ONLINE FIRST

The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis Revisited

Evidence of Genetic Moderation

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Context: Two recent meta-analyses assessed the set of studies exploring the interaction between a serotonin transporter promoter polymorphism (5-HTTLPR) and stress in the development of depression and concluded that the evidence did not support the presence of the interaction. However, even the larger of the meta-analyses included only 14 of the 56 studies that have assessed the relationship between 5-HTTLPR, stress, and depression.

Objective: To perform a meta-analysis including all relevant studies exploring the interaction.

Data Sources: We identified studies published through November 2009 in PubMed.

Study Selection: We excluded 2 studies presenting data that were included in other larger studies.

Data Extraction: To perform a more inclusive meta-analysis, we used the Liptak-Stouffer *z* score method to combine findings of primary studies at the level of significance tests rather than the level of raw data.

Data Synthesis: We included 54 studies and found strong evidence that 5-HTTLPR moderates the relationship between stress and depression, with the 5-HTTLPR *s* allele associated with an increased risk of developing depression under stress ($P=.00002$). When stratifying our analysis by the type of stressor studied, we found strong evidence for an association between the *s* allele and increased stress sensitivity in the childhood maltreatment ($P=.00007$) and the specific medical condition ($P=.0004$) groups of studies but only marginal evidence for an association in the stressful life events group ($P=.03$). When restricting our analysis to the studies included in the previous meta-analyses, we found no evidence of association (Munafò et al studies, $P=.16$; Risch et al studies, $P=.11$). This suggests that the difference in results between meta-analyses was due to the different set of included studies rather than the meta-analytic technique.

Conclusion: Contrary to the results of the smaller earlier meta-analyses, we find strong evidence that the studies published to date support the hypothesis that 5-HTTLPR moderates the relationship between stress and depression.

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THE PRINCIPAL FUNCTION OF the serotonin transporter is to remove serotonin from the synapse, returning it to the presynaptic neuron where the neurotransmitter can be degraded or rereleased at a later time. A polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) has been found to affect the transcription rate of the gene, with the short (*s*) allele transcriptionally less efficient than the alternate long (*l*) allele. In 2003, Caspi and colleagues[1] used a prospective, longitudinal design to examine the relationship between 5-HTTLPR, stress, and depression in a large birth cohort and found a significant interaction between 5-HTTLPR and both stressful life events (SLEs) and childhood maltreatment in the development of depression. In this cohort, subjects carrying the less functional 5-HTTLPR *s* allele reported greater sensitivity to stress.

The Caspi et al study has been cited more than 2000 times in the scientific literature and generated a great deal of excitement around the potential of gene × environment interaction studies.[2] To date, there have

been 55 follow-up studies exploring whether 5-HTTLPR moderates the relationship between stress and depression, with some studies supporting the association between the 5-HTTLPR *s* allele and greater stress sensitivity and others not. Two recent meta-analyses have assessed a subset of these studies and concluded that there is no evidence supporting the presence of the interaction.[3,4]

Since their publication, these meta-analyses have generated substantial debate and intense criticism. Some of the discussion has revived the long-standing debate about whether exploring epidemiological interaction effects, in general, will produce worthwhile results.[5,6] The criticism specific to this genetic association, however, has largely revolved around the fact that only a subset of the studies investigating the relationship between 5-HTTLPR, stress, and depression were included in the meta-analyses.[7-12] In fact, while 56 primary data studies have assessed whether 5-HTTLPR moderates the relationship between stress and depression, the Munafò et al[3] and Risch et al[4] meta-analyses included only 5 and 14 of those studies, respectively.[13-51] Further, Uher and McGuffin[11] have demonstrated that the larger Risch et al meta-analysis included a significantly greater proportion of negative replication studies than positive replication studies.

There are multiple reasons that the studies included in the meta-analyses were limited. First, the primary study data needed for traditional meta-analysis were often not available, either in the original publications or in follow-up e-mail inquiries to study authors. For instance, Munafò and colleagues reported that 15 studies met criteria for inclusion in their meta-analysis. However, they were only able to obtain the primary study data needed for inclusion for 5 of those studies. There is no evidence that the studies that were able to be included in the meta-analyses were of higher "quality" than those not included.

A second reason why many studies were not included in the Risch et al and Munafò et al meta-analyses is that both meta-analyses focused exclusively on studies that explored an interaction between 5-HTTLPR and SLEs in the development of depression. The original Caspi et al article, however, not only reported an interaction between 5-HTTLPR and SLEs, but also an interaction between 5-HTTLPR and childhood maltreatment stress. Nine studies have attempted to replicate this interaction with childhood maltreatment, but these studies were not included in the meta-analyses.

Some observers have noted that the SLE study design may have limited power to detect genetic moderation effects because they are susceptible to a set of potential biases: (1) impaired recall of stressors by subjects, (2) highly variable stressors between subjects, and (3) the reduced statistical power inherent to tests of statistical interaction.[10,48] A newer class of studies has attempted to bypass these potential problems by focusing on specific populations that have experienced a substantial, specific stressor. Eighteen studies have used such a specific stressor design to assess whether 5-HTTLPR moderates the relationship between stress and depression, but like the childhood maltreatment studies, these studies were excluded from the previous meta-analysis.

In this investigation, rather than focus on a specific class of studies, we sought to perform a meta-analysis on the entire body of work assessing the relationship between 5-HTTLPR, stress, and depression. Unfortunately, the different classes of studies generally used different study designs to explore this question, rendering it very difficult to combine the studies into a single traditional meta-analysis. An approach useful in situations where equivalent raw data are not available across all studies is to combine the studies at the level of significance tests.[52] The Liptak-Stouffer *z* score method is a well-validated method for combining *P* values across studies and has been used widely across genomics and biostatistics.[53-59] Herein, we use the Liptak-Stouffer *z* score method to combine the results from studies investigating whether the 5-HTTLPR variant moderates the relationship between stress and depression.

METHODS

STUDIES

Potential studies were identified from previous meta-analyses and review articles and through PubMed at the National Library of Medicine, using the search terms depression or depressed and "serotonin transporter" or 5-HTTLPR and stress or maltreatment.[3,4,10] We subsequently checked the reference sections of the identified publications and contacted authors through e-mail to identify additional studies in press or review. We considered all English-language studies published by November 2009 assessing whether 5-HTTLPR moderates the relationship between stress and depression. Two studies were excluded because their data were part of another larger study included in the analysis.[12,60] In total, data from 54 publications met inclusion criteria and were included in the analysis.

To identify study design characteristics that might influence the ability to detect the interaction effect between 5-HTTLPR and life stress, we used 2 different grouping methods set out in a recent review article[10] and assessed the presence of the association within each group. First, we stratified studies by the type of stressor studied (childhood maltreatment, specific medical conditions, and SLEs). When publications reported results for multiple types of stressors that matched different groups, we included the study in each relevant group.[1,49,61-64] Second, we stratified studies by the method of stress assessment (objective, interview, and self-report questionnaire).

QUALITY ASSESSMENT

We evaluated the methodological quality of the included studies by applying an 11-item quality checklist, derived from the STREGA (Strengthening the Reporting of Genetic Association Studies) and STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklists.[65,66] Specifically, the quality criteria were (1) clear statement of objectives and hypothesis, (2) clear eligibility criteria for study participants, (3) clear definition of all variables, (4) replicability of statistical methods, (5) assessment of Hardy-Weinberg equilibrium, (6) assessment of ethnicity, (7) addressing the problem of mixed ethnicities statistically (if applicable), (8) sufficient descriptive data (age, sex, ethnicity), (9) statement of genotype frequencies, (10) sample in Hardy-Weinberg equilibrium, and (11) consideration of population stratification.

