999]. We address these points by performing supplementary analyses that assess the specific hypotheses they raise.

MATERIALS AND METHODS

Samples

The participants comprised an older and younger cohort of twins (detailed in the Supplementary Information Table I supp.) from the Australian NHMRC Twin Register and are of predominantly (97%) Northern European ancestry. All provided written informed consent under study protocols approved by the Queensland Institute of Medical Research Human Research Ethics Committee. During the period 1988–1990 study participants were mailed an extensive Health and Lifestyle Questionnaire (HLQ) containing items addressing SLEs and depression (N = 16,154, response rate 82%). The symptoms of anxiety and depression in the older cohort are typical of the Australian population [Kendler et al., 1986], although the level of education completed is higher, particularly for males [Baker et al., 1996]. Over the period 1992–2000, participants were interviewed by telephone using a version of the SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism) modified for use in Australia (N = 16,302 response rate 83%), a comprehensive psychiatric interview assessing the physical, psychological, and

social manifestations of alcoholism and psychiatric disorders in adults [Bucholz et al., 1994, 1995; Hesselbrock et al., 1999] according to DSM-IV criteria [American-Psychiatric-Association, 1987]. Participants (N = 4,949) have subsequently provided a blood (or buccal/saliva in ~4% of cases) sample. Summary statistics characterizing the cohorts are provided in Table I supp. The "SSAGA" case–control sample in Wray et al. [2009] were unrelated individuals from the cohorts reported here. The 824 MZ twins used in the study of Wray et al. [2008] were also included in the present study. Study participants were middle-aged: mean = 32.3; SD = 13.6; range = 18–95 years; and were 60% female.

Genotyping

Both 5HTTLPR and rs25531 were genotyped using the assays described in Wray et al. [2009]. The assay for 5HTTLPR (which includes duplicate genotyping of each sample) was found to be more accurate than the original assays [Heils et al., 1996] which generate considerable bias towards S allele identification [e.g., Kaiser et al., 2002; Yonan et al., 2006]. Our data provided extensive opportunity for quality control checking: in total 6,607 DNA samples were genotyped (4,949 were the twins analyzed herein), of which 764 samples were duplicates (0.45% were discordant), 857 were from MZ twin pairs (0.16% were discordant), and some were from nuclear families [see Wray et al., 2009]. When only one individual from an MZ twin was genotyped, missing genotypes were imputed from the genotype of their co-twin: this recovered 264 and 280 genotypes for 5HTTLPR and rs25531, respectively. After exclusion of identifiable errors, genotyping call rates were 96.9% for 5HTTLPR and 95.4% for rs25531, somewhat lower than normal for our laboratory, but nonetheless much improved over the original assay for 5HTTLPR. In total, 1,232 individuals had been genotyped for the study reported by Gillespie et al. [2005] using the standard protocols available at the time. Of these, 16% had changed genotypes using the new assay. Most inconsistencies (35%) were for samples typed as SS by the earlier assay but SL by the improved assay [expected to be 22% by chance alone: the frequency of SS genotypes reported in Gillespie et al., 2005], consistent with observations of L allele dropout by others [Heils et al., 1996; Kaiser et al., 2002; Yonan et al., 2006].

For 5HTTLPR, the genotype frequencies were 19%, 49%, and 32% for SS, SL, and LL, respectively of 5HTTLPR, and for rs25531 these were 86% and 14% for L_aL_a and L_aL_g, respectively. The genotypes were combined as five two-locus genotypes: SS, SL_g, SL_a, L_gL_a, and L_aL_a, after removing S_g genotypes [Wray et al., 2009]. Based on the relative frequencies of SL_g (6%) and SL_a (43%) we estimate that only 0.9% of the total sample are expected to have genotype L_gL_g or 12% of those genotyped as L_gL_a. As the rare L_g alleles are functionally equivalent to S alleles [Hu et al., 2006; Wendland et al., 2006], we constructed a 5HTTLPR + rs25531 variable (N = 4,886) with three levels, (1) SS and SL_g (25%), (2) SL_a and L_gL_a (51%), and (3) L_aL_a (24%).