Consistent with current guidelines, we did not weigh studies by quality scores or exclude studies with low-quality studies. Instead, we report the quality data extracted, so that they

are available for readers to evaluate (eTable, http://www.archgenpsychiatry.com).[67,68] Further, to assess whether our results were influenced by studies rated as lower quality through this measure, we repeated our overall meta-analysis with only studies with a quality score higher than the median.[69]

P VALUE EXTRACTION

Two investigators (K.K. and S.S.) independently extracted the relevant *P* value from each study. There were no cases of disagreement between the 2 investigators. When several *P* values were provided (because of the use of several depression scales or separate *P* values for different subsets of samples), we used a weighted mean *P* value for our analyses. For studies with nonsignificant results that did not provide exact probabilities, a *P* value of 1 (no association in either direction) was assumed. When an article reported analyses that matched different groups of our study, we incorporated the mean of the *P* value of each group into the overall analysis.

STATISTICAL ANALYSIS

The Liptak-Stouffer *z* score method was used to combine studies at the level of significance tests, weighted by study sample size. First, all extracted *P* values were converted to 1-tailed *P* values, with *P* values less than .50 corresponding to greater *s* allele stress sensitivity and *P* values more than .50 corresponding to greater *l* allele stress sensitivity.

Next, these *P* values were converted to *z* scores using a standard normal curve such that *P* values less than .50 were assigned positive *z* scores and *P* values more than .50 were assigned negative *z* scores. Subsequently, these *z* scores were combined by calculating

$$z_W = \frac{\sum_{i=1}^{k} w_i z_i}{\sqrt{\sum_{i=1}^{k} w_i^2}}$$

where the weighting factor w_i corresponds to the individual study sample sizes, k corresponds to the number of total studies, and z_i corresponds to the individual study *z* scores. The outcome of this test, z_w, follows a standard normal distribution and the corresponding probability can be obtained from a standard normal distribution table. We used this procedure on the overall sample as well as on each of the individual study subgroups. To assess whether our results were substantially influenced by the presence of any individual study, we conducted a sensitivity analysis by systematically removing each study and recalculating the significance of the result. Further, to compare our method of combining studies at the significance test level with the method of combining studies at the raw data level used in the previous meta-analyses, we performed an analysis with only the studies included in the previous meta-analyses.[3,4]

To assess the possibility that results of the meta-analysis were affected by publication bias, we calculated the fail-safe N for our overall analysis, the number of unpublished studies that would have to exist to change the outcome of the Liptak-Stouffer test from significant to nonsignificant. Because the commonly used analytic approximation[70] method of calculating the fail-safe N has been criticized for inadequate reliability and accuracy, we used a more rigorous, direct computational approach.[71] Specifically, we calculated the number of studies with a *P* value equal to .50 and a sample size of 755 (the average sample size in the studies we analyzed) that we would need to incorporate into the weighted Liptak-Stouffer analysis to obtain a nonsignificant outcome. The ratio between the fail-safe N and the number of studies actually published estimates the potential for publication bias to influence our results. We also considered the effect of false-positive findings due to small sample size by calculating the number of smallest studies that could be deleted before the analysis would reveal a nonsignificant result.

RESULTS

OVERALL META-ANALYSIS

Our initial search identified 148 publications. Of these studies, we identified 54 studies that included 40 749 subjects meeting criteria for inclusion (**Table 1**). We found strong evidence that 5-HTTLPR moderates the relationship between stress and depression, with the *s* allele associated with an increased risk of developing depression under stress ($P = .00002$) (**Figure**). The significance of the result was robust to sensitivity analysis, with the overall *P* values remaining significant when each study was individually removed from the analysis ($1.0 \times 10^{-6} < P < .00016$). When we restricted our analysis to those studies with a study "quality" score higher than the median, the *P* value remained significant (3.2×10^{-10}). Further, there was evidence for genetic moderation among both the group of studies that used categorical measures of depression ($P = .03$; n = 17) and the group of studies that used continuous measures of depression ($P = .001$; n = 23).

SUBGROUP STRATIFICATION

When stratifying our analysis by the type of stressor studied, we found strong evidence for an association between the *s* allele and increased stress sensitivity in the childhood maltreatment group ($P = .00007$) and the specific medical condition group ($P = .0004$) and marginal evidence for an association in the SLEs group ($P = .03$) (**Tables 2**, **3**, and **4**, respectively). The removal of individual studies did not lead to changes in the significance of the outcome in studies of childhood maltreatment ($7.4 \times 10^{-6} < P < .00014$) or specific medical conditions ($.00017 < P < .0068$). However, the result among studies of SLEs became nonsignificant after the exclusion of any 1 of several studies[1,35,38,40,76] ($.013 < P < .062$).

When stratifying our analysis by the stress assessment method, we found strong evidence for an association between the *s* allele and increased stress sensitivity among the objective measure group ($P = .000003$) and the interview assessment group ($P = .0002$) and marginal evidence in the self-report questionnaire group ($P = .042$). The removal of individual studies did not lead to changes in the significance of the outcome in studies assessing stress with objective measures ($8.7 \times 10^{-7} < P < .000029$) or with interview assessments ($4.0 \times 10^{-6} < P < .0014$). However, the result among studies assessing stress with self-report questionnaires became nonsignificant after the exclusion of several studies[23,25,35,38,40,73,76] ($.018 < P < .093$).

Table 1. Description of 5-HTTLPR, Stress, and Depression Studies Included in the Overall Meta-Analysis