Measures

Clinical diagnosis of major depression (ClinDep). The depression items from the SSAGA telephone interview were prepared for ordinal regression and IRT analyses. The former used an ordinal scale of clinical depression (ClinDep) with four levels, the latter analyzed two gateway and seven symptom items (all yes/no): the details of both are presented in Table I. Prevalence rates for lifetime MD were 15% and 20% in males and females, respectively, as detailed in the Supplementary Information Table I supp. For all analyses, we then excluded participants whose onsets of ClinDep occurred before SLE reporting, removing 62% of the participants with subclinical depression (see Table I) or greater. This only left participants with onsets (which represented an increase from zero symptoms) that occurred only during or after SLE reporting.

Suicidality. The SSAGA telephone interview assessed lifetime history of suicide ideation, hereafter referred to as suicidality [Statham et al., 1998]. Details of both the ordinal scale used in the regression analyses and the IRT items (all yes/no) are presented in Table I. One or more items were endorsed by 25% of males and 24% of females. As with ClinDep, we exclude participants whose onsets of suicidality occur before SLE reporting.

Self report depression (SelfRepDep). The HLQ included 14 items from the Delusion Symptoms States Inventory [DSSI; Bedford et al., 1976] scales, and 19 from the 90-item Symptom Checklist [Derogatis et al., 1973] rephrased to match the DSSI: "Recently I have had . . ." rather than "In the past two weeks . . ." A factor analysis [see Gillespie et al., 1999] derived nine depression items (SelfRepDep) scored on a four-point scale: (0) "not-at-all," (1) "a little," (2) "a lot," (3) "unbearably." The IRT analyses treated these individually; the ordinal regressions used their summed score which was skewed so recoded to the four-point ordinal scale giving approximately equal frequencies: 0 = 0, 1 = 1, 2 = 2 or 3, and 3 = 4 or more.

Stressful life events (SLEs) experienced in the past 12 months. The HLQ included a total of 40 items in three SLE inventories (personal, social problems, and network) which were adapted from the List of Threatening Experiences (LTE) proposed by Brugha et al. [1985]. Based on a preliminary factor analysis, 19 items (personal and social problems) were summed to create a personal life events (PLE) factor and 11 items were summed creating a network life events (NLE) factor (see Table I). Frequency histograms are presented in Figure 1 supp. The average interval between the 12-month reporting window for SLE and the first onset of depression is 4.2 years (SD = 1.0; range 1.1–10.0) after the end of the reporting window. To allow our data to be included in future meta-analyses relevant summary statistics [Munafo et al., 2008] are presented in the Supplementary Information Table IV supp.

Statistical Analyses

Ordinal regression analyses. The ordinal regression models predicting the outcomes of ClinDep, suicidality, and SelfRepDep were as follows:

$$\begin{aligned}\text{Logit}(\text{outcome}) = {} & \beta_0 + \beta_1(\text{sex}) + \beta_2(\text{age}) \\ & + \beta_3(\text{PLE}) + \beta_4(\text{NLE}) \\ & + \beta_4(\text{genotype}) + \beta_5(\text{PLE} \times \text{genotype}) \\ & + \beta_6(\text{NLE} \times \text{genotype})\end{aligned}$$

where the β weights were in log(odds) units and sex was coded males = 0, females = 1. Analyses were run for two genotype

TABLE I. Each Measure, the Individual Items and Coding Used for the Ordinal Regression and Item Response Theory Analyses

Clinical depression (ClinDep)[a]	
Item response theory analyses	
Gateway items	1. Have you experienced two or more weeks of feeling depressed/down? 2. Have you experienced two or more weeks in which you were a lot less interested in most things or unable to enjoy the things usually enjoyed?
Postgateway items	1. Change in appetite/weight 2. Trouble with sleep 3. Fidgety/restless or talking/moving slowly 4. Loss of energy/tired 5. Feeling guilty/a bad person/a failure/worthless 6. Trouble thinking/concentrating/making decisions 7. Feeling suicidal
Ordinal regression analyses	
0 No depression	Those answering "no" to both of the gateway items
1 Subclinical depression	Those answering "yes" to at least one of the gateway items
2 DSM-IV major depression[b]	Those qualifying for a DSM-IV major depression diagnosis (MD)
3 Severe depression	Those qualifying for DSM-IV diagnosis and who were also either prescribed medication, hospitalized, or were completely unable to function for two or more days
Suicidality	
Item response theory analyses	
Gateway item	1. Have you had thoughts of suicide?
Postgateway items	1. Did these persist for more than a day? 2. Did you have a plan?
Gateway item	2. Have you attempted suicide?
Postgateway items	1. More than once? 2. Did you really want to die?
Ordinal regression analyses	
0	No thoughts
1	Thoughts of suicidality
2	Thoughts persisting for more than a day and/or a plan and attempts because feeling depressed
Stressful life events (SLE) experienced in the past 12 months	
Personal SLE (PLE) factor[b]	
12-item inventory of personal life events	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
7-item social problem inventory	Serious problems in relationships with a spouse, other family member, close friend, neighbor, someone living with them (e.g., child or elderly parent), their twin, or a workmate or co-worker
Network SLE (NLE) factor[b]	
21-item network life events (NLE) inventory	Had participant's close relative or friend, died, suffered a serious illness/injury, or suffered a serious personal crisis