Source, Year	No. of Participants	Female, %	Mean Age, y	Study Design	Stressor	Stress Assessment Method	Depression Measure	Reported Findings[a]	Averaged 1-Tailed *P* Value[b]	Liptak-Stouffer *P* Value After Study Exclusion
Mössner et al,[51] 2001	72	46	NA	Exposed only	Parkinson disease	Objective	Hamilton Depression Rating Scale	Positive	.0125	1.90×10^{-5}
Caspi et al,[1] 2003	845	48	26	Longitudinal	Child maltreatment	Objective	Diagnosis of depression	Positive	.0100	4.20×10^{-5}
Eley et al,[72] 2004	374	58	16	Case-control	Adverse family environment	Self-report questionnaire	MFQ	Partially positive	.2575	1.95×10^{-5}
Grabe et al,[73] 2005	973	69	52	Cross-sectional	Number of chronic diseases	Self-report questionnaire	von Zerssen Complaints Scale	Partially positive	.2503	2.16×10^{-5}
Kendler et al,[19] 2005	549	NA	35	Longitudinal	Stressful life events	Interview	Diagnosis of depression	Positive	.0070	3.27×10^{-5}
Nakatani et al,[28] 2005	2509	25	64	Exposed only	Acute myocardial infarction	Objective	Zung Self-Rating Depression Scale	Positive	.0075	1.62×10^{-4}
Jacobs et al,[20] 2006	374	100	27	Longitudinal	Stressful life events	Self-report questionnaire	SCL-90	Positive	.0200	2.51×10^{-5}
Kaufman et al,[18] 2006	196	51	9	Cross-sectional	Child abuse	Objective	MFQ	Partially positive	.0225	2.12×10^{-5}
Ramasubbu et al,[30] 2006	51	35	60	Exposed only	Stroke	Objective	Diagnosis of depression	Positive	.0130	1.86×10^{-5}
Sjöberg et al,[21] 2006	198	63	17	Cross-sectional	Psychosocial circumstances in family	Interview	Depression Self-Rating Scale	Partially positive/ opposite	.4721	1.76×10^{-5}
Surtees et al,[74] 2006	4175	47	60	Cross-sectional	Childhood adversities/ stressful life events	Self-report questionnaire	Diagnosis of depression	Negative	.5000	1.33×10^{-6}
Taylor et al,[63] 2006	110	57	21	Cross-sectional	Childhood adversities	Self-report questionnaire	BDI	Partially positive	.0268	1.95×10^{-5}
Wilhelm et al,[75] 2006	127	67	48	Longitudinal	Stressful life events	Interview	Diagnosis of depression	Partially positive	.1178	1.89×10^{-5}
Zalsman et al,[64] 2006	79	68	38	Case-control	Stressful life events	Interview	Hamilton Depression Rating Scale	Partially positive	.2233	1.81×10^{-5}
Cervilla et al,[76] 2007	737	72	49	Case-control	Stressful life events	Self-report questionnaire	Diagnosis of depression	Positive	.0143	3.62×10^{-5}
Chipman et al,[61] 2007	2094	52	23	Cross-sectional	Stressful life events	Self-report questionnaire	Goldman Depression Scale	Negative	.3400	1.60×10^{-5}
Chorbov et al,[77] 2007	236	100	22	Longitudinal	Traumatic events	Self-report questionnaire	Diagnosis of depression	Opposite	1.0000	1.10×10^{-5}
Cicchetti et al,[22] 2007	339	46	17	Cross-sectional	Child abuse	Objective	ASEBA	Partially positive	.2518	1.94×10^{-5}
Dick et al,[35] 2007	956	NA	NA	Family-based association study	Problems with work, relationship, or health	Self-report questionnaire	Diagnosis of depression	Positive	.0040	5.37×10^{-5}
Kilpatrick et al,[14] 2007	589	64	≥60 (77%)	Cross-sectional	Hurricane exposure + low social support	Objective	Diagnosis of depression	Positive	.0015	3.94×10^{-5}
Kim et al,[78] 2007	732	NA	≥65	Cross-sectional	Stressful life events	Interview	Diagnosis of depression	Negative	0.0385	3.11×10^{-5}
Kraus et al,[36] 2007	139	49	42	Exposed only	Interferon alfa treatment	Objective	Hospital Anxiety and Depression Scale	Negative	.5650	1.73×10^{-5}
Mandelli et al,[15] 2007	670	68	48	Case-only	Stressful life events	Interview	Diagnosis of depression	Positive	.0112	3.50×10^{-5}
Middeldorp et al,[79] 2007	367	68	39	Longitudinal	Stressful life events	Self-report questionnaire	Anxiety-Depression Rating Scale	Negative	.5000	1.73×10^{-5}

(continued)

Table 1. Description of 5-HTTLPR, Stress, and Depression Studies Included in the Overall Meta-Analysis (continued)

Source, Year	No. of Participants	Female, %	Mean Age, y	Study Design	Stressor	Stress Assessment Method	Depression Measure	Reported Findings[a]	Averaged 1-Tailed *P* Value[b]	Liptak-Stouffer *P* Value After Study Exclusion
Otte et al,[29] 2007	557	15	68	Exposed only	Coronary disease	Objective	Diagnosis of depression	Partially positive	.0275	2.86×10^{-5}
Scheid et al,[16] 2007	568	100	20-34	Cross-sectional	Stressful life events	Self-report questionnaire	CES-D	Negative	.0800	2.50×10^{-5}
Brummett et al,[37] 2008	288	75	58	Cross-sectional	Alzheimer caregiving	Objective	CES-D	Positive	.0015	2.64×10^{-5}
Kohen et al,[26] 2008	150	37	60	Exposed only	Stroke	Objective	Geriatric Depression Scale	Positive	.0225	2.03×10^{-5}
Lazary et al,[38] 2008	567	79	31	Cross-sectional	Stressful life events	Self-report questionnaire	Zung Self-Rating Depression Scale	Positive	.0025	3.67×10^{-5}
Lenze et al,[27] 2005	23	87	77	Exposed only	Hip fracture	Objective	Diagnosis of depression	Positive	.0068	1.81×10^{-5}
Power et al,[80] 2010	1421	NA	≥65	Cross-sectional	Stressful life events	Self-report questionnaire	MINI, CES-D	Negative	.6200	1.10×10^{-5}
Wichers et al,[39] 2008	394	100	18-64	Cross-sectional	Childhood trauma	Self-report questionnaire	SCL-90; SCID depressive symptoms	Negative	.2000	2.03×10^{-5}
Aguilera et al,[23] 2009	534	55	23	Cross-sectional	Childhood trauma	Self-report questionnaire	SCL-90-R	Positive	.0001	4.63×10^{-5}
Araya et al,[34] 2009	4334	NA	7	Longitudinal	Stressful life events	Self-report questionnaire	SDQ emotional symptom 5-item subscale	Negative	.5000	1.03×10^{-6}
Aslund et al,[40] 2009	1482	48	17-18	Cross-sectional	Parental fighting and maltreatment	Self-report questionnaire	Depression Self-Rating Scale	Positive	.0078	7.68×10^{-5}
Bull et al,[41] 2009	98	36	46	Longitudinal	Interferon alfa and ribavirin treatment	Objective	Zung Self-Rating Depression Scale/BDI	Positive	.0150	1.95×10^{-5}
Coventry at al,[42] 2010	3243	60	32	Longitudinal	Stressful life events	Self-report questionnaire	Diagnosis of depression	Negative	.5000	4.33×10^{-6}
Bukh et al,[43] 2009	290	66	39	Case-only	Stressful life events	Interview	Diagnosis of depression	Negative	.0350	2.25×10^{-5}
Kim et al,[25] 2009	521	55	72	Longitudinal	No. of chronic health problems	Self-report questionnaire	Diagnosis of depression	Positive	.0050	3.27×10^{-5}
Laucht et al,[62] 2009	309	54	19	Cross-sectional	Stressful life events	Self-report questionnaire	Diagnosis of depression, BDI	Partially negative/ opposite	.7375	1.57×10^{-5}
Lotrich et al,[33] 2009	71	27	48	Exposed only	Interferon alfa treatment	Objective	BDI	Positive	.0250	1.88×10^{-5}
McCaffery et al,[44] 2009	977	21	59	Exposed only	Cardiovascular disease	Objective	BDI	Negative	.5000	1.57×10^{-5}
Ressler et al,[81] 2010	926	62	≥18	Cross-sectional	Childhood trauma	Self-report questionnaire	Diagnosis of depression (partially), BDI	Partially positive	.5000	1.59×10^{-5}
Ritchie et al,[82] 2009	942	58	65-92	Cross-sectional	Childhood adversities	Self-report questionnaire	Diagnosis of depression, CES-D, treatment with antidepressants	Partially opposite	.5390	1.51×10^{-5}

(continued)

STUDIES FROM PREVIOUS META-ANALYSES

When we restricted our analysis to the studies included in the 2 previous meta-analyses, we found no evidence of an association between 5-HTTLPR and stress sensitivity (Munafò et al studies, *P*=.16; Risch et al studies, *P*=.11).

PUBLICATION BIAS

To make the result of our overall analysis nonsignificant (*P*=.05), more than 729 unpublished or undiscovered studies with an average sample size (N=755) and a nonsignificant result (*P*=.50) would need to exist. This corresponds to a fail-safe ratio of 14 studies not in-

Table 1. Description of 5-HTTLPR, Stress, and Depression Studies Included in the Overall Meta-Analysis (continued)

Source, Year	No. of Participants	Female, %	Mean Age, y	Study Design	Stressor	Stress Assessment Method	Depression Measure	Reported Findings[a]	Averaged 1-Tailed *P* Value[b]	Liptak-Stouffer *P* Value After Study Exclusion
Wichers et al,[83] 2009	502	100	27	Longitudinal	Stressful life events	Self-report questionnaire	Diagnosis of depression, SCL-90-R	Partially positive	.3803	1.84×10^{-5}
Zhang et al,[45] 2009	792	54	33	Case-control	Stressful life events	Self-report questionnaire	Diagnosis of depression	Opposite	.9975	5.24×10^{-6}
Zhang et al,[84] 2009	306	38	NA	Exposed only	Parkinson disease	Objective	CES-D	Negative	.5000	1.74×10^{-5}
Hammen et al,[13] 2010	346	62	24	Longitudinal	Negative acute life events, chronic family stress	Interview	BDI	Partially positive	.3763	1.86×10^{-5}
Benjet et al,[46] 2010	78	100	12	Cross-sectional	Relational aggression	Self-report questionnaire	Children's Depression Inventory	Positive	.0050	1.94×10^{-5}
Goldman et al,[50] 2010	984	45	66	Longitudinal	Stressful life events	Interview	CES-D	Partially positive	.0203	4.19×10^{-5}
Grassi et al,[85] 2010	145	100	56	Exposed only	Breast cancer	Objective	Hospital Anxiety and Depression Scale	Negative	.5000	1.75×10^{-5}
Kumsta et al,[47] 2010	125	NA	11/15	Longitudinal	Institutionalization in Romanian orphanages	Objective	CAPA, Rutter Child Scale, SDQ	Positive	.0117	2.02×10^{-5}
Sen et al,[48] 2010	268	58	28	Longitudinal	Medical internship	Self-report questionnaire	PHQ	Positive	.0020	2.54×10^{-5}
Sugden et al,[49] 2010	2017	51	12	Longitudinal	Bullying victimization	Interview	ASEBA	Negative	.1603	2.94×10^{-5}
Total	**40 749**									
Average sample size	755									.00002

Abbreviations: ASEBA, Achenbach System of Empirically Based Assessment; BDI, Beck Depression Inventory; CAPA, Child and Adolescent Psychiatric Assessment; CES-D, Center for Epidemiologic Studies Depression Scale; MFQ, Mood and Feelings Questionnaire; MINI, Mini International Neuropsychiatric Interview; NA, not available; PHQ, Patient Health Questionnaire; SCID, Structured Clinical Interview for *DSM* Disorders; SCL-90, Symptom Checklist 90; SCL-90-R, Symptom Checklist 90 Revised; SDQ, Strengths and Difficulties Questionnaire.

[a] "Positive" indicates a significant ($P<.05$) interaction effect with the *s* allele, "Negative" indicates no interaction effect ($P>.05$), and "Opposite" indicates a significant ($P<.05$) interaction effect with the *l* allele.

[b] One-tailed *P* value, with smaller values indicating greater stress sensitivity among *s* allele subjects.

cluded in this meta-analysis for every included study. Additionally, we found that 45 of the 54 studies with the smallest sample sizes could be deleted before the outcome of the analysis would change to nonsignificant.

COMMENT

We found strong evidence that a serotonin transporter promoter polymorphism (5-HTTLPR) moderates the relationship between stress and depression, with the less functional *s* allele associated with increased stress sensitivity. This quantitative meta-analytic result is consistent with recent qualitative reviews on the same set of studies.[10,11] In addition, our results are consistent with a wide range of experimental neuroscience studies that have found increased stress reactivity among 5-HTTLPR *s* allele carriers.[87-89] Evidence from animal studies also supports that functional variation in the serotonin transporter (SERT) gene affects behavioral response to stress. Serotonin transporter knockout mice show increased hypothalamic-pituitary-adrenal axis activation in response to both physical and psychological stressors.[90,91] Developmentally, SERT knockout mice show impaired cortex-layer 4-barrel pattern formation and altered levels of a broad range of serotonin receptor subtypes, providing potential mechanisms through which SERT function may be affecting behavior.[92-94] Further, naturally occurring, low-functioning SERT gene variants in mice and nonhuman primates are associated with changes in central nervous system biochemistry as well as with behaviors linked to stress sensitivity.[95,96]

While our findings are consistent with this broad set of experimental neuroscience and animal studies, our findings are inconsistent with 2 other meta-analyses that have explored this association. The 2 most likely causes of the conflicting results between our meta-analysis and the previous meta-analyses are (1) the difference in meta-analytic technique used and (2) the different sets of included studies. To distinguish between these 2 possible causes, we applied our meta-analytic technique to the sets of studies used in the previous meta-analyses.[4] With these limited sets of studies, our meta-analytic technique produced the same nonsignificant result as the previous meta-analyses, suggesting that the difference in results be-

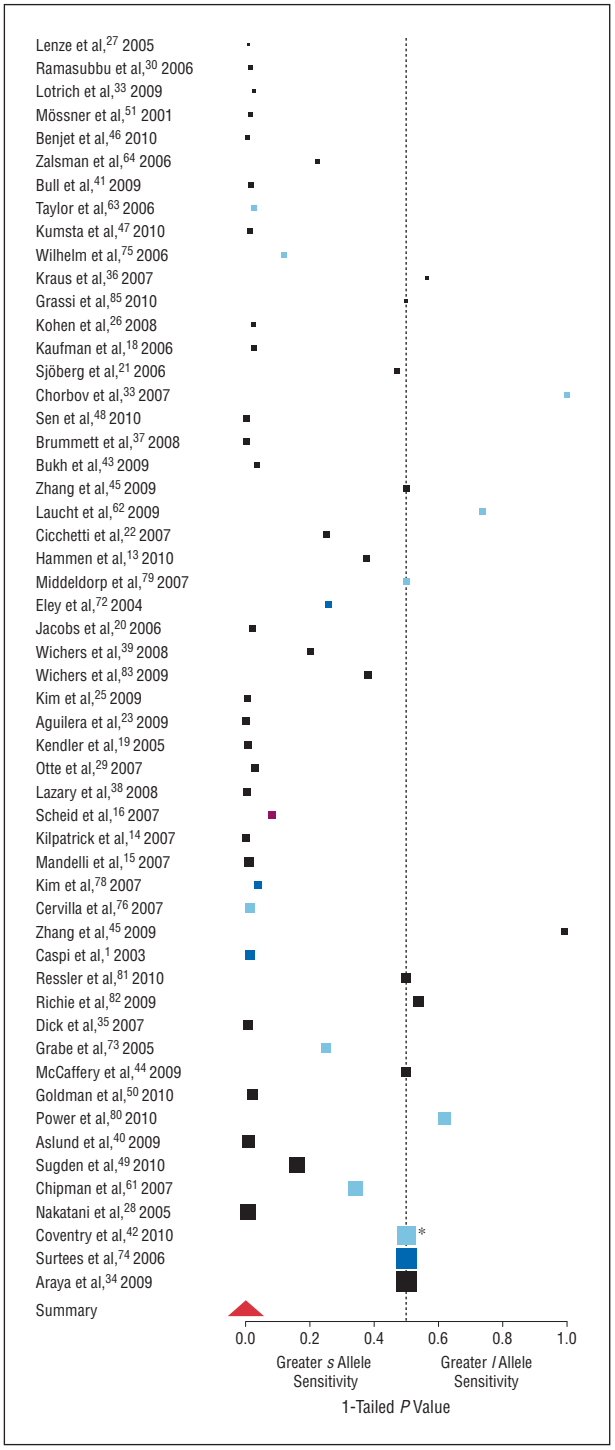

Figure. Forest plot for the 56 human observational studies assessing the relationship between 5-HTTLPR, stress, and depression. The boxes indicate the 1-tailed *P* value for each study, with lower values corresponding to greater stress sensitivity of *s* allele carriers and higher values, greater stress sensitivity of *l* allele carriers. The size of the box indicates the relative sample size. The triangle indicates the overall result of our meta-analysis. Purple indicates that the study was included only in the Munafò et al meta-analysis[3]; cyan, only in the Risch et al meta-analysis[4]; blue, both in the Munafò et al and the Risch et al meta-analyses; black, both in the Munafò et al and the Risch et al meta-analyses but the validity is questionable. *Participants were selected according to extremes of high and low neuroticism scores.[74] Because there is a strong positive correlation between depression and neuroticism, a large portion of variance in depression in such a sample will be explained by neuroticism alone.[86] The absence of individuals with intermediate neuroticism scores, for which the gene × environment effect may be strongest, might obscure any gene × environment interaction effect.