The measures include clinical depression (ClinDep), suicidality, and stressful life events (SLE). Self-reported depression (SelfRepDep) is presented in the text.

[a]Participants were removed from the ordinal regression and IRT analyses if depression was associated with the DSM-IV diagnostic exclusion criteria of prescription medication, drugs, alcohol, bereavement, or illness. For a conservative DSM-IV diagnosis, cases with postpartum onset were also removed. Further, depression differs from that used in Gillespie et al. [2005] in that: (1) when depression/suicidality occurred before the reporting of SLE, we excluded participants rather than coding them as having no depression/suicide, and (2) our diagnoses were for *first* rather than *most severe* episode of depression/suicidality. This ensured individuals were excluded if depression (both *first* and *most* severe episodes) occurred before SLE reporting, since 11% with major depression experienced one or more episodes that occurred before their *most severe* episode.

[b]The factors comprised the sum of the individual items.

classifications, (1) 5HTTLPR and (2) 5HTTLPR + rs25531 (see above), where both additive (SL intermediate to SS and LL) and S-dominant (SS and SL combined) models were fitted. The interaction terms were centered for all analyses.

Except where noted (footnote to Table II supp.), the formal test of equal regression slopes for each threshold on the ordinal scales could not be rejected and confirmed the validity of the ordinal models. We report the $\exp^{\beta}$ or odds ratio (the odds of increasing one unit in the outcome variable given a one unit increase in the fitted term when all other fitted terms are held constant) and significance (two-tailed) according to the Wald statistic. The interdependence between twin pairs was ignored in the ordinal regressions, yielding anti-conservative tests of main effects and interactions.

Item response theory (IRT) analyses. In the ordinal regression analyses, interview questions (items) are summed to create ordinal scores for depression. The summing of items does not necessarily optimize the information in the items. In contrast, IRT models a normally distributed latent liability based on the responses to the individual questionnaire items. This allows the "difficulty" (endorsement probability) and "discrimination" (extent to which items discriminate persons across the latent continuum) of each item to be taken into account. A review of the advantages of IRT over classical test theory is provided by Reise and Henson [2003]. One advantage is that IRT analyses model missingness of item responses by interpolating from the rest of the data. Therefore, individuals who were excluded from the ordinal regression analyses could be retained (Table II).

The IRT models were similar to those used in Wray et al. [2008] but were adapted to accommodate dizygotic as well as MZ pairs. The data were entered as twin pairs to account for their interdependence. IRT models were analyzed in the WinBUGS (Bayesian Inference Using the Gibbs Sampler) program [Lunn et al., 2000]. After a burn-in phase of 4,000 iterations, the characterization of the posterior distribution for the model parameters was based on 8,000 iterations from two independent Markov chains. The independent variables were those used in the ordinal regressions.

Replication of Caspi et al. [2003]. Our full dataset, and the methods used to analyze it, depart in a number of respects from those in the original report by Caspi et al. [2003]. Therefore, we also attempt to replicate their findings by restricting our data set and analyses to match theirs as closely as possible. To do this, we restricted age at time of SLE reporting to 23–29, removing 80% of the participants, and used a model which included sex, PLE (truncated here to a maximum of five events, not seven), an additive model for 5HTTLPR genotypes, and a PLE × 5HTTLPR interaction.

Supplementary analyses to address specific hypotheses of Uher and McGuffin [2008]. We present, in the Supplementary Material, the Materials and Methods and Results Sections of the analyses that address the specific hypotheses of Uher and McGuffin [2008], including the table of results (Table II supp.).