Table 2. Studies Included in the Childhood Maltreatment Group Meta-Analysis

Source, Year	Total No. of Participants	1-Tailed *P* Value	Fisher *P* Value After Study Exclusion
Caspi et al,[1] 2003	845	.010	5.38×10^{-4}
Kaufman et al,[18] 2006	196	.023	1.17×10^{-4}
Cicchetti et al,[22] 2007	339	.252	8.72×10^{-5}
Wichers et al,[39] 2008	394	.200	9.71×10^{-5}
Aguilera et al,[23] 2009	534	5.0×10^{-5}	8.31×10^{-4}
Aslund et al,[40] 2009	1482	.008	1.40×10^{-3}
Ressler et al,[81] 2010	926	.500	2.97×10^{-5}
Benjet et al,[46] 2010	78	.005	9.27×10^{-5}
Kumsta et al,[47] 2010	125	.012	1.03×10^{-4}
Sugden et al,[49] 2010	2017	.160	7.42×10^{-6}
Total	6936		
Average sample size	694		.00007

Table 3. Studies Included in the Specific Medical Condition Group Meta-Analysis

Source, Year	Total No. of Participants	1-Tailed *P* Value	Fisher *P* Value After Study Exclusion
Mössner et al,[51] 2001	72	.025	.00044
Grabe et al,[73] 2005	973	.250	.00041
Nakatani et al,[28] 2005	2509	.008	.00679
Ramasubbu et al,[30] 2006	51	.013	.00041
Kraus et al,[36] 2007	139	.565	.00035
Otte et al,[29] 2007	557	.028	.00104
Kohen et al,[26] 2008	150	.023	.00051
Lenze et al,[27] 2005	23	.007	.00038
Bull et al,[41] 2009	98	.015	.00046
Kim et al,[25] 2009	521	.005	.00145
Lotrich et al,[33] 2009	71	.025	.00042
McCaffery et al,[44] 2009	977	.500	.00017
Zhang et al,[84] 2009	306	.500	.00034
Grassi et al,[85] 2010	145	.500	.00035
Total	**6592**		
Average sample size	471		.0004

tween meta-analyses was due to the different set of included studies rather than the different meta-analytic technique.

The results of our secondary meta-analysis, where we stratified studies by stressor type, also support the hypothesis that the difference in results between our meta-analysis and the previous meta-analyses is due to the difference in the primary studies included. Both previous meta-analyses focused exclusively on SLEs and reported no evidence that 5-HTTLPR moderates the relationship between SLEs and depression. Herein, we were able to include 11 additional SLE studies not included in previous meta-analyses but still found only marginal evidence that 5-HTTLPR moderates the relationship between SLEs and depression.[6] In contrast, we found robust evidence that 5-HTTLPR moderates the relationship between both childhood maltreatment and specific stressors and depression.

One important variable that may help to account for the different results in the different stressor groups is the variability between studies within each group.[86] Within

Table 4. Studies Included in the Stressful Life Events Group Meta-Analysis

Source, Year	Total No. of Participants	1-Tailed *P* Value	Fisher *P* Value After Study Exclusion
Caspi et al,[1] 2003	845	.010	.054
Eley et al,[72] 2004	374	.258	.034
Kendler et al,[19] 2005	549	.007	.047
Jacobs et al,[20] 2006	374	.020	.040
Sjöberg et al,[21] 2006	198	.472	.032
Surtees et al,[74] 2006	4175	.500	.014
Taylor et al,[63] 2006	110	.028	.034
Wilhelm et al,[75] 2006	127	.118	.034
Zalsman et al,[64] 2006	79	.342	.033
Cervilla et al,[76] 2007	737	.014	.050
Chipman et al,[61] 2007	2094	.292	.039
Chorbov et al,[77] 2007	236	.99995	.025
Dick et al,[35] 2007	956	.004	.062
Kim et al,[78] 2007	732	.039	.046
Mandelli et al,[15] 2007	670	.011	.049
Middeldorp et al,[79] 2007	367	.500	.032
Scheid et al,[16] 2007	568	.080	.040
Lazary et al,[38] 2008	567	.002	.050
Power et al,[80] 2010	1421	.620	.026
Araya et al,[34] 2009	4334	.500	.013
Coventry et al,[42] 2010	3243	.500	.021
Bukh et al,[43] 2009	290	.035	.037
Laucht et al,[62] 2009	309	.500	.032
Ritchie et al,[82] 2009	942	.539	.030
Wichers et al,[83] 2009	502	.380	.033
Zhang et al,[45] 2009	792	.998	.016
Hammen et al,[13] 2010	346	.376	.034
Goldman et al,[50] 2010	984	.020	.055
Total	**26 921**		
Average sample size	961		.03

the childhood maltreatment and specific stressors groups, the designs of the primary studies were generally similar. In contrast, there is marked variation in study design between SLE studies. Some studies asked subjects about SLEs and depressive episodes that occurred decades earlier while others assessed SLEs and depressive episodes soon after they occurred.[33,75] Further, SLE studies vary substantially in what they consider a life event. Another potential reason for the difference in stressor subgroup results is the nature of the stressors studied. Most of the specific stressor studies focused on chronic stressors while the SLE studies focused on acute SLEs. Interestingly, 3 studies have explicitly looked at both acute and chronic stressors in their cohorts and all 3 have found that the evidence for moderating effects was stronger for chronic stressors.[13,19,48]

In addition to the type of stressor studied, the stress assessment method used by investigators emerged as an important variable in our analysis. In particular, we found that the evidence of genetic moderation was stronger among studies that used objective measures or in-person interviews to assess stress than among studies that used self-report questionnaires.

There are limitations to our study, most of which follow from the meta-analytic technique that we used. Because we combined studies at the level of *P* values, errors or bias present in the statistical tests performed in the primary studies could potentially affect the results of this meta-analysis. We guarded against finding false-positive results due to this potential bias by using an average of reported *P* values when authors performed separate tests on different sample subgroups or multiple depression measures. Further, when authors performed multiple tests but only reported the significance results for a subset of these tests, we assumed that $P=1$ for the unreported tests. The fact that we found a nonsignificant result when we applied our meta-analytic technique to the set of studies included in a previous nonsignificant meta-analysis suggests that statistical bias from primary studies did not unduly affect our results. Another drawback of using this meta-analytic method is that we were unable to estimate the magnitude of the genetic effect and, in particular, how the interaction effect size compares with any genetic main effect.[17]

Against this background, the present study suggests that there is cumulative and replicable evidence that 5-HTTLPR moderates the relationship between stress and depression. Our findings, particularly the identification of important study characteristics that influence study outcome (stressor type and stress assessment method), can provide guidance for the design of future gene × environment interaction studies. While there is certainly variation between study results, there is hope that a new generation of studies purpose-built for testing this specific hypothesis will improve replicability and shed light on sources of inconsistency. In the meantime, the results of our inclusive analysis of studies in this controversial area underscore the importance of including all relevant studies in meta-analyses and highlight the utility of incorporating environmental exposures in genetic association studies.