TABLE II. Number of (A) Participants at Each Level of the Dependent Variables (Clinical Depression [ClinDep], Suicidality or Self-Report Depression [SelfRepDep]) and (B) Monozygotic (MZ) and Dizygotic (DZ) Incomplete and Complete Pairs Used for Analysis of 5HTTLPR

	ClinDep	Suicidality	SelfRepDep
(A) Ordinal regression analyses			
0	2,470	3,179	2,287
1	249	97	670
2	124	90	581
3	80	NA	669
Total individuals[a]	2,923	3,366	4,207
(B) IRT analyses			
Complete MZ pairs	469	692	737
Complete DZ pairs	544	872	943
Incomplete MZ pairs	466	368	344
Incomplete DZ pairs	751	566	537
Total individuals[a]	3,243	4,062	4,241

The numbers used for analysis of rs25531 are not shown but the totals are presented in Table IV supp.
[a]The sample size was smaller for ordinal than IRT analyses because missingness of some items led to exclusion of participants.

RESULTS

The distributions of age, sex, PLE, SLE, self-report depression classes, clinical depression classes, and suicidality do not differ significantly between the full and genotyped sample (see Supplementary Information Table I supp.). The polychoric correlations of SelfRepDep with ClinDep and suicidality are 0.24 and 0.27, respectively, and between ClinDep and suicidality is 0.59.

For the IRT and ordinal regression analyses, the participant numbers for each level of the outcome variables are presented in Table II and results are presented in Table III. Coefficient values are different for the two analyses because they are on the odds scale for the regression analyses and in standard deviation units for the IRT analysis.

Main Effects

All main effects for PLE are significant ($P < 0.001$). For SelfRepDep, with each additional PLE the odds of being one SelfRepDep category higher are ~1.6 (from ordinal regressions) or 0.36 SD units on a normal underlying depression scale (from IRT analyses). Strikingly, for ClinDep and suicidality, these odds are ~1.3 and ~1.5, respectively or ~0.15 and ~0.23 SD units, respectively, even though ClinDep/suicidality represents clinical diagnoses measured 1–10 years after PLE, whereas SelfRepDep is measured in the period immediately after PLE. The association with NLE is in the same direction but only evident for ClinDep and SelfRepDep (not suicidality) where the effect is weaker (odds ratio of ~1.06 or ~0.04 SD units) and the *P*-value is inconsistent (ns to $P < 0.01$).

Neither of the main effects for the two genotype classifications (5HTTLPR and 5HTTLPR + rs25531) are associated with any of the outcomes (ClinDep, suicidality or SelfRepDep), irrespective of the mode of gene action (additive or dominant) or method of analysis (IRT or ordinal regression). The two exceptions (nominal $P < 0.05$), the additive and dominant models of

TABLE III. Using the Full Sample, the Effect Sizes of Fitted Terms Expressed in Standard Deviation Units From Item-Response Theory (IRT) Analyses and Odds Ratios From Ordinal Regression Analyses Predicting Clinical Depression (ClinDep), Suicidality, or Self-Report Depression (SelfRepDep) From Sex, Age, Genotype (5HTTLPR, 5HTTLPR + rs25531), Personal and Network Stressful Life Events (PLE and NLE) and the Two-Way Interactions Between These and Genotype (Both Additive[a] and S-Dominant[b] Genotypic Models Were Fitted)