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REFERENCES

1. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003;301(5631):386-389.
2. Holden C. Behavioral genetics: getting the short end of the allele. *Science*. 2003; 301(5631):291-293.
3. Munafò MR, Durrant C, Lewis G, Flint J. Gene X environment interactions at the serotonin transporter locus. *Biol Psychiatry*. 2009;65(3):211-219.
4. Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA*. 2009;301(23):2462-2471.
5. Zammit S, Owen MJ, Lewis G. Misconceptions about gene-environment interactions in psychiatry. *Evid Based Ment Health*. 2010;13(3):65-68.
6. Clayton DG. Prediction and interaction in complex disease genetics: experience in type 1 diabetes. *PLoS Genet*. 2009;5(7):e1000540.
7. Lotrich FE, Lenze E. Gene-environment interactions and depression. *JAMA*. 2009; 302(17):1859-1860, author reply 1861-1862.
8. Rutter M, Thapar A, Pickles A. Gene-environment interactions: biologically valid pathway or artifact? *Arch Gen Psychiatry*. 2009;66(12):1287-1289.
9. Kaufman J, Gelernter J, Kaffman A, Caspi A, Moffitt T. Arguable assumptions, debatable conclusions. *Biol Psychiatry*. 2010;67(4):e19-e20, author reply e21-e23.
10. Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psychiatry*. 2010;167(5):509-527.
11. Uher R, McGuffin P. The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. *Mol Psychiatry*. 2010;15(1):18-22.
12. Munafò MR, Durrant C, Lewis G, Flint J. Defining replication: a response to Kaufman and colleagues. *Biol Psychiatry*. 2010;67(4):e21-e23. doi:10.1016/j.biopsych .2009.09.035.
13. Hammen C, Brennan PA, Keenan-Miller D, Hazel NA, Najman JM. Chronic and acute stress, gender, and serotonin transporter gene-environment interactions predicting depression symptoms in youth. *J Child Psychol Psychiatry*. 2010; 51(2):180-187.
14. Kilpatrick DG, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Resnick HS, Roitzsch J, Boyle J, Gelernter J. The serotonin transporter genotype and social support and moderation of posttraumatic stress disorder and depression in hurricane-exposed adults. *Am J Psychiatry*. 2007;164(11):1693-1699.
15. 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*. 2007;10(4): 437-447.
16. Scheid JM, Holzman CB, Jones N, Friderici KH, Nummy KA, Symonds LL, Sikorskii A, Regier MK, Fisher R. Depressive symptoms in mid-pregnancy, lifetime stressors and the 5-HTTLPR genotype. *Genes Brain Behav*. 2007;6(5): 453-464.
17. Clarke H, Flint J, Attwood AS, Munafò MR. Association of the 5-HTTLPR genotype and unipolar depression: a meta-analysis. *Psychol Med*. 2010;40(11): 1767-1778.
18. Kaufman J, Yang BZ, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Krystal JH, Gelernter J. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biol Psychiatry*. 2006;59(8):673-680.
19. 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(5): 529-535.
20. Jacobs N, Kenis G, Peeters F, Derom C, Vlietinck R, van Os J. Stress-related negative affectivity and genetically altered serotonin transporter function: evidence of synergism in shaping risk of depression. *Arch Gen Psychiatry*. 2006;63(9): 989-996.
21. Sjöberg RL, Nilsson KW, Nordquist N, Ohrvik J, Leppert J, Lindström L, Oreland L. Development of depression: sex and the interaction between environment and a promoter polymorphism of the serotonin transporter gene. *Int J Neuropsychopharmacol*. 2006;9(4):443-449.
22. Cicchetti D, Rogosch FA, Sturge-Apple ML. Interactions of child maltreatment and serotonin transporter and monoamine oxidase A polymorphisms: depressive symptomatology among adolescents from low socioeconomic status backgrounds. *Dev Psychopathol*. 2007;19(4):1161-1180.
23. Aguilera M, Arias B, Wichers M, Barrantes-Vidal N, Moya J, Villa H, van Os J, Ibáñez MI, Ruipérez MA, Ortet G, Fañanás L. Early adversity and 5-HTT/BDNF genes: new evidence of gene-environment interactions on depressive symptoms in a general population. *Psychol Med*. 2009;39(9):1425-1432.
24. Stein MB, Schork NJ, Gelernter J. Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology*. 2008;33(2):312-319.
25. Kim JM, Stewart R, Kim SW, Yang SJ, Shin IS, Yoon JS. Modification by two genes of associations between general somatic health and incident depressive syndrome in older people. *Psychosom Med*. 2009;71(3):286-291.
26. Kohen R, Cain KC, Mitchell PH, Becker K, Buzaitis A, Millard SP, Navaja GP, Teri L, Tirschwell D, Veith R. Association of serotonin transporter gene polymorphisms with poststroke depression. *Arch Gen Psychiatry*. 2008;65(11):1296-1302.
27. Lenze EJ, Munin MC, Ferrell RE, Pollock BG, Skidmore E, Lotrich F, Rogers JC, Quear T, Houck P, Reynolds CF III. Association of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) genotype with depression in elderly persons after hip fracture. *Am J Geriatr Psychiatry*. 2005;13(5):428-432.
28. Nakatani D, Sato H, Sakata Y, Shiotani I, Kinjo K, Mizuno H, Shimizu M, Ito H, Koretsune Y, Hirayama A, Hori M; Osaka Acute Coronary Insufficiency Study Group. Influence of serotonin transporter gene polymorphism on depressive symptoms and new cardiac events after acute myocardial infarction. *Am Heart J*. 2005; 150(4):652-658.
29. Otte C, McCaffery J, Ali S, Whooley MA. Association of a serotonin transporter polymorphism (5-HTTLPR) with depression, perceived stress, and norepinephrine in patients with coronary disease: the Heart and Soul Study. *Am J Psychiatry*. 2007;164(9):1379-1384.
30. Ramasubbu R, Tobias R, Buchan AM, Bech-Hansen NT. Serotonin transporter gene promoter region polymorphism associated with poststroke major depression. *J Neuropsychiatry Clin Neurosci*. 2006;18(1):96-99.
31. Phillips-Bute B, Mathew JP, Blumenthal JA, Morris RW, Podgoreanu MV, Smith M, Stafford-Smith M, Grocott HP, Schwinn DA, Newman MF; Perioperative Genetics and Safety Outcomes Investigative Team. Relationship of genetic variability and depressive symptoms to adverse events after coronary artery bypass graft surgery. *Psychosom Med*. 2008;70(9):953-959.
32. McCaffery JM, Bleil M, Pogue-Geile MF, Ferrell RE, Manuck SB. Allelic variation in the serotonin transporter gene-linked polymorphic region (5-HTTLPR) and cardiovascular reactivity in young adult male and female twins of European-American descent. *Psychosom Med*. 2003;65(5):721-728.
33. Lotrich FE, Ferrell RE, Rabinovitz M, Pollock BG. Risk for depression during interferon-alpha treatment is affected by the serotonin transporter polymorphism. *Biol Psychiatry*. 2009;65(4):344-348.
34. Araya R, Hu X, Heron J, Enoch MA, Evans J, Lewis G, Nutt D, Goldman D. Effects of stressful life events, maternal depression and 5-HTTLPR genotype on emotional symptoms in pre-adolescent children. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B(5):670-682.
35. Dick DM, Plunkett J, Hamlin D, Nurnberger J Jr, Kuperman S, Schuckit M, Hesselbrock V, Edenberg H, Bierut L. Association analyses of the serotonin transporter gene with lifetime depression and alcohol dependence in the Collaborative Study on the Genetics of Alcoholism (COGA) sample. *Psychiatr Genet*. 2007; 17(1):35-38.
36. Kraus MR, Al-Taie O, Schäfer A, Pfersdorff M, Lesch KP, Scheurlen M. Serotonin-1A receptor gene HTR1A variation predicts interferon-induced depression in chronic hepatitis C. *Gastroenterology*. 2007;132(4):1279-1286.
37. Brummett BH, Boyle SH, Siegler IC, Kuhn CM, Ashley-Koch A, Jonassaint CR, Züchner S, Collins A, Williams RB. Effects of environmental stress and gender on associations among symptoms of depression and the serotonin transporter gene linked polymorphic region (5-HTTLPR). *Behav Genet*. 2008;38(1):34-43.
38. Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Juhasz G, Bagdy G. New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events, and depressive phenotype. *Biol Psychiatry*. 2008; 64(6):498-504.
39. Wichers M, Kenis G, Jacobs N, Mengelers R, Derom C, Vlietinck R, van Os J. The BDNF Val(66)Met x 5-HTTLPR x child adversity interaction and depressive symptoms: an attempt at replication. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B(1):120-123.
40. Aslund C, Leppert J, Comasco E, Nordquist N, Oreland L, Nilsson KW. Impact of the interaction between the 5HTTLPR polymorphism and maltreatment on adolescent depression: a population-based study. *Behav Genet*. 2009;39(5):524-531.
41. Bull SJ, Huezo-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, Miyazaki C, Alexander N, Hotopf M, Cleare AJ, Norris S, Cassidy E, Aitchison KJ, Miller AH, Pariante CM. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. *Mol Psychiatry*. 2009;14(12):1095-1104.