							Genotype		Geno. × PLE		Geno. × NLE	
Outcome	**Genotype**	**N**	**Sex**	**Age[c]**	**PLE**	**NLE**	**Dom[c]**	**Add[a]**	**Dom**	**Add**	**Dom**	**Add**
IRT analyses												
ClinDep	5HTTLPR	3,243	0.04	−0.02***	0.14***	0.04*	−0.02	−0.05	−0.02	−0.03	0.07	−0.00
	5HTTLPR + rs25531	3,206	0.04	−0.02***	0.15***	0.04*	−0.01	−0.04	0.01	0.00	0.01	0.01
Suicidality	5HTTLPR	4,062	0.06	−0.01***	0.22***	0.01	−0.02	−0.07	0.01	0.06	−0.03	−0.05
	5HTTLPR + rs25531	4,020	0.06	−0.01***	0.23***	−0.01	−0.03	−0.08	0.03	0.07	−0.01	−0.05
SelfRepDep	5HTTLPR	4,241	0.06**	−0.01**	0.36***	0.04**	0.04	0.06	−0.01	−0.01	0.04*	0.03
	5HTTLPR + rs25531	4,198	0.06**	−0.01**	0.36***	0.05**	0.05*	0.06*	−0.01	0.01	0.04*	0.04
Ordinal regressions												
ClinDep	5HTTLPR	2,923	1.17	0.97***	1.28***	1.05	0.98	0.95	0.96	0.98	1.02	1.00
	5HTTLPR + rs25531	2,888	1.17	0.97***	1.28***	1.06	0.96	0.92	0.98	0.99	1.03	1.02
Suicidality	5HTTLPR	3,366	1.34	0.96***	1.46***	0.95	0.93	0.87	1.03	1.13	0.93	0.91
	5HTTLPR + rs25531	3,327	1.36	0.96***	1.51***	0.92	0.86	0.83	1.08	1.14*	0.92	0.89
SelfRepDep	5HTTLPR	4,207	1.20**	1.00	1.58***	1.06**	1.05	1.08	0.98	0.99	1.04*	1.03
	5HTTLPR + rs25531	4,164	1.21**	1.00	1.58***	1.07**	1.04	1.05	0.98	1.00	1.03	1.03
Matched to Caspi et al. [2003]												
ClinDep	5HTTLPR	585	1.03		1.34***	1.00	1.00	0.81		1.03		
Suicidality	5HTTLPR	623	1.67		1.70***	1.00	1.00	1.34		1.07		

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
[a]Additive model (i.e., ordered genotypes).
[b]Model with a dominant S allele for 5HTTLPR and 5HTLPR + rs25531. The statistics for sex, age, and SLE are also from these models.
[c]In the IRT analyses age was a continuous covariate, but in the ordinal regressions it was coded 18–28, 28–38, >38 which ensure the regression slopes for each of the thresholds on the ordinal scales were equal.

5HTTLPR + rs25531 for SelfRepDep when run with the IRT analyses, are in the opposite direction to that expected (less depression with each additional S allele) and amount to little given multiple testing.

Two-Way Interactions

On the whole, estimates for two-way interactions between SLE (either personal or network) and the genotypes (either 5HTTLPR or 5HTTLPR + rs25531) on risk of ClinDep, suicidality or SelfRepDep are not significant, irrespective of the mode of action (additive or dominant). There are four exceptions with nominal $P < 0.05$ (Table III and the Supplementary Information Figure II supp.), and each is in a direction opposite to that expected from Caspi et al. [2003]: with 48 interactions terms in all, these are likely to occur by chance alone.

Replication of Caspi et al. [2003]

In the ordinal regression analyses designed to mimic Caspi et al. [2003], there was no evidence of either a main effect of 5HTTLPR or an interaction with SLE, but the reduced sample size necessarily means that these analyses have substantially less power than our full analyses.

DISCUSSION

Despite the intrinsic appeal of an interaction between SLEs and 5HTTLPR, and despite support from animal models demonstrating the biological plausibility of such an interaction, results from human studies fail to replicate convincingly the original finding of Caspi et al. [2003]. The meta-analyses by Munafo et al. [2008] and Risch et al. [2009] combines results from 5 and 14 studies, respectively that rigorously adhered to the conditions of the initial study, and conclude there is no evidence for an interaction. Our results strengthen these conclusions. Uher and McGuffin [2008] reviewed evidence from a range of quantitative population genetic studies, neurophysiological investigations, and experimental studies in animals and concluded that there is evidence for an interaction. All three studies are critical of many of the human studies that set out to replicate the finding and they make recommendations about design and analysis of future studies. We note however, that all fail to acknowledge potential problems with the genotyping assay of 5HTTLPR.

In pilot experiments conducted for this study, we recognized problems with the original PCR assay [Heils et al., 1996], as found by others [Kaiser et al., 2002; Yonan et al., 2006] and designed a new high-throughput assay [Wray et al., 2009]. The genotyping in duplicate of all samples, the genotyping of replicated samples, of

MZ twins and of family members, all serve to provide a high level of quality control in our study. We found, in a subsample genotyped by our group [Gillespie et al., 2005] using the original assay, on which much of the literature is based, 16% showed inconsistencies with the new genotyping assay. Without our levels of quality control, it is likely other studies using the original assay will have suffered similar genotyping inaccuracy.