42. Coventry WL, James MR, Eaves LJ, Gordon SD, Gillespie NA, Ryan L, Heath AC, Montgomery GW, Martin NG, Wray NR. Do 5HTTLPR and stress interact in risk for depression and suicidality? item response analyses of a large sample. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B(3):757-765.
43. Bukh JD, Bock C, Vinberg M, Werge T, Gether U, Vedel Kessing L. Interaction between genetic polymorphisms and stressful life events in first episode depression. *J Affect Disord*. 2009;119(1-3):107-115.
44. McCaffery JM, Duan QL, Frasure-Smith N, Barhdadi A, Lespérance F, Théroux P, Rouleau GA, Dubé MP. Genetic predictors of depressive symptoms in cardiac patients. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B(3):381-388.
45. Zhang K, Xu Q, Xu Y, Yang H, Luo J, Sun Y, Sun N, Wang S, Shen Y. The combined effects of the 5-HTTLPR and 5-HTR1A genes modulates the relationship between negative life events and major depressive disorder in a Chinese population. *J Affect Disord*. 2009;114(1-3):224-231.
46. Benjet C, Thompson RJ, Gotlib IH. 5-HTTLPR moderates the effect of relational peer victimization on depressive symptoms in adolescent girls. *J Child Psychol Psychiatry*. 2010;51(2):173-179.
47. Kumsta R, Stevens S, Brookes K, Schlotz W, Castle J, Beckett C, Kreppner J, Rutter M, Sonuga-Barke E. 5HTT genotype moderates the influence of early institutional deprivation on emotional problems in adolescence: evidence from the English and Romanian Adoptee (ERA) study. *J Child Psychol Psychiatry*. 2010;51(7):755-762.
48. Sen S, Kranzler HR, Krystal JH, Speller H, Chan G, Gelernter J, Guille C. A prospective cohort study investigating factors associated with depression during medical internship. *Arch Gen Psychiatry*. 2010;67(6):557-565.
49. Sugden K, Arseneault L, Harrington H, Moffitt TE, Williams B, Caspi A. Serotonin transporter gene moderates the development of emotional problems among children following bullying victimization. *J Am Acad Child Adolesc Psychiatry*. 2010;49(8):830-840.
50. Goldman N, Glei DA, Lin Y-H, Weinstein M. The serotonin transporter polymorphism (5-HTTLPR): allelic variation and links with depressive symptoms. *Depress Anxiety*. 2010;27(3):260-269.
51. Mössner R, Henneberg A, Schmitt A, Syagailo YV, Grässle M, Hennig T, Simantov R, Gerlach M, Riederer P, Lesch KP. Allelic variation of serotonin transporter expression is associated with depression in Parkinson's disease. *Mol Psychiatry*. 2001;6(3):350-352.
52. Hedges LV, Olkin I. *Statistical Methods for Meta-Analysis*. New York, NY: Academic; 1985.
53. Richards JB, Waterworth D, O'Rahilly S, Hivert MF, Loos RJ, Perry JR, Tanaka T, Timpson NJ, Semple RK, Soranzo N, Song K, Rocha N, Grundberg E, Dupuis J, Florez JC, Langenberg C, Prokopenko I, Saxena R, Sladek R, Aulchenko Y, Evans D, Waeber G, Erdmann J, Burnett MS, Sattar N, Devaney J, Willenborg C, Hingorani A, Witteman JC, Vollenweider P, Glaser B, Hengstenberg C, Ferrucci L, Melzer D, Stark K, Deanfield J, Winogradow J, Grassl M, Hall AS, Egan JM, Thompson JR, Ricketts SL, König IR, Reinhard W, Grundy S, Wichmann HE, Barter P, Mahley R, Kesaniemi YA, Rader DJ, Reilly MP, Epstein SE, Stewart AF, Van Duijn CM, Schunkert H, Burling K, Deloukas P, Pastinen T, Samani NJ, McPherson R, Davey Smith G, Frayling TM, Wareham NJ, Meigs JB, Mooser V, Spector TD; GIANT Consortium. A genome-wide association study reveals variants in ARL15 that influence adiponectin levels. *PLoS Genet*. 2009;5(12):e1000768.
54. Hwang D, Rust AG, Ramsey S, Smith JJ, Leslie DM, Weston AD, de Atauri P, Aitchison JD, Hood L, Siegel AF, Bolouri H. A data integration methodology for systems biology. *Proc Natl Acad Sci U S A*. 2005;102(48):17296-17301.
55. Leung M, Cheung C, Yu K, Yip B, Sham P, Li Q, Chua S, McAlonan G. Gray matter in first-episode schizophrenia before and after antipsychotic drug treatment: anatomical likelihood estimation meta-analyses with sample size weighting. *Schizophr Bull*. 2009;(Sep):16.
56. Majeti R, Becker MW, Tian Q, Lee TL, Yan X, Liu R, Chiang JH, Hood L, Clarke MF, Weissman IL. Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. *Proc Natl Acad Sci U S A*. 2009;106(9):3396-3401.
57. Liptak T. On the combination of independent tests. *Magyar Tudományos Akadémia Matematikai Kutató Intezetenek Kozlemenyei*. 1958;3:171-197.
58. Stouffer S, Suchman E, DeVinnery L, Star S, Williams R. *The American Soldier, Volume I: Adjustment During Army Life*. Princeton, NJ: Princeton University Press; 1949.
59. Koziol JA, Tuckwell HC. A Bayesian method for combining statistical tests. *J Statist Plann Inference*. 1999;78(1-2):317-323. doi:10.1016/S0378-3758(98)00222-5.
60. 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(1):101-111.
61. Chipman P, Jorm AF, Prior M, Sanson A, Smart D, Tan X, Easteal S. No interaction between the serotonin transporter polymorphism (5-HTTLPR) and childhood adversity or recent stressful life events on symptoms of depression: results from two community surveys. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B(4):561-565.
62. Laucht M, Treutlein J, Blomeyer D, Buchmann AF, Schmid B, Becker K, Zimmermann US, Schmidt MH, Esser G, Rietschel M, Banaschewski T. Interaction between the 5-HTTLPR serotonin transporter polymorphism and environmental adversity for mood and anxiety psychopathology: evidence from a high-risk community sample of young adults. *Int J Neuropsychopharmacol*. 2009;12(6):737-747.
63. Taylor SE, Way BM, Welch WT, Hilmert CJ, Lehman BJ, Eisenberger NI. Early family environment, current adversity, the serotonin transporter promoter polymorphism, and depressive symptomatology. *Biol Psychiatry*. 2006;60(7):671-676.
64. Zalsman G, Huang YY, Oquendo MA, Burke AK, Hu XZ, Brent DA, Ellis SP, Goldman D, Mann JJ. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *Am J Psychiatry*. 2006;163(9):1588-1593.
65. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, Scheet P, Gwinn M, Williamson RE, Zou GY, Hutchings K, Johnson CY, Tait V, Wiens M, Golding J, van Duijn C, McLaughlin J, Paterson A, Wells G, Fortier I, Freedman M, Zecevic M, King R, Infante-Rivard C, Stewart A, Birkett N. Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE Statement. *Hum Genet*. 2009;125(2):131-151.
66. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 2007;370(9596):1453-1457.
67. Detsky AS, Naylor CD, O'Rourke K, McGeer AJ, L'Abbé KA. Incorporating variations in the quality of individual randomized trials into meta-analysis. *J Clin Epidemiol*. 1992;45(3):255-265.
68. Kavvoura FK, Ioannidis JP. Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. *Hum Genet*. 2008;123(1):1-14.
69. Jüni P, Witschi A, Bloch R, Egger M. The hazards of scoring the quality of clinical trials for meta-analysis. *JAMA*. 1999;282(11):1054-1060.
70. Rosenthal R. The file drawer problem and tolerance for null results. *Psychol Bull*. 1979;86(3):638-641. doi:10.1037//0033-2909.86.3.638.
71. Pham B, Platt R, McAuley L, Klassen TP, Moher D. Is there a "best" way to detect and minimize publication bias? an empirical evaluation. *Eval Health Prof*. 2001;24(2):109-125.
72. Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, Plomin R, Craig IW. Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol Psychiatry*. 2004;9(10):908-915.
73. Grabe HJ, Lange M, Wolff B, Völzke H, Lucht M, Freyberger HJ, John U, Cascorbi I. 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(2):220-224.
74. 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(3):224-229.
75. Wilhelm K, Mitchell PB, Niven H, Finch A, Wedgwood L, Scimone A, Blair IP, Parker G, Schofield PR. Life events, first depression onset and the serotonin transporter gene. *Br J Psychiatry*. 2006;188:210-215.
76. Cervilla JA, Molina E, Rivera M, Torres-González F, Bellón JA, Moreno B, Luna JD, Lorente JA, Mayoral F, King M, Nazareth I, Gutiérrez B; PREDICT Study Core Group. 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. *Mol Psychiatry*. 2007;12(8):748-755.
77. Chorbov VM, Lobos EA, Todorov AA, Heath AC, Botteron KN, Todd RD. Relationship of 5-HTTLPR genotypes and depression risk in the presence of trauma in a female twin sample. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B(6):830-833.
78. Kim JM, Stewart R, Kim SW, Yang SJ, Shin IS, Kim YH, Yoon JS. Interactions between life stressors and susceptibility genes (5-HTTLPR and BDNF) on depression in Korean elders. *Biol Psychiatry*. 2007;62(5):423-428.
79. Middeldorp CM, de Geus EJ, Beem AL, Lakenberg N, Hottenga JJ, Slagboom PE, Boomsma DI. Family based association analyses between the serotonin transporter gene polymorphism (5-HTTLPR) and neuroticism, anxiety and depression. *Behav Genet*. 2007;37(2):294-301.
80. Power T, Stewart R, Ancelin ML, Jaussent I, Malafosse A, Ritchie K. 5-HTTLPR genotype, stressful life events and late-life depression: no evidence of interaction in a French population. *Neurobiol Aging*. 2010;31(5):886-887.
81. Ressler KJ, Bradley B, Mercer KB, Deveau TC, Smith AK, Gillespie CF, Nemeroff CB, Cubells JF, Binder EB. Polymorphisms in CRHR1 and the serotonin transporter loci: gene x gene x environment interactions on depressive symptoms. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B(3):812-824.

82. Ritchie K, Jaussent I, Stewart R, Dupuy AM, Courtet P, Ancelin ML, Malafosse A. Association of adverse childhood environment and 5-HTTLPR genotype with late-life depression. *J Clin Psychiatry*. 2009;70(9):1281-1288.
83. Wichers M, Geschwind N, Jacobs N, Kenis G, Peeters F, Derom C, Thiery E, Delespaul P, van Os J. Transition from stress sensitivity to a depressive state: longitudinal twin study. *Br J Psychiatry*. 2009;195(6):498-503.
84. Zhang JL, Yang JF, Chan P. No association between polymorphism of serotonin transporter gene and depression in Parkinson's disease in Chinese. *Neurosci Lett*. 2009;455(3):155-158.
85. Grassi L, Rossi E, Cobianchi M, Aguiari L, Capozzo M, Martinis E, Nanni MG, Lelli G, Schillani G, Biancosino B, Giraldi T. Depression and serotonin transporter (5-HTTLPR) polymorphism in breast cancer patients. *J Affect Disord*. 2010; 124(3):346-350.
86. Uher R, McGuffin P. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. *Mol Psychiatry*. 2008;13(2):131-146.
87. Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nat Neurosci*. 2005;8(6):828-834.
88. Heinz A, Smolka MN, Braus DF, Wrase J, Beck A, Flor H, Mann K, Schumann G, Büchel C, Hariri AR, Weinberger DR. Serotonin transporter genotype (5-HTTLPR): effects of neutral and undefined conditions on amygdala activation. *Biol Psychiatry*. 2007;61(8):1011-1014.
89. Munafò MR, Brown SM, Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biol Psychiatry*. 2008;63(9):852-857.
90. Li Q. Cellular and molecular alterations in mice with deficient and reduced serotonin transporters. *Mol Neurobiol*. 2006;34(1):51-66.
91. Li Q, Wichems C, Heils A, Van De Kar LD, Lesch KP, Murphy DL. Reduction of 5-hydroxytryptamine (5-HT)(1A)-mediated temperature and neuroendocrine responses and 5-HT(1A) binding sites in 5-HT transporter knockout mice. *J Pharmacol Exp Ther*. 1999;291(3):999-1007.
92. Mössner R, Schmitt A, Hennig T, Benninghoff J, Gerlach M, Riederer P, Deckert J, Lesch KP. Quantitation of 5HT3 receptors in forebrain of serotonin transporter deficient mice. *J Neural Transm*. 2004;111(1):27-35.
93. Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy DL, Lanfumey L, Hamon M, Martres MP. Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. *Eur J Neurosci*. 2000;12(7):2299-2310.
94. Persico AM, Mengual E, Moessner R, Hall FS, Revay RS, Sora I, Arellano J, DeFelipe J, Gimenez-Amaya JM, Conciatori M, Marino R, Baldi A, Cabib S, Pascucci T, Uhl GR, Murphy DL, Lesch KP, Keller F, Hall SF. Barrel pattern formation requires serotonin uptake by thalamocortical afferents, and not vesicular monoamine release. *J Neurosci*. 2001;21(17):6862-6873.
95. Barr CS, Newman TK, Schwandt M, Shannon C, Dvoskin RL, Lindell SG, Taubman J, Thompson B, Champoux M, Lesch KP, Goldman D, Suomi SJ, Higley JD. Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. *Proc Natl Acad Sci U S A*. 2004;101(33):12358-12363.
96. Carneiro AM, Airey DC, Thompson B, Zhu CB, Lu L, Chesler EJ, Erikson KM, Blakely RD. Functional coding variation in recombinant inbred mouse lines reveals multiple serotonin transporter-associated phenotypes. *Proc Natl Acad Sci U S A*. 2009; 106(6):2047-2052.