Our study sample for this analysis is one of the largest to date and uses both the original definition of 5HTTLPR and a re-definition of the L allele subdivided by the SNP rs25531, which based on evidence from functional studies [based on Hu et al., 2005; Wendland et al., 2006] is expected to be more associated with depression than 5HTTLPR alone. However, we find no evidence for a main effect of genotype nor an interaction between stressful events and genotype, either on the observed scale of disease using ordinal regression or on the underlying liability scale or risk to depression using IRT. This implies that individuals exposed to more stressful environments are no more susceptible to depression if they have either one or two S alleles than if they have the L allele. We observe this irrespective of whether we fit an additive or S-dominant model. Further, we attempted to replicate Caspi et al. [2003] by matching their sample and analyses as best we could but still found no evidence for an interaction. Finally, our data suggest that the reported interaction is no more likely for females alone, younger adults, or shorter intervals between SLE and depression/suicidality, despite the review by Uher and McGuffin [2008] suggesting effects were more likely in such subsamples. As there is no evidence for association in our study sample between depression/suicidality and either 5HTTLPR or 5HTTLPR + rs25531, we cannot make any conclusion about the value of including the rs25531 polymorphism. We recently reported an association between MD and rs6354 located ~15.5 kb from, and in linkage equilibrium ($r^2 = 0.01$) with, 5HTTLPR, although the association in the study sample used here (the SSAGA cohort) was not significant [Wray et al., 2009]. We repeated the G × E analyses presented here for genotypes of rs6354 but found no evidence for an interaction (results not presented).

The current findings are broadly consistent with earlier research published on a subset of the current data [Gillespie et al., 2005]. However, a criticism [Uher and McGuffin, 2008] of our earlier article [Gillespie et al., 2005] is that we failed to find an interaction, in part because there was only a weak association between SLE and depression ($P = 0.08$). The association in the current article is clearly stronger ($P < 0.001$), which in part is a result of the seven "problems getting along with" items being allocated as personal SLE, a decision based on a factor analysis of all SLE items (see Materials and Methods Section). In Gillespie et al. [2005] these items were included as network SLE. Nonetheless, our study does have limitations. Munafo et al. [2008] showed that the relationship between power and sample size is complex and depends on the size of the main effects and the proportion of individuals experiencing SLE. Based on our conservative power calculations, presented in Table II Supplementary, our largest sample (SelfRepDep) has substantial power to detect a G × E. However, our sample constructed to most closely match Caspi et al. [2003] has quite low power. These power calculations are the minimum we expect since we necessarily used binary variables for depression and SLE to compute power, and the power will be greater for the ordinal depression and continuous SLE scales used throughout this article. Second, despite our improved genotyping and subclassing by rs25531, we could not distinguish those with genotypes L_gL_g from those with genotype L_gL_a. However since we estimate that L_gL_g comprise only 0.9% of the total sample (or 12% of those genotyped as L_gL_a) we do not believe that being able to disentangle these genotypes would change our results. Third, self-reported depression, whilst assessed at the same time as SLE, assesses "recent" experiences so does not represent those of the 12-month duration of SLE reporting.

CONCLUSION

The strengths of the current study include the large sample [only one other study, to date, is of similar size; Surtees et al., 2006] and accurate genotyping, including subdivision of the 5HTTLPR L allele according to the SNP rs25531. We use different outcome variables (ClinDep, suicidality and SelfRepDep) and, to interpret interactions in the context of scale of measurement, we use IRT analyses in addition to ordinal regressions. Finally, we addressed specific hypotheses that result from reviews of previous studies and limited our analyses to females, younger subjects, or limiting the time frame between stressful events and depression. Yet despite the strengths of the current study, and within the context of a strong association between PLE and depression/suicidality, we fail to replicate the hypothesized interaction [Caspi et al., 2003] between stress and the 5HTTLPR genotypes beyond that likely to occur by chance alone. Previous reports of replication should be considered within the context of potential problems including, poor quality genotyping, inconsistent types of interactions, inconsistent grouping of genotypes, selective presentation of results, interactions arising from the scale of measurement, and publication bias.

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